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Effects of amino acids on the properties of white-fleshed and orange-fleshed Beauregard sweet potato starch

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EFFECTS OF AMINO ACIDS ON THE PROPERTIES OF WHITE-FLESHED AND ORANGE-FLESHED BEAUREGARD SWEET POTATO STARCH

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ABSTRACT

This study assessed the effects of amino acid additives, aspartic acid, leucine, lysine, and methionine, on the pasting and thermal characteristics of white-fleshed and orange-fleshed Beauregard sweet potato starches. Also, the white-fleshed and the orange-fleshed sweet potato starches were compared for any differences in their resistant starch and crystalline properties. This study was performed using Differential Scanning Calorimetery (DSC), Rapid Visco Analysis (RVA), X-ray Diffraction (XRD), and Resistant Starch Determination.

The orange-fleshed starch granules began to gelatinize at a lower temperature (56.8°C) than the white-fleshed starch (70.1°C), but the two starches needed the same amount of energy to gelatinize. Lysine increased the gelatinization temperature of the orange-fleshed starch. The addition of lysine and aspartic acid increased the gelatinization temperatures of the white-fleshed starch.

In comparing pasting characteristics, the orange-fleshed starch was found to be easier to cook, had a lower potential for retrogradation, and was less stable during heating than the white-fleshed starch. The RVA analysis showed that the charged amino acids, aspartic acid and lysine, had more of an affect on the two starches than did the neutral amino acids, leucine and methionine. Aspartic acid had similar effects on both starches, making them less stable during cooking and lowering the potential for retrogradation. Lysine, when added to the orange-fleshed sweet potato starch, decreased the breakdown, allowing for more stability during cooking.

The results of the resistant starch determination revealed that the white-fleshed sweet potato starch had significantly more resistant starch than the orange-fleshed starch in gelatinized and ungelatinized forms. Lysine increased the amount of resistant starch in

the orange-fleshed starch, while leucine and methionine decreased the resistant starch in the orange-fleshed and the white-fleshed starches, respectively.

The crystallinity patterns of the white-fleshed and orange-fleshed sweet potato starches were found to be the A-type pattern before gelatinization, and the B-type pattern after gelatinization. The addition of aspartic acid and methionine did not alter the crystalline pattern of either of the starches and caused a decrease in crystallinity, while the addition of leucine and lysine increased the crystallinity of the white-fleshed sweet potatoes.

CHAPTER 1

INTRODUCTION

Starch is one of the main components of the human diet, and represents the primary source of energy for humans. Starch can be collected from many vegetable crop sources including wheat, corn, potatoes, and rice, and is used as a storage molecule in plants. Starch can be extracted from many plant sources for use in a wide variety of foods. Starches from different plant sources exhibit different thermal and physicochemical properties. The products that a starch will be used in are determined by the properties of that particular starch. The starch's use is determined by several factors including the amylose/amylopectin ratio and the structure of the starch (Katayama et al., 2002 and Englyst, 2005). Starch's physical, thermal, and pasting properties are assessed and will determine its particular usefulness. Modifications can be made to the different starches in order to achieve a more useful end product; these include alterations to a starch's gelatinization temperature, and changes to the pasting characteristics. Much research has centered on the modifications of starches and the determination of factors that can change a starch's properties. It has been found that additives including proteins, lipids, and amino acids can change the properties of a starch (Liang and King, 2003).

Also, the modification of a starch's resistant starch content may prove to be very useful in terms of promoting the health aspects of a starch. Resistant starch is the starch that is resistant to digestion by enzymes within the body. This type of starch offers many health benefits such as a reduction in the risk of both cancer and heart problems and aid with problems of obesity (Sajilata et al., 2006). Modifications to produce more of this

type of starch have been attempted with the addition of amino acids in rice starch (An, 2005).

One vegetable crop that is used for starch production is the sweet potato (*Ipomoea batatas*). The sweet potato is the seventh most produced and consumed crop in the world behind wheat, rice, maize, potato, barley, and cassava. The sweet potato is grown in over 100 countries worldwide, and has become known as an "insurance crop" because it can outlast many other crops during droughts, floods, and other natural disasters and is able to grow in a wide variety of soil types and climates (Prakash, 1994 and Ishiguro et al., 2003).

The United States is 10th in the world in terms of sweet potato production, producing an estimated 600,000 tons of sweet potato annually. Within the United States, Louisiana produces 24% of the nation's sweet potatoes with almost all of the sweet potatoes produced in the state being a variety called the Beauregard sweet potato. This Louisiana crop accounts for over half of the state's vegetable crop income, which adds $100 million to the state's economy (Lucier et al., 2002). The sweet potato is consumed in a variety of ways from the whole fresh root, to canned products, to products such as chips and snacks made from the sweet potato's starch (Patrick, 1996).

This research studied two types of Beauregard sweet potato starch, one starch was from the orange-fleshed Beauregard sweet potato while the second type was extracted from the white-fleshed Beauregard sweet potato. These two starches were examined for differences in pasting and thermal characteristics, resistant starch content, and crystallinity. Also, amino acids, such as aspartic acid, leucine, lysine, and methionine were added to each starch in order to determine whether these amino acids affected the aforementioned properties of the starches. The orange-fleshed and white-fleshed sweet

potato starches were examined using a variety of methods: thermal properties by Differential Scanning Calorimetery (DSC), the pasting characteristics by Rapid Visco Analysis (RVA), the resistant starch content by resistant starch determination using the Megazyme method, and the crystallinity by X-ray Diffraction (XRD).

CHAPTER 2

LITERATURE REVIEW

2.1. CARBOHYDRATES

Carbohydrates are a major source of energy and nutrition in most people's daily diets (Annison and Topping, 1994). Currently, in the United States, the average daily diet is composed of 50% carbohydrates, 35% fat, and 15% protein (Higgins, 2004). Carbohydrates are beneficial to one's health only when they are unrefined such as in the case of whole grains and fruits and vegetables. However, many times the grains are refined in order to break down the cell walls before being used in many breakfast cereals and bakery products. The refining process greatly lowers the amount of dietary fiber present in the end product. Not only is fiber lost, but also many micronutrients are depleted, and the sugars and starches in the food are disrupted and made more easily digestible in the small intestine. The result of greater digestibility of carbohydrates in the small intestine leads to a greater glycemic response within the body (Englyst, 2005). A high or rapid glycemic response means that there is a large release of insulin following the consumption of a particular food. The insulin released in large quantities in the body prevents stored fat from being used and may also encourage the feeling of hunger. In contrast, unrefined carbohydrates promote a slow glycemic response, which decreases the amount of insulin released: this response reduces hunger, makes stored fat more accessible and could help in the overall management of weight (Tapsell, 2004).

2.1.1. Starch

Starch, a storage carbohydrate found in plant sources, is a polymer of D-glucose. It is found in granular form with the size and shape of the granules dependant on the particular plant species; some starches, such as corn starch, have small, spherical granules

around 2μm in diameter, while others, such as potato, are large and oval with a diameter of up to 100 μm. The size of the starch granule affects the functionality of the starch by altering characteristics such as swelling and digestibility (Moorthy, 2002). The starch granules are built up in layers around a central core, called a hilum (deMan, 1999). The layers alternate between amorphous and crystalline regions (Katayama et al., 2002). The granules range in crystallinity from 15 to 45% crystalline. The crystallinity of the starch can be observed through x-ray diffraction and is formed from the intertwining of amylopectin with linear chains of glucose into a double helix. Several forms of crystalline structure exist within the starch, A, B, C, and V types as well as intermediates between the four types (deMan, 1999). The various crystalline types differ in the packing of their double stranded helices as well as in the amount of associated water. The different types of crystalline structures are also individually associated with a particular source: A-type is found in cereal starches, B-type comes from tuber and high amylose starches, and C-type is found in legume starches (Annison and Topping, 1994).

The starch granules are composed of two polymers, amylose and amylopectin. The proportion of amylose to amylopectin depends on the source of the starch as well as many other factors including the conditions in which the starch has been held. The ratio of the two polymers and the way in which they interact affects the properties of the particular starch and how much resistant starch will be found within the food product, and will also have an effect on the digestibility of the particular starch (Murugesan et al, 1993 and Englyst, 2005). Native, unmodified starch is insoluble in water unless heat is applied to the system. When heated, the starch is solubilized in the water and the starch undergoes many changes, including gelatinization, pasting, retrogradation (Thomas and Atwell, 1998).

2.1.1.1. Gelatinization

The gelatinization of a starch is the first in a series of changes that occur to the starch upon heating with water. During this process, the starch granules begin to uptake the water and a disruption occurs in the molecular order of the starch. Gelatinization causes the starch granules to swell and, therefore, increasing the viscosity of the solution. This process is irreversible and can be seen by the loss of birefringence of the starch granules, which is a sign that the crystalline structure of the starch has been disrupted (Thomas and Atwell, 1998). The gelatinization properties of any particular starch will dictate what type of applications the starch could be used for (Katayama, 2002).

2.1.1.2. Pasting

If the process of gelatinization is allowed to continue, pasting will occur. Pasting is said to occur when the largest percentage of granules are swollen, but still intact. This is known as peak viscosity, when the mixture reaches its maximum viscosity. During this process, amylose leaches out of the starch granules followed by the release of some amylopectin as heating continues. After pasting, the starch granules begin to breakdown causing an increase in the release of both amylose and amylopectin, at this point, the structural integrity of the granules is lost (Thomas and Atwell, 1998). The pasting properties of a starch often determine how a starch will be used in industry (Katayama, 2002).

2.1.1.3. Retrogradation

Depending on the amylose content of the starch, either a paste or gel will form upon cooling. A high amylose content starch will set into a firm gel. The process of forming gels upon cooling is caused by the reassociation of amylose and, to a lesser extent, amylopectin. Amylose is the main component that is said to retrograde (Figure

2.1); its linear structure can reassociate tightly forming a harder, firmer gel (Thomas and Atwell, 1998). Starches that contain higher amylopectin to amylose ratios tend to retrograde much slower than starches that have a high percentage of amylose; this is due to the highly branched nature of amylopectin that takes longer periods of time to reassociate in a tight manner (Moorthy, 2002). Retrogradation can have a major effect on the overall quality and shelf-life stability of food products. Retrogradation is often an undesirable side effect of starch gels; this process when found in bread and other bakery products in known as staling and negatively effects the product (Katayama et al., 2002). A retrograded starch often exhibits the B-type crystalline pattern even when no amylose is present (i.e. waxy starch) (Annison and Topping, 1994).

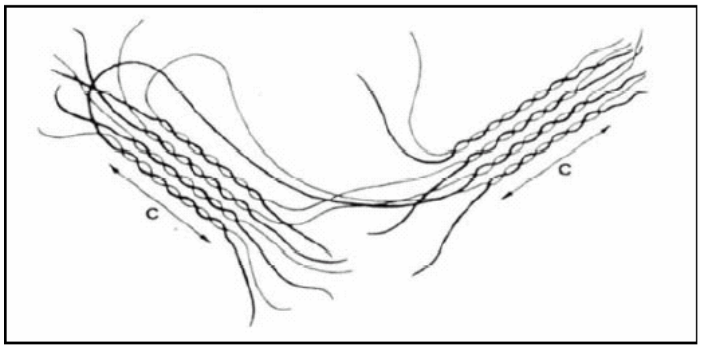

Figure 2.1. Schematic representation of retrogradation of amylose.
Adapted from Sajilata et al. (2006)

2.1.2. Amylose

Amylose is a fraction of starch which is composed of repeating glucose molecules linked with α-D(1-4) linkages (Figure 2.2.). Amylose is generally a straight chained or linear polysaccharide that can have a degree of polymerization of up to DP 6000 and a molecular mass of around 105 g/mol (Sajilata et al., 2006). The amylose content in native starch can range anywhere from 0 to almost 50%.

Amylose forms stiff and generally irreversible gels that will only reverse upon heating to autoclave temperatures (110-160˚C). These polymers do complex readily through the formation of hydrogen bonds between molecules. The tightly bonded structures and intimate associations promote the stiffness and irreversibility of the gels (Zobel, 1988a).

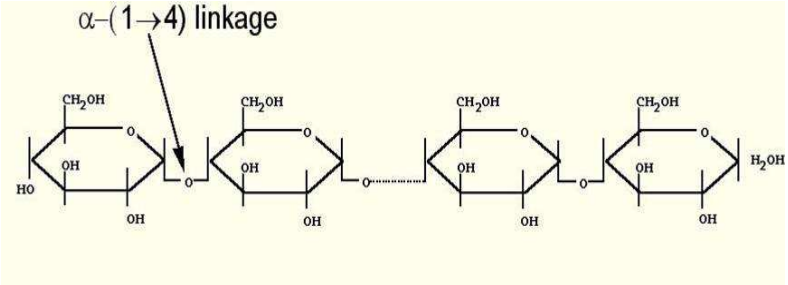

Figure 2.2. Structure of Amylose
Adapted from Nowjee (2004)

2.1.3. Amylopectin

Amylopectin is the second fraction that is found within starch. This polysaccharide is a polymer with glucose molecules linked together with α-D(1-4) and α-D(1-6) linkages (Figure 2.3.). Amylopectin is highly branched and has a degree of polymerization of DP 2 million and a molecular weight of around 109 g/mol making it one of the largest molecules found in nature. The structure of amylopectin is characterized by a central chain of glucose molecules held together with α-D(1-4) linkages with branches at every 20-25 glucose units that come off of the main chain with α-D(1-6) linkages (Sajilata et al., 2006). The amount of amylopectin present in a starch can be as low as 50% and as high as 100%. Starches with 100% amylopectin are known as waxy starches.

Amylopectin forms soft, reversible gels; these polymers do not complex readily. The softer gels are due to the highly branched nature of the polymer making interactions less favorable and fewer in number. The temperature required to reverse an amylopectin gel can range anywhere from room temperature to 90˚C, depending on the degree of polymerization and the number of branches of the particular amylopectin (Zobel, 1988a).

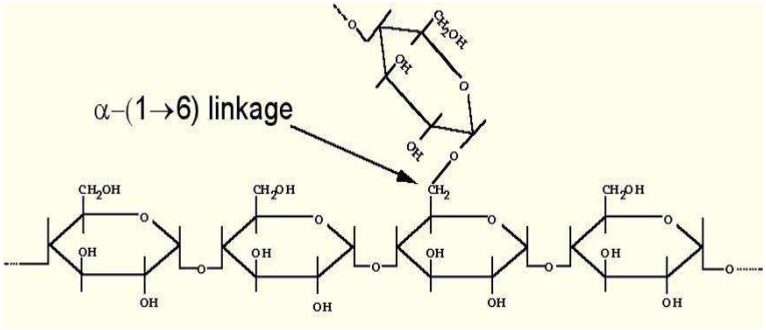

Figure 2.3. Structure of Amylopectin
Adapted from Nowjee (2004)

2.1.4. Effect of Protein on Starch

Liang and King (2003) found that amino acid additives affected the properties of rice starch. Positive, negative, and neutral amino acids were used in their study. The positive additives along with the negative ones showed a greater influence than the neutral amino acids on pasting properties. The various pasting properties include pasting temperature, peak viscosity, time to peak, minimum viscosity, and breakdown value, all of which reflect how the starch would act during processing and cooking. Overall, Liang and King (2003) concluded that adding various amino acids, depending mainly on charge, could influence the cooked and processed properties of foods that contain starch. Also, research has demonstrated a relationship between crystallinity patterns and the amount of resistant starch present. Through the use of X-ray diffraction, the crystallinity of starch

may be observed. A study by Botham et al. (1995) indicated that the crystal structure of resistant starch was very similar to that of the amylose fraction within starch. An increase in the amount of amylose present within a food translates to a possible increase in the amount of resistant starch; therefore, an increase in the crystallinity of a starch could indicate the presence of more resistant starch (Botham et al., 1995).

The addition of amino acids to starch was shown to influence the starch's crystallinity; these changes in the crystallinity may be due to an increase in the amount of resistant starch (Botham et al., 1995 and Liang and King, 2003). If the samples with added amino acids did contain more resistant starch than the native samples, these modified starches could have a greater impact on health by promoting the fiber-like effects of resistant starch (Liang and King, 2003). Also, Hamaker and Griffin (1993) studied deproteinized starch, and found that these starches had a higher viscosity due to a greater amount of swelling. The proteins were found to have an inhibitory role when it came to the swelling potential of the granules. Ito et al. (2004) reported that charged molecules such as amino acids could interact electrostatically with the starch granules and possibly changing their thermal stability. The researchers also found that the charged amino acids, both positive and negative, had a greater effect on the gelatinization characteristics of the starch then did the neutral amino acids (Ito et al., 2004).

2.1.5. Resistant Starch

Resistant starch is one form of starch that acts more like dietary fiber than other starches (Goldring, 2004). Resistant starch is any starch that passes through the small intestine undigested and moves through to the large intestine where it is then used as a substrate for fermentation (Higgins, 2004). The term resistant is used to indicate that the starch is unharmed and neither degraded by digestive enzymes nor by stomach acids.

Although it may act like fiber within the body, resistant starch has several advantages that fiber does not provide. Resistant starch does not retain much water and therefore can be used in places where fiber may impart a soggy texture such as in cookies, crackers, and other low-moisture food products. Also, resistant starch has a smooth mouthfeel, unlike fiber, which is gritty, and does not mask or alter flavors and textures of foods (Ranhotra, 1996). Resistant starches are useful in the production of low-carbohydrate foods and also products targeting special populations such as diabetics (Brown, 2004).

2.1.5.1. Types of Resistant Starch

There exist four types or sub classifications of resistant starch; these are RS1, RS2, RS3, and RS4. RS1 is a starch that is inaccessible, physically, to digestion. This includes partly milled grains and seeds and also some very dense starchy products. This form of resistant starch can be measured by the difference in the amount of glucose released during enzymatic digestion from homogenized and non-homogenized food samples. RS1 can be used in a wide variety of food products because it is very heat stable during the cooking process. See Figure 2.4 for a representation of RS1. RS2 is found in granular sources that have not been gelatinized and are resistant to enzymatic digestion. RS2 is measured as the difference between the glucose responses during enzyme digestion of a boiled homogenized food versus that of an unboiled non-homogenized food. This type of resistant starch can be found in raw vegetables such as bananas and potatoes. See Figure 2.5 for the structure of RS2. RS3 is starch, mainly amylose, which has gelatinized and retrograded to become indigestible upon cooling. Most bakery products that are moist heated contain substantial amounts of RS3. This fraction can be measured as the starch that is resistant to degradation both by boiling and enzymatic digestion. See Figure 2.1. for a representation of RS3.

RS4 is starch that is resistant to digestion because of some chemical modification including the formation of bonds other than the α-D(1-4) and α-D(1-6) linkages. Most modified starches can be included in this fraction of resistant starch (Goldring, 2004 and Sajilata et al., 2006). All products containing starch have resistant starch in them, but the amount and form (i.e., RS1, RS2, etc.) depends on many factors including storage times and temperatures, the methods used to process the products, and the sources from which the starch was obtained (Brown, 2004). Also, several extrinsic factors affect the resistant starch. These include the amount and thoroughness of chewing, transit time within the gastrointestinal tract, amount of starch present, other food ingested concurrently with the resistant starch, and the concentration of amalyse in the body (Englyst, 1992). Some common foods that contain resistant starch include grains, vegetables, cereals, seeds, legumes, and nuts (Goldring, 2004).

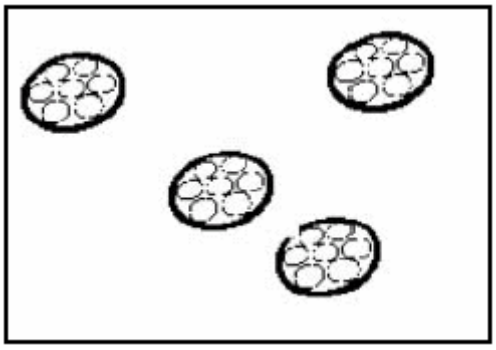

Figure 2.4. Structure of RS1.

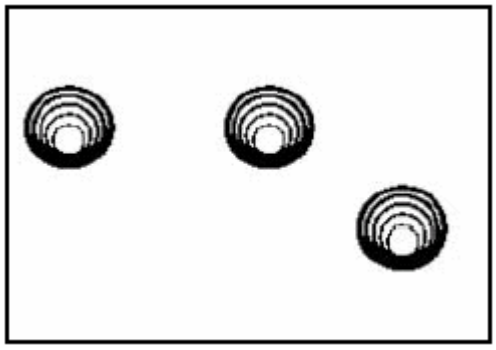

Figure 2.5. Structure of RS2.

Both figures adapted from Sajilata et al.(2006).

2.1.5.2. Legal and Health Issues

In animal studies, high amounts of resistant starch administered orally caused several effects including a decrease in both body weight and food intake, but these effects are not considered to be adverse. The intake of resistant starch does not decrease mineral

retention in humans, but at high levels an increase in flatulence was reported (Goldring, 2004). In order to have any beneficial effects on health, an estimated 15-20g/day of resistant starch is necessary in the diet (Brown, 2004). Resistant starch does not have any legal definitions attached, and must only appear on food labels if the resistant starch was induced through chemical modifications. In these circumstances, the term "chemically modified starch" must be included. The addition of resistant starch does not affect the carbohydrate content within a food product, and may reduce the caloric value if the resistant starch is included in and categorized as an insoluble fiber (Goldring, 2004).

Resistant starch has many of the same health benefits as dietary fiber, but has been found to be more appealing than fiber when used in food products. Resistant starch may reduce the risk of cancer in the digestive tract, lower lipid levels in the blood, and also helps with constipation and osteoporosis (Goldring, 2004). Resistant starch acts as a prebiotic in the body, this means it serves as a substrate for the growth and proliferation of probiotic bacteria. These bacteria live in the gastrointestinal tract, are beneficial to the host and can improve the host's overall health. Along with all of these benefits, resistant starch offers better taste, texture, and appearance in foods than does added dietary fiber (Brown, 2004). Resistant starch can be very useful in a low-carbohydrate diet as it produces a small glycemic response and also when carbohydrates are replaced with resistant starch in a food product, the total calories of the food may be reduced since the resistant starch travels through the body undigested (Goldring, 2004).

2.2. SWEET POTATO

The sweet potato is a storage root that belongs to the family *Convolvulaceae*, the morning glory family. Although its name may suggest otherwise, the sweet potato has no relation to the regular potato tuber. The sweet potato originated in Central America

where it was domesticated over 5000 years ago (CIP, 2006). This root is grown in over 100 countries due to its adaptability to many different soil types and growing conditions, and is currently the seventh most important food crop in the world after wheat, rice, maize, potato, barley, and cassava (Prakash, 1994 and Ishiguro et al., 2003). Also, the sweet potato has a shorter growth period than other tuber or root crops and can be grown year round under the proper conditions. This particular crop has become known for its usefulness in times of crisis and as such has come to be known as an "insurance crop" (FAO, 1990). The sweet potato has been used throughout history during famines when staple crops have fallen prey to disease. This root has a long shelf life and can be stored at room temperature for up to nine months once cured (Adam, 2005).

The sweet potato, due to its high nutritive qualities, is being used in health campaigns all over the world. Some of these include fighting childhood blindness and other diseases such as measles and malaria due to lack of vitamin A in sub-Saharan Africa and south and west Asia. The sweet potato is also used to help nourish newly weaned children in Peru who do not get proper nourishment once off of breast milk (Mukherjee, 2002 and Espinola et al, 1998). The sweet potato offers a host of macro and micronutrients as well as fiber. Beta-carotene, a precursor to vitamin A, is found in abundance in the sweet potato, along with high levels of many antioxidants. Also, the sweet potato is a good source of complex carbohydrates, vitamin C, vitamin B6, iron, and potassium and is low in fat (Tsou, 1992). These vitamins and minerals have been shown to help prevent many types of cancer, reduce the risk of heart attack, and protect the body from infection. Antioxidants such as vitamin C have been shown to decrease the number of free radicals within the body. This reduction decreases the risk eye problems, like cataracts, many cancers, and can help slow down the aging process (Patrick, 1996). Table

2.1. summarizes the nutritional aspects of both the average white and orange-fleshed sweet potato. Table 2.2. shows the amino acid content of sweet potatoes.

Table 2.1. Nutritional quality of White and Orange-flesh sweet potato as % of RDA/100g fresh weight. Adapted from Tsou (1992).

	White-Fleshed Sweet Potato Starch	Orange-Fleshed Sweet Potato Starch
Nutrient	% of RDV	% of RDV
Protein	0.63	0.79
Riboflavin	1.73	1.37
Thiamin	0.80	0.79
Calcium	0.65	1.83
Iron	2.19	2.39
Vitamin A	-	238
Vitamin C	6.08	7.97

Table 2.2. Amino Acid Content of Sweet Potatoes, reported as mg/g of crude protein. Adapted from FAO (1990).

Amino Acid	Histidine	Leucine	Lysine	Methionine + Cysteine
mg/g	13	54	34	28
Amino Acid	Phenylalanine + Tyrosine	Threonine	Tryptophan	Valine
mg/g	62	38	14	45

2.2.1. Sweet Potato Industry

Approximately 600,000 tons of sweet potatoes are produced annually in the United States, making it 10th in the world in terms of sweet potato production. Within the United States, North Carolina, Louisiana, and California are the top 3 states to produce sweet potatoes. About 24 percent of the nations sweet potatoes are grown in Louisiana, which accounts for over half of the state's vegetable cash income and translates to $100 million for the states economy. Much of the roots grown in Louisiana are sent for processing, mainly canning (Lucier et al, 2002). The Beauregard variety of sweet potato is the major variety grown in Louisiana, accounting for almost one hundred percent of the

sweet potato crop. This variety is often referred to as a yam even though it bears no relation to the true yam tuber. This nomenclature is used to differentiate the Louisiana sweet potato which has a more moist flesh from the dry sweet potatoes grown elsewhere in the country. There are two "yam" processors located in Louisiana as well as thirty fresh market shippers (Patrick, 1996). When the roots are processed, much waste is created in the form of peelings and rejected sweet potatoes. Colston and Smallwood (1974) monitored a sweet potato processor in North Carolina where they found that 33% of the raw potato brought into the facility ended up as waste. The mostly organic waste is harbored in lagoons. At present, there is not much use for this waste and it must be discarded, but much of this waste could be used to produce sweet potato starch in a very cost effective manner and would also eliminate the unnecessary waste of so many sweet potato pieces.

2.2.2. Sweet Potato Uses

The sweet potato is used within several different markets around the United States and the world. The fresh roots can be prepared several different ways and are eaten whole. Also, the unprocessed sweet potato can be used in a myriad of ways within recipes, such as in casseroles, salads, sauces, soups, desserts, and as a dipping vegetable. The processed sweet potato flesh can be found in French fries, patties, and twice baked potatoes and also as a dehydrated product. Canned sweet potatoes are very popular and can be found sliced, candied, or mashed. Also, many baby foods now contain sweet potato (Lucier et al., 2002). Twenty percent of the sweet potatoes produced throughout the world are used for their starch (Ishiguro et al., 2003). Sweet potato starch can be found on the list of ingredients for many food products including breads, cookies,

noodles, crackers, pies, cakes, and chips. Alcohol can also be made through the distillation of sweet potatoes (Lucier et al., 2002).

2.2.3. Sweet Potato Starch

Sweet potato starch is a very important food product material around the world with an emphasis of use in Asian countries (Moorthy, 2002). The usefulness of sweet potato starch ranges from one variety to another. The starch's use is determined by several factors including the amylose/amylopectin ratio, granule size, and the structure of the starch (Katayama et al., 2002). The sweet potato starch granules vary in shape from polygonal, round to oval with diameters ranging from 2 to 25 μm (Moorthy, 2002). The average sweet potato starch granule has an amylose content of around 18% (Tsou, 1992). Sweet potato starch has been characterized as having various x-ray diffraction patterns ranging from A, C, and an intermediate between the A and C types. Also, the absolute crystallinity of this starch is reportedly around 38%. Depending on variety, sweet potato starch has a known range of amylose content of 8.5-38%, a gelatinization temperature of 63-79˚C, and a pasting temperature of 58.5 to 90˚C (Moorthy, 2002).

Much research has been performed on the sweet potato across the world including much research on gelatinization, pasting, and retrogradation, and also on the various factors that can influence the properties of the sweet potato starch. Kaur et al. (2006) studied the gelatinization patterns of sweet potato starch and found that starches with high amylose content had a higher gelatinization temperature and a lower enthalpy than starches with lower amylose contents. The researchers reasoned that the correlation between amylose content with gelatinization temperature and enthalpy suggests that there must be a higher percentage of amorphous regions rather than crystalline regions within the amylose. This lack of crystalline regions raises the gelatinization temperature (Kaur

et al., 2006). The crystallinity of a starch granule imparts stability to the system that can be disturbed only through higher temperatures. Also, a correlation between the harvesting of sweet potatoes, early to late during the sweet potato growth period, and the enthalpy needed to gelatinize the starch has been noticed.

Research found that the earlier a sweet potato was harvested in its growing season, the lower the enthalpy will be to produce a gelatinized sample (Moorthy, 2002). Jangchud et al. (2003) found that the peak temperatures of pasting varied between sweet potato starches because of the variety of starch granule sizes that exist. Starches with larger granules were correlated with lower pasting temperatures, but also with an increase in the amount of swelling observed (Jangchud et al., 2003). Collado et al. (1999) found that the pasting viscosity and amylose content of sweet potatoes was negatively correlated. Moorthy (2002) reported that sweet potato starches having lower amylose contents or those starches with smaller amylopectin molecules retrograded slower than those starches having a high amylose content.

CHAPTER 3

EFFECTS OF AMINO ACID ADDITIVES ON GELATINIZATION CHARACTERISTICS OF SWEET POTATO STARCHES BY USING DIFFERENTIAL SCANNING CALORIMETRY (DSC)

3.1. INTRODUCTION

Starch granules are insoluble in cold water, but when heat is added to the system, the granules begin to swell and absorb some of the water and the starch begins to solubilize in the water. At this point the birefringence that had been characteristic of the granules under a light microscope disappears, and the irreversible process of gelatinization is said to occur. During gelatinization, the crystalline structure of the starch granule is disrupted and the molecular order is lost (deMan, 1999 and Thomas and Atwell, 1998). The viscosity of the starch and water solution begins to increase during this process. This increase in viscosity makes the starch solution, now a paste, suitable for use in various food products (Thomas and Atwell, 1998).

The gelatinization characteristics of a starch are very important in the function that starch will play in a particular food. It determines cooking behavior as well as the characteristics of the food in which it is found (Tester and Morrison, 1990). The temperature at which gelatinization occurs can be influenced by many factors, including heating rate, presence of other compounds, pH, and the size, shape, and source of the starch granules. Starches containing granules of larger sizes generally gelatinize at a lower temperature than starches that have smaller sized granules (deMan, 1999). Also, the plant source from which the starch is extracted can influence the gelatinization temperature. The gelatinization temperatures of roots and tubers are generally lower than those of cereal and grain starches (Thomas and Atwell, 1998).

Differential Scanning Calorimetry (DSC) is a useful method in analyzing the thermal properties of various substances, including the gelatinization of starch. The DSC measures the onset temperature, the peak temperature, and the conclusion temperature of gelatinization as well as the total enthalpy needed to gelatinize the sample. Different varieties of the same sample species can have considerable variations in their gelatinization temperatures. Collado et al. (1999) studied forty-four types of sweet potato native to the Philippines and found that there existed vast ranges in the DSC characteristics of these potatoes. Onset temperature had a range of 61.3-70°C, peak temperature had a range of 70.2-77°C, and a range of 80.7-88.5°C was observed for the conclusion temperature.

It has been shown that the addition of amino acids to a starch can affect the starch's gelatinization characteristics. Liang (2001) found that the addition of various amino acids to rice starch increases the gelatiniztion parameters of the starch including the peak temperature and conclusion temperature. These effects were most often seen with charged amino acids including aspartic acid and lysine (Liang, 2001). An (2005) studied the effects on gelatinization when lysine was added to rice starch. She found that the gelatinization characteristics, onset temperature, peak temperature, and conclusion temperature, increased due to this addition, while the total enthalpy needed to gelatinize the starch decreased. Ito et al. (2004) also found that it was possible to regulate the gelatinization temperatures of potato starch through the addition of amino acids. The researchers found that the addition of neutral amino acids had little effect on the potato starch, but adding charged amino acids such as lysine cause significant increases in the gelatinization characteristics of the starch.

Sweet potatoes were used in this research because of the large-scale production of sweet potatoes worldwide. The sweet potato is also a very hardy crop that can withstand many climates and growing conditions. The sweet potato is an excellent source of starch, but this starch has gone largely unstudied when it comes to the addition of various additives.

The objectives of this study were 1) to determine the effect of various amino acid additives on the thermal properties of sweet potato starches and 2) to investigate the differences between the white and orange flesh sweet potato starches through the use of DSC.

3.2. MATERIALS AND METHODS

3.2.1. Materials

Sweet potato starch was extracted from white and orange-fleshed Beauregard sweet potatoes grown at the Louisiana State University AgCenter research station and were harvested in October 2006. The amino acids used in this study were purchased from Sigma Chemical Company (St. Louis, Missouri). The amino acids used included one positive (Lysine), one negative (Aspartic Acid), one neutral (Leucine), and one sulfur-containing amino acid (Methionine). These particular amino acids were chosen based on past research (Liang, 2001 and An, 2005).

3.2.2. Sweet Potato Starch Extraction

White-fleshed and orange-fleshed Beauregard sweet potatoes were peeled and sliced. Then in batches of 400g the sweet potatoes were blended at high speed in a Waring Blender for 2 minutes with 500mL of distilled water. The resulting mixture was then passed through a 150μm sieve. The pulp left atop the sieve was further washed with 500mL of distilled water. Three batches were combined before the next step. The filtrate

(approximately 3000mL) was divided between four 800mL centrifuge bottles. These bottles were then centrifuged at 3000 x g at 2˚C for 10 minutes in a Thermo Electronic Corporation Sorvall RC 6 Plus Centrifuge (Waltham, MA) fitted with a Sorvall SLC-4000 Super-Lite rotor, after this, the liquid was discarded and the orange layer manually scraped off of the starch. The bottles were refilled with distilled water, the starch resuspended, and centrifuged in the same manner. Each batch was centrifuged and washed with distilled water a total of four times. After the fourth centrifugation, the precipitate (starch) was removed from the bottle, frozen at -80˚C, and freeze dried to a fine powder. All batches were combined to form a uniform sample. This same process was then repeated for the white-fleshed Beauregard sweet potatoes. The sweet potato starch was stored in hermetically sealed plastic bags.

3.2.3. Proximate Analysis

White-flesh and orange-flesh Beauregard sweet potato starch was examined for lipid content using chloroform methanol (method 983.23, AOAC 1995), protein content using thermal conductivity on a Model 2410 Nitrogen Analyzer (Perkin Elmer, Norwalk, CT) (method 992.15, AOAC 1995), ash content using a Phoenix Microwave Ashing System (CEM, Matthews, NC) (method 920.153, AOAC 1995), and moisture content using a SMART System 5 (CEM, Matthews, NC) (method 985.14, AOAC 1995). The carbohydrate content was determined by using the formula: 100- (% protein + % fat + % moisture + % ash) = % carbohydrate. Trace metal content of the native white-fleshed and orange-fleshed sweet potato starch was quantified through the use of ICP (Inductively Coupled Plasma). Each starch sample was analyzed in duplicate. The replicates were then averaged.

3.2.4. Amylose Content Determination

Quantifying the amylose content of the sweet potato starch was done following the Megazyme Amylose/Amylopectin Assay Procedure (Megazyme International, Ireland). This method is based on the method developed by Yun and Matheson (1990). All reagent solutions/suspensions, buffers, and solvents were prepared beforehand following the instructions given by Megazyme.

Twenty to twenty-five mg of starch sample were accurately weighed into 10mL screw capped tubes. The analyses were performed in triplicate on the white and orange sweet potato starches that had no additives added. One mL of DMSO was added to the tubes while it was gently mixed on low speed on a vortex mixer. The tubes were capped and heated in a boiling water bath until the samples were completely dispersed (about 1 minute). The contents of the sealed tubes were vigorously mixed at high speed on a vortex mixer, after which the tubes were placed in a boiling water bath and heated for 15 minutes with intermittent high-speed stirring on a vortex mixer. The tubes were then stored at room temperature for 5 minutes and 2mL of 95% ethanol were added with continuous stirring on a vortex mixer. A further 4mL of ethanol were added; the tubes were capped and inverted to mix. The tubes were allowed to stand for 15 minutes at room temperature to allow a starch precipitate to form. The tubes were centrifuged at 2000g for 5 minutes, the supernatant discarded and the tubes were drained on tissue paper for 10 minutes, ensuring that all of the ethanol had drained. The starch pellet was used in the subsequent amylose and starch determinations. Two mL of DMSO were added to the starch pellets. The tubes were placed in a boiling water bath for 15 minutes and mixed occasionally. On removing the tubes from the boiling water bath, 4mL of Concanavalin A solvent (30 mL of a 600mM, pH 6.4 sodium acetate buffer diluted to 100mL with

distilled water) were immediately added, the tubes were mixed thoroughly and then the tube contents were quantitatively transferred to 25mL volumetric flasks. The contents were diluted to volume with Concanavalin A solvent, this mixture is Solution A.

One mL of Solution A, from the above section, was transferred to a 2.0mL Eppendorf microfuge tube, 0.5mL of Concanavalin A solution (200mg ConA, a lectin protein, in 50mL ConA solvent) was added, then the tubes were capped and gently mixed by repeated inversion. The tubes were allowed to stand for 1 hour at room temperature, and then centrifuged at 14,000g for 10min in a microfuge at room temperature. One mL of the supernatant was transferred to 15mL centrifuge tubes. Three mL of 100mM sodium acetate buffer, pH 4.5 were then added. This reduced the pH to 5. The contents were mixed; the tubes were lightly stoppered and heated in a boiling water bath for 5min to denature the Con A. The tubes were placed in a water bath at 40°C and allowed to equilibrate for 5 minutes, then 0.1 mL of amyloglucosidase (3300U)/α-amylase (500U) enzyme mixture was added and the tubes were incubated at 40°C for 30 minutes. The tubes were centrifuged at 2000g for 5 minutes. To 1.0mL aliquots of the supernatant, 4mL of GOPOD Reagent (glucose oxidase (>12,000U) plus peroxidase (>650U) and 4-aminoantipyrine (80mg) diluted in 20mL of GOPOD Reagent Buffer (potassium phosphate buffer (1M,pH 4.7), p-hydroxybenzoic acid (0.22M) and sodium azide (0.02% w/w))) was added. The tubes were then incubated at 40°C for 20 minutes. A Reagent Blank was made by adding 1.0mL of 100mM sodium acetate buffer to 4.0mL of GOPOD Reagent; the D-Glucose Controls were made by adding 0.1mL of D-glucose standard solution (1mg/mL) and 0.9mL of sodium acetate buffer to 4.0mL of GOPOD reagent. The Reagent Blank and the D-Glucose Controls were incubated concurrently with the

starch samples. The absorbance of each sample and the D-glucose controls were read at 510nm against the reagent blank.

3.2.5. Differential Scanning Calorimeter Analysis

A Differential Scanning Calorimeter (DSC) Q10 (TA Instruments, New Castle, DE) was used to determine the gelatinization properties of the sweet potato starch samples. Ten mg of sample were weighed and placed into aluminum DSC pans. Twenty μL of distilled water was then added to each pan, making a 1:2 ratio of starch to water. For the samples containing amino acids, 6% starch weight basis additive solutions were made and were added to the DSC pans in place of the distilled water (Liang and King, 2003). The amino acid solutions were made by combining 300mg of amino acid with 10mL of distilled water. The amino acid solutions were then mixed and allowed to equilibrate for 10 minutes before use. One pan containing only 20 μL of distilled water served as a reference. The pans were then placed within the DSC apparatus. The procedure began by equilibrating the samples at 25˚C then they were heated to 150˚C at 5˚C/minute ramp. Afterwards, the graphs were analyzed to identify any patterns or trends relating to the amino acid additives used. All DSC analyses were performed in triplicate.

3.2.6. Statistical Analysis

SAS (Statistical Analysis System) software (version 8.0) was used to analyze the DSC data. Standard deviation, ANOVA (Analysis of Variance), and Tukey's Studentized Range (HSD) were used to examine the effects of the amino acid additives on the white and orange sweet potato starches, on a $p \leq 0.05$ level. The abbreviations used were White for the white-flesh sweet potato starch, Orange for the orange-flesh sweet potato starch, NOAA for no amino acid additives, ASPA for aspartic acid, LEU for

leucine, LYS for lysine, METH for methionine, OT for onset temperature, PT for peak temperature, CT for conclusion temperature, and EN for enthalpy.

3.3. RESULTS AND DISCUSSION

3.3.1. Proximate Analysis

The results of the proximate analyses on both sweet potato starches are shown in Table 3.1. The orange-fleshed sweet potato starch had a higher amount of both fat and ash, but had a lower total amount of carbohydrates. Both starches did contain a fairly small level of lipid and no protein, but the vast majority of the product was in carbohydrate form. Moorthy (2002) reported on the chemical composition of various sweet potato varieties. He found that on average, sweet potato starch contained a range of 0.006- 0.26 % fat and 0.05- 1.3% ash. The results from the present study reveal values close to these known ranges; however, the fat contents of 0.38 and 0.31 for the orange and white sweet potato starches, respectively were slightly higher and the values for ash, 0.002 and 0.0 % for the orange-fleshed and white-fleshed sweet potato starches, respectively, were slightly lower than the known ranges. Table 3.2. shows the results of the trace mineral analysis.

Table 3.1. Proximate Analysis Results

Sample	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate (%)	Amylose (%)
Orange-fleshed Sweet Potato Starch	4.96	0.38	0.00	0.002	97.13	4.59± 0.82
White-fleshed Sweet Potato Starch	2.98	0.31	0.00	0.00	98.19	14.43± 0.46

Table 3.2. Trace Mineral Analysis Results

	White-Fleshed Sweet Potato Starch	Orange-Fleshed Sweet Potato Starch
Aluminum (ppm)	3.34 ± 0.45	3.96 ± 0.46
Boron (ppm)	1 ± 0.00	1.06 ± 0.06

(Table 3.2. continued)

Calcium (%)	0.01 ± 0.00	0.04 ± 0.00
Copper (ppm)	1.14 ± 0.09	4.64 ± 0.62
Iron (ppm)	1.44 ± 0.30	1.54 ± 0.26
Magnesium (%)	0 ± 0.00	0 ± 0.00
Molybdenum (ppm)	1 ± 0.00	1 ± 0.00
Phosphorus (%)	0.02 ± 0.00	0.01 ± 0.00
Potassium (%)	0.02 ± 0.00	0.01 ± 0.00
Sodium (ppm)	29.02 ± 4.22	16.81 ± 0.06
Sulfur (%)	0 ± 0.00	0 ± 0.00
Zinc (ppm)	1.07 ± 0.07	1.11 ± 0.11

3.3.2. Amylose Content

The orange Beauregard sweet potatoes yielded a starch with 4.59% amylose, while the white sweet potato starch contained 14.43 % amylose, Table 3.1. These two amylose values were significantly different ($p<0.05$). Several researchers cite that the amylose content of orange-fleshed sweet potatoes is around 20% (Jangchud et al., 2003 and Moorthy, 2002). This is much higher value than was found in this research, although none of the other studies had been carried out specifically on Beauregard sweet potatoes. Kitahara et al. (2005) analyzed several varieties of Japanese sweet potatoes and found them to contain between 13.6 and 16.2% amylose content.

3.3.3. Differential Scanning Calorimeter Analysis

3.3.3.1. Effects of Amino Acids on Orange-Fleshed Sweet Potato Starch

For the orange-fleshed sweet potato starch, only lysine seemed to have an effect on the gelatinization characteristics of the starch (Table 3.3, Figure 3.1). Moorthy (2002) reported the range of known gelatinization temperatures of sweet potatoes: onset temperature of 61.3-70°C, peak temperature of 70.2-77°C, conclusion temperature of 80.7-88.5°C, and an enthalpy of 10-18.6J/g. The orange-fleshed sweet potato starch values with no amino acid additives falls below the range for both onset and peak

temperatures, but is well within the range for the conclusion temperature and enthalpy. This result is also true for all of the orange-fleshed sweet potato starches that contained amino acids. For onset temperature, there was no difference observed when aspartic acid, leucine, or methionine were added. For this parameter, only lysine had an effect on the starch, which was to increase the onset temperature by 2.2°C. Lysine was also the only amino acid to have an effect on the peak temperature of the orange-fleshed sweet potato starch. In this case, the lysine also had an increasing effect on the peak temperature, with an increase of 3.2°C. The final temperature and enthalpy were not affected by the addition of any of the amino acid additives. These results correlated with findings from An (2005). An (2005) studied the effects of lysine on rice starch, and found that the lysine increased the gelatinization temperatures of both untreated, ozone treated, and oxygen treated rice starch samples. Ito et al. (2004) added lysine, glycine, alanine, and leucine to potato starch in order to determine their effects on gelatinization. The researchers found that all of the charged amino acids, both positive and negative, had similar effects, which was to increase the gelatinization temperatures. They also found that the neutral amino acids had only a weak effect on the gelatinization properties of the starch, if any. Ito et al. (2004) used the amino acids on a 10% starch basis, whereas the amino acids in the present study were used on a 6% starch basis. These results correspond with those in the present study for the positively charged lysine and the neutral acids leucine and methionine, but do not account for aspartic acid that is negatively charged. According to past research, since aspartic acid is a charged amino acid an effect on the gelatinization would be expected, but the results show that for sweet potato, aspartic acid does not have a significant effect on the gelatinization characteristics of the starch (Ito et al., 2004). From these results, it is clear that only the positively

charged lysine had significant effects on the orange sweet potato starch in terms of gelatinization.

Table 3.3. Effects of Amino Acid Additives on Gelatinization of Orange-fleshed Sweet Potato Starch[1]

Amino Acid Additive	Onset Temperature (°C)	Peak Temperature (°C)	Final Temperature (°C)	Enthalpy (J/g)
No Amino Acid	$56.81 \pm 1.21_{b}$	$67.69 \pm 0.51_{b,c}$	$82.88 \pm 2.99_{a}$	$12.51 \pm 3.65_{a}$
Aspartic Acid	$58.09 \pm 1.01_{a,b}$	$68.66 \pm 0.29_{b}$	$82.55 \pm 0.57_{a}$	$10.86 \pm 0.26_{a}$
Leucine	$57.52 \pm 0.15_{a,b}$	$67.67 \pm 0.2_{b,c}$	$79.71 \pm 1.11_{a}$	$9.73 \pm 0.74_{a}$
Lysine	$59.04 \pm 0.22_{a}$	$70.89 \pm 0.22_{a}$	$83.92 \pm 0.81_{a}$	$10.88 \pm 0.67_{a}$
Methionine	$56.77 \pm 0.54_{b}$	$66.42 \pm 0.85_{c}$	$80.93 \pm 1.53_{a}$	$10.70 \pm 1.80_{a}$

[1] Means with the same letter in each column are not significantly different $p>0.05$.

3.3.3.2. Effects of Amino Acids on White-Fleshed Sweet Potato Starch

Similar results were seen in the white sweet potato starch as in the orange-fleshed sweet potato starch in terms of effects of the additives on the starch (Table 3.4, Figure 3.2). The white-fleshed sweet potato starch, however, was within the known range of gelatinization for sweet potatoes as reported by Moorthy (2002). All of the treatment groups with amino acid additives also fell within the ranges of known gelatinization temperatures. The onset temperature of gelatinization was affected by two amino acids, aspartic acid and lysine, which raised the temperature by 2 and 3°C, respectively. Aspartic acid and lysine were also seen to affect the peak temperature, by the same amount as the onset temperature 2 and 3°C, respectively. Both lysine and aspartic acid are charged amino acids. These results correlate to the research of Ito et al. (2004) that found that charged amino acids, both positive and negative, had the effect of raising the gelatinization temperatures of potato starch. They had also found that neutral amino acids had only weak effects on the potato starch, as seen here with leucine and

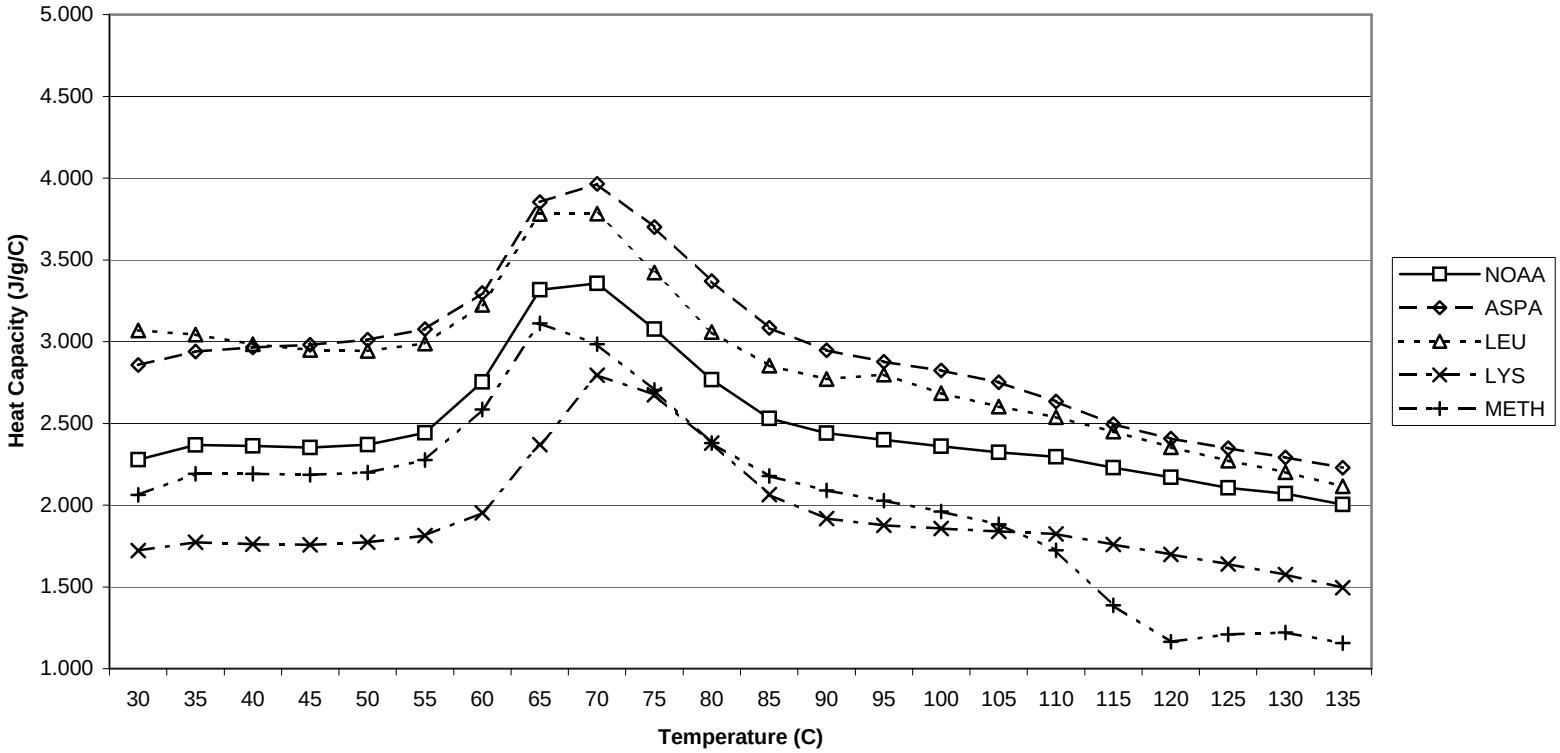

Figure 3.1. Effects of Amino Acid Additives on the Gelatinization of Orange-Fleshed Sweet Potato Starch.

caused an increase in the gelatinization characteristics, especially the addition of the aspartic acid on a 6% dry starch basis. As in the orange-fleshed sweet potato starch, there was no effect seen by the amino acids on either the final temperature or the enthalpy of the white-fleshed sweet potato starch.

Table 3.4. Effects of Amino Acid Additives on Gelatinization of White-fleshed Sweet Potato Starch[1]

Amino Acid Additive	Onset Temperature (°C)	Peak Temperature (°C)	Final Temperature (°C)	Enthalpy (J/g)
No Amino Acid	$70.13 \pm 0.10_{c}$	$76.66 \pm 0.07_{c}$	$85.58 \pm 0.64_{a,b}$	$11.61 \pm 0.46_{a}$
Aspartic Acid	$72.03 \pm 0.76_{b}$	$78.68 \pm 0.25_{b}$	$86.77 \pm 0.70_{a}$	$10.58 \pm 1.74_{a}$
Leucine	$70.70 \pm 0.36_{c}$	$76.65 \pm 0.39_{c}$	$84.07 \pm 1.18_{b}$	$9.02 \pm 1.10_{a}$
Lysine	$73.21 \pm 0.22_{a}$	$79.61 \pm 0.23_{a}$	$87.10 \pm 1.45_{a}$	$10.70 \pm 1.78_{a}$
Methionine	$69.67 \pm 0.21_{c}$	$76.01 \pm 0.39_{c}$	$83.84 \pm 0.47_{b}$	$10.48 \pm 0.52_{a}$

[1]Means in the same column with the same letter are not significantly different at $p>0.05$.

3.3.3.3. Comparison of Gelatinization Characteristics of White-Fleshed and Orange-Fleshed Sweet Potato Starches

In comparing the white-fleshed to the orange-fleshed sweet potato starch, it is obvious that even with no additives, differences exist in the onset temperature and the peak temperature of gelatinization (Table 3.5). The onset temperature of the white-fleshed sweet potato starch was 14°C higher than that of the orange-fleshed sweet potato starch, and its peak temperature was 11°C higher (Figure 3.3). Kitahara et al. (2005) found that the phosphate content of sweet potato starches was correlated positively with the gelatinization temperature. This could mean that the white-fleshed sweet potato starch contains more phosphate groups than the orange-fleshed sweet potato starch, and that the attached phosphate groups could be raising the gelatinization temperatures. In measuring

the phosphate contents of both the white and orange-fleshed sweet potato starches, the white-fleshed starch was found to contain 0.02% phosphate while the orange-fleshed starch contained 0.01%. These values are very similar, however the white-fleshed sweet potato starch does contain more phosphate than the orange-fleshed sweet potato starch and the increased phosphate could have a greater effect in raising the gelatinization temperatures of the white-fleshed sweet potato starch. Kitahara et al. (2005) also found that the gelatinization temperature of sweet potato starch was positively correlated with the amount of apparent amylose in the starch. The white- fleshed sweet potato starch in the present research was found to have an amylose content of 14.43% whereas the orange-fleshed sweet potato starch had only 4.59% amylose. Our research also suggests that the gelatinization temperatures decreased with decreasing amylose content. There were no statistically significant differences between the white-fleshed and orange-fleshed sweet potato starch with regard to the conclusion temperature or the enthalpy needed for gelatinization. There may also possibly exist a difference in either the size or the shape of the white-fleshed and orange-fleshed sweet potato starch granules. Larger granules tend to swell at lower temperatures than smaller starch granules (Kaur et al., 2006). Taking this fact into account, the orange-fleshed sweet potato starch may have larger granules, which could have induced a lower gelatinization temperature than the white-fleshed sweet potato starch, or the orange-fleshed sweet potato starch may have granules of a different and more accessible shape than the white-fleshed sweet potato starch.

The addition of the amino acids to the white and orange sweet potato starches made no noticeable changes to the comparison of the two starches except for the conclusion temperature of gelatinization (Figures 3.4-3.7). All of the onset temperatures

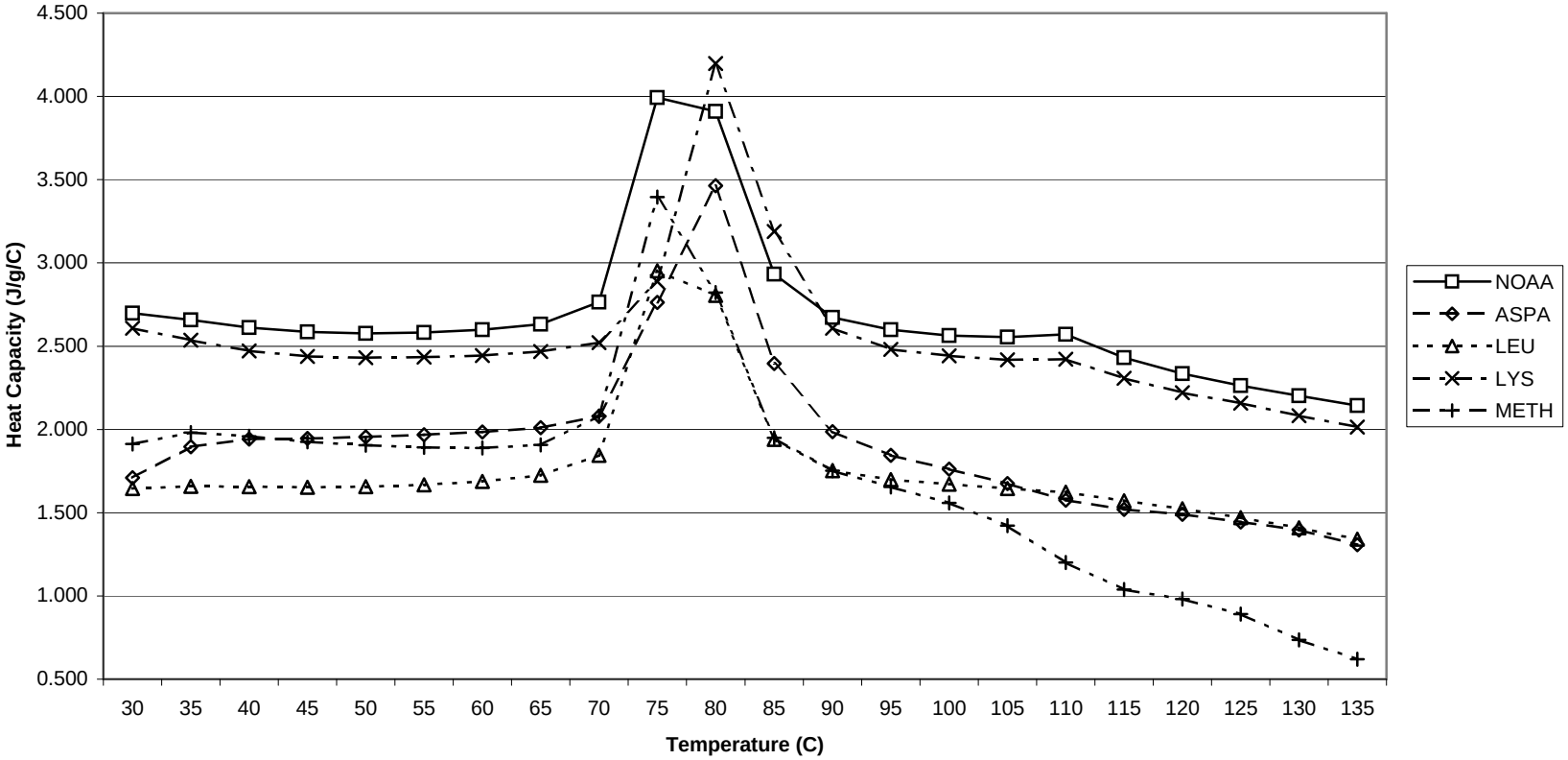

Figure 3.2. Effects of Amino Acid Additives on the Gelatinization of White-Fleshed Sweet Potato Starch.

Table 3.5. Comparing Gelatinization Properties of Orange-fleshed and White-fleshed Sweet Potato Starches with Amino Acid Additives.[1]

Onset Temperature (°C)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange	$56.81 \pm 1.21_a$	$58.09 \pm 1.01_a$	$57.52 \pm 0.15_a$	$59.04 \pm 0.22_a$	$56.77 \pm 0.54_a$
White	$70.13 \pm 0.10_b$	$72.03 \pm 0.76_b$	$70.70 \pm 0.36_b$	$73.21 \pm 0.22_b$	$69.67 \pm 0.21_b$
Peak Temperature (°C)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange	$67.69 \pm 0.51_a$	$68.66 \pm 0.29_a$	$67.67 \pm 0.2_a$	$70.89 \pm 0.22_a$	$66.42 \pm 0.85_a$
White	$76.66 \pm 0.07_b$	$78.68 \pm 0.25_b$	$76.65 \pm 0.39_b$	$79.61 \pm 0.23_b$	$76.01 \pm 0.39_b$
Final Temperature (°C)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange	$82.88 \pm 2.99_a$	$82.55 \pm 0.57_a$	$79.71 \pm 1.11_a$	$83.92 \pm 0.81_a$	$80.93 \pm 1.53_a$
White	$85.58 \pm 0.64_a$	$86.77 \pm 0.70_b$	$84.07 \pm 1.18_b$	$87.10 \pm 1.45_b$	$83.84 \pm 0.47_b$
Enthalpy (J/g)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange	$12.51 \pm 3.65_a$	$10.86 \pm 0.26_a$	$9.73 \pm 0.74_a$	$10.88 \pm 0.67_a$	$10.70 \pm 1.80_a$
White	$11.61 \pm 0.46_a$	$10.58 \pm 1.74_a$	$9.02 \pm 1.10_a$	$10.70 \pm 1.78_a$	$10.48 \pm 0.52_a$

[1]For each parameter, means with the same letter in each column are not significantly different at $p>0.05$.

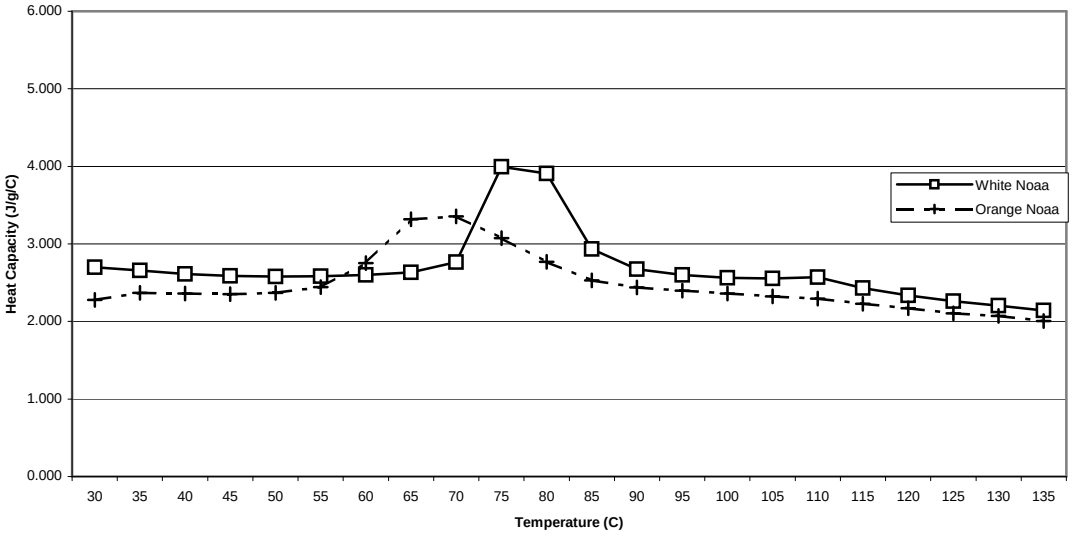

Figure 3.3 DSC Analysis of White-Fleshed and Orange-Fleshed Sweet Potato Starches without Added Amino Acids.

and peak temperatures with and without amino acids added were significantly different between the white-fleshed and orange-fleshed sweet potato starches. The addition of the amino acids resulted in a significantly lower ($p \leq 0.05$) the final temperature of the orange-fleshed sweet potato starch in all cases compared to the white-fleshed sweet potato starch with amino acids. When the enthalpies of gelatinization were compared, none of the treatment groups or the control were found to be significantly different.

3.4. CONCLUSION

This study showed that there are apparent differences in the white-fleshed and orange-fleshed sweet potato starches in terms of their gelatinization characteristics. The orange-fleshed sweet potato starch granules begin to gelatinize at a lower temperature and also completed gelatinization at a lower temperature than those of the white-fleshed sweet potato starch. Both the orange-fleshed and the white-fleshed sweet potato starches used similar amounts of energy to gelatinize as seen by their similar enthalpies.

The addition of amino acids did affect both of the sweet potato starches. The orange-fleshed sweet potato starch was mostly affected by the addition of lysine, a positively charged amino acid, which increased the gelatinization temperature. The addition of leucine, aspartic acid, and methionine had no apparent impact on the orange-fleshed sweet potato starch. The white-fleshed sweet potato starch, however, was affected by both lysine and aspartic acid, positive and negative amino acids, respectively. Both of these amino acids had the same effect on the starch, which was to increase the gelatinization temperature. Overall, the two starches used were more affected by charged amino acids than by the neutral ones.

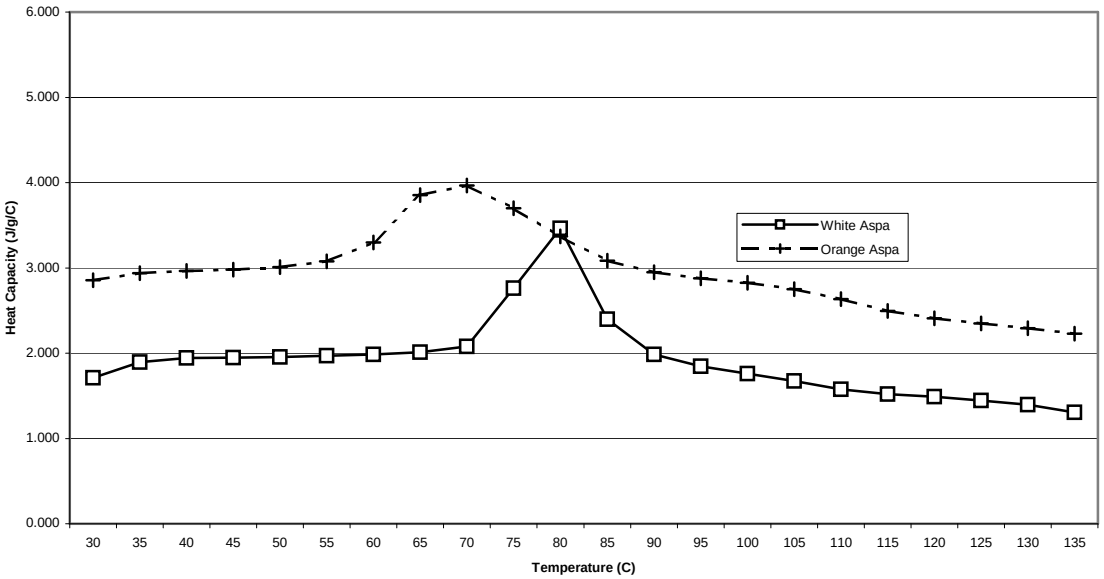

Figure 3.4. DSC Analysis of White-Fleshed and Orange-Fleshed Sweet Potato Starches with Aspartic Acid.

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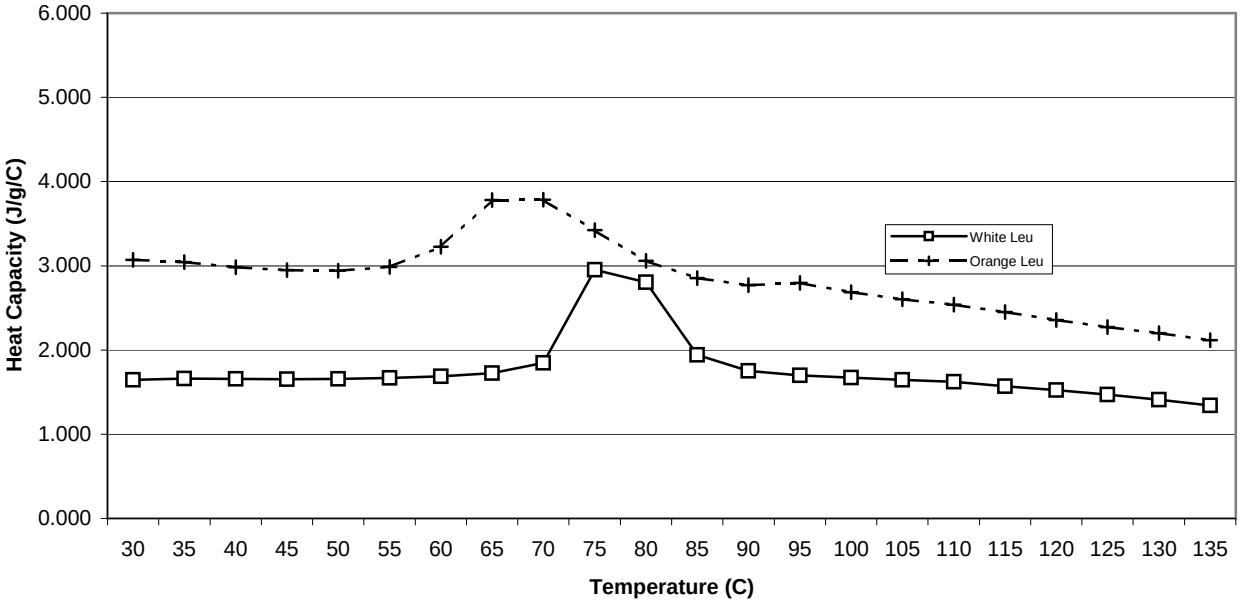

Figure 3.5. DSC Analysis of White-Fleshed and Orange-Fleshed Sweet Potato Starches with Leucine.

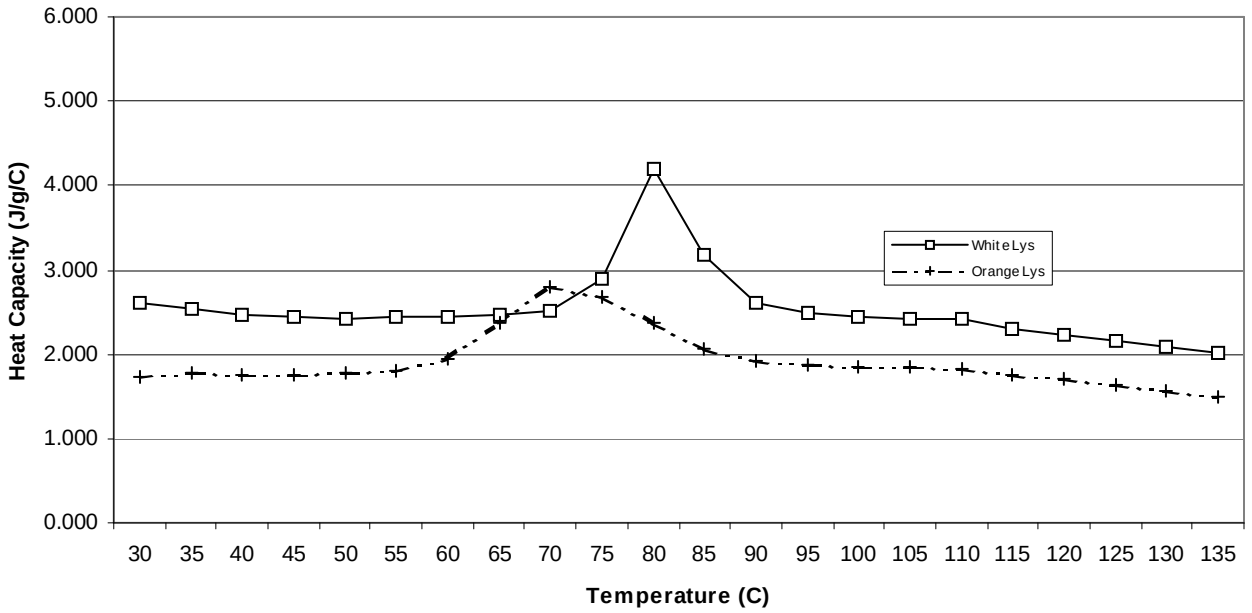

Figure 3.6 DSC Analysis of White-Fleshed and Orange-Fleshed Sweet Potato Starches with Lysine.

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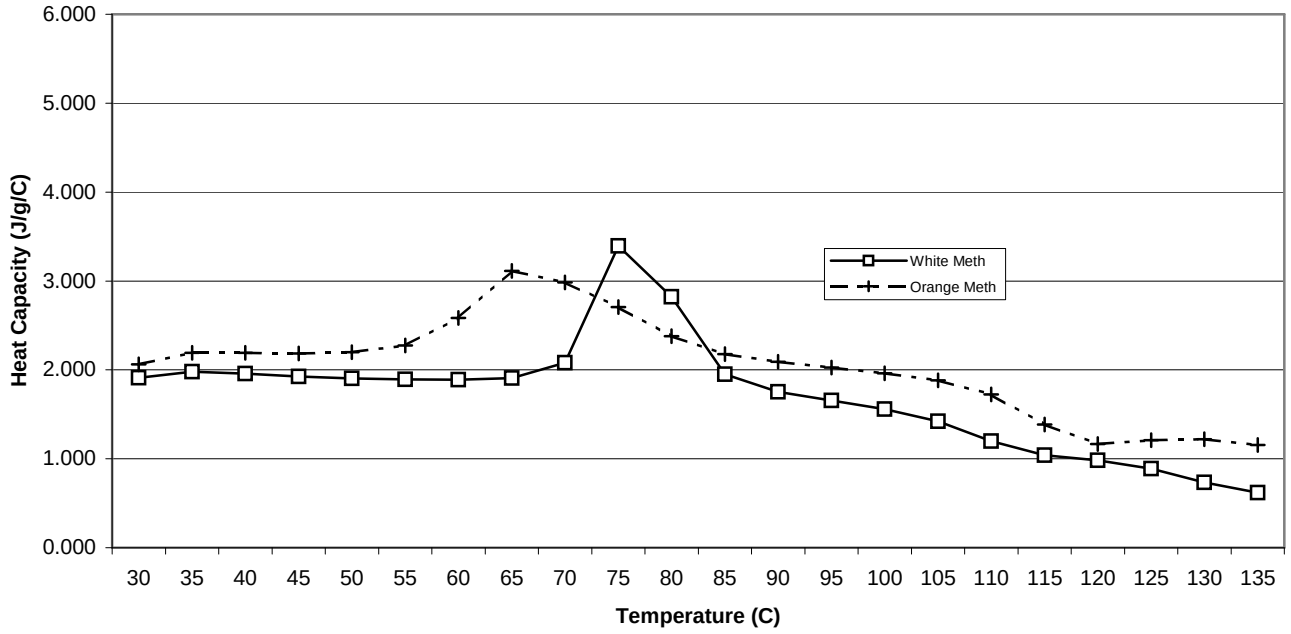

Figure 3.7 DSC Analysis of White-Fleshed and Orange-Fleshed Sweet Potato Starches with Methionine.

CHAPTER 4

EFFECTS OF AMINO ACID ADDITIVES ON PASTING CHARACTERISTICS OF SWEET POTATO STARCHES USING RAPID VISCO ANALYZER (RVA)

4.1. INTRODUCTION

The pasting of a starch occurs after gelatinization, but can also be viewed as a continuation of the gelatinization of a starch. As heating continues after gelatinization, the starch granules become even more swollen causing an increase in the viscosity of the starch paste. The starch is said to be fully pasted when the largest percentage of granules, swollen but still intact, are present; this is also known as the peak viscosity. During the swelling of the starch granules, amylose as well as amylopectin leach out from the granules. After continued heating, the starch granules gradually rupture and breakdown. Once the heating of the starch is complete and cooling begins, the polymers of starch that were released from the starch granules now begin to reassociate. This process is known as retrogradation. Crystalline structures are formed, mainly from amylose molecules in the short term, followed later on by amylopectin (Thomas and Atwell, 1998).

Several different methods exist that can be used to measure the pasting and potential for retrogradation in various starches. These include Rapid Visco Analyzer (RVA) and Brabender viscoamylography (BV). RVA has been shown to be a better method of quantifying pasting characteristics than BV because it couples a small sample size, around 3 grams, with a rapid run time. BV, because of its large sample sizes, can cause errors during the analysis. The RVA gives viscosity curves that show the various pasting characteristics of the starch as they would occur during processing. (Qian and Kuhn, 1999).

Several studies have shown that proteins and added amino acids may influence the pasting characteristics of starches. Hamaker and Griffin (1993) studied the effects of deproteinization on the pasting of starch granules. They found that the removal of proteins from starch caused the starch to have greater viscosity upon pasting because the granules without the protection of proteins were more fragile and allowed a greater amount of water to enter the granule causing increased swelling. An (2005) researched the effects of added amino acids on rice starch and found that the addition of charged amino acids, including aspartic acid and lysine, resulted in changes to the pasting characteristics. The results from An (2005) agreed with those from Liang and King (2003) who also found that the addition of charged amino acids effected the pasting characteristics of rice starch, while the addition of neutral amino acids did not effect the pasting of the starch nearly as much as the charged amino acids.

Sweet potatoes were used in this research in order to assess the effects of additives on the pasting properties of the sweet potato starch. The sweet potato is an excellent source of starch, but this starch has gone largely unstudied.

The objectives of this study were 1) to determine the effect of various amino acid additives on the pasting properties of sweet potato starches and 2) to investigate the differences between the white and orange-fleshed sweet potato starches through the use of RVA.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Sweet potato starch was extracted from white-fleshed and orange-fleshed Beauregard sweet potatoes grown at the LSU research station that were harvested in October 2006. The amino acids used in this study were purchased from Sigma Chemical

Company (St. Louis, Missouri). The amino acids used included one positive (Lysine), one negative (Aspartic Acid), one neutral (Leucine), and one sulfur-containing amino acid (Methionine). These particular amino acids were chosen based on past research (Liang, 2001 and An, 2005).

4.2.2 Sweet Potato Starch Extraction

See section 3.2.2. in Chapter 3.

4.2.3. Proximate Analysis

See section 3.2.3. in Chapter 3.

4.2.4. Amylose Determination.

See section 3.2.4. in Chapter 3.

4.2.5. Rapid Visco Analyzer Analysis

A Rapid Visco Analyzer 3D (Newport Scientific, Warriewood, Australia) was used to determine pasting properties. Samples were made for the RVA on a 7% dry weight starch basis, based on preliminary study, plus amino acid additives on a 6% basis of the starch (Liang, 2001). Water was added to a total of 28g (starch, amino acid, and water). The following formulas were used to determine the amount of starch:

(7/100) = (x/28) x= 1.96g dry starch

100g- moisture content= theoretical dry starch weight

1.96/ theoretical dry starch weight= grams of wet starch

grams of wet starch x 6% = grams of amino acid

28- (starch + amino acid)= grams of water

The actual moisture content of the starch was determined by using a moisture analyzer. The combined water, starch and amino acids were mixed several times to ensure proper combination of the water and starch. The sample was then placed into the RVA, which

was programmed using the thirteen-minute method of Shin et al. (2004), which is specific to sweet potatoes. The procedure started by holding the starch for 1 minute at 50˚C then the mixture was heated to 95˚C at a ramp of 12˚C/minute, the starch was then held at 95˚C for 2.5 minutes, and was cooled to 50˚C at 12˚C/ minute. Throughout the process, the rotating speed of the RVA was kept constant at 160 rpm. The following table illustrates the procedure used:

Table 4.1. RVA Procedure.

Process	Time (minutes)
Hold 50°C	1:00
Ramp 12°/min from 50-95°C	4:45
Hold at 95°C	7:15
Ramp 12°/min from 95-50°C	11:00
Hold 50°C	13:00

The measurements for time, temperature, and viscosity were collected and analyzed. The RVA measured several points including: peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), time to peak (Ptime), and pasting temperature (PT). Total setback (TSB) and breakdown (BD) were calculated using the formulas: FV-MV= TSB and PV-MV= BD. All samples were analyzed in triplicate. The gelatinized samples were then freeze dried and stored in air tight containers for use in the resistant starch and x-ray diffraction procedures in the following chapters.

4.2.6. Statistical Analysis

SAS (Statistical Analysis System) software (version 8.0) was used to analyze the RVA data. Standard deviation, ANOVA (Analysis of Variance), and Tukey's Studentized Range (HSD) were used to examine the effects of the amino acid additives on the white and orange sweet potato starches, on a $p \leq 0.05$ level. The abbreviations used were White for the white-flesh sweet potato starch, Orange for the orange-flesh

sweet potato starch, NOAA for no amino acid additives, ASPA for aspartic acid, LEU for leucine, LYS for lysine, METH for methionine, P1 for peak viscosity, T1 for minimum viscosity, BD for breakdown, FV for final viscosity, SB for total setback, Ptime for time to peak, and PT for pasting temperature.

4.3. RESULTS AND DISCUSSION

4.3.1. Effects of Amino Acid Additives on the Pasting Characteristics of Orange-Fleshed Sweet Potato Starch

Four different amino acids were added to the orange-fleshed sweet potato starch in order to determine whether they would affect the pasting characteristics of the starch (Table 4.2, Figure 4.1). Aspartic acid and lysine, the two charged amino acids used, had the greatest effect on pasting overall. This result agreed with the results obtained by Liang and King (2003) and An (2005), who found that the use of charged amino acids on rice starch caused a greater effect on pasting characteristics as compared to neutral amino acids. In this study, aspartic acid, a negatively charged amino acid, decreased the peak viscosity (PV) of the control by 19.23 RVU, decreased the minimum viscosity (MV) by 37.86 RVU, and increased the breakdown (BD) by 18.64 RVU as compared to the no amino acid control. Aspartic acid also decreased the final viscosity (FV) by 52.64 RVU, decreased the total setback (TS) by 14.78 RVU, and increased the pasting time (Ptime) by 0.05 minutes as compared to the control without amino acids (Table 4.2, Figure 4.1). The results for the effects of aspartic acid on sweet potato starch agree with those results from Liang and King (2003). The increased breakdown of the orange-fleshed sweet potato starch with added aspartic acid signifies a decrease in the cooking stability of the starch (Bean, 1986). Total setback has been correlated with the potential for retrogradation in starches, and a lowering of the total setback could mean that there is less chance for

retrogradation (Qian and Kuhn, 1999). This decrease in the retrogradation of the starch upon cooling may make the starch more suitable for use in some products, such as bakery goods, that could be negatively affected by staling.

An (2005) also found that the addition of aspartic acid on a 6% dry starch basis to rice starch caused similar effects as those seen in this study. The positively charged amino acid, lysine, caused a decrease in PV of 33.31 RVU, a decrease in MV of 24.36 RVU, and a decrease in BD of 8.87 RVU as compared to the no amino acid control (Table 4.2, Figure 4.1). Lysine also caused a decrease in FV of 21.62 RVU, an increase in Ptime of 0.09 minutes, and an increase in pasting temperature (PT) of 1.74°C as compared to the control without amino acids. The increase in the pasting temperature of the starch with added lysine shows that the starch granules will begin to swell at a higher temperature than the control starch, possibly causing a slightly longer cooking time. The decrease in the breakdown of the starch signifies that the paste will be more stable to shear during cooking (Bean, 1986). The other two amino acids, leucine and methionine, both neutral in charge, showed little or no pasting property changes on the orange-fleshed sweet potato starch compared to the control (Table 4.2). The leucine did increase the Ptime by 0.04 minutes, but methionine did not show any statistically significant increases or decreases in any of the pasting characteristics.

Table 4.2. Effects of amino acid additives on the pasting characteristics of orange-fleshed sweet potato starch[1].

	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Peak Viscosity (RVU)	$223.67 \pm 2.70_a$	$204.44 \pm 2.29_b$	$220.36 \pm 1.79_a$	$190.36 \pm 0.54_c$	$220.69 \pm 0.77_a$
Minimum Viscosity (RVU)	$126.19 \pm 0.86_a$	$88.33 \pm 0.00_c$	$128.69 \pm 0.46_a$	$101.83 \pm 4.26_b$	$128.92 \pm 1.95_a$

(Table 4.2. continued)

Breakdown (RVU)	97.47± 1.85$_{b}$	116.11± 2.29$_{a}$	91.67± 1.79$_{b,c}$	88.53± 4.47$_{c}$	91.78± 1.51$_{b,c}$
Final Viscosity (RVU)	172.31± 3.15$_{a}$	119.67± 0.96$_{c}$	179.81± 3.08$_{a}$	150.69± 2.82$_{b}$	180.14± 3.94$_{a}$
Total Setback (RVU)	46.11± 2.36$_{a}$	31.33± 0.96$_{b}$	51.11± 2.79$_{a}$	48.86± 1.72$_{a}$	51.22± 2.65$_{a}$
Pasting Time (min)	4.41± 0.02$_{c}$	4.46± 0.00$_{a,b}$	4.45± 0.02$_{b}$	4.50± 0.00$_{a}$	4.44± 0.02$_{b,c}$
Pasting Temperature (°C)	73.18± 0.26$_{b,c}$	73.50± 0.30$_{b}$	73.08± 0.29$_{b,c}$	74.92± 0.15$_{a}$	72.72± 0.03$_{c}$

[1]Means with the same letter in each row are not significantly different at p>0.05.

4.3.2. Effects of Amino Acid Additives on the Pasting Characteristics of White-Fleshed Sweet Potato Starch

The white-fleshed sweet potato starch responded differently to the added amino acids than did the orange-fleshed sweet potato starch. Like the orange-fleshed sweet potato, however, the charged amino acids caused the greatest effects, but the neutral acids also affected the white-fleshed sweet potato starch (Table 4.3, Figure 4.2). The greater effects of the charged amino acids than those of the neutral amino acids were also seen by Liang and King (2003) and An (2005) on rice starches. The added aspartic acid caused a decrease in PV of 41.94 RVU, a decrease in MV of 50.25 RVU, an increase in breakdown of 8.31 RVU compared to control without amino acids. A decrease in FV of 70.17 RVU, a decrease in TS of 19.91 RVU, a decrease in Ptime of 0.13 minutes, and an increase in PT of 0.65°C were also seen with aspartic acid compared to the control without amino acids. The decrease in pasting viscosity shows that this starch was modified into a thinner pasting starch. The decrease of both the pasting time and the minimum viscosity for this starch could translate into a faster cooking time and a product that is easier to cook (Liang and King, 2003). The increase in breakdown shows that the starch may be less stable during cooking than was the control starch without amino acids (Bean, 1986).

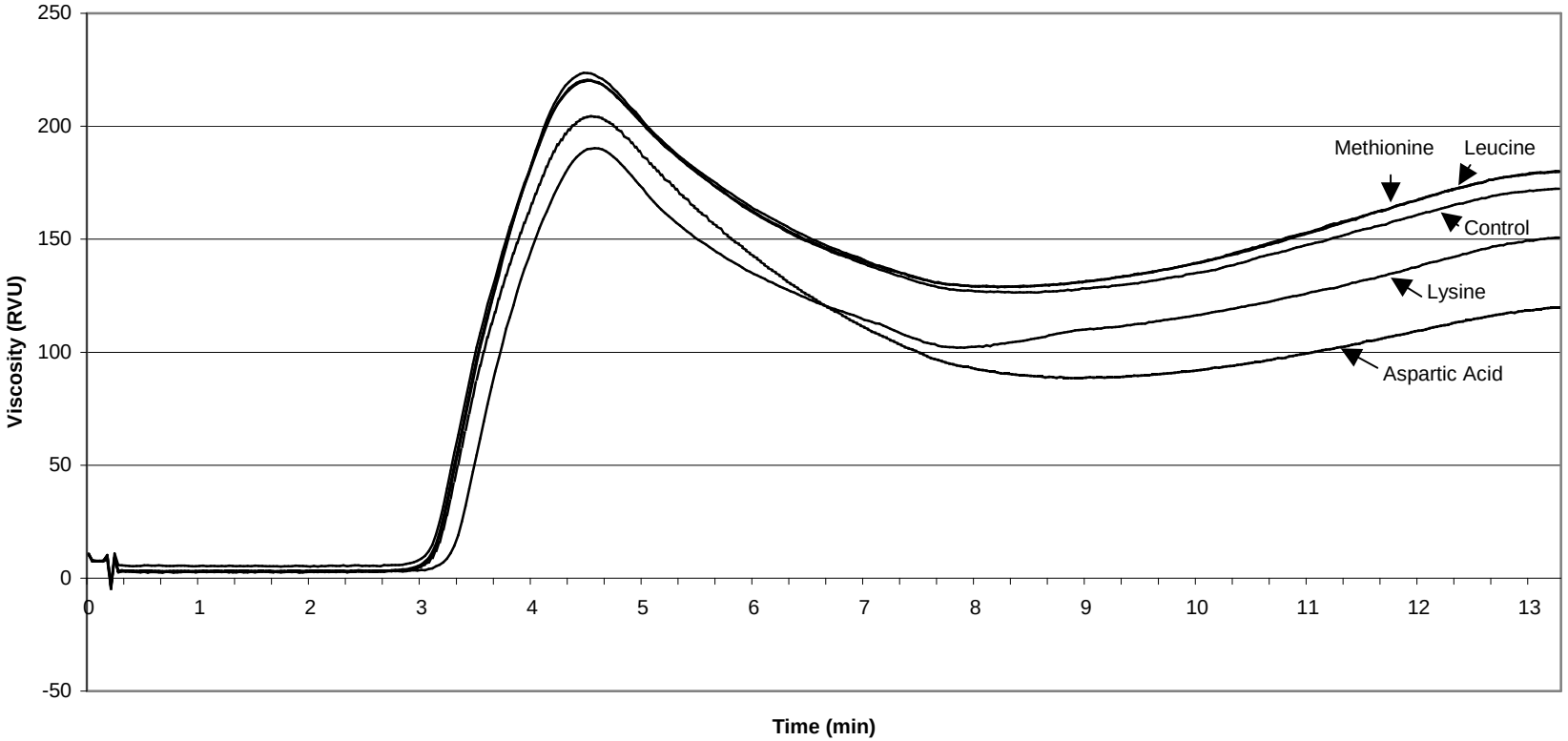

Figure 4.1. Effects of amino acid additives on the pasting characteristics of orange-fleshed sweet potato starch.

When lysine was added to the white-fleshed sweet potato starch, a decrease in PV of 29 RVU was observed, as well as a decrease in MV of 28.27 RVU, a decrease in FV of 28.17 RVU, and an increase in PT of 1.86°C. Liang and King (2003) found that charged amino acids added to rice starch had the ability of decreasing the cooking stability of the starch as well as lowering the tendency for retrogradation. Our study showed that the positively charged lysine had no effect on the cooking stability of the starch as seen through the breakdown. For the possibility of retrogradation, lysine, again, had no effect on the total setback of the starch, but aspartic acid added to the starch did decrease the starch's total setback and its chance for retrogradation. The neutral leucine also caused several changes in the pasting characteristics of the white-fleshed sweet potato starch compared to the control without amino acids, including decreases in PV (14.08 RVU), MV (5.8 RVU), BD (8.28 RVU), and an increase in TS (5.53 RVU). The starch with added leucine showed a decrease in the breakdown, making it possibly more stable during cooking, but also showed an increase in the total setback, which correlates to an increase in the potential for retrogradation (Bean, 1986). Methionine also caused a couple changes in pasting characteristics compared to the control with a decrease in PV of 12.55 RVU and a decrease in FV of 5.63 RVU. The effects caused by leucine and methionine, however, were not as large as those caused by the two charged amino acids.

Table 4.3. Effects of amino acid additives on the pasting properties of white-fleshed sweet potato starch[1].

	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Peak Viscosity (RVU)	$221.44 \pm 1.34_a$	$179.50 \pm 4.17_d$	$207.36 \pm 3.31_b$	$192.44 \pm 4.63_c$	$208.89 \pm 1.47_b$

(Table 4.3. continued)

Minimum Viscosity (RVU)	138.69± 0.76$_a$	88.44± 0.76$_d$	132.89± 1.88$_b$	110.42± 3.92$_c$	133.42± 0.85$_{a,b}$
Breakdown (RVU)	82.75± 1.84$_b$	91.06± 3.42$_a$	74.47± 1.88$_c$	82.03± 5.13$_{b,c}$	75.47± 0.79$_{b,c}$
Final Viscosity (RVU)	189.92± 0.38$_a$	119.75± 1.61$_d$	189.64± 1.79$_a$	161.75± 1.75$_c$	184.06± 0.42$_b$
Total Setback (RVU)	51.22± 1.11$_b$	31.31± 0.86$_c$	56.75± 1.36$_a$	51.33± 2.35$_b$	50.64± 0.43$_b$
Pasting Time (min)	4.55± 0.02$_a$	4.42± 0.04$_b$	4.51± 0.04$_a$	4.51± 0.02$_a$	4.51± 0.04$_a$
Pasting Temperature (°C)	79.62± 0.26$_c$	80.27± 0.06$_b$	79.42± 0.03$_c$	81.48± 0.06$_a$	79.45± 0.05$_c$

[1]Means with the same letter in each row are not significantly different at p>0.05.

4.3.3. Comparison of Pasting Characteristics of White-Fleshed and Orange-Fleshed Sweet Potato Starch.

When the orange-fleshed and white-fleshed sweet potato starches were directly compared, the only pasting parameter that was not different between the two types of sweet potato starch without amino acids was peak viscosity. The similar measure of peak viscosity shows that the two starches could have similar thickness during cooking. All other characteristics measured, MV, BD, FV, TS, Ptime, and PT, were significantly different at a p value of ≤ 0.05 (Table 4.4, Figure 4.3). The orange-fleshed sweet potato starch had a lower minimum viscosity (12.5 RVU), lower pasting time (0.14min), and a lower pasting temperature (6.44°C). These three characteristics work synergistically to make the starch easier to cook, than the white-fleshed sweet potato starch, which had higher MV, Ptime, and PT (Bean, 1986). The white-fleshed sweet potato starch had a lower breakdown by 14.72 RVU, which shows that this starch is more stable during cooking than the orange-fleshed starch. The white-fleshed sweet potato starch also had a

higher setback than the orange-fleshed sweet potato starch. This characteristic has been found to correlate with the potential for retrogradation, so the white-fleshed sweet potato starch would be more susceptible to retrogradation than the orange-fleshed starch.

A possible explanation for the differences between the pasting characteristics of the white and orange-fleshed sweet potato starches could be the large difference in amylose content. The white-fleshed sweet potato starch contains 14.4% amylose, while the orange-fleshed starch only contains 4.6% amylose. The amount of amylose present in a starch has been negatively correlated with breakdown and positively correlated with pasting temperature and setback (Juliano et al., 1964 and Noda et al., 2003). These correlations could explain why the white-fleshed sweet potato starch has a lower breakdown, and higher pasting temperature and total setback than the orange-sweet potato starch.

The addition of amino acids to the white-fleshed and orange-fleshed sweet potato starches did change their pasting characteristic relationship. For the peak viscosity, the addition of lysine reduced both the white-fleshed and orange-fleshed starches by 9 and 13.3RVU, respectively. These changes, however, did not produce any statistical differences between the two starches. The addition of leucine, aspartic acid, and methionine did cause a significant difference in the peak viscosity between the orange-fleshed and white-fleshed sweet potato starches. The minimum viscosity was unchanged for both starches with the addition of leucine and methionine. Aspartic acid and lysine lowered the pasting characteristics of minimum viscosity, total setback, and pasting time of both starches to a point where no significant difference was seen between the two starches in those pasting parameters. The addition of leucine and methionine caused a decrease to the breakdown of both the white-fleshed and orange-fleshed starches,

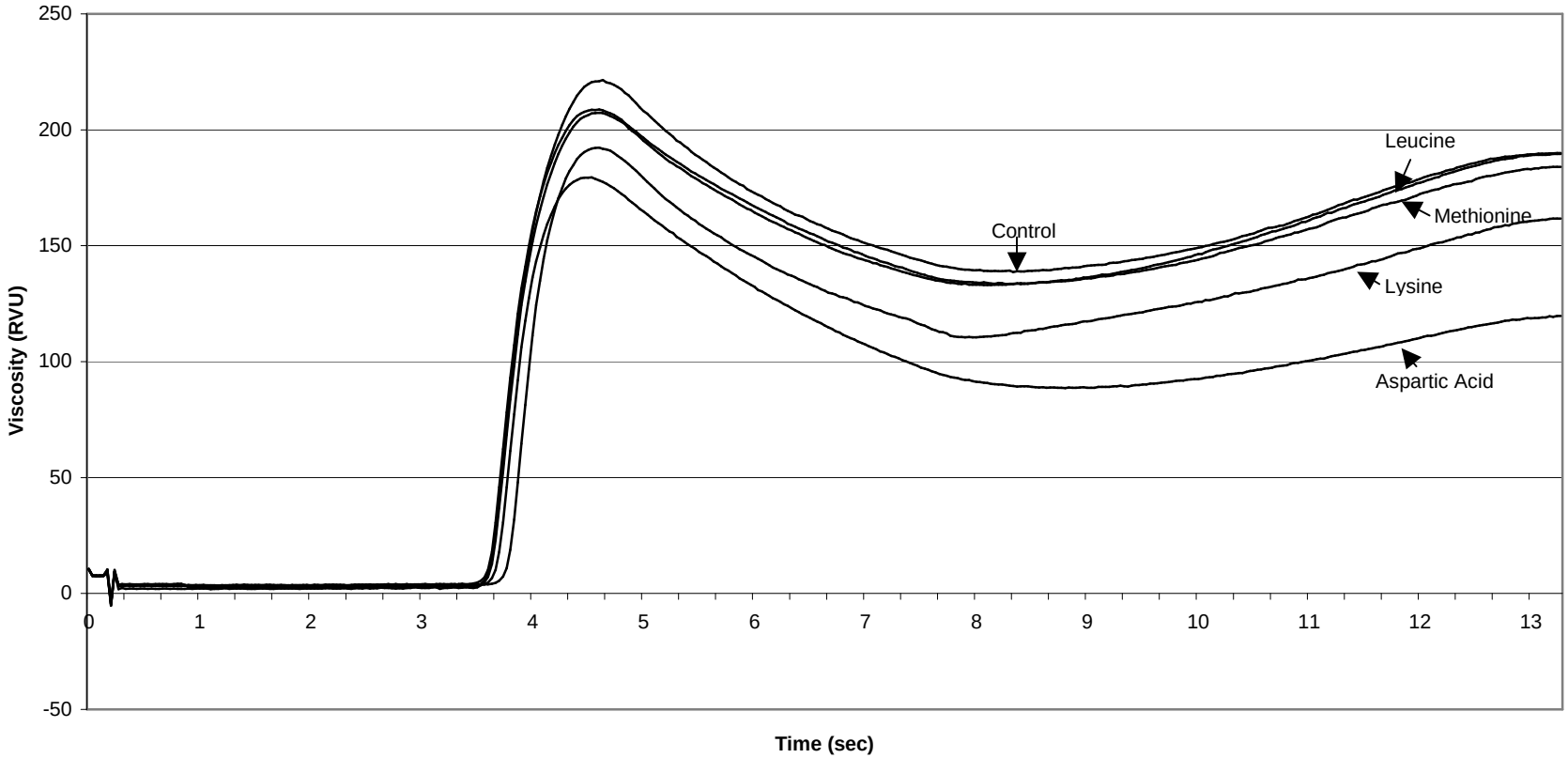

Figure 4.2. Effects of amino acid additives on the pasting characteristics of white-fleshed sweet potato starch.

Table 4.4. Comparing pasting properties of orange-fleshed and white-fleshed sweet potato starches with added amino acids[1]

Peak Viscosity (RVU)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed sweet potato starch	223.67± 2.70$_a$	204.44± 2.29$_a$	220.36± 1.79$_a$	190.36± 0.54$_a$	220.69± 0.77$_a$
White-fleshed sweet potato starch	221.44± 1.34$_a$	179.50± 4.17$_b$	207.36± 3.31$_b$	192.44± 4.63$_a$	208.89± 1.47$_b$
Minimum Viscosity (RVU)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed sweet potato starch	126.19± 0.86$_a$	88.33± 0.00$_a$	128.69± 0.46$_a$	101.83± 4.26$_a$	128.92± 1.95$_a$
White-fleshed sweet potato starch	138.69± 0.76$_b$	88.44± 0.76$_a$	132.89± 1.88$_b$	110.42± 3.92$_a$	133.42± 0.85$_b$
Breakdown (RVU)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed sweet potato starch	97.47± 1.85$_a$	116.11± 2.29$_a$	91.67± 1.79$_a$	88.53± 4.47$_a$	91.78± 1.51$_a$
White-fleshed sweet potato starch	82.75± 1.84$_b$	91.06± 3.42$_b$	74.47± 1.88$_b$	82.03± 5.13$_a$	75.47± 0.79$_b$
Final Viscosity (RVU)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed sweet potato starch	172.31± 3.15$_a$	119.67± 0.96$_a$	179.81± 3.08$_a$	150.69± 2.82$_a$	180.14± 3.94$_a$
White-fleshed sweet potato starch	189.92± 0.38$_b$	119.75± 1.61$_a$	189.64± 1.79$_b$	161.75± 1.75$_b$	184.06± 0.42$_a$
Total Setback (RVU)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed sweet potato starch	46.11± 2.36$_a$	31.33± 0.96$_a$	51.11± 2.79$_a$	48.86± 1.72$_a$	51.22± 2.65$_a$
White-fleshed sweet potato starch	51.22± 1.11$_b$	31.31± 0.86$_a$	56.75± 1.36$_b$	51.33± 2.35$_a$	50.64± 0.43$_a$
Pasting Time (min)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed sweet potato starch	4.41± 0.02$_a$	4.46± 0.00$_a$	4.45± 0.02$_a$	4.50± 0.00$_a$	4.44± 0.02$_a$
White-fleshed sweet potato starch	4.55± 0.02$_b$	4.42± 0.04$_a$	4.51± 0.04$_a$	4.51± 0.02$_a$	4.51± 0.04$_a$

(Table 4.4. continued)

Pasting Temperature(°C)					
	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed sweet potato starch	$73.18\pm 0.26_a$	$73.50\pm 0.30_a$	$73.08\pm 0.29_a$	$74.92\pm 0.15_a$	$72.72\pm 0.03_a$
White-fleshed sweet potato starch	$79.62\pm 0.26_b$	$80.27\pm 0.06_b$	$79.42\pm 0.03_b$	$81.48\pm 0.06_b$	$79.45\pm 0.05_b$

[1]For each parameter, means with the same letter in each column are not significantly different at $p>0.05$.

resulting in a significant difference, while lysine decreased the orange-fleshed sweet potato starch breakdown but did not affect the white-fleshed starch. The addition of lysine minimized the original differences between the two starches, resulting in no difference in all of the pasting parameters except for the final viscosity and pasting temperature. The addition of aspartic acid and methionine changed the orange-fleshed sweet potato starch final viscosity so that there was no difference in final viscosity left between the two starches. For the total setback of the starches, the addition of lysine and aspartic acid served to eliminate the original differences in the total setback between the two starches. The same was also true for the pasting time, but for this parameter all four amino acids, aspartic acid, lysine, leucine and methionine, removed the original differences that existed between the two types of starch. In considering the pasting temperature, all of the amino acids had minimal effects on the orange-fleshed and white-fleshed sweet potato starches; however, these changes were not great enough to alter the original difference between the two starches.

4.4. CONCLUSION

This study showed that both positive and negative amino acids have greater effects on the pasting properties of both white-fleshed and orange-fleshed sweet potato

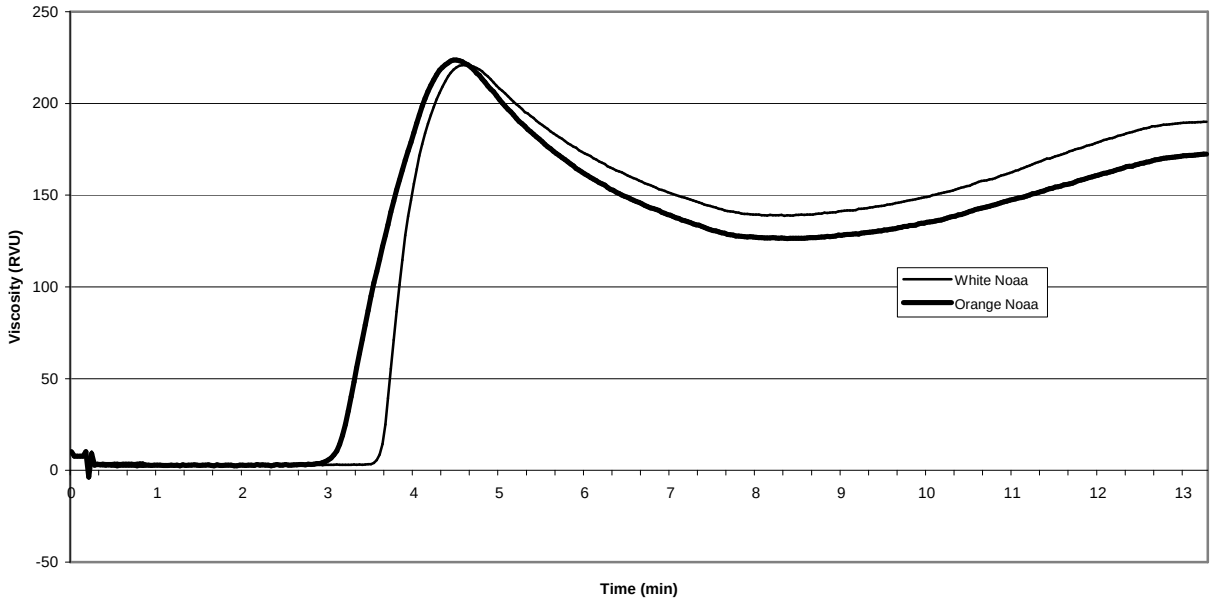

Figure 4.3. RVA analysis of white-fleshed and orange-fleshed sweet potato starch without added amino acids.

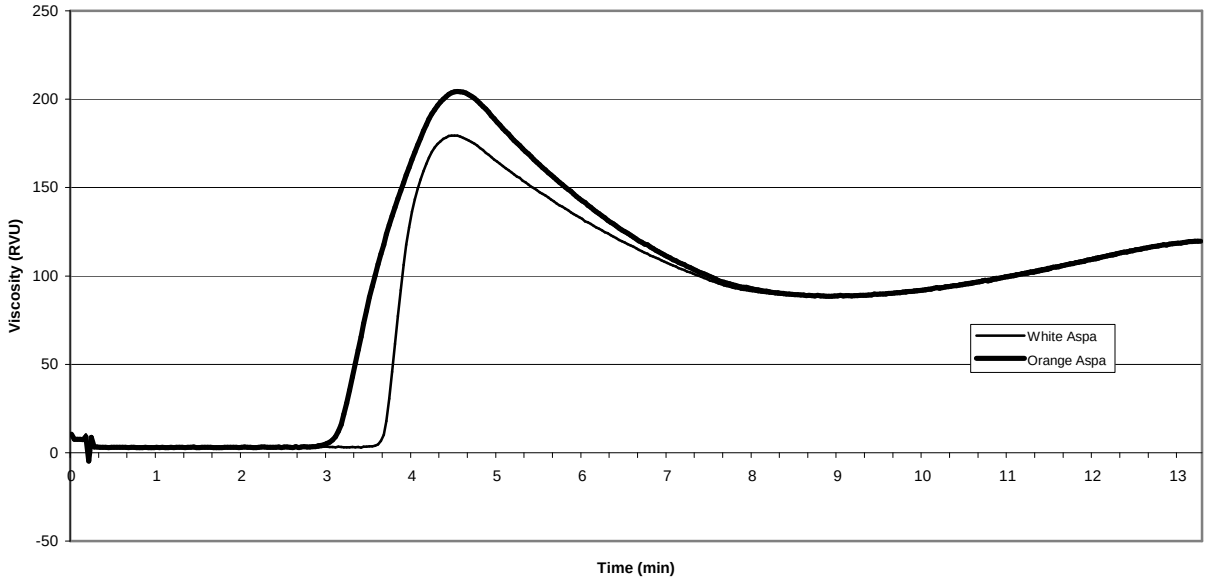

Figure 4.4. RVA analysis of white-fleshed and orange-fleshed sweet potato starch with aspartic acid.

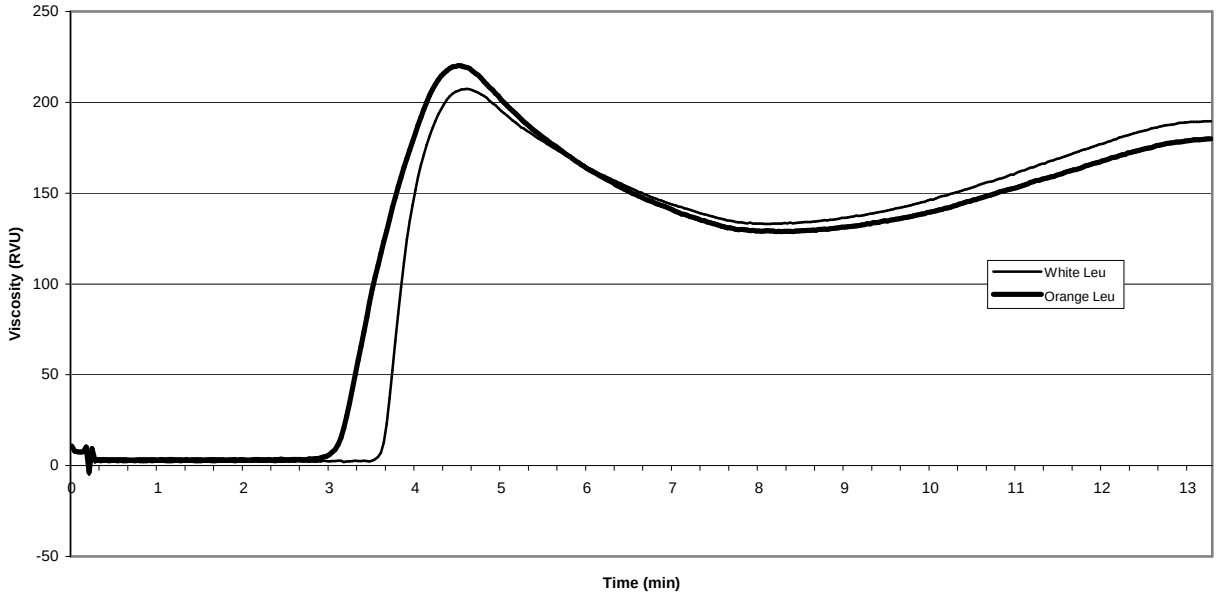

Figure 4.5. RVA analysis of white-fleshed and orange-fleshed sweet potato starch with leucine.

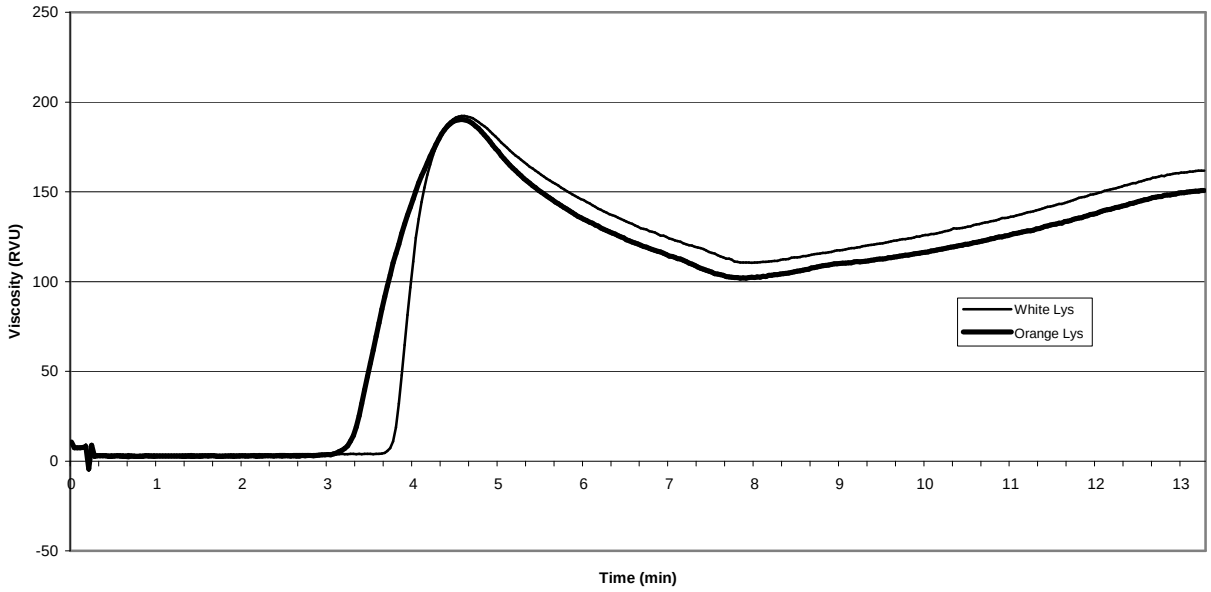

Figure 4.6. RVA analysis of white-fleshed and orange-fleshed sweet potato starch with lysine.

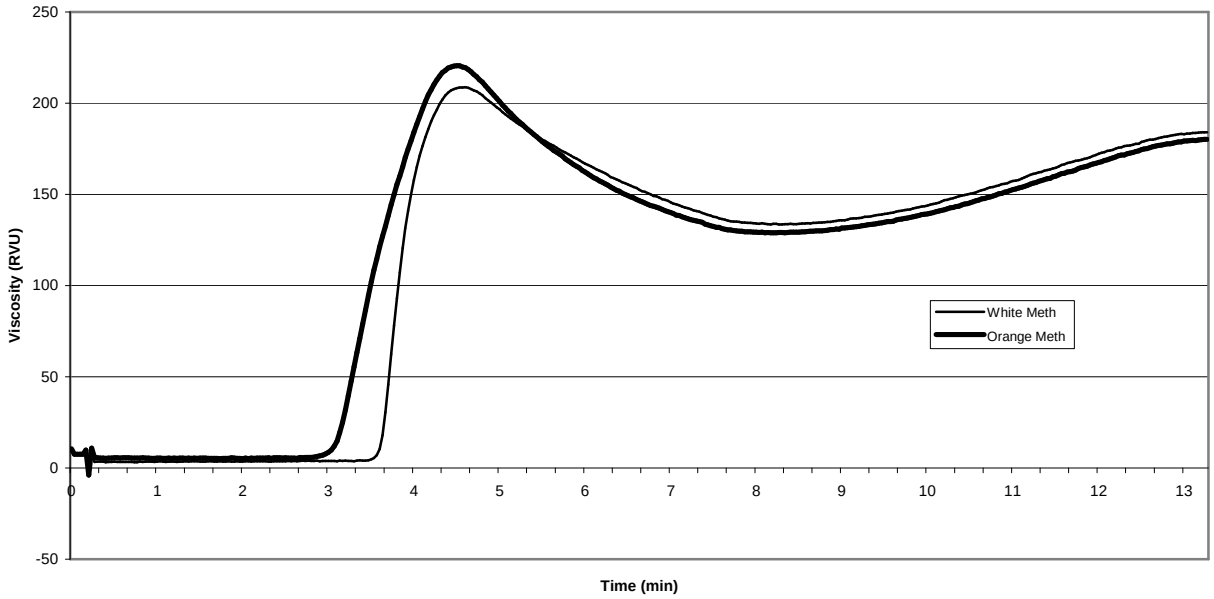

Figure 4.7. RVA analysis of white-fleshed and orange-fleshed sweet potato starch with methionine.

starch than did the neutral amino acids. The aspartic acid made a starch that was less stable during cooking and had a lower potential for retrogradation in the white and orange-fleshed sweet potato starches. Lysine, in the orange-fleshed sweet potato starch, decreased the breakdown, making a starch that is more resistant to shear during cooking. The lysine, however, did not affect the setback in either of the starches or the breakdown in the white-sweet potato starch.

The white-fleshed and orange-fleshed sweet potato starches were found to be profoundly different in all of the pasting characteristics except for peak viscosity where no difference was observed. The orange-fleshed sweet potato starch was found to be easier to cook and had a lower possibility of retrogradation, but had a higher breakdown which makes it less stable during cooking than the white-fleshed sweet potato starch.

CHAPTER 5

EFFECTS OF AMINO ACID ADDITIVES ON THE FORMATION OF RESISTANT STARCH

5.1. INTRODUCTION

Starch can be classified into three groups: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). These groups are differentiated based on the time it takes each to be digested by enzymatic hydrolysis of pancreatic amylase and amyloglucosidase at 37°C. The RDS is digested within 20 minutes, while the SDS is only digested within 120 minutes of incubation. The RS is the starch left over after the 120-minute incubation period (Englyst et al., 1992).

The resistant starch can also be broken down into fractions. Four distinct fractions of RS exist: RS1, RS2, RS3, and RS4. RS1 is starch that cannot be digested because it is physically inaccessible to the digestive enzymes. This includes partly milled grains and seeds and also dense starchy foods. This type of RS is heat stable during cooking, which lends itself to be very useful in a wide array of foods. RS2 is starch in certain granular forms that are inaccessible to digestion. The compact structure of the granules makes it impossible for the enzymes to completely break down the starch. Ungelatinized starch is an example of RS2. RS3 is the most resistant of the resistant starch fractions to digestion by enzymes and is formed upon cooling after gelatinization is complete. This starch is formed from the reassociation (retrogradation) of amylose molecules in the starch after they have leached out of the starch granule during the gelatinization process. Finally, RS4 is starch that is resistant to digestion because of the formation of novel chemical bonds other than α-(1-4) and α-(1-6) linkages that were formed within the molecules. This fraction of resistant starch is formed through chemical treatments (Sajilata et al., 2006).

Several methods exist to determine the overall amount of resistant starch within starch samples. These include the Megazyme method (AOAC method 2002.02), a method proposed by Champ et al. (2003), a method by Berry (1986), and another method developed by Saura-Calixto et al. (1993). The Megazyme method centers on enzymatic digestion at 37°C. This method has been shown to be the most reproducible and repeatable. This method, however, does not separate the resistant starch that is quantified into fractions, only one total percentage of resistant starch is determined (Sajilata et al., 2006).

Much research has centered around the modification of resistant starch contents of various starches. Some of the methods employed partial acid hydrolysis, heat-moisture treatments, and the addition of amino acids (Shin et al., 2004 and An, 2005). An (2005) studied the effects of amino acid additives to various rice starch samples. An (2005) found that none of the amino acids tested influenced the percentage of resistant starch, that had been measured at a level of 5.37% in non-treated rice starch. However, she did find that on rice starch treated with ozone or oxygen, the amino acids did change the total amount of resistant starch. The level of resistant starch either increased or decreased depending on the amino acid used (An, 2005).

Sweet potatoes were used in this research because the sweet potato is an excellent source of starch, but the resistant starch contents and modifications thereof have not been well researched to date.

The objective of this research was to study the effects of the addition of certain amino acids on the amount of resistant starch in white-fleshed and orange-fleshed sweet potato starch.

5.2. MATERIALS AND METHODS

5.2.1. Materials

Sweet potato starch was extracted from white and orange-fleshed Beauregard sweet potatoes grown at the LSU AgCenter research station that were harvested in October of 2006. The amino acids used in this study were purchased from Sigma Chemical Company (St. Louis, Missouri). The amino acids used included one positive (Lysine), one negative (Aspartic Acid), one neutral (Leucine), and one sulfur-containing amino acid (Methionine). These particular amino acids were chosen based on past research (Liang, 2001 and An, 2005).

5.2.2. Sweet Potato Starch Extraction

See Chapter 3 for details on the starch extraction procedures.

5.2.3. Resistant Starch Determination Procedure

To determine resistant starch content in each sample the Megazyme procedure (Megazyme International Ireland Limited, Bray, Ireland) was used. This method is an approved AOAC method (method 2002.02) and also an approved AACC method (method 32-40). The samples used had been previously gelatinized in the presence of amino acids on a 6% dry starch weight basis in the RVA (see Chapter 4) and were subsequently freeze dried, ground with a mortar and pestle, and kept at room temperature in hermetically sealed containers. A 100 mg sample was weighed into a screw cap tube, and gently tapped to ensure that the entire sample fell to the bottom. Four mL of pancreatic α-amylase (Pancreatin, 10g, 3 Ceralpha Units/mg) (10mg/mL) containing amyloglucosidase (AMG) (3U/mL) was then added. The tubes were tightly capped and then mixed on a vortex mixer and attached horizontally in a shaking water bath. The tubes were incubated at 37˚C with continuous shaking for exactly 16 hours. Then the

tubes were removed from the water bath and a paper towel was used to remove excess surface water. The tube caps were removed and the contents treated with 4.0mL of ethanol (99%) with stirring on a vortex mixer. The tubes were centrifuged at 1500g (approx. 3000rpm) for 10 minutes non-capped. All supernatants were decanted and the pellets were re-suspended in 2mL of 50% ethanol with stirring on a vortex mixer. A further 6mL of 50% of ethanol was added, the tubes were mixed and centrifuged again at 1500g for 10 minutes. The supernatants were decanted and then the suspension and centrifugation steps were repeated once more. The supernatants were carefully decanted and the tubes inverted on absorbent paper to drain excess liquid. A magnetic stirrer bar and 2mL of 2M KOH were added to each tube and the pellets were re-suspended by stirring for approximately 20 minutes in an ice/water bath over a magnetic stirrer. Eight mL of 1.2M sodium acetate buffer (pH 3.8) were added to each tube with stirring on the magnetic stirrer. Immediately, 0.1mL of AMG (300U/mL) was added and mixed well. The tubes were then placed in a water bath at 50˚C. The tubes were incubated for 30 minutes with intermittent mixing on a vortex mixer. For samples containing <10% resistant starch, the tubes were then directly centrifuged at 1500g for 10 minutes. For samples containing >10% resistant starch, the contents of the tubes were transferred to a 100mL volumetric flask with the use of a water wash bottle. The contents of the flask was adjusted to 100mL with distilled water and mixed well. An aliquot of this diluted sample was then centrifuged at 1500g for 10 minutes. 0.1 mL aliquots of either the diluted or undiluted supernatants were transferred into glass test tubes, treated with 3.0mL of Glucose Determination Reagent (GOPOD) and incubated at 50˚C for 20 minutes. A reagent blank was made by mixing 0.1mL of 0.1M sodium acetate buffer (pH

4.5) and 3.0mL of GOPOD reagent. The absorbance of each solution was measured at 510nm against the reagent blank.

The calculations for the percent of resistant starch were performed as follows:

Samples containing > 10 % resistant starch:

$= \Delta E \times F \times 100/0.1 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 90$

Samples containing < 10% resistant starch:

$= \Delta E \times F \times 10.3/0.1 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 9.27$

Where:

ΔE= absorbance read against reagent blank

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

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

1/1000= conversion from micrograms to milligrams

W= dry weight of sample analyzed

100/W= factor to present RS as a percentage of sample weight

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

10.3/0.1= volume correction (0.1mL taken from 10.3mL) for samples containing 0-10% RS where the incubation solution is not diluted and the final volume is about 10.3mL

5.2.4. Statistical Analysis

SAS (Statistical Analysis System) software (version 8.0) was used to analyze the DSC data. Standard deviation, ANOVA (Analysis of Variance), and Tukey's Studentized Range (HSD) were used to examine the effects of the amino acid additives

on the formation of resistant starch of the white-fleshed and orange-fleshed sweet potato starches, on a $p \leq 0.05$ level. The abbreviations used were White for the white-flesh sweet potato starch, Orange for the orange-flesh sweet potato starch, NOAA for no amino acid additives, AA for aspartic acid, LEU for leucine, LYS for lysine, METH for methionine.

5.3. RESULTS AND DISCUSSION

The ungelatinized, native white-fleshed and orange-fleshed sweet potato starches had significant amounts of resistant starch, 39.8 and 20.7% respectively (Table 5.1). These two values were significantly different from one another, with the white-fleshed sweet potato starch containing much more resistant starch than the orange-fleshed starch. When the starches were gelatinized, through the use of a Rapid Visco Analyzer, the amount of resistant starch decreased dramatically in both the white-fleshed and orange-fleshed sweet potato starches. The resistant starch content of the white-fleshed sweet potato starch fell from 39.8% in the ungelatinized starch to 8.26% in the gelatinized starch. The resistant starch content of the orange-sweet potato starch decreased from 20.6% to 4.8% in the native starch to the gelatinized starch, respectively. Although a significant portion of resistant starch is eliminated through the gelatinization process, not many foods include ungelatinized starch (Annison and Topping, 1994). Any cooking process will completely gelatinize a starch, making the preservation of the high level of pregelatinized resistant starch rather improbable. When a starch is heated and gelatinized, the crystalline structure of the starch granules is disrupted resulting in a loss of the natural resistant starch that is normally present. The disturbance of the crystalline structure changes the way in which the starch is process in the body, allowing for a greater degree of absorbance of the starch granules (Annison and Topping, 1994). Shin et al. (2004) found that the resistant starch content for gelatinized and cooled sweet potato starch was

5.4%. The resistant starch content was measured using the enzymatic-gravimetric AOAC method for the determination of insoluble dietary fiber with slight modifications in order to isolate resistant starch. This result compares favorably with the amount of resistant starch found in our gelatinized orange-fleshed sweet potato starch.

Table 5.1. Effects of amino acid additives on the resistant starch content of orange-fleshed and white-fleshed sweet potatoes[1,2,3] Reported as a percentage (%) of total starch.

Starch	Native Starch	No Amino Acid	Aspartic Acid	Leucine	Lysine	Methionine
Orange-fleshed Sweet Potato Starch	20.65 ± 1.7^{a}	$4.78\pm 0.51^{a}{}_{b}$	$5.01\pm 0.50^{a}{}_{a,b}$	$3.92\pm 0.59^{a}{}_{c}$	$5.74\pm 0.40^{a}{}_{a}$	$5.57\pm 0.35^{a}{}_{a,b}$
White-fleshed Sweet Potato Starch	39.82 ± 2.7^{b}	$8.26\pm 0.48^{b}{}_{a}$	$8.42\pm 0.64^{b}{}_{a}$	$8.91\pm 0.36^{b}{}_{a}$	$8.26\pm 0.75^{b}{}_{a}$	$6.81\pm 0.26^{b}{}_{b}$

[1]All non-native starches are gelatinized starches.
[2]Means with the same subscript letter in each row are not significantly different at $p>0.05$. These values compare the amino acid additives within the same starch.
[3] Means with the same superscript letter in each column are not significantly different at $p>0.05$. These values compare the amino acid additives between the two starch types.

The gelatinized starch samples with no amino acids added were significantly different on a $p\leq 0.05$ level. The differences in the amounts of resistant starch in the gelatinized without amino acid additive treated white-fleshed and orange-fleshed sweet potato starches may be due to the difference in the amylose content of the two starches. The amylose content of the white-fleshed sweet potato starch (Chapter 3, Table 3.1) was significantly higher than that of the orange-fleshed sweet potato starch. The amylose contents were 14.4% for the white-fleshed starch, while only 4.6% for the orange-fleshed sweet potato starch. Amylose is the main component of short-term retrogradation, and retrograded starch is one type of resistant starch found after a heating and cooling cycle (Sajilata et al., 2006).

The addition of amino acids to the gelatinized orange-sweet potato starch served to both increase and decrease the amount of resistant starch, or had no effect, depending on the amino acid used. The addition of the positively charged lysine caused a significant increase in the amount of resistant starch while the addition of the neutral leucine caused a significant decrease in the resistant starch compared to the control without amino acids. Aspartic acid and methionine had no effect on the percentage of resistant starch in the orange-fleshed sweet potato starch. An (2005) studied the effects of amino acid additives on the resistant starch content of rice starch. She found that none of the amino acids used, aspartic acid, leucine, and lysine, significantly affected the percentage of resistant starch in the rice starch samples without further treatment. However, An (2005) did find that the addition of leucine to rice white starch isolate caused a significant decrease in the amount of resistant starch. In the white-fleshed sweet potato starch, the addition of amino acids mostly did not affect the amount of resistant starch. Methionine was the only amino acid that had any effect on the percentage of resistant starch in the white-fleshed sweet potato starch, where a significant decrease in the resistant starch was found.

5.4. CONCLUSION

This study showed that the gelatinization of both white-fleshed and orange-fleshed sweet potato starches results in a major decrease in the amount of resistant starch, and that the white-fleshed sweet potato starch contains significantly more resistant starch than the orange-fleshed sweet potato starch in both the gelatinized and ungelatinized forms. Also the addition of the amino acid, aspartic acid had no overall effect on the resistant starch content in either of the two sweet potato starches tested. The addition of lysine served to significantly increase the resistant starch content of the orange-fleshed sweet potato starch, which may serve to make sweet potato starch containing foods

healthier. However, the addition of leucine to the orange-fleshed sweet potato starch decreased the amount of resistant starch present. The addition of methionine to the white-fleshed sweet potato starch also decreased the amount of resistant starch, making both of these combinations unfavorable.

CHAPTER 6

EFFECTS OF AMINO ACID ADDITIVES ON THE CRYSTALLINITY OF WHITE AND ORANGE-FLESHED SWEET POTATO STARCH USING X-RAY DIFFRACTION (XRD)

6.1. INTRODUCTION

The starch granule consists of both amorphous and crystalline regions (Zobel, 1988b). Native starches are said to have between 15 and 45% crystalline material. The crystallinity of a granule is determined by the extent of helical structures formed from the association of amylopectin molecules. The crystal structure of the starch granules can be observed through X-Ray Diffraction (XRD) (Yadav et al., 2004). The crystal structure and pattern of crystallinity of starches is characteristic of the plant source from which they were obtained (Englyst et al., 1992). These are A, B, C, and V types. The A-type pattern results from monoclinic cells, while the B-type pattern is caused by two double helices within the starch granules. The C-type pattern is a combination of the A and the B patterns. The A-type patterns are generally regarded as patterns for cereal starches, including maize, rice and wheat. The B patterns are often found in tubers, while the C patterns of starch are typical to legumes, roots, and some seed starches. The V-type pattern results from amylose-lipid complexes within the starch granules (Zobel, 1988b).

The four types of X-ray diffraction pattern have a unique set of peaks that are characteristic to each specific type. The A-type patterns have three peaks, 5.8, 5.2, and 3.8 Angstroms (A), each of these peaks are relatively strong in intensity. The B-type patterns can be distinguished by a peak between 15.8 and 16.0A, one at 5.9A that is medium in intensity, a strong peak at 5.2A, and a doublet consisting of 4.0 and 3.7A with medium intensity. The C-type pattern mimics that of the A-type pattern, but with the

addition of a fairly strong peak at 16.0A. The V-type pattern shows the amylose-lipid complex with peaks at 12, 6.8, and 4.4A (Zobel, 1988ab)

B and C-type patterns tend to be more resistant to pancreatic amylase and, therefore, more resistant to digestion within the body (Englyst et al., 1992). V-type patterns have been correlated with the staling of bread and retrogradation of a gelatinized starch, but the B-pattern has also been suggested as being the crystalline pattern for retrogradation. This occurs when the amylose that had previously leached out of the starch granules during gelatiniztion, reassociates to form tight knit groups (Dragsdorf and Varriano-Marston, 1980, and Annison and Topping, 1994). Mahadevamma and Tharanathan (2006) found that the x-ray diffraction pattern of rice changed from an A-type pattern in the native starch to a V-type pattern after gelatinization due to the disruption of the crystalline patterns during heating. Their results were based on the XRD pattern of several processed rice products including parboiled rice, puffed rice, and rice flakes.

Modifications of the XRD patterns of starches have been performed through heat moisture treatments, differences in growth temperature of sweet potatoes, various gelatinization temperatures, and the addition of outside materials such as amino acids (Genkina et al., 2003, Kitahara et al., 2005, An, 2005, and Liang and King, 2003). All of these factors can influence the types of XRD patterns of starches. The addition of amino acids resulted in various effects on the XRD pattern of rice starches. An (2005) studied the effect of lysine on rice starch and found that the lysine changed the crystalline structure from an A to a A+B pattern and enhanced the overall crystallinity. Liang and King (2003) added aspartic acid, gutamic acid, lysine, arginine, leucine, and alanine to rice starch to test the effects of these amino acids on the crystalline properties of the

starch. They found that, overall, the addition of the amino acids caused an increase in the V-type pattern of crystallinity and some of the amino acids caused in increase in the crystallinity of the starch (Liang and King, 2003).

Sweet potatoes were studied in order to assess the changes in crystallinity due to gelatinization. Also, the addition of amino acids to the sweet potato starch has not been well researched.

The objectives of this study were 1) to compare the white-fleshed and orange-fleshed sweet potato starch XRD profiles and 2) to assess the influence of amino acid additives on the crystallinity of the two sweet potato starches.

6.2. MATERIALS AND METHODS

6.2.1. Materials

Sweet potato starch was extracted from white-fleshed and orange-fleshed Beauregard sweet potatoes grown at the LSU AgCenter research station that were harvested in October of 2006. The amino acids used in this study were purchased from Sigma Chemical Company (St. Louis, Missouri). The amino acids used included one positive (Lysine), one negative (Aspartic Acid), one neutral (Leucine), and one sulfur-containing amino acid (Methionine). These particular amino acids were chosen based on past research (Liang, 2001 and An, 2005).

6.2.2. Sweet Potato Starch Extraction

See Chapter 3 for details on the procedure for starch extraction.

6.2.3. X-ray Diffraction Analysis

The starch samples used were both gelatinized and ungelatinized. Both white-fleshed and orange-fleshed native sweet potato starch samples were tested. The rest of the samples were from the starch gels collected after Rapid Visco Analysis (see Chapter

4). These samples had already been pasted in the presence of 6% amino acids and cooled. They were then freeze dried and ground into a powder with a mortar and pestle.

The starch samples were placed in a desiccator that contained a saturated sodium chloride solution and had a 75% relative humidity (An, 2005). The samples were stored overnight. One gram of gelatinized freeze-dried samples was placed in a 10x25mm pellet and hermetically sealed with a hydraulic press. The X-ray diffraction patterns were obtained using a Siemens D5000 X-ray diffraction instrument set with conditions of 40KV, 30mA, and with a scanning angle of 2 θ set from 2˚ to 36˚ at a scanning rate of 0.6˚/minute. Relative crystallinity (RC) of the starch was determined by the method of Hermans and Weidinger (1948), as described by Nara et al (1978), i.e., the area of the crystalline fraction (ac) is divided by the diffraction area for a 100% crystalline substance (Ac). In this study, the area of the crystalline fraction in raw starch XRD pattern was used as the value of Ac (Dragsdorf and Varriano-Marston, 1980). X-ray patterns were designated according to the d-spacings and intensities given by Zobel (1988a,b). The diffraction patterns were then recorded and compared. The XRD experimentation was performed as a single analysis of each sample.

6.3. RESULTS AND DISCUSSION

6.3.1. XRD Patterns of Native White-Fleshed and Orange-Fleshed Sweet Potato Starches

The native sweet potato starches, both the white-fleshed and orange-fleshed, showed a clear A type XRD pattern with peaks at 5.8, 5.2, and 3.8A (Figure 6.1). The A pattern in XRD is generally regarded as the pattern for native cereal starches (Zobel, 1988a). Moorthy (2002) reported that ungelatinized sweet potato starches do exhibit A type patterns under XRD. The white-fleshed sweet potato starch was 34.4% more crystalline than orange-fleshed sweet potato starch, but both graphs were almost identical

when it came to intensities of peaks, placement of peaks, and pattern type. The increased relative crystallinity of the white-fleshed sweet potato starch may be due to the fact that the white-fleshed starch contained a much higher percentage of resistant starch than did the orange-fleshed sweet potato starch. The white-fleshed starch contained 39.8% resistant starch while the orange-fleshed sweet potato starch had only 20.6% resistant starch in the ungelatinized, native starch form. An increase in crystallinity has been associated with an increase in the amount of resistant starch present within a starch sample (Botham et al., 1995).

6.3.2. Effects of Gelatinization on the XRD Patterns of White-Fleshed and Orange-Fleshed Sweet Potato Starches

After gelatinization was complete through Rapid Visco Analysis, the starch samples changed dramatically in their crystalline patterns and overall relative crystallinity. The white-fleshed sweet potato starch exhibited a loss of the peaks at 5.8 and 3.8A, and a diminished peak at 5.2A, while a new peak emerged weakly at 4.0A after gelatinization (Figure 6.2). This new pattern is best described as a B type pattern, although it is missing the characteristic B-pattern peak at 16.0A. Although, retrograded starch is sometimes seen with a V-type pattern, the gelatinized starches in this study were found to have patterns more consistent with B-type patterns. Annison and Topping (1994) reported that the normal pattern of retrograded starch is in the B form. The gelatinization process caused a loss in relative crystallinity of 59.6% in the white-fleshed sweet potato starch (Table 6.1). During gelatinization both amylose and amylopectin leach out of the crystal structure of the starch granules. Eventually, with enough heating, the starch granules are completely disrupted and fall apart. The gelatinization process

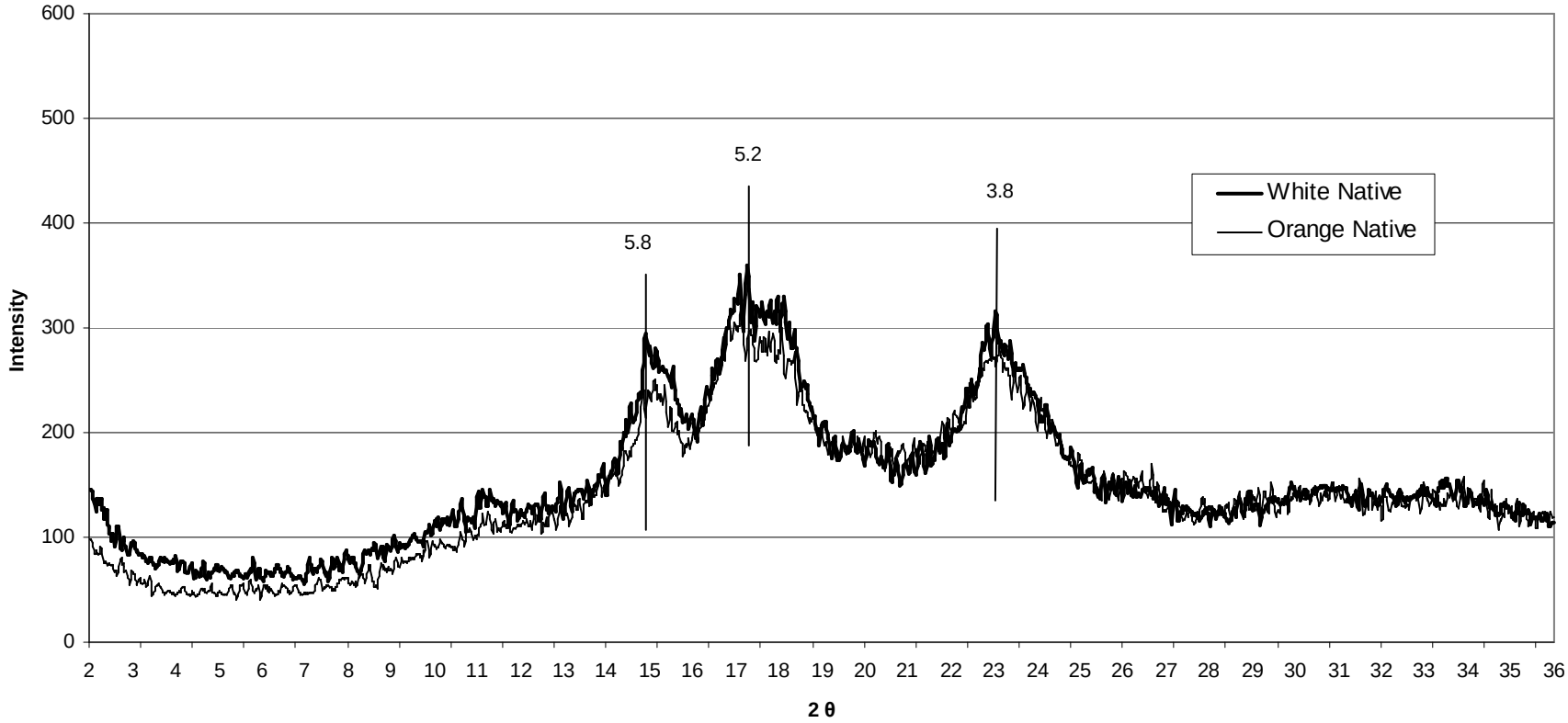

Figure 6.1. The XRD graph of native white-fleshed and orange-fleshed sweet potato starches.

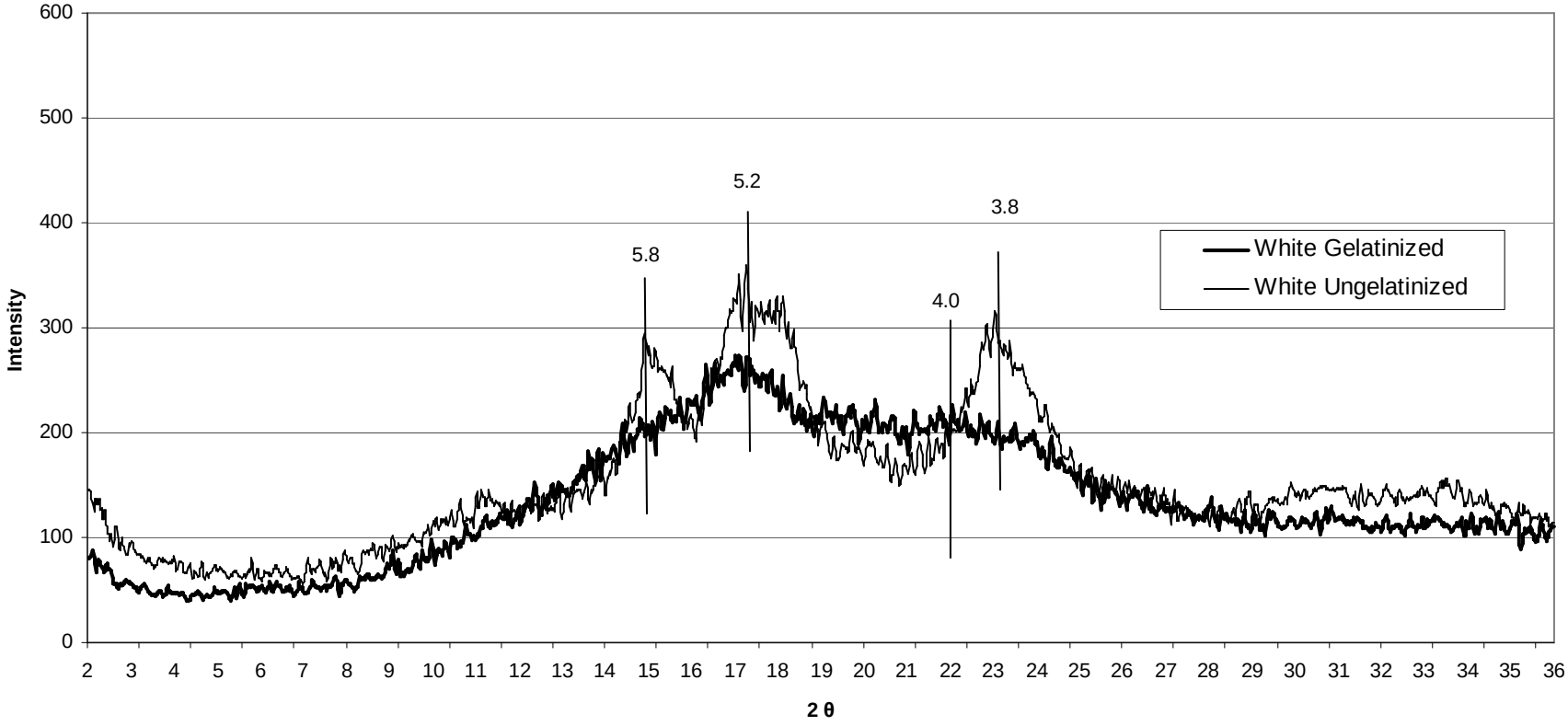

Figure 6.2. The XRD graph comparing white-fleshed gelatinized to the white-fleshed ungelatinized sweet potato starch.

alters the crystalline structure and relative crystallinity in a negative way (Thomas and Atwell, 1998). Liang and King (2003) found that after gelatinization, rice starch lost 47% relative crystallinity.

Table 6.1. Relative crystallinities of native and gelatinized white-fleshed and orange-fleshed sweet potato starches.

Starch	Native	Gelatinized
Orange-Fleshed Sweet Potato Starch	100%	76.6%
White-Fleshed Sweet Potato Starch	100%	40.4%

When the orange-fleshed sweet potato starch was gelatinized, the A type pattern of the native starch was lost (Figure 6.3). As in the white-fleshed sweet potato starch, the 5.8 and 3.8A peaks were lost, a new peak at 4.0A was gained and the peak at 5.2A was also diminished. This crystalline pattern can best be described as a B-type pattern, although it is missing the characteristic peak at 16.0A that the B-patterns usually have. After undergoing gelatinization, the orange-fleshed sweet potato starch lost 23.4% relative crystallinity (Table 6.1).

When examined together, the gelatinized white-fleshed and orange-fleshed sweet potato starch XRD patterns are almost identical (Figure 6.4). Both graphs show the same two peaks at 5.2 and 4.0A. The gelatinized orange-fleshed sweet potato starch was 19.6% more crystalline than the white-fleshed sweet potato starch (Table 6.1). This could be because the white-fleshed sweet potato starch contained a greater percentage of amylose in the native form. During gelatinization, the amylose is first to leach out of the starch granule and could have caused a much greater decrease in crystallinity in the white-fleshed sweet potato starch granules.

6.3.3. Effects of Amino Acid Additives on the Crystallinity of White-Fleshed and Orange-Fleshed Sweet Potato Starches

The amino acids aspartic acid, leucine, lysine and methionine were added to both the orange-fleshed and white-fleshed sweet potato starches and gelatinized prior to assessing their effects on the crystallinity of those starches. For the white-fleshed starch, the addition of aspartic acid did not change the crystalline pattern of the gelatinized starch, and had the effect of decreasing the overall relative crystallinity by 2.5% (Table 6.2).

Table 6.2. Relative crystallinities of the gelatinized orange-fleshed and white-fleshed sweet potato starches with added amino acids.

Starch	Control	Aspartic Acid	Leucine	Lysine	Methionine
Orange-Fleshed Sweet Potato Starch	100%	85.8%	99.3%	73.7%	95.9%
White-Fleshed Sweet Potato Starch	100%	97.5%	125.7%	145.9%	97.7%

A decrease in crystallinity is an unfavorable occurrence, since it could signify a decrease in the amount of resistant starch (Botham et al., 1995). Liang and King (2003) found that the addition of aspartic acid to rice starch did not affect the overall crystallinity of the starch but did induce new peaks at 3.7 and 3.4A. Figure 6.6 shows the differences in the graphs of the gelatinized white-fleshed sweet potato starch with no added amino acids and that of the gelatinized white-fleshed starch with added leucine at 6% dry weight basis. The leucine changed the crystalline pattern of the starch by decreasing the intensity of the 5.2 and 4.0A peaks and by adding a novel peak at 16.0A. This XRD graph, with peaks at 16.0, 5.2, and 4.0A is also a B-type pattern. In addition, the overall relative crystallinity was increased 25.7% (Table 6.2). The addition of methionine caused a decrease in relative crystallinity of 2.3% with the formation of a peak at 16.0A

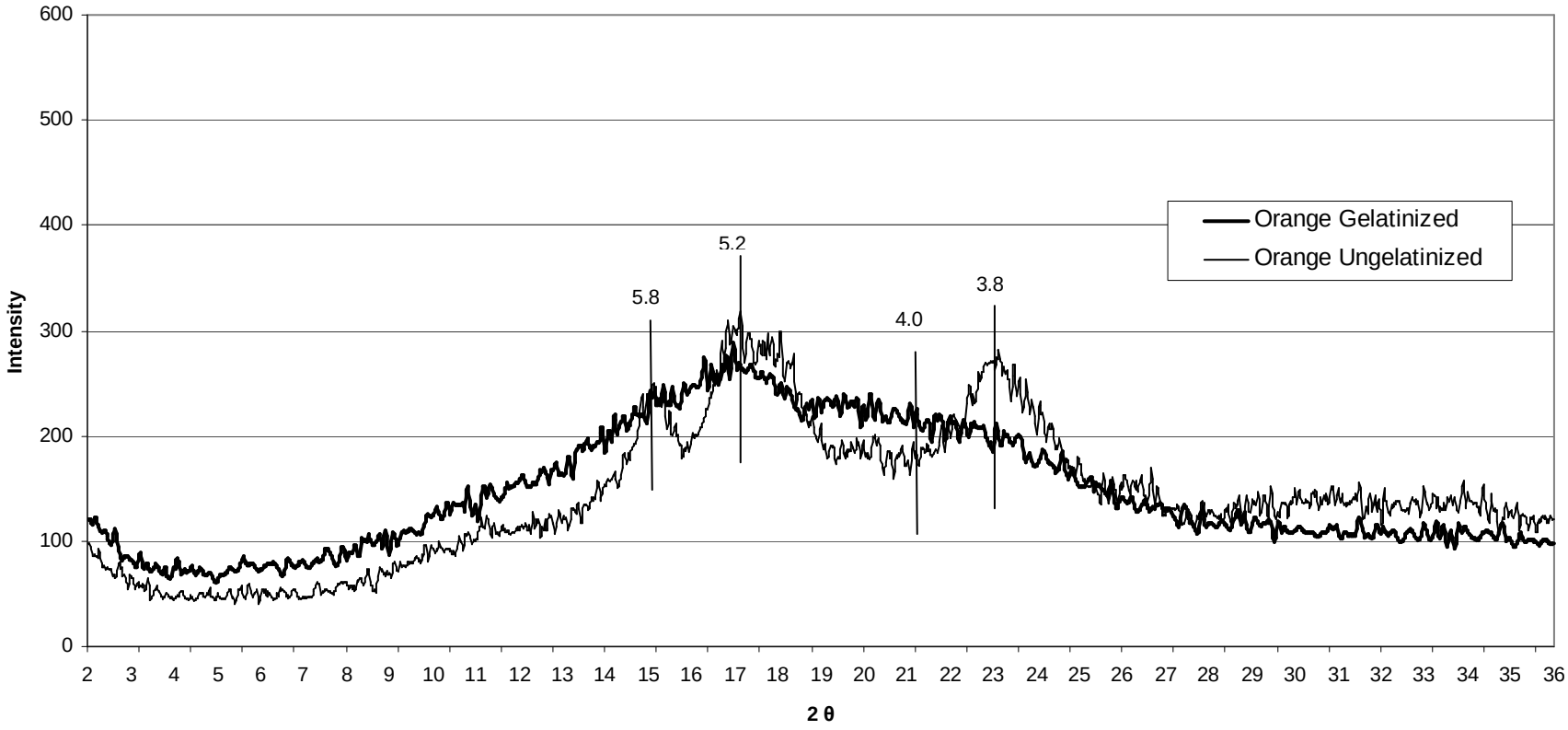

Figure 6.3. The XRD graph comparing orange-fleshed gelatinized to the orange-fleshed ungelatinized sweet potato starch.

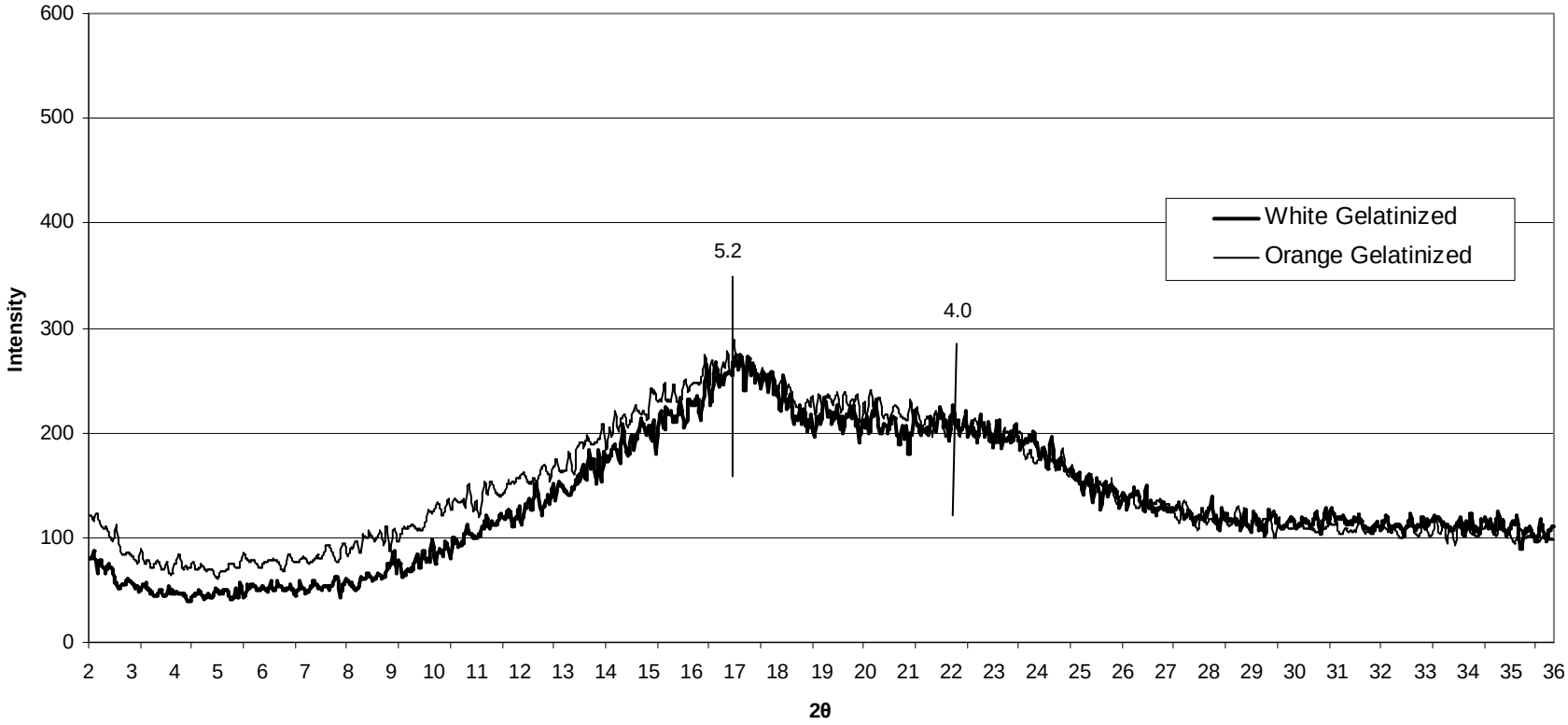

Figure 6.4. The XRD graph of gelatinized white-fleshed and orange-flesh sweet potato starch.

(Table 6.2, Figure 6.7). When lysine was added to the white-fleshed sweet potato starch, an increase in the intensity of the peaks at 5.2 and 4.0A was observed, as well as the appearance of a small peak at 16.0A (Figure 6.8). This pattern is still a B-type pattern. An (2005) found that the addition of lysine at 6% dry starch basis to rice starch induced the formation of two peaks, 5.2 and 4.0A. This result agrees with the results of the present study. The lysine also induced an increase in relative crystallinity of 45.9% over the crystallinity of the gelatinized white-fleshed sweet potato starch with no added amino acids (Table 6.2). This could suggest an increase in resistant starch, but the lysine did not cause any fluctuation in the amount of total resistant starch present in the white-fleshed sweet potato starch (Botham et al., 1995). An (2005) also found that the addition of lysine to rice starch caused an increase in the overall relative crystallinity of the starch.

When the amino acids were added to the orange-fleshed sweet potato starch, only leucine caused any large effects on the crystallinity of the starch. The addition of aspartic acid did not change the crystalline pattern of the starch and decreased the relative crystallinity by 14.2% (Table 6.2, Figure 6.9). Lysine and methionine, when added to the orange-fleshed sweet potato starch caused the formation of a small peak at 16.0A, but decreased the relative crystallinity by 26.3 and 4.1%, respectively (Table 6.2, Figures 6.10 and 6.11). This decrease in relative crystallinity could translate to a starch that is more easily digestible and an overall decrease in the percentage of resistant starch. The addition of leucine caused a change in the crystalline pattern, as well as only a slight decrease in relative crystallinity of 0.7% (Table 6.2). The peak at 5.2A was decreased slightly, while a new very strong peak appeared at 16.0A (Figure 6.12). This pattern is also a B-type pattern.

6.4. CONCLUSION

White-fleshed and orange-fleshed sweet potato starch, in the native form, show A-type crystalline patterns under X-ray diffraction. The gelatinization of both white-fleshed and orange-fleshed sweet potato starches results in a loss of overall relative crystallinity. Also, the addition of various amino acids can affect the overall pattern and relative crystallinity of the two sweet potato starches. Aspartic acid did not change the crystalline pattern of the gelatinized white-fleshed or orange-fleshed sweet potato starches, but did cause a large decrease in the overall relative crystallinity. The addition of lysine, and methionine to the white-fleshed and orange-fleshed sweet potato starch had similar effects including the evolution of a new weakpeak at 16.0A. Leucine, added to the both sweet potato starches, induced the formation of a strong peak at 16.0A and increased the relative crystallinity in the white-fleshes sweet potato starch, while slightly decreasing the relative crystallinity in the orange-fleshed sweet potato starch.

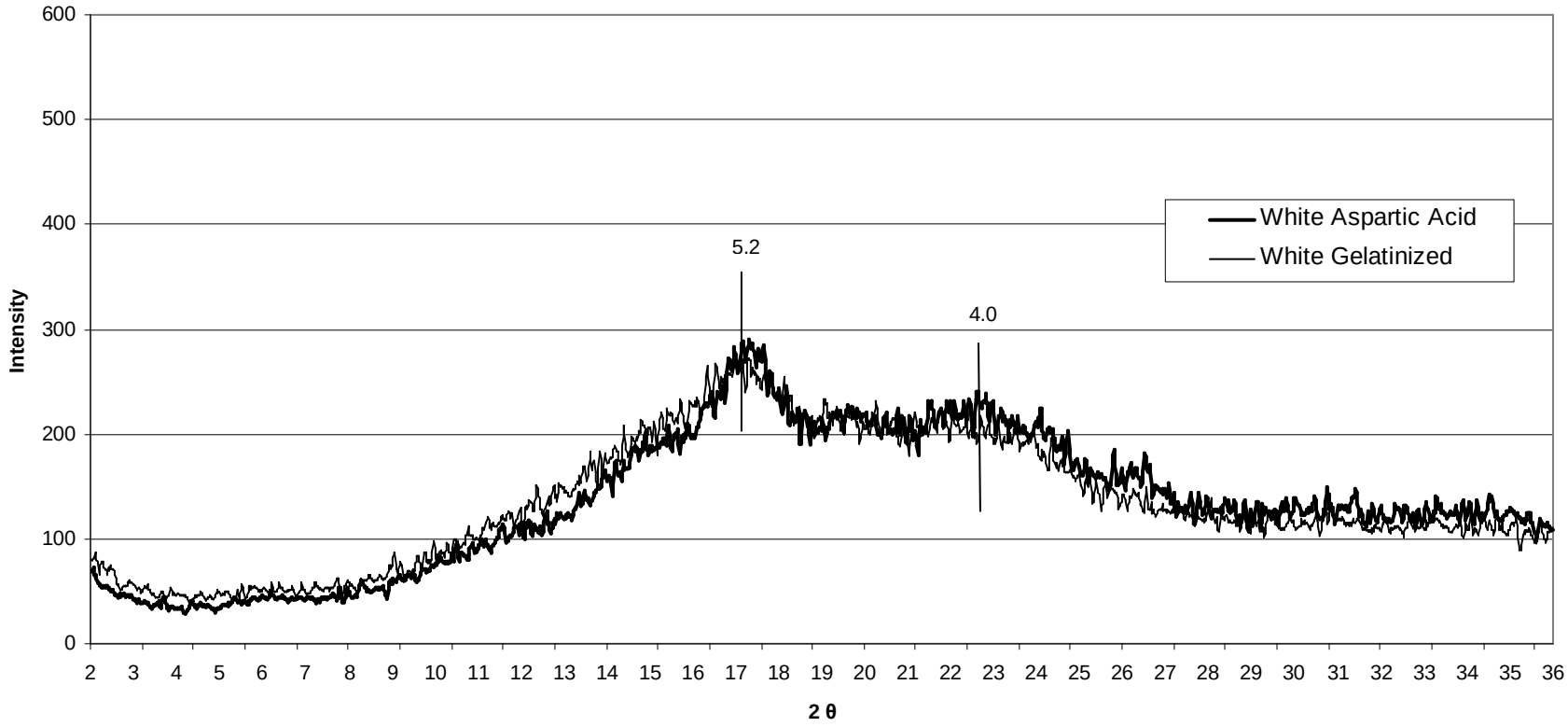

Figure 6.5. Comparison of white-fleshed sweet potato starch with added aspartic acid to the control (no added amino acids).

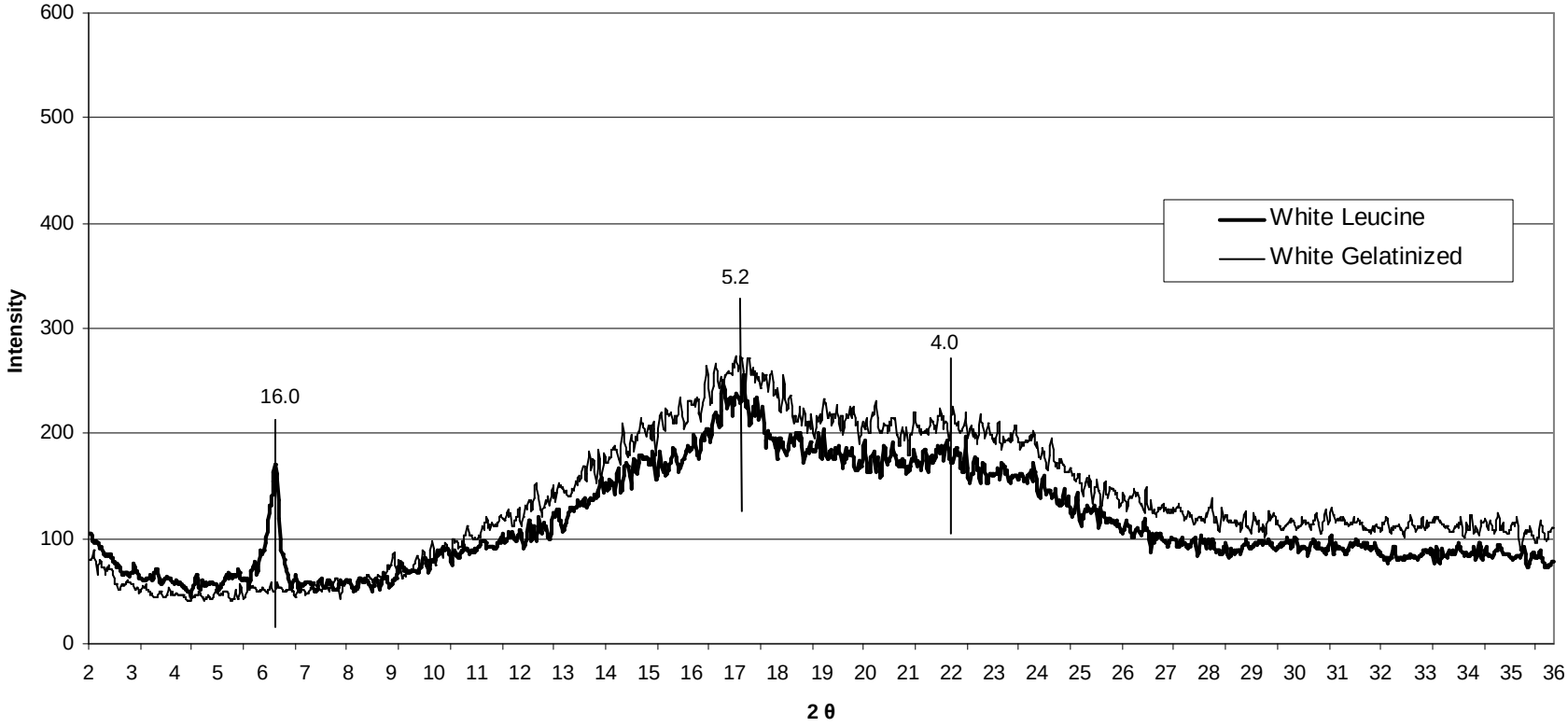

Figure 6.6. Comparison of white-fleshed sweet potato starch with added leucine to the control (no added amino acids).

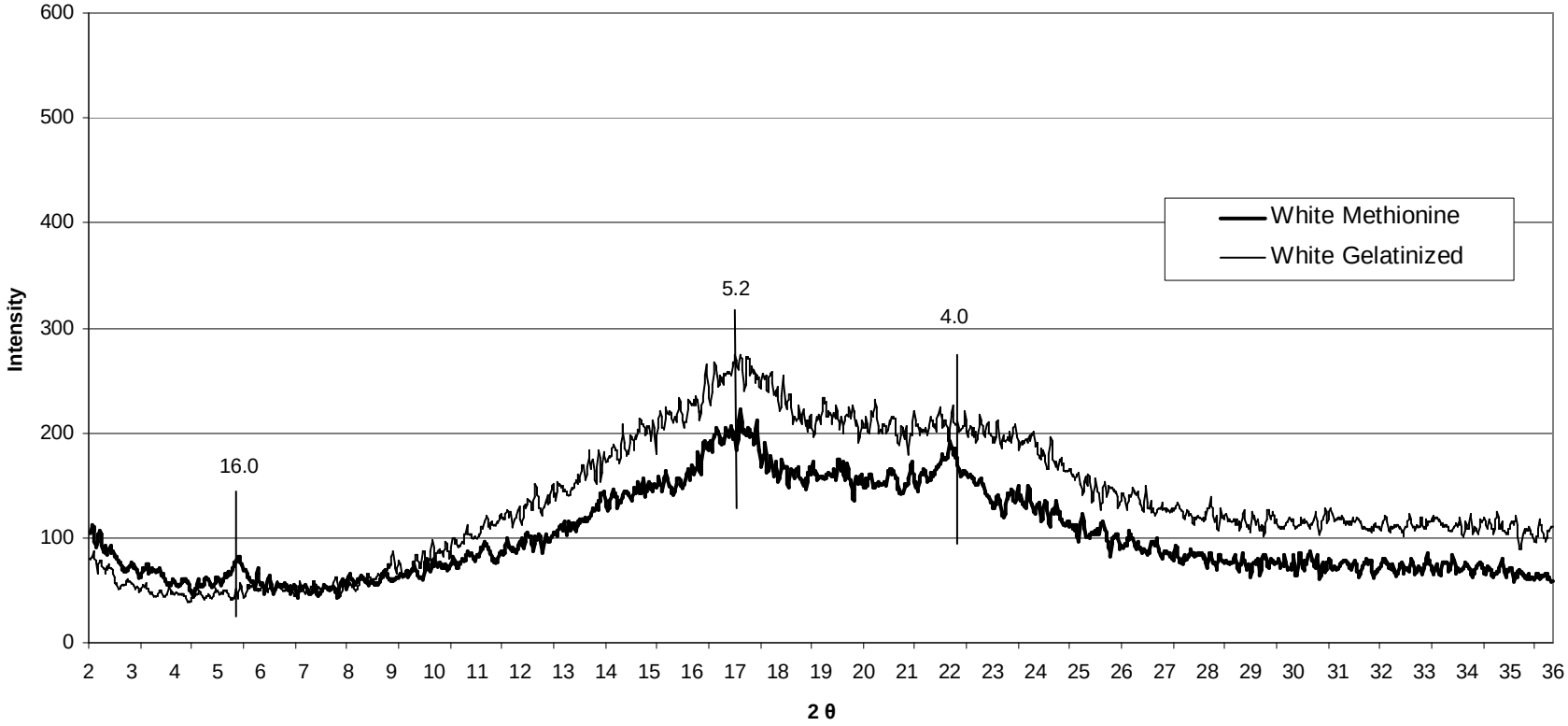

Figure 6.7. Comparison of white-fleshed sweet potato starch with added methionine to the control (no added amino acids).

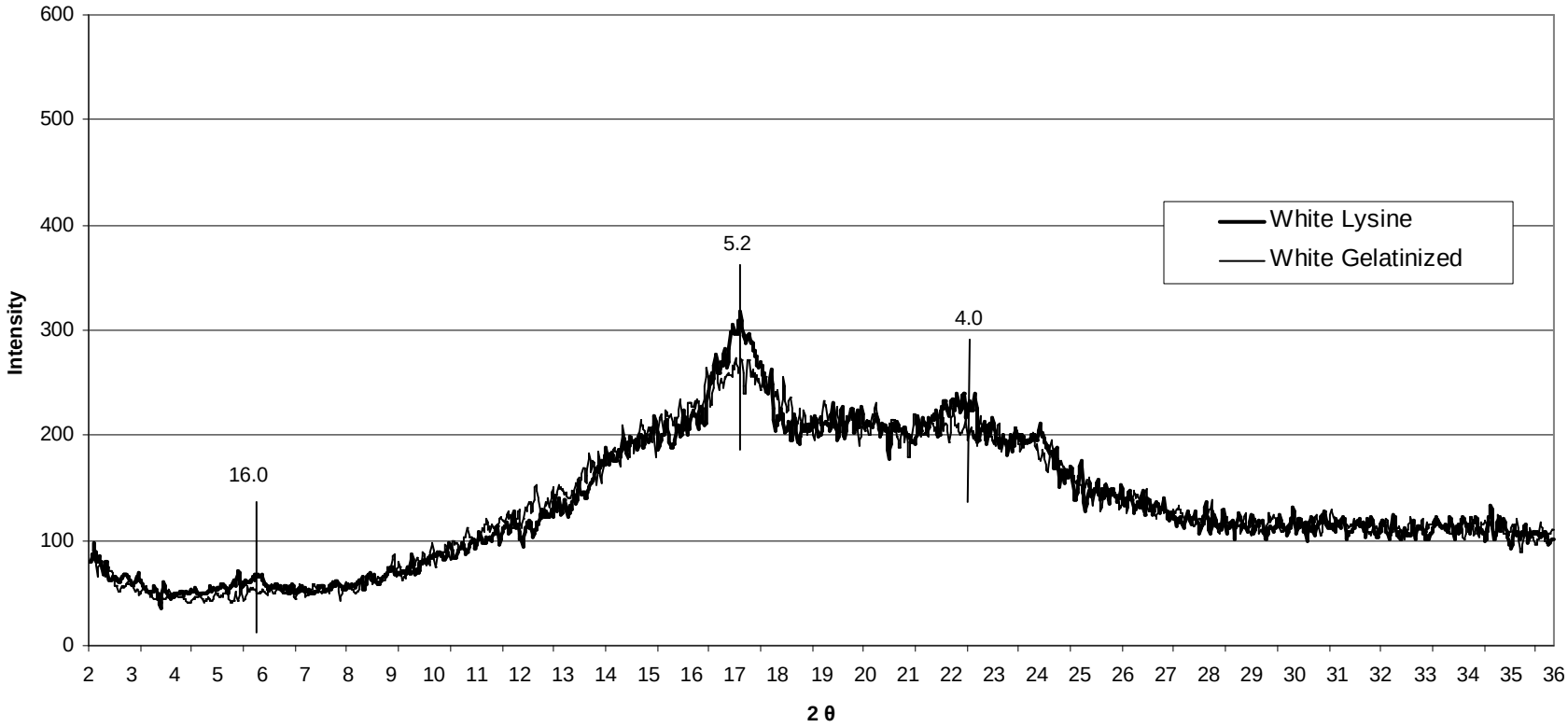

Figure 6.8. Comparison of white-fleshed sweet potato starch with added lysine to the control (no added amino acids).

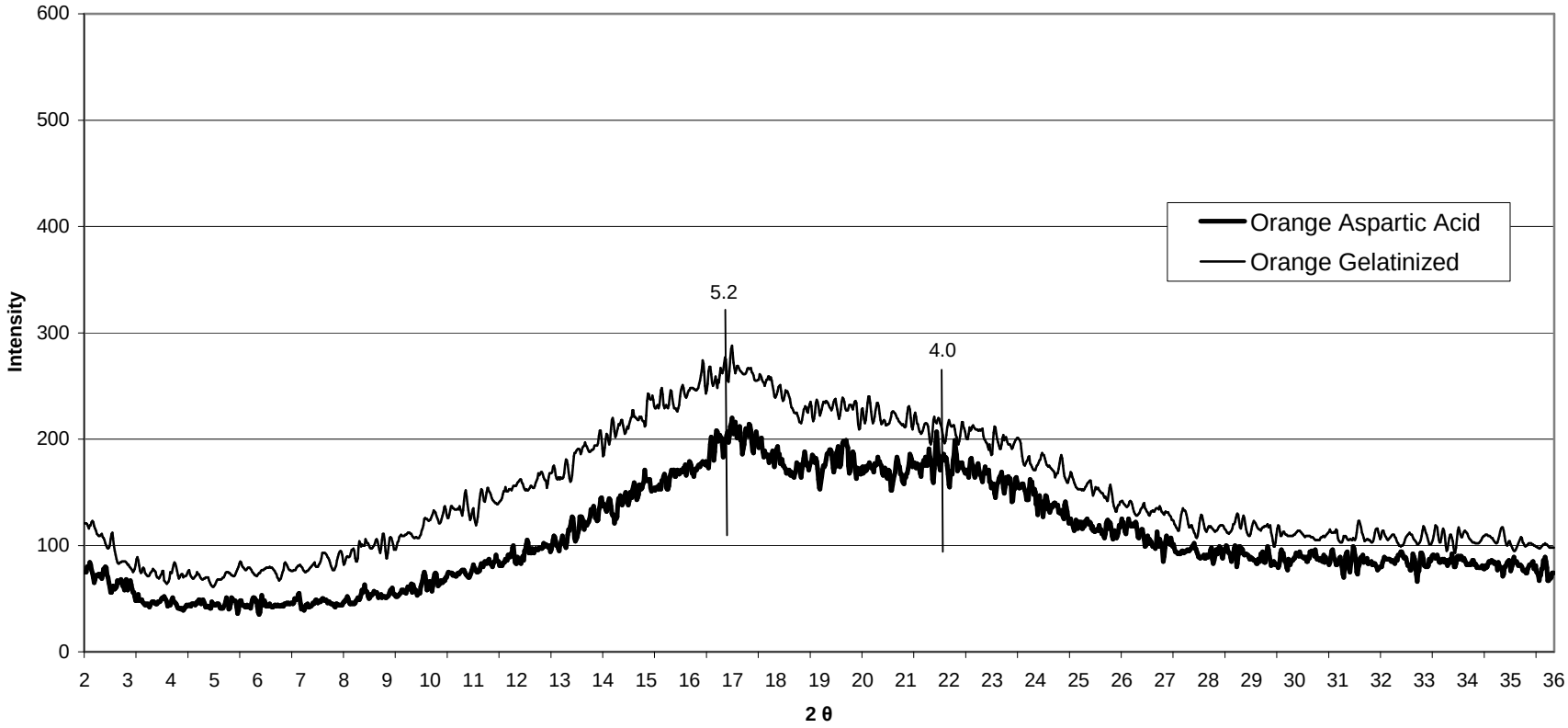

Figure 6.9. Comparison of orange-fleshed sweet potato starch with added aspartic acid to the control (no added amino acid).

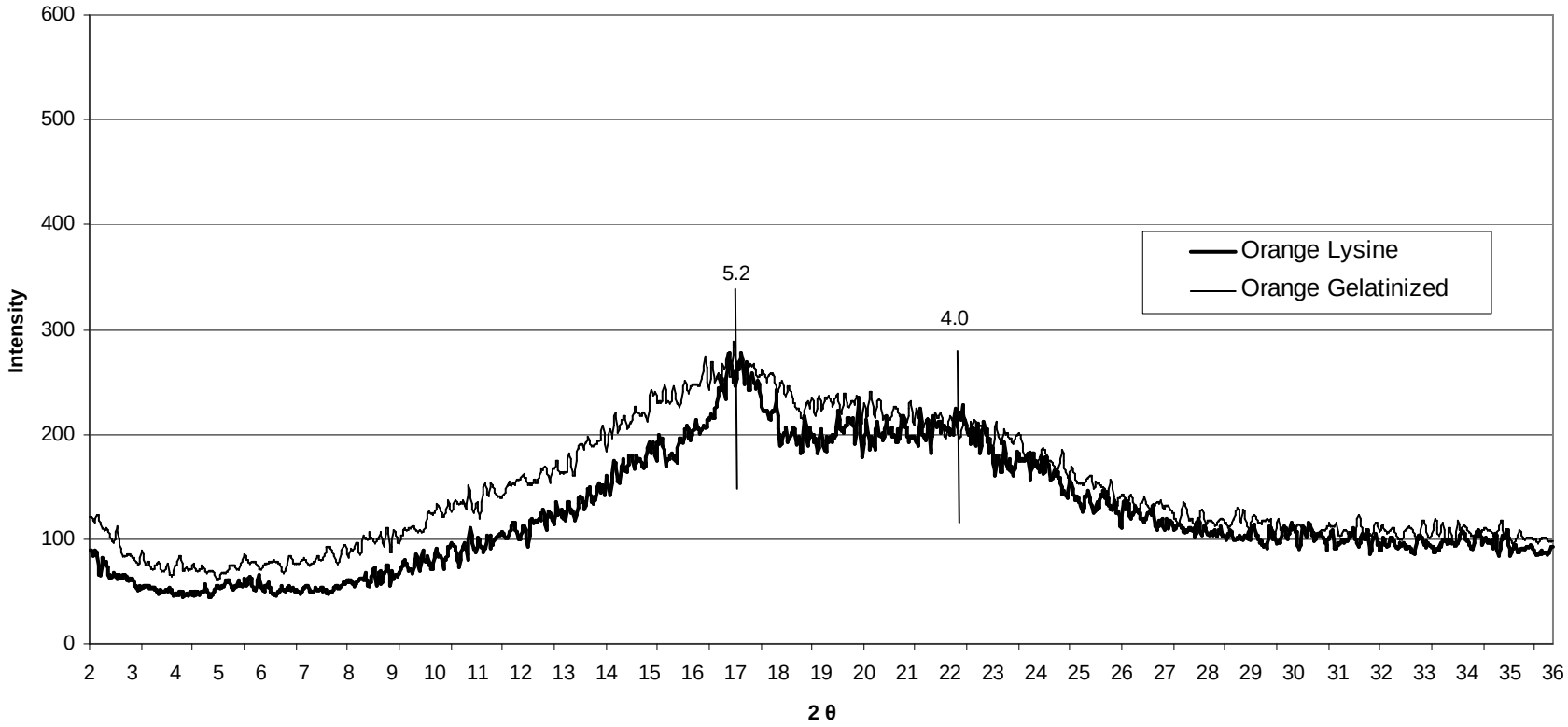

Figure 6.10. Comparison of orange-fleshed sweet potato starch with added lysine to the control (no added amino acid).

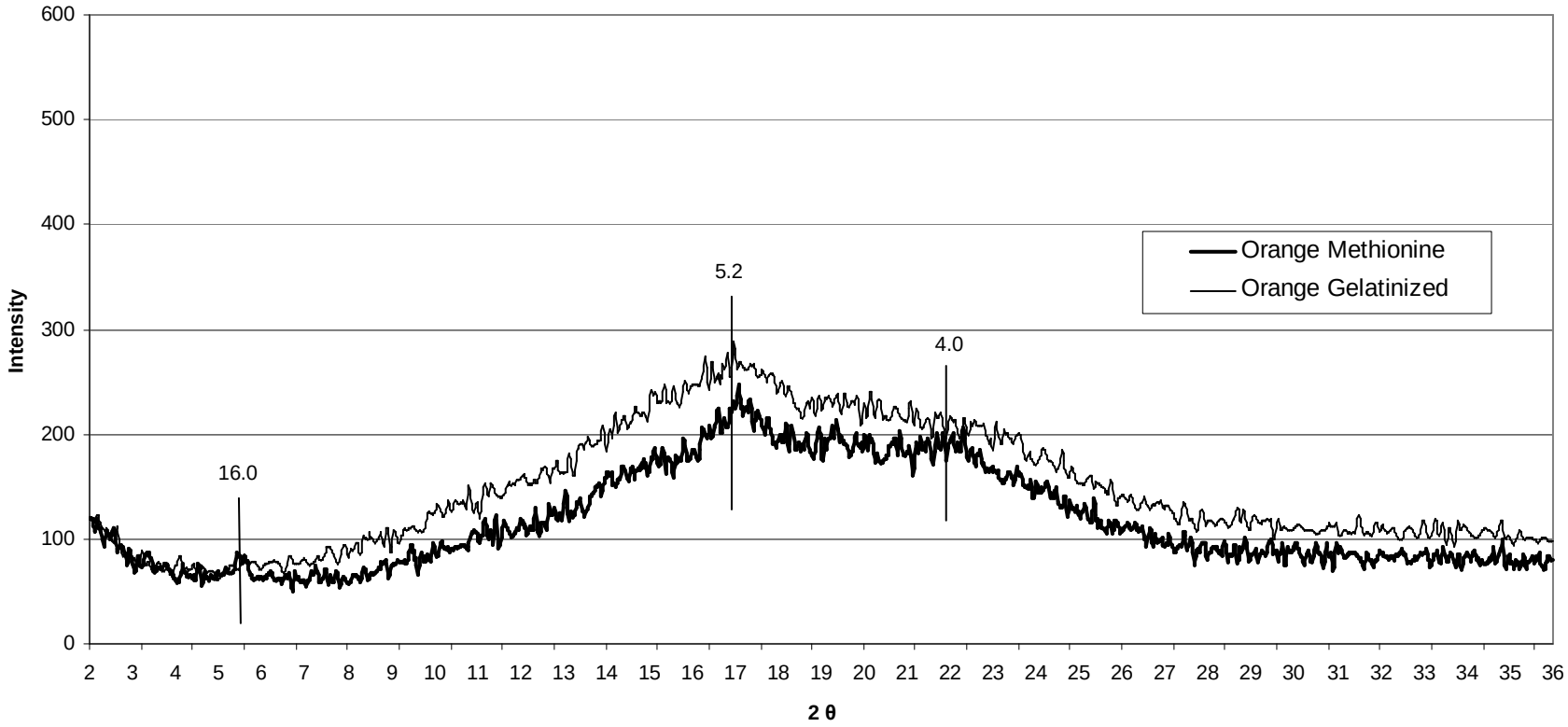

Figure 6.11. Comparison of orange-fleshed sweet potato starch with added methionine to the control (no added amino acid).

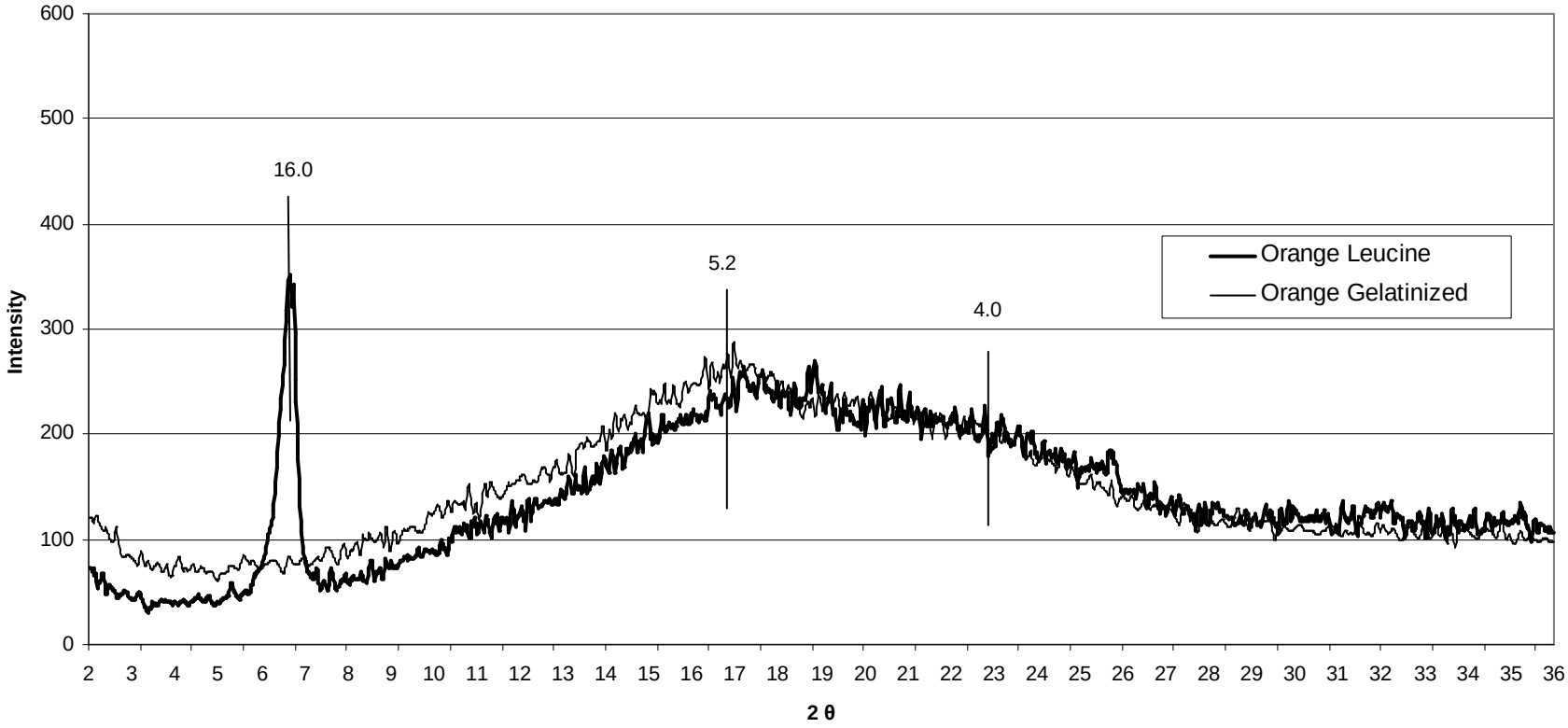

Figure 6.12. Comparison of orange-fleshed sweet potato starch with added leucine to the control (no added amino acid).

CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The sweet potato is a very useful crop in terms of the variety of food products that can be made from the flesh and starch of the root. The extraction of starch could be an outlet for much of the rejected waste cuts of sweet potato produced by the sweet potato processors. This could include misshapen, small, or blemished pieces of sweet potato that are unfit to be sold as the whole root and cannot be used in canning.

The comparison of the orange-fleshed sweet potato starch to that of the white-fleshed starch showed many differences between the two starches. The orange-fleshed sweet potato starch exhibited a lower gelatinization temperature than the white-fleshed starch, but both starches needed the same amount of energy to complete gelatinization. The white-fleshed starch had a much larger amylose content than did the orange-fleshed sweet potato starch; the relationship between amylose content and retrogradation was supported by our study using RVA. The orange-fleshed starch was found to be easier to cook with a lower potential for retrogradation but was also found to be less stable to shear during cooking than the white-fleshed sweet potato starch. The white-fleshed sweet potato starch had more resistant starch than the orange-fleshed starch in both gelatinized and ungelatinized forms. In terms of crystallinity, both the white-fleshed and orange-fleshed sweet potato starches exhibited an A-type pattern in their native forms with a shift to a B-type pattern after gelatinization. The orange-fleshed sweet potato starch was found to be more crystalline after gelatinization than the white-fleshed starch.

Although most of the amino acids tested, did not significantly increase the amount of resistant starch in either of the white-fleshed or orange-fleshed sweet potato starches to

produce a healthier starch, the additives did affect other characteristics of the starches that could make the starches more suitable for cooking and processing. Lysine was the only additive found to increase the amount of resistant starch in the orange-fleshed sweet potato starch. Lysine, also, was the amino acid with the greatest effect on the thermal characteristics for both the white-fleshed and orange-fleshed starches. The lysine served to increase the gelatinization temperatures for both starches; aspartic acid raised these temperatures, as well, but only in the white-fleshed sweet potato starches. The addition of aspartic acid and lysine, the two charged amino acids, caused significant alterations to the pasting properties of both the white-fleshed and orange-fleshed sweet potato starches. The aspartic acid decreased the stability of both starches to shear during cooking and also lowered the potential for retrogradation. Lysine, in the orange-fleshed sweet potato starch, made the starch more stable during cooking. The addition of leucine and lysine, to a lesser extent, caused changes in the crystalline pattern of the gelatinized orange-fleshed and white-fleshed sweet potato starches. All of the amino acids added to the orange-fleshed sweet potato starch decreased the relative crystallinity, which makes the starch more susceptible to digestion by enzymes. The addition of leucine and lysine to the white-fleshed starch caused large increases in the relative crystallinity of the starch, making the starch harder to digest.

Overall, the charged amino acids used, lysine and aspartic acid, caused the most changes to both the white-fleshed and orange-fleshed sweet potato starches. More research should be done examining the effects of different levels of amino acid additives, and possibly other charged amino acids. Also, scanning electron microscopy (SEM) could be done to the white-fleshed and orange-fleshed sweet potato starches in order to determine whether a difference exists in the starch granule shapes and sizes.

REFERENCES

Adam, Katherine. 2005. Sweetpotato: Organic Production. *ATTRA: The National Sustainable Agriculture Information Service*.

American Association of Cereal Chemists. 2000. AACC Approved Methods, 10th ed. AACC, St. Paul, MN, USA.

An, Hee-Joung. 2005. Effects of Ozonation and Addition of Amino Acids on Properties of Rice Starches. PhD Dissertation. Louisiana State University. Pp164.

Annison G, Topping DL. 1994. Nutritional Role of Resistant Starch: Chemical Structure vs. Physiological Function. *Ann. Rev. Nutr*. 14:297-320.

Association of Official Agricultural Chemists. 1995. AOAC Official Method of Analysis, 16th ed. AOAC International, Arlington, VA, USA.

Bean, Maura M. 1986. Rice Flour—Its Functional Variations. *Cereal Foods World*. 477-481.

Berry, C.S. 1986. Resistant Starch: Formation and Measurement of Starch that Survives Exhaustive Digestion with Amylolytic Enzymes during the Determination of Dietary Fibre. *Journal of Cereal Science*. 4:301-314.

Botham, R.L, P. Cairns, V.J. Morris, S.G. Ring, H.N. Englyst, and J.H. Cummings. 1995. A physicochemical characterization of chick pea starch resistant to digestion in the human small intestine. *Carbohydrate Polymers*. 26: 85-90.

Brown, Ian L. 2004. Applications and Uses of Resistant Starch. *Journal of AOAC International*. 87(3): 727-32.

Centro International de la Papa. 2006. About Sweet Potato. www.CIPotato.org. 16 June 2006.

Champ, Marine, Anna-Maria Langkilde, Fred Brouns, Bernd Kettlitz, and Yves Le Bail-Collet. 2003. Advances in dietary fibre characterization. *Nutrition Research Reviews*. 16:143-161.

Collado, L.S., R.C. Mabesa, H. Corke. 1999. Genetic variation in the physical properties of sweet potato starch. *Journal of Agricultural Food Chemistry*. 47: 4195-4201.

Colston, N.V. and C. Smallwood. 1974. Waste Control in the Processing of Sweet Potatoes. *Environmental Protection Agency*.

DeMan, John. 1999. Polysaccharides. *Principles of Food Chemistry*. 3rd Edition. Pp. 183-187. Aspen Publishers Inc. Gaithersburg, Maryland

Dragsdorf, R.D., and E. Varriano-Marston. 1980. Bread staling: x-ray diffraction studies on bread supplemented with α-Amylases from different sources. *Journal of Cereal Chemistry*. 57(5):310-314.

Englyst KN and Englyst HN. 2005. Carbohydrate Bioavailability. *British Journal of Nutrition*. 94:1-11.

Englyst, H.N., S.M. Kingman, and J.H. Cummings. 1992. Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*. 46:33-50.

Espinola, N, H. Creed-Kanashiro, M.E. Ugaz, M. van Hal, G. Scott. 1998. Development of a Sweetpotato-Based Instant Weaning Food for Poorly Nourished Children Six Months to Three Years Old. *Impact on a Changing World.* CIP Program Report 1997-98. p. 295-301.

Food and Agriculture Organization. 1990. Roots, tubers, plantains, and bananas in human nutrition. *FAO Corporate Document Repository*.

Goldring, M. 2004. Resistant Starch: Safe Intakes and Legal Status. *Journal of AOAC International*. 87(3): 733-9.

Hermans, P.H. and Weidinger, A. 1948. Quantitative X-ray investigations on the crystallinity of cellulose fibers, a background analysis. *Journal of Applied Physics*. 19:491-506.

Higgins, JA. 2004. Resistant Starch: Metabolic Effects and Potential Health Benefits. *Journal of AOAC International*. 87(3):761-8.

Ishiguro, Koji, Takahiro Noda, and Osamu Yamakawa. 2003. Effect of Cultivation Conditions on Retrogradation of Sweetpotato Starch. *Starch*. 55:564-568.

Ito, Azusa, Makoto Hattori, Tadashi Yoshida, Koji Takahashi. 2004. Reversible Regulation of Gelatinization of Potato Starch with Poly(lysine) and Amino Acids. *Starch*. 56:570-575.

Jangchud, Kamolwan, Yuthana Phimolsiripol, and Vichai Haruthaithanasan. 2003. Physicochemical Properties of Sweet Potato Flour and Starch as Affected by Blanching and Processing. *Starch*. 55: 258-264.

Juliano, Bienvenido, Gloria Bautista, Joaquin Lugay, Aurora Reyes. 1964. Studies on the Physicochemical Properties of Rice. *Rice Quality*. 12(2): 131-138.

Karim, A.A., L.C. Toon, V.P.L Lee, W.Y. Ong, A. Fazilah, and T. Noda. 2007. Effects of phosphorus contents on the gelatinization and retrogradation of potato starch. *Journal of Food Science*. 72(2):132-138.

Kitahara, Kanefumi, Satoshi Fukunaga, Kenji Katayama, Yasuhiro Takahata, Yoshinori Nakazawa, Masaru Yoshinaga, and Toshihiko Suganuma. 2005. Physicoshemical Properties of Sweetpotato Starches with Different Gelatinization Temperatures. *Starch*. 57: 473-479.

Katayama, Kenji, Kozo Komae, Kaoru Kohyama, Tsuneo Kato, Seiji, Tamiya, and Katsumi Komaki. 2002. New Sweet Potato Line having low Gelatinization Temperature and altered starch structure. *Starch*. 54: 51-57.

Kaur, Lovedeep, Jaspreet Singh, and Narpinder Singh. 2006. Effect of cross-linking on some properties of potato (Solanum Terosum L.) starches. *Journal of the Science of Food and Agriculture*. 86: 1945-1954.

Liang, Xiaoming. 2001. Effects of Lipids, Amino Acids, and Beta-Cyclodextrin on Gelatinization, Pasting, and Retrogradation Properties of Rice Starch. PhD Dissertation. Louisiana State University. Pp. 161.

Liang X. and J.M. King. 2003. Pasting and Crystialline Property Differences of Commercial and Isolated Rice Starch with Added Amino Acids. *Journal of Food Science*. 68(3):832-8.

Lucier, Gary, Jane Allshouse, and Biing-Hwan Lin. 2002. Sweet Potatoes: Getting to the Root of Demand. *Agricultural Outlook November 2002.* p. 13-17.

Mahadevamma, S. and R. N. Tharanathan. 2006. Processed rice starch characteristics and morphology. *Eur Food Res Technol*. 225(3-4): 1438-2377.

McPherson, A.E., and J. Jane. 1999. Comparison of waxy potato with other root and tuber starches. *Carbohydrate Polymers*. 40: 57-70

Megazyme Resistant Starch Assay Procedure. 2002. Megazyme International Ireland Ltd. AOAC Methods 2002.02 and 32-40.

Moorthy, Subramony Narayana. 2002. Physicochemical and Functional Properties of Tropical Tuber Starches: A Review. *Starch*. 54: 559-592.

Mukherjee, P.K. and S. Ilangantileke. 2002. Dietary Intervention with Orange Fleshed Sweet potato (Impomoea batatas(L.) Lam.) to Alleviate Vitamin A Deficiency in South and West Asia. *Proceedings of the 1st International Conference on Sweetpotato*. Acta Horticulturae. 583: 205-210

Murugesan, Gurunathan, Kiyoshi Shibanuma, and Susumu Hizukuri. 1993. Characterisation of hot-water-soluble components of starches. *Carbohydrate Research*. 242: 203-15.

Nara, S., Mori, A., Komiya, T. 1978. Study on Relative Crystallinity of Moist Potato Starch. *Starch/Starke*. 30:111.

Noda, Takahiro, Shogo Tsuda, Motoyuki Mori, Shigenobu Takigawa, Chie Matsuura-Endo, Katsuichi Saito, Wichramasinghe Hetti Arachichige Mangalika, Akihiro Hanaoka, Yasuyuki Suzuki, and Hiroaki Yamauchi. 2003. The effect of harvest dates on the starch properties of various potato cultivars. *Food Chemistry*. 86: 119-125.

Noda, Takahiro, Shogo Tsuda, Motoyuki Mori, Shigenobu Takigawa, Chie Matsuura-Endo, Sun-Ju Kim, Naoto Hashimoto, and Hiroaki Yamauchi. 2005. Determination of the phosphorus content in potato starch using an energy-dispersive X-ray fluorescence method. *Food Chemistry*. 95:632-637.

Nowjee, Nitin C., 2004. Melt processing and foaming of starch. University of Cambridge. www.cheng.cam.ac.uk.

Patrick, Ruth and Mike Cannon. 1996. Louisiana Yams. LSU AgCenter Research and Extension.

Prakash, C.S. 1994. Sweet Potato Biotechnology: Progress and potential. *Biotechnology and Development Monitor*.18: 1819-22.

Qian, Jian Ya, and Manfred Kuhn. 1999. Evaluation on gelatinization of buckwheat starch: a comparative study of Brabender viscoamylography, rapid visco-analysis, and differential scanning calorimetry. *Eur Food Res Technol*. 209:277-280.

Ranhotra, G.S., J.A. Gelroth, and B.K. Glaser. 1996. Energy value of resistant starch. *Journal of Food Science*. 61(2): 454-459.

Sajilata, M.G., Rekha Singhal, Pushpa Kulkarni. 2006. Resistant Starch—A Review. *Comprehensive Reviews in Food Science and Food Safety*. 5: 1-17.

Saura-Calixto, F., I. Goni, L. Bravo, and E. Manas. 1993. Resistant Starch in Foods: Modified Method for Dietary Fiber Residues. *Journal of Food Science*. 58(3):642-643.

Shin, SangIck, Jaeeon Byun, Kwan Hwa Park, and Tae Wha Moon. 2004. Effect of Partial Acid Hydrolysis and Heat-Moisture Treatment on Formation of Resistant Tuber Starch. *Cereal Chemistry*. 81(2):194-198.

Statistical Analysis Software. 2006. SAS 9.1.3. Service Pack 4. SAS Institute, Cary, NC, USA.

Tapsell, Linda C., 2004. Diet and Metabolic Syndrome: Where Does Resistant Starch Fit In? *Journal of AOAC International*. 87(3):756-60.

Tester, R.F. and W.R. Morrison. 1990. Swelling and gelatinization of cereal starches. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*. 67(6): 551-557.

Thomas, David, and William Atwell. 1998. Gelatinization, Pasting, and Retrogradation. *Starches*. Eagen Press. Pp. 25-30.

Tsou, S.C.S. and T.L. Hong. 1992. The Nutrition and Utilization of Sweet potato. In *Sweet Potato Technology for the Twenty-first Century*, ed. Chapter 4. Tuskegee University: Tuskegee University Press.

Yadav, Ramesh, Manisha Guha, R.N. Tharanathan, and R.S. Ramteke. 2004. Changes in characteristics of sweet potato flour prepared by different drying techniques. *Swiss Society of Food Science and Technology*. 39: 20-26.

Yun, S.H. and N.K. Matheson. 1990. Estimation of amylose content of starches after precipitation of amylopectin by concannain A. *Starch*. 42: 302-305.

Zobel, H.F. 1988a. Molecules to granules: a comprehensive starch review. Starch/Starke 40(1988): 44-50.

Zobel, H.F. 1988b. Starch crystal transformations and their industrial importance. Starch/Starke 40(1988): 1-7.

APPENDIX 1
DSC RAW DATA AND SAS CODE

Sweetpotato	Additive	Onset Temp	Peak Temp	Concl. Temp	Enthalpy
white	noaa	70.19	76.7	85.4	11.68
white	noaa	70.19	76.58	86.29	12.03
white	noaa	70.01	76.69	85.04	11.11
white	aspa	72.6	78.61	85.96	8.61
white	aspa	71.17	78.96	87.24	11.19
white	aspa	72.31	78.47	87.1	11.93
white	leu	71.03	76.93	85.39	10.16
white	leu	70.75	76.82	83.69	7.97
white	leu	70.32	76.2	83.12	8.94
white	lys	73.02	79.72	88.38	12.07
white	lys	73.16	79.76	87.38	11.34
white	lys	73.45	79.35	85.53	8.684
white	meth	69.65	76.4	84.33	10.69
white	meth	69.48	75.63	83.79	10.87
white	meth	69.89	76.01	83.4	9.89
orange	noaa	55.68	67.48	86.25	16.7
orange	noaa	56.67	68.27	81.84	10.77
orange	noaa	58.09	67.31	80.56	10.06
orange	aspa	58.38	68.88	82.55	10.93
orange	aspa	58.92	68.77	83.12	10.57
orange	aspa	56.96	68.34	81.98	11.07
orange	leu	57.67	67.8	78.43	8.873
orange	leu	57.52	67.78	80.27	10.2
orange	leu	57.38	67.44	80.42	10.11
orange	lys	59.23	71.1	83.69	10.53
orange	lys	58.8	70.66	84.82	11.66
orange	lys	59.09	70.91	83.26	10.46
orange	meth	56.77	67.32	82.54	12.69
orange	meth	57.31	66.33	80.75	9.17
orange	meth	56.24	65.62	79.49	10.24

```
dm "clear log; clear output";
options nodate nonumber;
data DSC;
input sweetpotato $ additives $ OT PT CT EN;
cards;
white  noaa  70.19  76.7   85.4   11.68
white  noaa  70.19  76.58  86.29  12.03
white  noaa  70.01  76.69  85.04  11.11
white  aspa  72.6   78.61  85.96  8.61
white  aspa  71.17  78.96  87.24  11.19
```

```
white   aspa   72.31   78.47   87.1    11.93
white   leu    71.03   76.93   85.39   10.16
white   leu    70.75   76.82   83.69   7.97
white   leu    70.32   76.2    83.12   8.94
white   lys    73.02   79.72   88.38   12.07
white   lys    73.16   79.76   87.38   11.34
white   lys    73.45   79.35   85.53   8.684
white   meth   69.65   76.4    84.33   10.69
white   meth   69.48   75.63   83.79   10.87
white   meth   69.89   76.01   83.4    9.89
orange  noaa   55.68   67.48   86.25   16.7
orange  noaa   56.67   68.27   81.84   10.77
orange  noaa   58.09   67.31   80.56   10.06
orange  aspa   58.38   68.88   82.55   10.93
orange  aspa   58.92   68.77   83.12   10.57
orange  aspa   56.96   68.34   81.98   11.07
orange  leu    57.67   67.8    78.43   8.873
orange  leu    57.52   67.78   80.27   10.2
orange  leu    57.38   67.44   80.42   10.11
orange  lys    59.23   71.1    83.69   10.53
orange  lys    58.8    70.66   84.82   11.66
orange  lys    59.09   70.91   83.26   10.46
orange  meth   56.77   67.32   82.54   12.69
orange  meth   57.31   66.33   80.75   9.17
orange  meth   56.24   65.62   79.49   10.24
;
proc sort; by sweetpotato additives;
proc means n mean std maxdec=2; by sweetpotato additives;
var OT PT CT EN;
proc anova; by sweetpotato;
class additives;
model OT PT CT EN = additives;
means additives/tukey lines;
run;
```

APPENDIX 2
RVA RAW DATA AND SAS CODE

SweetPotato	Additive	P1	T1	BD	FV	SB	Ptime	PT
white	noaa	222	137.8333	84.1667	190.3333	52.5	4.5293	79.55
white	noaa	222.4167	139	83.4167	189.5833	50.5833	4.5622	79.4
white	noaa	219.9167	139.25	80.6667	189.8333	50.5833	4.5626	79.9
white	aspa	179.5	88.3333	91.1667	119.4167	31.0833	4.3985	80.2
white	aspa	175.3333	87.75	87.5833	118.3333	30.5833	4.3993	80.3
white	aspa	183.6667	89.25	94.4167	121.5	32.25	4.4637	80.3
white	leu	203.75	131.4167	72.3333	189.6667	58.25	4.4641	79.4
white	leu	208.0833	132.25	75.8333	187.8333	55.5833	4.5293	79.4
white	leu	210.25	135	75.25	191.4167	56.4167	4.5293	79.45
white	lys	188.4167	106.5833	81.8333	160.5	53.9167	4.4963	81.55
white	lys	191.4167	114.4167	77	163.75	49.3333	4.4974	81.45
white	lys	197.5	110.25	87.25	161	50.75	4.5293	81.45
white	meth	208.1667	132.6667	75.5	183.6667	51	4.5293	79.4
white	meth	207.9167	133.25	74.6667	184	50.75	4.4644	79.5
white	meth	210.5833	134.3333	76.25	184.5	50.1667	4.53	79.45
orange	noaa	220.75	125.25	95.5	168.6667	43.4167	4.4311	72.9
orange	noaa	224.1667	126.4167	97.75	174.25	47.8333	4.3985	73.4
orange	noaa	226.0833	126.9167	99.1667	174	47.0833	4.3996	73.25
orange	aspa	201.8333	88.3333	113.5	118.75	30.4167	4.4637	73.8
orange	aspa	206.0833	88.3333	117.75	119.5833	31.25	4.4641	73.2
orange	aspa	205.4167	88.3333	117.0833	120.6667	32.3333	4.4648	73.5
orange	leu	222.3333	128.9167	93.4167	178.4167	49.5	4.4641	73.25
orange	leu	219.9167	128.1667	91.75	177.6667	49.5	4.4311	72.75
orange	leu	218.8333	129	89.8333	183.3333	54.3333	4.4644	73.25
orange	lys	190.75	104.4167	86.3333	153.0833	48.6667	4.4974	75.05

orange	lys	189.75	104.1667	85.5833	151.4167	47.25	4.497	74.95
orange	lys	190.5833	96.9167	93.6667	147.5833	50.6667	4.4974	74.75
orange	meth	220.9167	127.75	93.1667	180.5833	52.8333	4.4311	72.7
orange	meth	219.8333	127.8333	92	176	48.1667	4.4644	72.7
orange	meth	221.3333	131.1667	90.1667	183.8333	52.6667	4.4315	72.75

```
dm "clear log; clear output";
options nodate nonumber;
data RVA;
input sweetpotato $ additives $ P1 T1  BD    FV    SB    Ptime PT;
cards;
white   noaa    222     137.8333        84.1667 190.3333        52.5    4.5293  79.55
white   noaa    222.4167        139     83.4167 189.5833        50.5833 4.5622  79.4
white   noaa    219.9167        139.25  80.6667 189.8333        50.5833 4.5626  79.9
white   aspa    179.5   88.3333 91.1667 119.4167        31.0833 4.3985  80.2
white   aspa    175.3333        87.75   87.5833 118.3333        30.5833 4.3993  80.3
white   aspa    183.6667        89.25   94.4167 121.5   32.25   4.4637  80.3
white   leu     203.75  131.4167        72.3333 189.6667        58.25   4.4641  79.4
white   leu     208.0833        132.25  75.8333 187.8333        55.5833 4.5293  79.4
white   leu     210.25  135     75.25   191.4167        56.4167 4.5293  79.45
white   lys     188.4167        106.5833        81.8333 160.5   53.9167 4.4963  81.55
white   lys     191.4167        114.4167        77      163.75  49.3333 4.4974  81.45
white   lys     197.5   110.25  87.25   161     50.75   4.5293  81.45
white   meth    208.1667        132.6667        75.5    183.6667        51      4.5293  79.4
white   meth    207.9167        133.25  74.6667 184     50.75   4.4644  79.5
white   meth    210.5833        134.3333        76.25   184.5   50.1667 4.53    79.45
orange  noaa    220.75  125.25  95.5    168.6667        43.4167 4.4311  72.9
orange  noaa    224.1667        126.4167        97.75   174.25  47.8333 4.3985  73.4
orange  noaa    226.0833        126.9167        99.1667 174     47.0833 4.3996  73.25
orange  aspa    201.8333        88.3333 113.5   118.75  30.4167 4.4637  73.8
orange  aspa    206.0833        88.3333 117.75  119.5833        31.25   4.4641  73.2
orange  aspa    205.4167        88.3333 117.0833        120.6667        32.3333 4.4648  73.5
orange  leu     222.3333        128.9167        93.4167 178.4167        49.5    4.4641  73.25
orange  leu     219.9167        128.1667        91.75   177.6667        49.5    4.4311  72.75
orange  leu     218.8333        129     89.8333 183.3333        54.3333 4.4644  73.25
orange  lys     190.75  104.4167        86.3333 153.0833        48.6667 4.4974  75.05
orange  lys     189.75  104.1667        85.5833 151.4167        47.25   4.497   74.95
orange  lys     190.5833        96.9167 93.6667 147.5833        50.6667 4.4974  74.75
orange  meth    220.9167        127.75  93.1667 180.5833        52.8333 4.4311  72.7
orange  meth    219.8333        127.8333        92      176     48.1667 4.4644  72.7
orange  meth    221.3333        131.1667        90.1667 183.8333        52.6667 4.4315  72.75
;
proc sort; by sweetpotato additives;
proc means n mean std maxdec=2; by sweetpotato additives;
var P1 T1  BD    FV    SB    Ptime PT;
proc anova; by sweetpotato;
class additives;
model P1 T1     BD    FV    SB    Ptime PT = additives;
means additives/tukey lines;
run;
```

APPENDIX 3
AMYLOSE RAW DATA AND SAS CODE

Sweet Potato	Amylose	Sweet Potato	Amylose
white	14.5	orange	3.91
white	13.73	orange	4.15
white	14.52	orange	4.18
white	14.21	orange	4.01
white	15.15	orange	5.72
white	14.47	orange	5.56

```
dm "clear log; clear output";
options nodate nonumber;
data amylose;
input sweetpotato $ amylose @@;
datalines;
white 14.5 orange     3.91
white 13.73 orange    4.15
white 14.52 orange    4.18
white 14.21 orange    4.01
white 15.15 orange    5.72
white 14.47 orange    5.56
;
proc sort; by sweetpotato;
proc means n mean std maxdec=2; by sweetpotato; var amylose;
proc anova;
class sweetpotato;
model amylose=sweetpotato;
means sweetpotato/tukey lines;
run;
```

APPENDIX 4
RESISTANT STARCH RAW DATA AND SAS CODE

Additive	White	Orange
NOAA	8.36	4.89
NOAA	7.52	5.4
NOAA	8.88	4.36
NOAA	7.91	4.31
NOAA	8.42	4.36
NOAA	8.46	5.37
AA	9.22	5.27
AA	8.08	4.54
AA	8.73	4.72
AA	8.47	4.45
AA	8.68	5.55
AA	7.36	5.55
LEU	9.48	3.9
LEU	9.04	4.69
LEU	8.41	2.96
LEU	8.82	4.36
LEU	8.99	3.71
LEU	8.71	3.92
LYS	8.46	6.11
LYS	7.84	5.64
LYS	7.53	5.25
LYS	7.76	5.36
LYS	9.59	6.25
LYS	8.36	5.84
METH	6.96	4.99
METH	6.69	5.87
METH	6.52	5.43
METH	7.26	5.97
METH	6.74	5.48
METH	6.68	5.67

```
dm "clear log; clear output";
options nodate nonumber;
data resistant_starch1;
input additives $ white orange;
datalines;
NOAA  8.36  4.89
NOAA  7.52  5.4
NOAA  8.88  4.36
NOAA  7.91  4.31
NOAA  8.42  4.36
NOAA  8.46  5.37
AA    9.22  5.27
AA    8.08  4.54
AA    8.73  4.72
```

```
AA    8.47  4.45
AA    8.68  5.55
AA    7.36  5.55
LEU   9.48  3.9
LEU   9.04  4.69
LEU   8.41  2.96
LEU   8.82  4.36
LEU   8.99  3.71
LEU   8.71  3.92
LYS   8.46  6.11
LYS   7.84  5.64
LYS   7.53  5.25
LYS   7.76  5.36
LYS   9.59  6.25
LYS   8.36  5.84
METH  6.96  4.99
METH  6.69  5.87
METH  6.52  5.43
METH  7.26  5.97
METH  6.74  5.48
METH  6.68  5.67
;
proc sort; by additives;
proc means n mean std maxdec=2; by additives; var white orange;
proc anova;
class additives;
model white orange=additives;
means additives/tukey lines;
run;
```

VITA

Stephanie Helen Lockwood was born in Monroe, Louisiana, in 1984. She graduated from Rhodes College in Memphis, Tennessee, with a Bachelor of Arts degree in biology in December 2005. She then studied food science at Louisiana State University in Baton Rouge, Louisiana, where she is currently a candidate for a master's degree. Stephanie will receive the master's degree in food science in August 2007.