EFFECT OF MICROWAVE HEAT-MOISTURE AND ANNEALING TREATMENTS ON BUCKWHEAT STARCH CHARACTERISTICS

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ABSTRACT

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ABSTRACT

Buckwheat is a non-glutinous pseudo-cereal that has a long and traditional history as a food source in Asia, Europe, and the United States and has many beneficial health aspects but has suffered from declining production within the past years. In order to prevent further decline of buckwheat production new products will need to be developed for the consumer market and more research will need to be conducted to study the effect of different processing parameters on

buckwheat characteristics. This study focused on the effect of microwave heat-moisture and annealing processes on buckwheat starch that had been dried to three moisture levels: 32.3%, 40.0%, and 44.4%. Starch samples were analyzed using a differential scanning calorimeter, a colorimetric amylose leaching tests, and an x-ray diffractometer. Additional moisture levels starch treatment groups, 13.2% and 26.8%, were produced for the x-ray diffraction test. Differential scanning calorimetry (DSC) and colorimeter amylose leaching tests were analyzed on SPSS 11.0 for Windows. DSC data indicated that moisture level had a significant effect on onset melting temperature ($p < 0.01$), peak melting temperature ($p < 0.01$), and enthalpy of fusion ($p < 0.05$). In addition, heat treatment ($p < 0.01$) and interaction of moisture with heat treatment ($p < 0.05$) both had a significant effect on amylose leaching results. Significant differences within each test were found mainly at the 44.4% moisture level. X-ray diffraction readings showed a stable d-space placement for all treatment groups. Intensity visibly increased with decreased moisture level and with heat treatment for the 40.0% and 44.4% moisture level starches. Resistance to amylose leaching and melting at higher temperatures for higher moisture level buckwheat starch was attributed to increased networking among amylose and amylopectin components in the buckwheat starch.

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CHAPTER I

INTRODUCTION

Introduction

Buckwheat (*Fagopyrum esculentum* Moench*)* is a non-glutinous pseudo-cereal that is consumed mainly in China, Japan, and Eastern Europe, but could be profitable in the United States if new uses were found for buckwheat products (Edwardson, 1996). It has a starch composition similar to cereals, but has higher amounts of amino acids lysine, methionine, and cystine which is more typical of legumes (Qian, Rayas-Duarte, & Grant, 1998; Zheng, Sosulski, & Tyler, 1998). In order to learn more about processing buckwheat into consumer products, it is important to find out how its major components such as starch react to different processing techniques. Most processing techniques involve the use of heat and moisture. The effects of several heat and/or moisture processing techniques, such as boiling, baking in bread, and dry-heat, on buckwheat starch composition and characteristics have been studied (Skrabanja, Elmståhl, Kreft, & Björck, 2001; Skrabanja, Laerke, & Kreft, 1998). One area that has yet to be studied is the effect of microwave annealing and heat-moisture treatments on buckwheat starch properties.

Annealing is a heat moisture process that uses treatment of starch at intermediate or excess moisture (40% moisture content and above) at a temperature below the gelatinization temperature (Jacobs & Delcour, 1998). The theory behind annealing is that it could cause changes in the molecular structures within the starch, creating structures that are more resistant to gelatinization (Stute,

1992). In a study by Hoover and Vasanthan (1994b) it was found that annealing led to greater resistance to gelatinization in that amylose leaching decreased and gelatinization temperature increased, especially for starches high in amylose. Since buckwheat is high in amylose content (Qian, Rayas-Duarte, & Grant, 1998) annealing could prove useful in making the starch more resistant to gelatinization. Heat moisture treatment is a process that uses treatment of starch at low moisture (35% or below) at a temperature below the gelatinization temperature (Jacobs & Delcour, 1998). The theory behind heat-moisture treatment is that it changes the crystalline structure of the starch, creating crystalline forms more resistant to gelatinization (Stute, 1992). In a study by Hoover and Vasanthan (1994a) it was found that heat-moisture treatment led to an increased gelatinization temperature and decreased amylose leaching.

Some studies have been conducted using annealing and heat moisture treatments that lasted up to 72 and 95 hours (Hoover & Vasanthan, 1994b; Stute, 1992). Since today's processing techniques require faster modes of treatment, a microwave with a probe was used to process the starch. The effects of the annealing and heat-moisture treatments were studied using a differential scanning calorimeter, an x-ray diffractometer, and an amylose leaching colorimetric method.

Hypothesis

The hypothesis for this study was that microwave annealing and heat-moisture treatments would manipulate buckwheat starch granules so as to make

them more resistant to breaking apart under the influence of additional heat and moisture. This hypothesis was tested using a differential scanning calorimeter (DSC), an x-ray diffractometer, and an amylose leaching colorimetric method.

Problem Statement

This study explored the effects that microwave annealing and heat-moisture treatment have on buckwheat starch properties. Several factors were involved in the microwave heating processes: moisture content of the starch, temperature at which the starch was heated, and amount of time that the starch was heated. To minimize interactions that could take place between buckwheat starch and other components in buckwheat, such as protein and lipids, the buckwheat starch was isolated from a buckwheat flour milling fraction that was produced from the starchy endosperm of the buckwheat plant. Moisture level was established at 32.3%, 40.0%, and 44.4%, and microwave heating parameters were set at 6 minutes at 150ºF (65.6ºC) and 10% power so as to heat the starch to allow for changes within the granule but not dry out the starch granules (dextrinize) or cause them to gelatinize.

Two mechanical and one chemical testing process were used in developing and testing the heat-moisture and annealing treatments. A DSC was used to establish at what temperatures the buckwheat granules underwent physical changes. The other instrumental test was an x-ray diffraction examination of the crystalline structures within the different starch samples. The chemical test involved the use of a starch-iodine colorimetric method which measures the

amount of amylose that has leached out of a granule after excess heat and moisture have been supplied.

Two-way analysis of variance was used to determine the influence that microwave heat-moisture and annealing treatments had on starch crystalline pattern, starch granule melting characteristics, and amylose leaching. Tests were repeated to enhance statistical significance. Data was analyzed using an SPSS 11.0 for Windows statistical analysis program.

Objectives

1. The first objective was to isolate buckwheat starch from buckwheat fancy flour (Minn-Dak Growers Ltd., Fargo, ND) and dry it to different moisture contents.
2. The second objective was to determine the temperature at which to heat the buckwheat in the microwave using a differential scanning calorimeter.
3. The third objective was to construct and conduct heat-moisture and annealing heating regimens in the microwave using the resources obtained from objectives one and two.
4. The fourth objective was to study the heat-moisture treated and annealed starch using the differential scanning calorimeter, the X-ray diffractometer, and an amylose leaching colorimetric method in order to determine whether starches resistant to

further heat and moisture were formed with annealing and heat-moisture treatment.

Use of Findings

Annealing and heat-moisture treatment are hydrothermal (heat and water) treatments that could have significant effects on the properties of the buckwheat starch. Microwave technology allows for faster heating of food items, decreasing the amount of time needed to process the food. The results of this experiment could help to:

1. Build knowledge of buckwheat starch behavior and its interaction with different heat/moisture processes
2. Establish new procedures for using microwave dielectric technology for annealing and heat-moisture treatments to create modified starches.
3. Encourage further study into the development of new products from buckwheat starch using the findings of this study.

CHAPTER II

REVIEW OF LITERATURE

Buckwheat: From Pseudocereal Food Source to Neutraceutical

Buckwheat (*Fagopryum esculentum*) is derived from the Anglo-Saxon boc (beech) and whoet (wheat) because it resembles the beech nut (Edwardson, 1996). However, buckwheat is neither a nut nor a cereal like wheat, but rather a pseudocereal whose history dates back over 1000 years. Cereals at their most basic structure are "one-seeded" fruits containing a small embryonic germ and a larger, starchy endosperm surrounded by an outer aleurone layer and a hull (Hoseney, 1994). Like cereals, the seed of the buckwheat plant contains a germ, endosperm, aleurone layer, and a hull. However, buckwheat is not a part of the cereal or grain family (Gramineae) but rather comes from the same family as rhubarb (Polygonaceae) (Hoseney, 1994; Saeger & Dyck, 2001). Buckwheat can grow to be anywhere from two to five feet and produces white or pink blossoms with five petals (Saeger & Dyck, 2001). Buckwheat can be divided into groups of species: annual and multiennal (Li & Zhang, 2001). The buckwheat used for this experiment is of the annual species – *Fagopyrum esculentum* Moench.

Although it contains the same tissue components as cereals, buckwheat has different tissue features. Buckwheat is a dicotyledon as are peas and beans, while grains like wheat and corn are monocots (Starr, 2000). These different features are visible for monocots and dicots in the actual appearance of the plants as well as the way in which they grow after germination. Dicotyledons contain

two cotyledons or “seed leaves” which store and absorb food for the plant during germination and primary growth. Monocotyledons contain only a single cotyledon. The foliage of dicotyledons contains netlike vascularization whereas the foliage of a monocot contains parallel veining. The vascular structures of dicotyledons are organized in a ring-like structure in the stem whereas the vascular structures of a monocot are dispersed in the stem. The buckwheat grain consists of a triangular seed with two cotyledons running through the endosperm and surrounding it - see Figure 1 (Steadman, Burgoon, Lewis, Edwardson, & Obendorf, 2001).

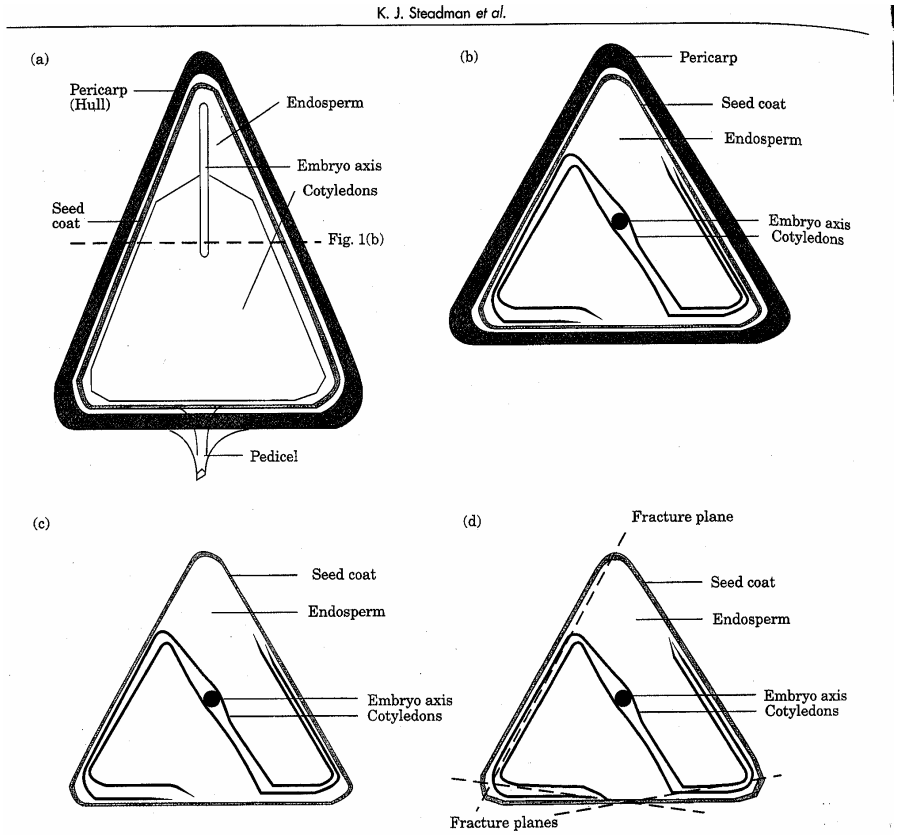

Figure 1 Schematic drawings of buckwheat achene and groat: vertical cross-section of achene (a), horizontal cross-section of achene (b), horizontal cross-section of groat (c), and theoretical fracture-planes for bran milling fraction obtained from groat (d). Dotted line in (a) represents the plane of horizontal cross-section in (b–d).

Figure 1: Diagram of a Buckwheat Groat/Achene
Reprinted from *Journal of Cereal Science*, 33, Steadman, K.J., Burgoon, M. S., Lewis, B. A., Edwardson, S. E., & Obendorf, R. L, Buckwheat seed milling fractions: description, macronutrient composition and dietary fibre, 271-278, 2001, with permission from Elsevier Science.

When studying cereals, it is also important to consider their internal composition. Most grains contain 60-75% carbohydrate, 8-16% protein, and varying levels of lipid, although most contain between 2-3% (Hoseney, 1994). In a study by Zheng, Sosulski, and Tyler (1998) dehulled buckwheat groats were found to contain 75% starch, 13.9% protein, and 2.3% lipid. An estimate of the whole groat by Steadman et al. (2001) stated that groat starch contained 55% starch, 12% protein, and 4% lipid. Most of the protein and lipid were found in the bran and embryo tissue. Unlike wheat and other cereals, buckwheat does not

contain gluten, a protein used in building volume in breads; however, this may be advantageous for people with celiac disease who are intolerant to a component of gluten and therefore must avoid items with gluten in them (Saeger & Dyck, 2001). In the study by Zheng et al. (1998) the amino acid profile of buckwheat was found to be different from grains and similar to that of other dicotyledons such as soybeans with higher amounts of lysine, methionine, cystine, arginine, and aspartic acid. Steadman et al. also found that buckwheat groats contained about 7.0 g/100 g DW total dietary fiber; of which 2.2 g/100 g DW was insoluble and 4.8 g/100 g DW was soluble. The total dietary fiber content and soluble fiber content were similar to oats.

As with grains, in order for buckwheat to be used as a food product, it must first be milled. In the most basic milling process, the outer hull is removed from the seed to produce a groat. The hulls of the buckwheat can be sold for special pillows (Pomeranz, 1983). The groat can then be ground further into several fractions with varying levels of the aleurone layer remaining (Minn-Dak Growers, Ltd., 1999). Coarsely ground groats are called grits and can be used for porridges or in breads. Roasted groats (kasha) are used in Eastern European ethnic dishes (Minn-Dak Growers, Ltd., 1999; Vinning, 2001). Buckwheat flour made from the aleurone layer of the groats is called Farinetta™ and can be used in breads, bakery products, and pancakes (Minn-Dak Growers, Ltd., 1999). Flour made from the entire buckwheat groat (Supreme flour) can be used in breads, bakery products, extruded snacks, pancakes, and pasta. Fancy flour made from

the whiter endosperm portion contains high amounts of starch and can be used in many starchy food products including soba noodles – a Japanese staple.

In addition to being used as a direct food source, buckwheat blossoms also provide nectar for honey bees (Saeger & Dyck, 2001). Buckwheat is ideal in that its blooms last up to a month later in the year than other honey-producing crops, providing a later harvest for beekeepers. The honey from buckwheat nectar tends to be darker and taste stronger than other honeys.

Buckwheat can also be used as a feed source for livestock and wildlife (Saeger & Dyck, 2001). Buckwheat grains can be ground and mixed with grains to use as feed. Inedible buckwheat hulls can be used for poultry litter.

Aside from its food potential, buckwheat crops are also useful for ground maintenance. Due to its size buckwheat is useful as a "smother crop." (Saeger & Dyck, 2001). A "smother crop" is a crop used to eradicate weeds. Buckwheat is especially potent against sowthistle, Canada thistle, quackgrass, creeping Jenny, Russian knapweed, leafy spurge, and perennial peppergrass. Buckwheat takes little time to grow (10-12 weeks) which makes it ideal as an emergency crop for crops that fail. In addition to a short life cycle, buckwheat also helps to revitalize soil by aerating the soil with its shallow and fibrous root system, by acidifying the soil, and by adding calcium and phosphorus back to the soil if the buckwheat crop is mulched into the soil as green manure.

Buckwheat production has experienced peaks and troughs, especially in the western hemisphere. Buckwheat is an ancient plant whose origins lie in China where it was believed to have been first cultivated around 900 AD (Pomeranz,

1983). About 500 years later it was introduced in Europe and brought over to the Americas during the early colonial period (Saeger & Dyck, 2001). Today buckwheat is grown in several areas throughout the world including India, Tibet, Bhutan, China, Japan, Russia, Australia, Canada, the United States, Germany, Poland, Slovenia, Italy, and the Ukraine with Russia being the highest producer followed by China (Edwardson, 1996; Li & Zhang, 2001).

Despite its past history as a food, feed, and ground enhancement product, buckwheat production has seen a decline within the United States over the last 100 years. Once grown extensively in the Northeast and North central states where production peaked at more than 100 million acres in 1866, production diminished to about 25,300 acres by 1997 (Saeger & Dyck, 2001; National Agricultural Statistics Service, 1997). More recent records on buckwheat production in the United States are hard to find aside from some individual state records (see Table 1). Most buckwheat production now takes place in Minnesota, Montana, New York, North Dakota, Pennsylvania, South Dakota, and Washington, and it is usually grown under contract (Edwardson, 1996; Vinning, 2001).

Table 1: Buckwheat Production (Acreage) Records 1997-2002

Source/State	1997[a]	2002[b]
National	25 299	46 636.5
Illinois	393	294.6
Iowa	n/a	542.3
Maryland	166	1.6
Michigan	351	592.4
Minnesota	6 719	5 805.5
Montana	367	75.7
New Hampshire	3	n/a
New York	2 423	1 838.7
North Dakota	5 857	29 469.6
Ohio	345	878.7
Oregon	420	379.5
Pennsylvania	1 587	1 581.7
South Dakota	3 507	1 110.6
Washington	2 557	2 882.5
West Virginia	46	13.0
Wisconsin	341	361.1
All other states	n/a	809

[a] Obtained from National Agricultural Statistics Service, 1997 Census of Agriculture.
[b] Obtained from Rice, Tom. Food Grains Analysis Group. EPAS/FSA. (February 6, 2003). Email Correspondence.

Several factors account for the decline of production in buckwheat in the United States. One factor is the lack of financial support such as crop insurance and a government supported loan program (Vinning, 2001). In a loan program growers are assured of at least a floor price return for their crops. Another factor is the variability in production. Edwardson (1996) in his review of current research stated that production varies unpredictably from cultivar to cultivar and from plant to plant. Even though the plants blossom profusely, only 10-20% produce seed. Buckwheat plants may produce anywhere from 10 to over 200 seeds. Buckwheat seed also does not ripen evenly (Saeger & Dyck, 2001). This

creates a variety of yields from only 200 kg/ha to over 3,000 kg/ha (Edwardson, 1996). Research into breeding more reliable varieties has been slow in the western hemisphere, although newer breeds from Canadian programs have shown improvement over older varieties and Russian and Chinese production have benefited from research efforts (Saeger & Dyck, 2001; Li & Zhang, 2001).

In addition to financial support and production problems, domestic markets for buckwheat products have declined over the years. Although buckwheat can still be used as a nutritional source of food for humans and animals, as well as a nutritive crop for fields, growers have switched to more profitable crops such as flax and canola oil (Vinning, 2001; Edwardson, 1996). After one year of storage buckwheat is considered to be of inferior quality (Saeger & Dyck, 2001). Products made from buckwheat tend to be darker in color and have a more "full-bodied taste" which some consumers find disagreeable. Livestock feed made from buckwheat does have a lower quality than that of other feed cereals. Buckwheat may also elicit some allergic reactions in both humans and animals if consumed in large quantities.

Despite its domestic decline as a staple food and feed source, recent research into the neutraceutical aspects of buckwheat is providing a new perspective for future buckwheat products. Buckwheat has been found to contain several natural components that make it advantageous for use with diabetes and cardiovascular disease patients. One component that buckwheat groats have been found to contain are phytochemicals such as flavonoids which may have antioxidant properties. Dietrych-Szostak and Oleszek (1999) found that whole

buckwheat contained six known flavonoids – rutin, orientin, vitexin, quercentin, isovitexin, and isoorientin - with most being concentrated in the hull and only rutin and isovitexin being found in dehulled buckwheat seeds. Oomah and Mazza (1996) in their study of Canadian buckwheat found that flavonoid content varied with cultivar and environment and that buckwheat also contained components other than flavonoids which gave it antioxidant properties.

Another group of phytochemicals associated with buckwheat are fagopyritols. Steadman, Burgoon, Schuster, Lewis, Edwardson, and Obendorf (2000) defined fagopyritols as "galactosyl derivatives of D-*chiro*-inositol" which have potential use for glycemic control in type II diabetics. The researchers found that fagopyritols were located in aleurone tissue which makes up the outer endosperm, as well as in the embryo. The highest content of fagopyritols was found in bran milling from groats, with lesser amounts found in supreme and fancy flour millings.

Buckwheat protein has also been found to be beneficial. In a study by Kayashita, Shimaoka, Nakajoh, Yamazaki, and Kato (1997) rats fed whole buckwheat protein products had lower plasma cholesterol levels than rats fed casein. These results were attributed to higher neutral sterol excretion and lower buckwheat digestibility compared to casein. Tomotake, Shimaoka, Kayashita, Yokoyama, Nakajoh, and Kato (2000) also conducted a study comparing the effect that buckwheat protein, casein, and soy protein had on gallbladder excretions and plasma cholesterol in hamsters. They found that consumption of

buckwheat protein elicited higher sterol secretion, lower plasma and liver cholesterol levels, and fewer instances of gallstones than soy protein or casein.

Increases in buckwheat usage as a food source because it not only provides nutrition but also neutraceutical advantages may result in an increase in its production in the western hemisphere as well as throughout the world. However, to process buckwheat on a large scale it is important to consider the way that its components interact with common processing factors such as heat and moisture. Knowledge of the effect of different processing techniques on buckwheat starch will aid in the conversion of starch into consumer products that retain nutritional quality while providing satisfactory sensory qualities.

The Nature of Starch

Starch is a component that exists in cereals, legumes, and tubers. Starch at its most basic configuration consists of small granules which contain two molecules – amylose and amylopectin (Hoseney, 1994). Granules come in several shapes including round, elliptical, polyhedral, and polygonal. The shape depends on the plant source and the part of the plant that is being examined. The two components of starch granules, amylose and amylopectin, are chains of glucose, a basic sugar, bonded together. Amylose is composed of α 1→4 linkages of glucose with minor branching. It forms random coils or semi-helical configurations. Due to its less structured configuration, amylose molecules are easily leached out of granules and broken down by amylase enzymes. Amylopectin is a molecule with α 1→4 linkages and α 1→6 linkages which

branch off the main chain. See Figure 2 for illustrations of amylose and amylopectin. Amylopectin branches form helical pairs of structures that bind with themselves to form ordered, crystalline regions. The ordering of the crystalline regions creates the appearance of a "maltese cross" in the granule when seen under photomicrographs, a phenomonen called birefringence. Between crystalline regions are found less ordered, amorphous regions where some amylose and amylopectin branches may reside (Jacobs & Delcour, 1998).

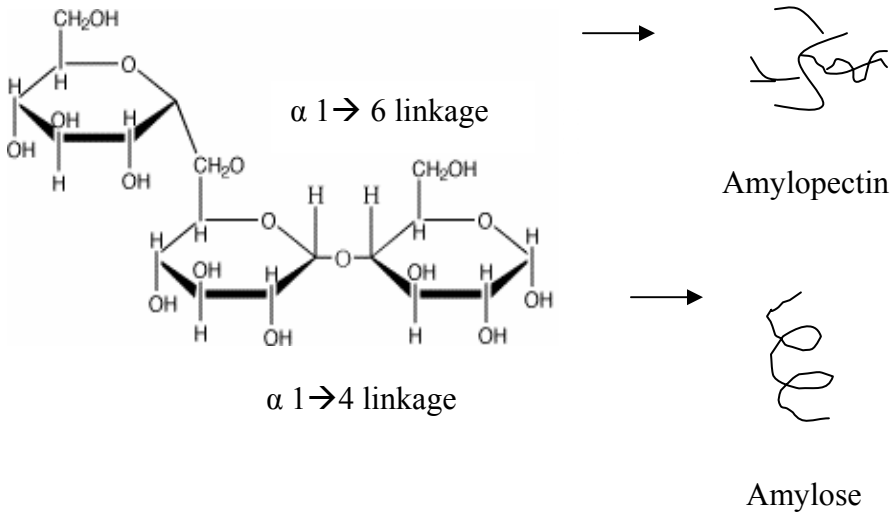

Figure 2. Starch Structure and Amylose and Amylopectin Formations

Starch crystallinity is arranged in one of four ways (Shelton & Lee, 2000). These arrangements determine how the granules react to processing conditions. One type of crystallinity is called A-type is found in cereal starches which have less than 40% amylose and contains crystalline regions with amylopectin parallel helical structures. B-type crystallization is found in tuber, root, and high amylose starches, as well as starches that have retrograded after processing and also consist of crystalline regions with parallel amylopectin helical structures. The

main difference between A- and B-types lies in the increased water content in B-type starches (8 vs. 36 water molecules) (Stute, 1992). C-type crystallinity is considered a mixture of A- and B-type crystallinity. V-type crystallinity is found in granules containing high amounts of amylose complexed with lipids (Jacobs & Delcour, 1998).

Crystallinity can be examined using X-ray diffraction methods. X-ray diffraction involves the use of x-ray technology (Pomeranz and Meloan, 2000). X-rays are produced when an anode target is subjected to 5,000-10,000 volts. The resulting X-rays are applied to a sample. If the sample contains a crystalline structure, such as starch, the X-rays may be diffracted. The defracted X-rays are measured on a detector and the spacing between the different diffractions used to characterize the crystalline structures. The X-rays are read as a series of peaks relating to relative intensity over diffraction angles. Peak intensity relates to amount of crystalline region in the granule (Cullity, 1978; Stute, 1992). Several studies have been conducted using X-ray diffraction to characterize the crystalline structures of starch (Hoover & Vasanthan, 1994a; Hoover & Vasanthan, 1994b; Stute, 1992). Stute (1992) found that heat-moisture treatment of potato starch granules resulted in a shift from B-type to A-type and C-type crystalline structures. Hoover and Vasanthan (1994a) found that heat-moisture treatment also resulted in a B-type to an A-type and B-type mixture shift for non-cereal starches, while cereal starch X-ray patterns remained unchanged except for an increase in diffraction intensities. In another study, Hoover and Vasanthan (1994b) also found that annealing different starches did not cause changes in X-ray patterns but

did cause an increase in X-ray intensities for some starches, particularly cereals and higher amylose content legume starches.

A study by Qian, Rayas-Duarte, and Grant (1998) examined the composition of buckwheat starch and compared it to corn and wheat starch. The researchers found that buckwheat starch was round and polygonal with some holes and pits on the surface, and was 1.6 to 2.4 times smaller than wheat or corn. They also found that buckwheat starch contained a higher amount of apparent amylose (46.6%) compared to corn (28.5%) and wheat (27.5%). Zheng, Sosulski, and Tyler (1998) also examined the buckwheat starch and compared it to rice and corn starch. The researchers found buckwheat to have A-type crystalline behavior like rice and corn, but with a higher degree of crystallinity than either cereal.

When starch granules are subjected to a certain amount of heat and water, they undergo a change called gelatinization (Hoseney, 1994). During gelatinization, amylose escapes from the starch granule and binds with water molecules forming a gel. The amylopectin regions also solubilize and lose their ordered effect, thus losing birefringence. The process begins when energy in the form of heat is supplied to the starch molecules, giving them energy to become more mobile (Fennema, 1996). The molecules most affected by this initial energy are those in the amorphous region. When the molecules reach the glass transition temperature (T_g) they become less rigid or "glass-like" and become more like rubber. As more energy is supplied the molecules gain even more movement until they reach the melting temperature (T_m) where the molecules become "fluid-like" and leach out of the granules. Depending on the amount and distribution of

water, starch granules will tend to break apart in stages (Donovan, 1979). In the discussion of his experimentation on starch-water systems, Donovan explained that in intermediate and excess water systems (moisture greater than 45%), water interacts with amylose molecules in some parts of the amorphous regions, causing the granules to swell and surface crystal structures to be stripped off the granules in regions with high swelling at lower temperatures. As moisture content decreased and water was more evenly distributed, the result was lower overall swelling and higher energy needed to break the crystals apart.

Transitions in starch crystallinity brought on by the addition of heat can be studied through thermal analysis on a differential scanning calorimeter (DSC) (Schenz & Davis, 1998). The breaking of the starch crystal is considered an endothermic reaction since energy is absorbed to break the bonds between the molecules. This change in heat can be detected by comparing the heat absorbed by a starch sample to that of a blank reference. A differential scanning calorimeter consists of a two separate heating units on which are placed sample pans containing a reference (usually water, buffer, or an empty pan) and an experimental sample. Each unit also contains a sensor which is used to ensure a controlled rate of heat application and to record how much energy it would take to keep both reference and experimental sample at the same temperature. These readings translate into endothermic peaks which show at what temperatures starch crystalline regions break apart, when they are at their peak, when the process ends, and how much energy it took to cause this transition. Sample amounts are small, usually 6 to 12 milligrams. Aluminum, hermetically sealed pans are used to

prevent error due to evaporation of water from the samples. DSC's can be programmed to heat the samples from 1 to 10 degrees per minute. Both heating rate and sample size have a direct effect on the length of the crystalline transition period. Keeping both of these items constant would help to reduce variability in DSC readings

According to a study by Qian, Rayas-Duarte, and Grant (1998) buckwheat starch's primary gelatinization peak was at 68.4ºC, between wheat starch (61.2ºC) and corn starch (69.9ºC). Buckwheat starch was found to have a higher water binding capacity compared to the other starches, but a lower degree of swelling, lower amylose leaching, retrogradation, and syneresis. The higher water binding capacity was attributed to the smaller granule size. The lower degree of swelling, retrogradation, and syneresis were attributed to amylose-lipid complexes within the starch and strong micellar networks inside the granules.

Two other studies which examined buckwheat starch found similar gelatinization temperatures for buckwheat starch, one which also examined water binding characteristics found them to be similar to those stated in Qian et al. (1998), but observed higher syneresis rates (Li, Lin, & Corke, 1997; Zheng, Sosulski, & Tyler, 1998).

Another method to test the resistance of starch to gelatinization is to conduct an amylose leaching test. A colorimetric test involving the use of iodine and an ultraviolet-visible spectrophotometer was developed by Chrastil (1987). Chrastil found that by heating a starch and water mixture in a 95ºC water bath for thirty minutes, then adding an iodine-potassium iodide solution to the mixture, a

colorimetric reaction would occur that could be used to detect how susceptible the starch was to amylose leaching. Iodine is able to complex with the amylose on the inside of the helical structure which causes a blue color to form. The starch is defatted in order to prevent complexation of the iodine solution with lipid chains which would result is an inaccurately high reading.

Ultraviolet – visible spectrophotometry was used to read and quantify colorimetric reactions through comparison of transmission/absorption of light through sample holders (Penner, 1998). The machine used in this experiment was a double-beam spectrophotometer. Using narrow window slits, concave mirrors which split visible light into different wavelengths, and gratings which diffract different wavelengths at different angles, a specific wavelength of light may be chosen to shine through the sample. In this experiment a wavelength of 620 nm was chosen because it is the λmax for the starch-iodine complex. In a double-beam spectrophotometer an additional rotating mirror is used along with a sample holder containing a blank sample (distilled water) (Harris, 2003). The rotating mirror constantly switches between the two samples so that the light that is absorbed in the sample can be constantly compared to the light that is absorbed in the blank sample. Absorbance of light is measured as the logarithm of the light entering the sample to that exiting the sample. In order to determine the amount of amylose that leached out of the granules a calibration curve was found by preparing and reading samples containing 0-100% amylose that had been mixed with amylopectin (Chrastil, 1987). Amylopectin does not react with iodine.

Several studies have used amylose leaching to determine the stability of starches that have been heat and moisture treated. In two studies Hoover and Vasanthan (1994a; 1994b) used a modified method of Chrastil (1987) to determine the extent of amylose leaching of cereal, legume, and tuber starches which had been heat-moisture treated and annealed. They found that annealing starches caused a marked decrease in amylose leaching, particularly in starches with high amounts of amylose such as lentils and oats. They also found that heat-moisture treatment caused a marked decrease in amylose leaching but more so in tuber and legume starches than cereal starches.

Other components of a cereal/legume/tuber also interact with starch, affecting the susceptibility of the granule to gelatinization. These interactions are visible on DSC endotherms (readings). In a study by Szczodrak and Pomeranz (1992) starch-lipid interaction in high-amylose (43-49%) barley caused an increase in initial DSC readings from 58-85ºC to 89-110ºC. Complexation of amylose starch with lipids was also found to prevent amylose-amylose interaction. Liu, Arntfield, Holley, and Aime (1997) found similar findings with pea starch. Lipids are able to complex with amylose by hiding within the helical complexes formed by amylose.

Starch granules are also known to interact with proteins. In a study by Eliasson and Tjerneld (1990) wheat proteins were found to adsorb unto wheat starch granules, potato starch, and maize starch. Adsorption increased with initial increased starch temperature due possibly to formation of starch gels or changes in the nature of the granule surface. Fardet, Abecassis, Hoebler, Baldwin,

Buléon, Bérot, and Barry (1999) in their study of protein and starch interactions in pasta products found that starch became entrapped in protein "networks" which rendered them less accessible to water.

Fornal, Smietana, Soral-Smietana, Fornal, and Szpendowski (1985) in their research of buckwheat starch granule interaction with proteins and lipids in an extrusion process with milk proteins found that the protein and lipids did interact with the starch. Addition of milk protein and extrusion temperature increased the degree of gelatinization and decreased the swelling power of the starch granule. Starch-lipid formations did take place but were greatest at lower extrusion temperatures (100ºC).

In order to fully understand a starch, it is important to study its interaction with common processing factors such as heat and moisture. Heat and moisture treatments can have effects on characteristics that relate to digestion and stability under adverse storage conditions. It is also important to look at the manner in which heat and moisture treatments are elicited as more efficient processes, such as microwave technology, are being used to process foods in less time than conventional oven heating methods.

Microwave Technology

Unlike ovens which rely on conduction (transfer of energy from metal or food molecule to food molecule) and convection methods (transfer of energy from liquid or air to food molecule) to heat food, microwaves heat food using dielectric energy (Fellows, 2000). Dielectric energy affects food components that contain

positive and negative poles (dipoles), particularly water, and a common component of food. Microwaves are able to create an environment where a moving electrical field is created, which causes the dipolar molecules to continually turn back and forth, creating frictional heat. Heating depends on distribution of water and other dipolar molecules such as salt. Unlike with conduction and convection methods the surface of the food is less warm than within the food due to evaporative loss of water. The temperature just below the surface, however, is much warmer and from there heat is conducted to the center of the food (Buffler, 1992).

Microwave ovens use a magnetron which produces electrons that are sent through a waveguide and scattered in the heating chamber where they contact food items (Fellows, 2000). Magnetrons provide bursts of energy at variable powers (load) for variable lengths of time (time base) and create fields that move from top to bottom, side to side, and front to back in the heating compartment (Buffler, 1992). To prevent microwaves from concentrating in only a few areas of the food, creating hot or cold spots, most microwave systems are equipped with stirrers or turntables to produce an even exposure of the food item to the microwaves. Sensors are also used, though they may be inaccurate up to ±8ºF (3ºC). Microwaves are best used for thawing, tempering, dehydrating, and baking, but not blanching or pasteurization (Fellows, 2000).

Microwave ovens, like any other heating equipment, work on the concept of power, “rate at which work is done” or, in other words, “the rate at which energy is expended or utilized” (Buffler, 1992). Many factors may affect the

power supplied to the food including the load and time base. These factors are influenced by the temperature and the power level at which the microwave is set. The shape of the food may also have an effect on the power supplied to it as items that are flat or square experience more corner heating than oval or circular foods. Individual food dielectric constants also play a part in the amount of power absorbed by the food (Miller, Gordon, & Davis, 1991). Dielectric constants look at the interaction between the material being heated and the microwave energy (K'), as well as its ability to dissipate energy as heat (K"). These constants are affected by the charge of the components of the food, the environment in which it is in, and the presence of water in the food.

Several researchers have investigated the effect of microwave energy on starch properties. Khan, Johnson, and Robinson (1979) studied the effect of water content and heating time in a microwave oven on the degradation of wheat starch flour. They found that water had a direct relationship with sugar production in that sugar production increased with increased starch hydration. Heating time also had a direct effect up to a point with total soluble sugar increasing except at high water and heating time where total soluble sugar was reduced due to sugar destruction. Glucose concentrations also increased with increased amounts of water and heating time. Sumnu, Ndife, & Bayindirli (1999) studied the effect of water, sugar, and protein on starch gelatinization in wheat starch that was microwaved. They found that wheat starch gelatinized even before applying heat at a 2:1 (w/w, water:starch) concentration. Of the three components, sugar had

the most significant effect on starch gelatinization and significantly interacted with protein and water to prohibit gelatinization.

Zylema, Grider, Gordon, and Davis (1985) compared the effect of microwave dielectric heating and conduction/convection heating in an oil bath on heating rate (up to 65ºC and 85ºC), microstructure, and swelling of wheat starch systems with 1:2 to 1:8 starch-to-water ratios. They found that heating time did not vary between the two types of heating but microwave heating did result in more uniform gelatinization at both the 65ºC and 85ºC temperatures in 1:2, 1:4, and 1:8 starch systems. In microwave 1:1 and oil bath 1:1-1:4 systems chalky regions were formed where the granules were not as swollen as in the gelled regions. In microwave 1:4 and 1:8 and oil bath 1:8 starch systems watery regions also formed in which granules swelled similar to those found in the gelled regions. Water concentration was found to play a great role in helping to distribute heat transfer by increasing microwave coupling with the starch and helping to conduct heat throughout the starch.

The effect of convection and microwave heat methods on wheat granule swelling was also studied by Goebel, Grider, Davis, & Gordon (1984). Varying levels of water:starch concentrations from 1:1 (w/w) starch:water to 5:95 were heated to 75ºC using the 177ºF convection and low/medium microwave mode of a convection/microwave oven. The researchers found that heating in both applications was uneven, forming distinct regions that were described as gel, chalky, watery gel, chalky gel, soft gel, paste, watery paste, and chalky paste. Studying the different regions under a scanning electron microscope and

light/polarized light microscope the researchers found that with increased water ratios there was higher swelling, and, looking at both convection and microwave modes, starch from chalky regions of samples heated using the convection mode had higher starch swelling than those heated using the microwave mode. Except for the 1:4 water: starch ratio level, little difference was noted between samples heated at low and medium microwave mode. Differences between convection and microwave mode heated samples decreased as water:starch ratio increased. The researchers stated that the advanced swelling in higher water:starch ratio samples was probably due to the longer heating period that these samples had as noted by the longer periods of time it took higher water:starch ratio samples to heat to 75ºC.

Yiu, Weisz, and Wood (1991) compared microwave heating of regular and quick-cooking oats to that of conventional boiled oats. Both samples were hydrated to a 1:8 starch-to-water ratio and were kept at temperatures between 90-95ºC for 1 minute and 20 minutes. The researchers found, when studying the starch samples from the different cooking techniques that oat starch granules remained intact even after 20 minutes heating while those of boiled oatmeal fragmented. However, this was attributed to the boiled oatmeal being stirred more.

Although microwave technology usage with heat and moisture treatment of some types of starch had been explored, the effect of microwave heat/moisture treatment had yet to be studied with buckwheat starch. Buckwheat starch with its

high amylose content was an excellent candidate for starch manipulation using microwave heat moisture and annealing processes.

CHAPTER III

METHODOLOGY

Buckwheat Starch Isolation

In order to obtain an accurate evaluation of the effect that microwave heat moisture treatment and annealing treatment had on buckwheat starch, the starch first had to be isolated from the buckwheat fancy flour. According to Minn-Dak Growers, Ltd. (1999), the fancy flour milling fraction used in this experiment contained 72.0% carbohydrate, 9.3% protein, 1.9% fat, 2.2% fiber, and 1.2% ash. The non-starch components were removed to avoid interactions between the starch and other components (lipid, protein) such as were recorded in experiments described in the literature review.

The first step in starch isolation involved the defatting of the flour. This was performed using petroleum ether. A total of 800 grams of buckwheat fancy flour (Minn-Dak Growers, Ltd., Fargo, ND) was mixed with 4 liters (1:5 w/v) of petroleum ether (ACROS Organic, Fischer Scientific, Chicago, IL). Petroleum ether is one of several chemicals that can be used for fat extraction. It is especially effective with extracting hydrophobic lipids and is safer and less expensive than other fat extractors such as ethyl ether (Min & Steenson, 1998). Due to the large amount of flour that was defatted, the flour was divided into four 1,500-mL Pyrex beakers each filled with 200 grams of flour and 1,000 mL of petroleum ether. In order to continually disperse the flour in the petroleum ether each beaker contained a large stir bar and was placed on an electronic stirrer (Corning Hot Plate Stirrers PC-351, PC-320, Pelco International, Redding, CA)

set from medium to high speed for the two-hour duration. This was done to prevent the buckwheat flour from settling and thereby preventing the petroleum ether from contacting and extracting the lipid from the flour. After two hours of stirring, each beaker was filtered using several large plastic filters fitted with Whatman® 24.0 cm filter paper placed over 1,000 or 1,500 mL Pyrex beakers. Liquid was poured off first until little remained except the flour which was dried in Pyrex evaporation dishes number 3180 overnight under the chemical hood. Evaporation dishes were weighed previous to use in order to help quantify the amount of defatted flour obtained from the defatting process.

The protein was removed using a centrifugation technique similar to Qian, Rayas-Duarte, & Grant (1998). Due to the limited amount of the sample that could be centrifuged at one time the starch isolation was performed in several batches. Defatted buckwheat flour (~ 30 g) was steeped in 0.2% NaOH (1:6 w/v) in 250-mL Erlenmeyer flasks and placed in a 45ºC water bath (VWR Scientific Product, Chicago, IL) for 90 minutes. Each flask was stirred with a glass stirring rod in order to suspend the starch in the NaOH prior to placing it in the water bath. The flour/NaOH mixture was then blended in an Osterizer blender (Milwaukee, WI) for 2 minutes and sieved through US no 70 (0.208 mm, 65 inch) mesh to remove larger particles. The flour mixture was weighed into counterbalancing centrifuge bottles and run at 3,000 rpm (~1464 x *g*) for 15 minutes on a Dupont Sorvall® RC 285 with GSA rotor (Kendro Laboratory Products, Newtown, CT) at 25-35ºC. The supernatant was discarded and the top brown-yellow protein layer removed with a metal spatula and water from a distilled water bottle. The white

starch layer was resuspended in distilled water, centrifuged, decanted, and cleaned of the top brown-yellow protein layer. This was repeated until there was no longer any visible protein present (usually two to three times). The starch was then resuspended in distilled water and adjusted to within a pH range of 6.5-7.0 using 1 M HCl and a calibrated Sargent-Welch pH 6050 meter (Skokie, IL). The starch was then washed two to three times with distilled water and dried at ambient temperature under a fume hood for specified lengths of time. Each time the protein isolation was performed, one-third of the starch was immediately placed in a Qorpak container (VWR International, West Chester, PA), capped, and sealed with parafilm wax (American Can Company, Greenwich, CT) and refrigerated at 4ºC. A second-third was capped and refrigerated after 12 hours drying at ambient temperature under a fume hood. The last portion was capped and refrigerated after 24 hours drying at ambient temperature under a fume hood. At the end of the starch isolation process, the starch moisture samples from the different isolation batches were combined using a KitchenAid food processor (model #KFPM65OWH, St. Joseph, MI).

Percent moisture content of the different samples was determined by a two-hour drying method. Five two-gram samples from each type of moisture were weighed into recorded and tared aluminum weigh boats and placed in a 105ºC mechanical oven (Lindberg Blue M, M014505A-1, Ashville, NC) for two hours. The boats were cooled in a Pyrex dessicator and then weighed again. Percent moisture was determined for starch samples using the following calculation.

$$\frac{\text{Weight of original sample (g)} - \text{Weight of sample post drying (g)}}{\text{Weight of original sample}} \times 100$$

Microwave Heat-Moisture and Annealing Treatments of Buckwheat Starch

Preliminary tests were run to determine the gelatinization temperature (using the differential scanning calorimeter) and amount of time to appropriately microwave the isolated buckwheat starch. Since the purpose of heat-moisture treatment and annealing treatment is to heat the starch below the gelatinization temperature with less than 35% and at least 40% moisture content respectively, the microwave temperature had to be such to allow for changes to occur within the starch granule without allowing the starch granule to break and amylose to leach out.

Microwave tests were performed in triplicate in a 900 Watt (IEC 705-1988 method) SHARP Carousel II Convection Microwave Oven (R-9H83, Mahwah, NJ). Approximately 10 grams of each sample was placed in 50-mL centrifuge tubes placed in 50-mL Pyrex containers (for stability) and microwaved at 10% power at 65.6ºC (150ºF) for six minutes. The temperature for heat treating the starch was determined per literature research (Qian, Rayas-Duarte, & Grant, 1998) and preliminary testing. A temperature probe attachment was placed in the center of the sample with no parts of the probe touching the sides of the container. The probe was used to monitor the internal temperature of the sample and ensure that it did not increase over the desired temperature while heating. Once the sample reached the desired internal temperature it was held for the specified length of

time. After the microwaving was completed, samples were immediately capped, wrapped with parafilm wax to prevent moisture loss or gain, and placed in a 25ºC water bath to prevent further heating. Once cooled, granules were separated by applying a mortar and pestle to the contents of the centrifuge tubes. Visible gelatinized starch granules were removed.

X-ray Diffraction Evaluation of Starch Crystalline Structure

X-ray diffraction is a method used to characterize the crystalline structure of a material (Pomeranz and Meloan, 2000). X-rays consist of high energy waves created when a high concentration of electrons hits a heavy target, causing the electrons to penetrate the atoms of the target and give off high energy waves. These waves then penetrate a sample such as a starch granule where they are diffracted by crystalline layers. The spacing of the crystalline layers may be examined by the distance (d) between the wavelengths that are diffracted. The intensity of the d-spacing peaks relates to the concentration of the crystalline phase within the starch granule (Cullity, 1978).

X-ray diffraction was performed on a Scintag PAD-X Advanced Diffraction System X_1 (Thermo ARL, Waltham, MA). A small amount of buckwheat starch powder was placed in a plastic x-ray sample holder and flattened with a piece of glass in order to entirely fill the holder and to make the sample level with the edges of the holder to reduce scanning errors. The buckwheat was scanned through the 2θ range of 0-40º using MDI Data Scan 3.2 software (Livermore, CA). The angles used were similar to those described in

Hoover and Vasanthan (1994a) and are typical for x-ray diffraction starch analyses. D-spacing and intensities were examined for the samples using MDI Jade 6.5 software (Livermore, CA) which contained a manual cursor function that gave d-spacing and intensity data at selected points.

For this procedure a starch sample with 13.247 ± 0.041% moisture and a sample with 26.809 ± 0.331% moisture were created in order to have a more complete view of the effect of moisture level and heat treatment on x-ray diffraction analyses of buckwheat starch. Preparations were similar to previous air temperature drying with 30 grams of buckwheat starch from the lowest and highest moisture level starches placed in evaporation dishes at ambient temperature for approximately 24 hours. New moisture levels were determined as previously described.

Differential Scanning Calorimeter Evaluation of Buckwheat Starch

During each test session, three samples of the heated buckwheat starch at the three moisture levels and one sample of the unheated starch at the three moisture levels were run through a differential scanning calorimeter (model Q10-0088, TA Instruments, New Castle, DE) that had TA-Instruments Q-Series and TA Instruments Universal Analysis 2000 programs. The DSC was set to 25 mL/min N_2 flow and programmed to heat and record from 40-200 ºC with a ramp of 10ºC per minute. Between 7.5-8.0 mg samples were weighed into hermetic aluminum pans and sealed with a TA Instruments Blue Sample Press. An empty,

hermetically sealed aluminum pan was used for a reference because no additional water was added to the samples.

DSC was used to observe and measure the temperature ranges at which the starch underwent melting as well as the amount of energy (enthalpy, J/g) required in the melting process. Onset temperature (T_o) was determined by extrapolation. This is the preferred measurement by some researchers and corrects for interpretation of primary deviation from the baseline, although it was difficult to determine the baseline on some readings (Schenz & Davis, 1998). The peak temperature (T_p) was determined as the temperature at which the DSC reading had reached maximum endothermic transition. Enthalpy of fusion was determined by the software as the area of the transition peak from selected onset temperature to conclusion temperature of transition. The mean of the onset of the melting transition, the peak, and the enthalpy of fusion were calculated for each treatment group. The DSC was used to determine the effect that moisture level and heat treatment had on starch melting characteristics.

Amylose Leaching Colorimetric Measurement

Amylose leaching was carried out in a similar manner to a procedure described by Hoover and Vasanthan (1994b). The method was a modified version of the procedure described by Chrastil (1987). Approximately 20 mg of heat and moisture treated starch was placed in centrifuge test tubes. Then 6 mL of water was added and the tubes weighed for centrifuge counterweighing purposes. The tubes were placed with caps slightly ajar in a 95ºC water bath (Fisher

Scientific Model 10L-M Iostemp Water Bath, Hanover Park, IL) for 30 minutes. After the thirty minutes the tubes were placed in a 25ºC water bath to cool. After cooling the tubes were placed in a Dupont Sorvall® (Kendro Laboratory Products, Newtown, CT) RC 285 with SA-600 rotor at 25-35ºC and run at 2,000 rpm (412 x *g*) for 10 minutes. After this, 1 mL of supernatant was withdrawn and placed in a small 25-mL Erlenmeyer flask. From this flask, 0.10 mL was withdrawn and added to 5 mL of 0.5% tricholoracetic acid and 0.05 mL of 0.01 N I_2-KI solution and mixed. After allowing the samples to sit at room temperature for thirty minutes they were run on a Varian/Cary double-beam spectrophotometer (Walnut Creek, CA) with Simple Scans software at 620 nm. A calibration curve was prepared using absorption readings from standards containing 0-100% amylose with amylopectin and Graphical Analysis software (Vernier Software & Technology, Beaverton, CA). The calibration curve from the standards was used to determine the percent of amylose that had leached out of the starch granules.

Ultraviolet-visible spectrophotometry is a quantitative analytical method that can be used to determine unknown concentrations of a known molecule in a solution (Penner, 1998). Ultraviolet and visible spectrophotometry deals with the interaction between energy from ultraviolet and/or visible light sources and a solution. Energy that strikes molecules in the solution causes the electrons in those solutions to move up the electron orbitals or, in other words, become "excited." This interaction causes a loss in energy that is transmitted through the sample. The relationship between the amount of energy entering and leaving the sample can be studied as transmittance or absorbance. Transmittance compares

the light entering and exiting the sample. Absorbance is the negative log of transmittance and measures the amount of energy that remains in the sample. In double-beam spectrophotometry two samples are read alternatively, a reference sample containing distilled water and the sample solution being studied (Harris, 2003). This method corrects for errors due to light beam intensity and detector response.

Statistical Analysis Procedure

Experiments for differential scanning calorimeter and amylose leaching testing were performed three times (test sessions) with three samples from each of the three heat treatment groups for a total of nine samples overall. Control samples of buckwheat starch from each of the moisture levels were also tested for comparison. For the differential scanning calorimeter only one control sample from each moisture level was taken each time a test session was conducted as previous scans had been made repeatedly on the unheated starch samples. This resulted in having less control samples for the 40.0% moisture level starch since fewer previous scans had been taken of this starch. For the x-ray diffraction procedure heated and unheated samples with different percent moisture levels were examined. Some treatment groups were examined several times, while most were examined once.

In order to determine the effect of heat and moisture treatment on amylose leaching and the melting temperature parameters of the starch the results were analyzed using two-way analysis of variance (ANOVA) set at an alpha level of

0.05 on SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). Independent sample t-tests and least significant difference (LSD) was also used to differentiate samples.

CHAPTER IV

RESULTS

Buckwheat Percent Moisture Level Results

Prior to and after heat treatment of the buckwheat starch the moisture levels were determined using the mechanical oven method discussed in the methodology. From the original starch three levels of starch hydration were produced using ambient temperature drying at 0 hours, 12 hours, and 24 hours after starch isolation. The moisture content in the samples were 32.261 ± 0.336%, 40.017 ± 0.149%, and 44.379 ± 0.079%. These values matched the criteria described in Jacobs and Delcour (1998) for heat-moisture treatment (< 35%) and annealing (> 40%). After microwave heat treatment, nine samples were taken from each sample set and tested for changes in hydration level in the mechanical oven. The resulting moisture levels were 30.745 ± 0.469%, 38.954 ± 0.179%, and 43.335 ± 0.309%.

X-Ray Diffraction Results

Several x-ray diffraction measurements were taken of unheated and heated buckwheat starch at moisture levels of 13.2%, 26.8%, 32.3%, 40.0%, and 44.4%. Heat treatment involved microwaving the starch at the same parameters as the other tests. All graphs were smoothed using MDI Jade 6.5 in order to better read and compare graphs. Figures 3 and 4 illustrate the difference in x-ray diffraction between moisture levels for unheated and heated samples. The following Figures 5-9 illustrate the difference in x-ray diffraction between the unheated and heated samples for each moisture level. For the unheated 44.4% moisture level two

different graphs were presented by the x-ray diffraction machine. Table 2 reports the d-spacing angles at which the crystalline layer in the starch refracted the x-ray and intensities for the two major peaks found on each graph. Most graphs peaked at 3.8 Å and 5.0 Å with intensities increasing with less moisture for unheated samples (Figure 3), but less so with heated samples (Figure 4). Within each moisture level changes in intensity were not seen with heating except for starch samples with moisture levels 40.0% and 44.4% (Figures 8 and 9).

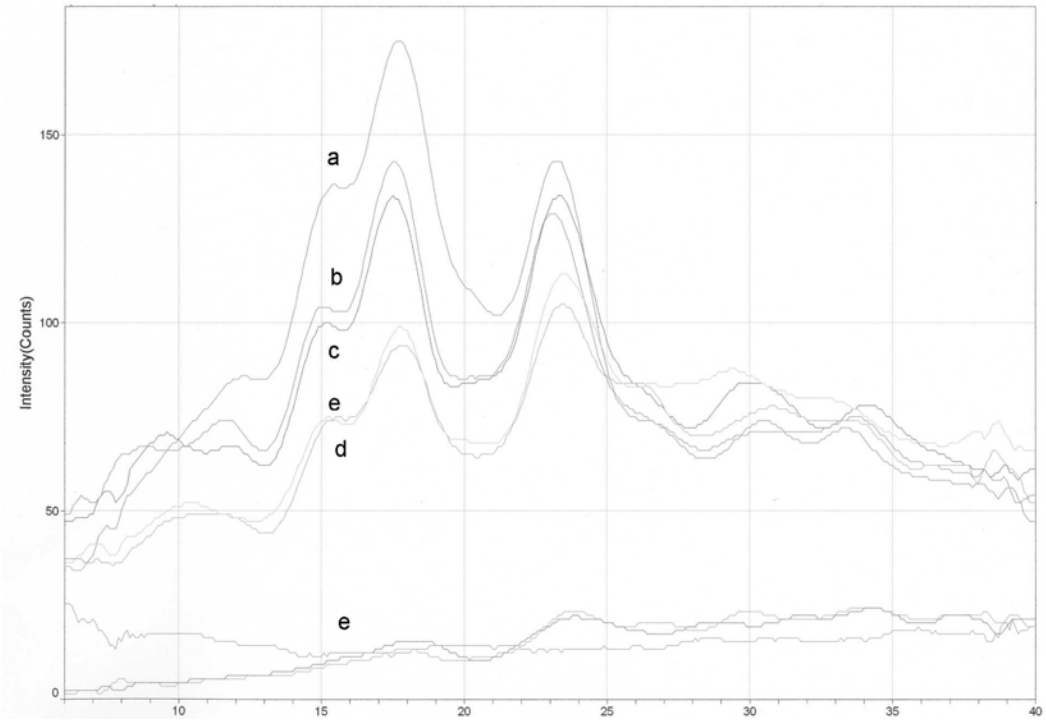

Figure 3. X-ray Diffraction Reading for Unheated Buckwheat Starch
Moisture levels: a - 13.2%, b - 26.8%, c – 32.3%, d – 40.0%, e. 44.4% (2)

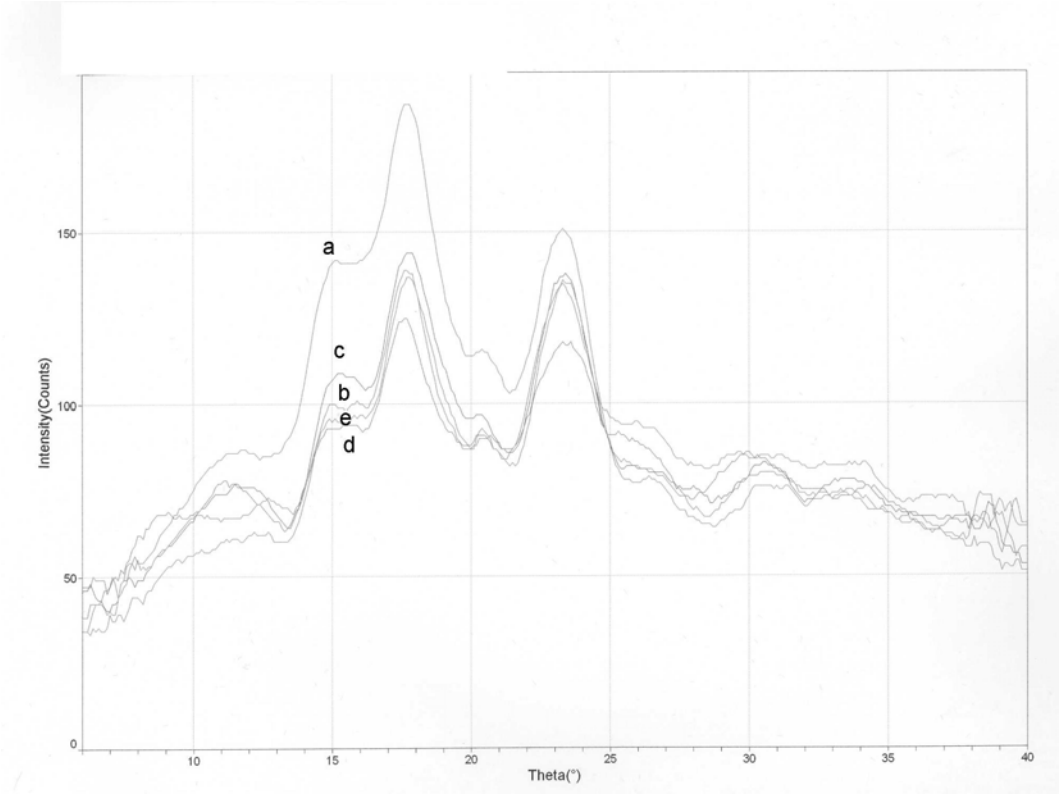

Figure 4. X-ray Diffraction Reading for Heated Buckwheat Starch
Moisture levels: a – 13.2%, b – 26.8%, c – 32.3%, d – 40.0%, e. 44.4%

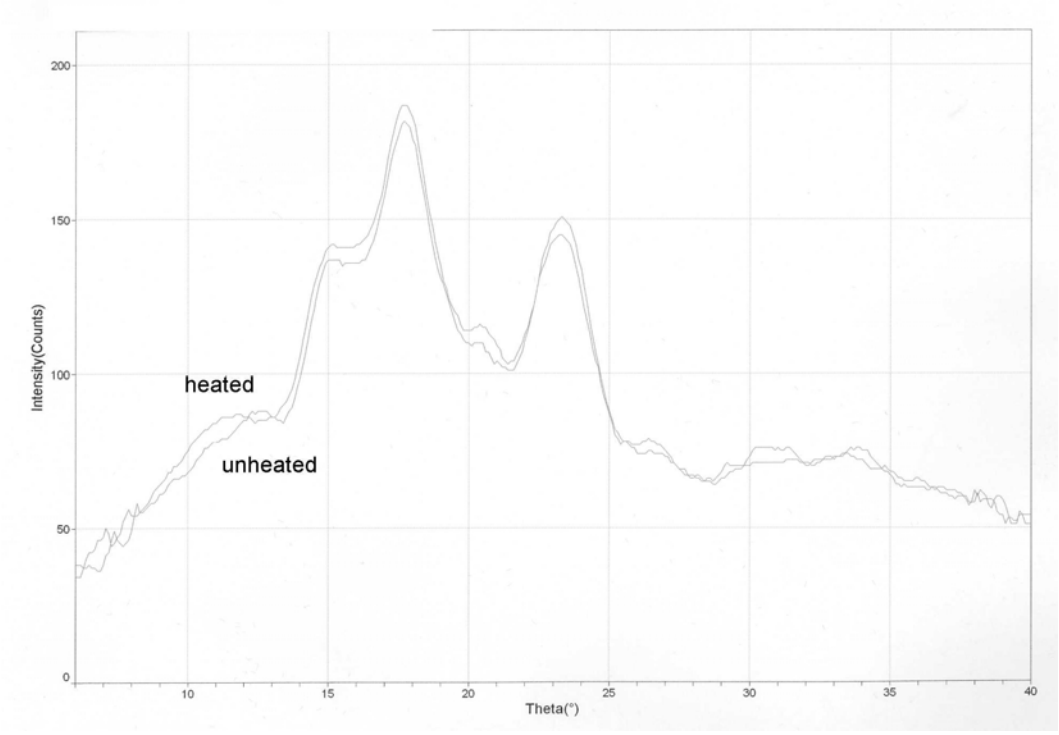

Figure 5. X-ray Diffraction Reading for Unheated and Heated 13.2% Moisture Level Buckwheat Starch

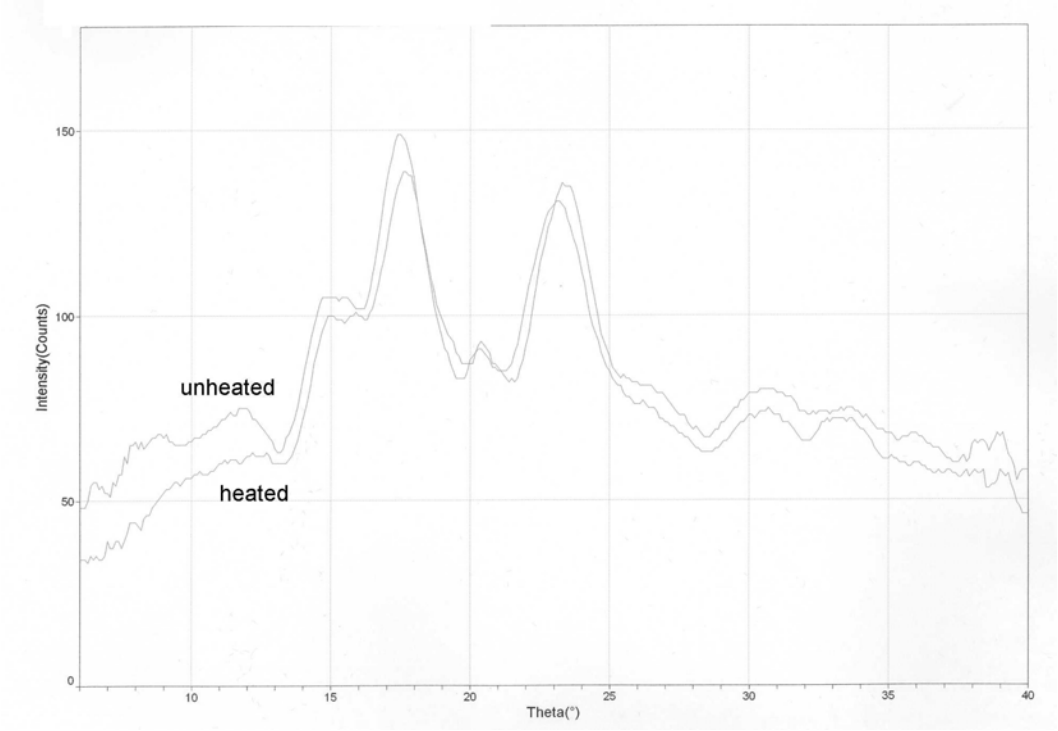

Figure 6. X-ray Diffraction Reading for Unheated and Heated 26.8% Moisture Level Buckwheat Starch

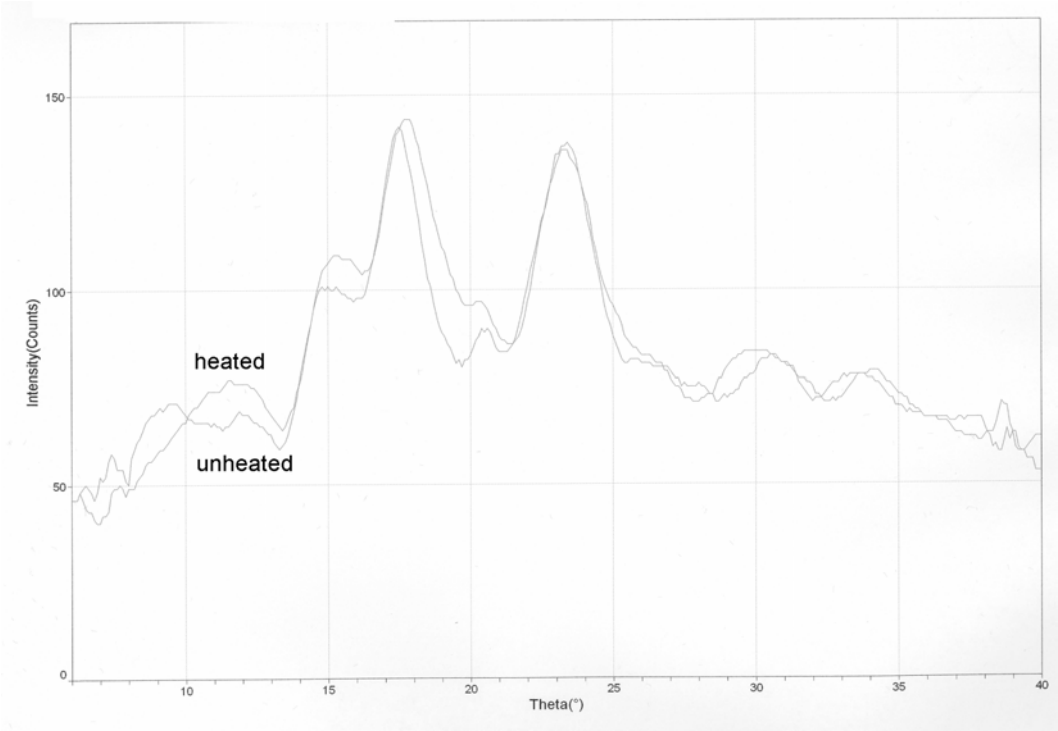

Figure 7. X-ray Diffraction Reading for Unheated and Heated 32.3% Moisture Level Buckwheat Starch

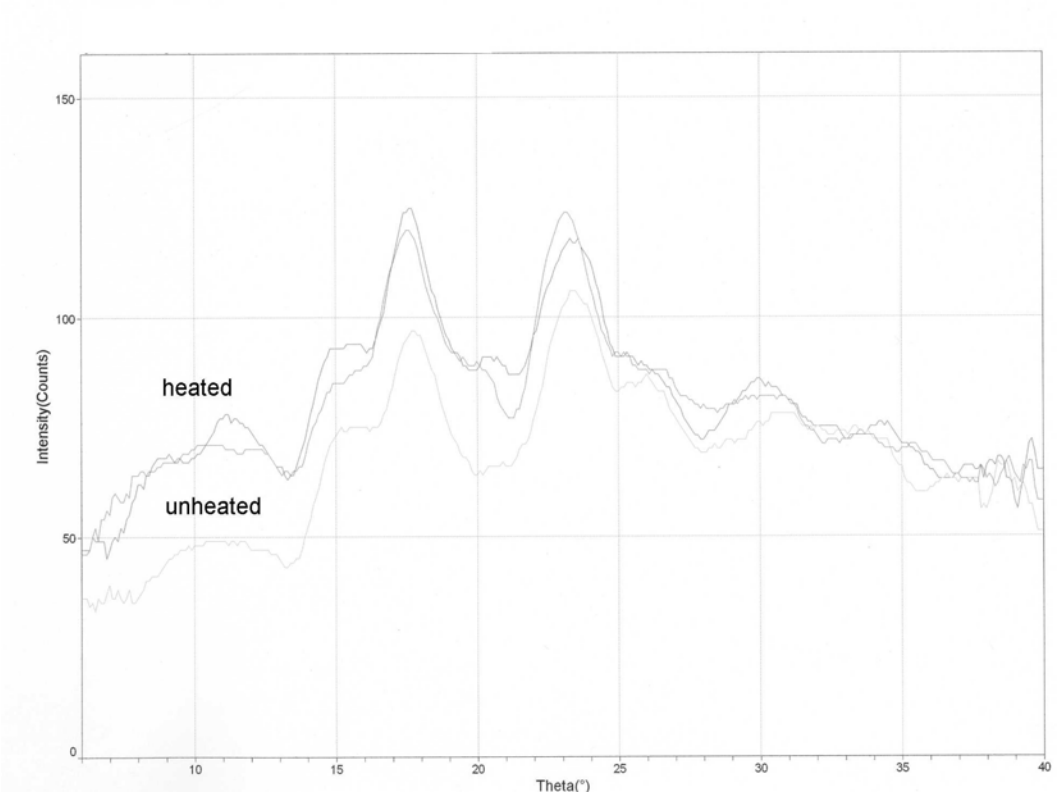

Figure 8. X-ray Diffraction Reading for Unheated and Heated 40.0% Moisture Level Buckwheat Starch

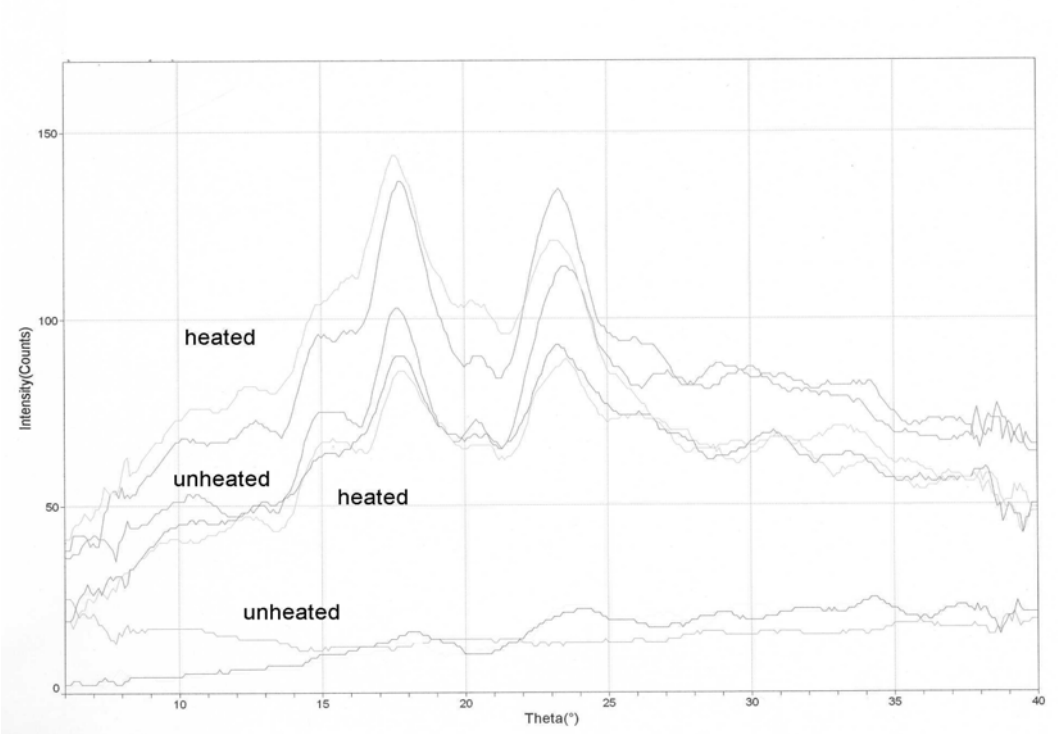

Figure 9. X-ray Diffraction Reading for Unheated and Heated 44.4% Moisture Level Buckwheat Starch

Table 2. X-ray Diffraction Results for Heated and Unheated Buckwheat Starch at Various Moisture Levels

Moisture Level (%)	Microwave Time (Minutes)	First Peak D-space (Å)	First Peak Intensity (Counts)	Second Peak D-space (Å)	Second Peak Intensity (Counts)
13.2	0	3.8711	146	5.0087	182
13.2	6	3.8194	151	5.0213	187
26.8	0	3.8414	131	5.0987	149
26.8	6	3.8267	136	5.0213	139
32.3	0	3.8194	136	5.0727	142
32.3	6	3.8049	138	4.9961	144
40.0	0	3.7977	106	4.9961	97
40.0*	6	3.8304	120	5.0341	120
44.4**	0	3.7834	114	5.0087	103
44.4*	6	3.8159	110	5.0120	114

* Reflects the average of two or more readings taken for starches with this treatment. Most starches were only run one time for each treatment group.
**Only the one, unheated 44.4% moisture level starch graph with readable peaks was recorded in this table.

Differential Scanning Calorimeter Results

Data analysis for differential scanning calorimeter (DSC) readings are shown in Table 3. All data analyses were set at an alpha level of 0.05. In onset temperature a two-way analysis of variance (ANOVA) indicated that moisture level did have a significant effect on mean onset melting temperature $F(2, 51) = 6.053$, $p < 0.01$ with a large effect size (Eta = 0.212). According to least significant difference (LSD) analysis, the 44.4% moisture level starch had a significantly higher mean onset temperature than the 32.3% moisture level starch ($p < 0.01$) while there was no significant difference between the 44.4% and 40.0% moisture level starches ($p = 0.072$) and the 32% and 40% moisture level starch ($p = 0.147$). Application of microwave heating did not have a significant effect on

mean onset melting temperature $F(1, 51) = 0.255$, $p = 0.616$ with a small effect size of (Eta = 0.006). The combined effect of moisture and microwave heat also did not have a significant effect on mean onset temperature $F(2, 51) = 1.289$, $p = 0.285$ with a moderate effect size (Eta = 0.054). According to Levene's test of equality of error variances there was no significant error variance among variables $F(5, 45) = 1.726$, $p = 0.148$ meaning that the variance was the same for each treatment group. Overall, moisture level did cause the 44.4% starch to have significantly higher onset melting temperature readings than the 32.3% starch but not the 40.0% starch. The application of heat did not have an influence on or interact with moisture level to have an influence on onset melting temperatures.

Two-way ANOVA data analysis of peak melting temperature also found moisture level to have a significant effect on mean peak melting temperature $F(2, 51) = 7.710$, $p < 0.01$ with a large effect size (Eta = 0.255). According to LSD analysis, the 44.4% moisture level had a significantly higher mean peak temperature than the 40.0% moisture level starch ($p < 0.05$) and the 32.3% moisture level starch ($p < 0.001$). There was no significant difference between the 32.3% and 40.0% moisture level starch ($p = 0.127$). Application of microwave heating did not have a significant effect on the mean peak melting temperature $F(1, 51) = 0.767$, $p = 0.386$ with a small effect size (Eta = 0.017). The combined effect of moisture and microwave heat also did not have a significant effect on mean peak melting temperature $F(2, 51) = 0.515$, $p = 0.601$ with a small effect size (Eta = 0.022). According to Levene's test of equality of error variances there was a significant error variance among variables $F(5, 45) =$

3.203, $p < 0.05$. This means that there was significant difference in variances across the different treatment groups and as such this could have an effect on mean peak melting temperature readings. As with onset melting temperatures, peak melting temperatures were influenced by moisture but not heating and had higher variances in readings which could have affected the two-way ANOVA analysis.

For two-way ANOVA of DSC enthalpy of fusion, two different analyses were run, one with the entire data set including samples that were suspected of being partially melted (had melting endotherm peaks with enthalpy < 100 J/g) and one without these samples. Suspected partially melted samples were found in every treatment group except for 32.3% moisture level starch, 0 minutes microwave heat treatment. The most suspected partially melted samples were found in treatment group 44.4%, 6 minutes microwave treatment with 3 samples. In the two-way ANOVA of mean DSC enthalpy of the entire set of starches at different moisture levels was found to have a significant effect on mean DSC enthalpy $F(2, 51) = 4.220$, $p < 0.05$ with a large effect size (Eta = 0.158) whereas heating was not found to have a significant effect $F(1, 51) = 1.044$, $p = 0.371$ with small effect size (Eta = 0.018). Interaction between moisture level and microwave heating also did not have a significant effect on DSC enthalpy $F (2, 51) = 1.044$, $p = 0.360$ with a moderate effect size (Eta = 0.044). In an LSD analysis of the moisture levels, 44.4% moisture level starch was found to have a significantly higher mean DSC enthalpy than 32.3% moisture level starch ($p < 0.01$). However, 44.4% moisture level buckwheat starch did have a significantly

higher mean DSC enthalpy than 40.0% moisture level starch ($p = 0.252$) and 40.0% moisture level starch did not have a significantly higher level mean DSC enthalpy than 32.3% moisture level starch ($p = 0.116$). Levene's test of equality of error variances did find that the error variance was not equal across groups ($p < 0.01$) which means that the variances could have had an effect on the mean DSC readings.

When the suspected partially melted sample data was eliminated moisture level was found to have a significant effect on mean DSC enthalpy $F(2, 44) = 83.072$, $p < 0.001$ with a large effect size (Eta = 0.814) while microwave heating did not have a significant effect on mean DSC enthalpy $F(1, 44) = 0.002$, $p = 0.964$ with a small effect size (Eta = 0.00) and interaction between moisture level and microwave heating also did not have a significant effect on mean DSC enthalpy $F(2, 44) = 0.387$, $p = 0.681$ with a small effect size (Eta = 0.020). In an LSD analysis of the moisture levels the 44.4% moisture level starch was found to have a significantly higher mean DSC enthalpy than the 40.0% and 32.3% moisture level starches ($p < 0.001$) and the 40.0% moisture level starch was found to be significantly higher than the 32.3% moisture level starch ($p < 0.001$). Levene's test of equality of error variances found that the error variance was not significantly different across groups ($p = 0.951$). Overall the removal of the suspected partially melted starch samples helped to reduce error due to variance and indicated a greater significant difference between the different moisture level starches.

Table 3. Differential Scanning Calorimeter Results

Moisture Level (%)	Microwave Time (Minutes)	Onset Melting Temperature (ºC)	Peak Melting Temperature (ºC)	Enthalpy of Melting (J/g)*
32.3	0	95.06 ± 9.66^{a}	113.80 ± 2.42^{a}	318.60 ± 62.76^{a} $(318.60 \pm 62.76)^{a}$
32.3	6	93.15 ± 9.84^{a}	118.36 ± 12.91^{a}	300.46 ± 110.55^{a} $(333.04 \pm 55.23)^{a}$
40.0	0	101.46 ± 10.09^{a}	123.46 ± 12.65^{a}	403.46 ± 187.49^{a} $(475.26 \pm 72.67)^{b}$
40.0	6	99.32 ± 9.30^{a}	121.54 ± 11.65^{a}	431.11 ± 175.89^{a} $(484.22 \pm 79.58)^{b}$
44.4	0	103.20 ± 11.85^{b}	128.08 ± 9.12^{b}	580.74 ± 222.84^{b} $(652.95 \pm 55.86)^{c}$
44.4	6	112.31 ± 17.35^{b}	133.96 ± 15.67^{b}	419.62 ± 316.82^{b} $(626.70 \pm 78.89)^{c}$

Data corrected for gelatinized samples are indicated with parentheses. All data is given as mean and standard deviation. Subscripts within the same column denote significant difference among data of at least $p < 0.05$. For onset and peak $n = 9$ except for 40.0% at 0 minutes where $n = 6$.
*For corrected enthalpy 32.3%, 0 minute $n = 9$, 32.3% 6 minutes, 40.0% 6 minutes, and 44.4% 0 minutes $n = 8$, 40.0% 0 minute $n = 5$, 44.4% 6 minutes $n = 6$.

Figures 10-12 are representative DSC of buckwheat starches at the different moisture levels. As percent moisture increased, the DSC endotherm peaks widened (increasing enthalpy) and shifted toward higher temperatures (increasing onset and peak melting temperature).

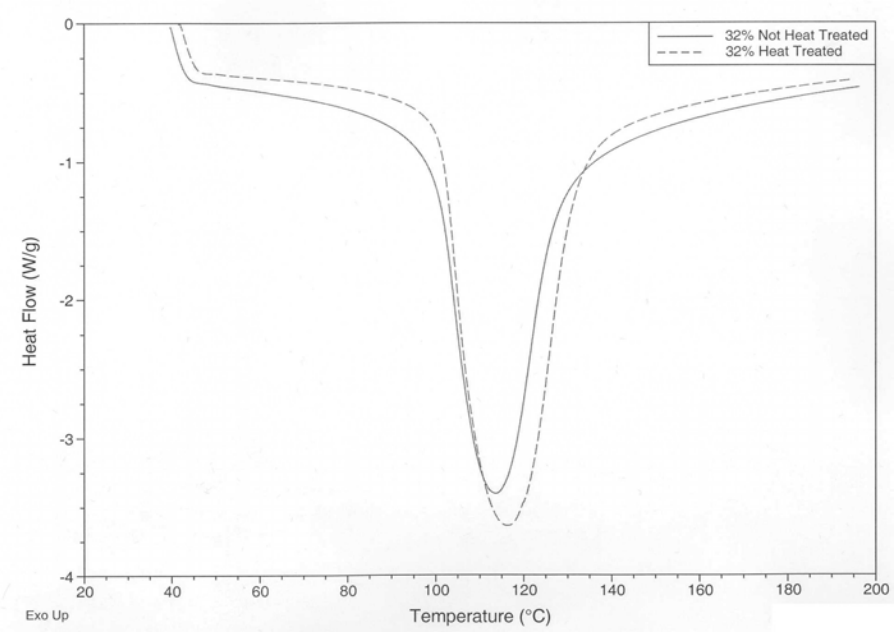

Figure 10. Representative DSC Scan of 32.3% Moisture Level Buckwheat Starch

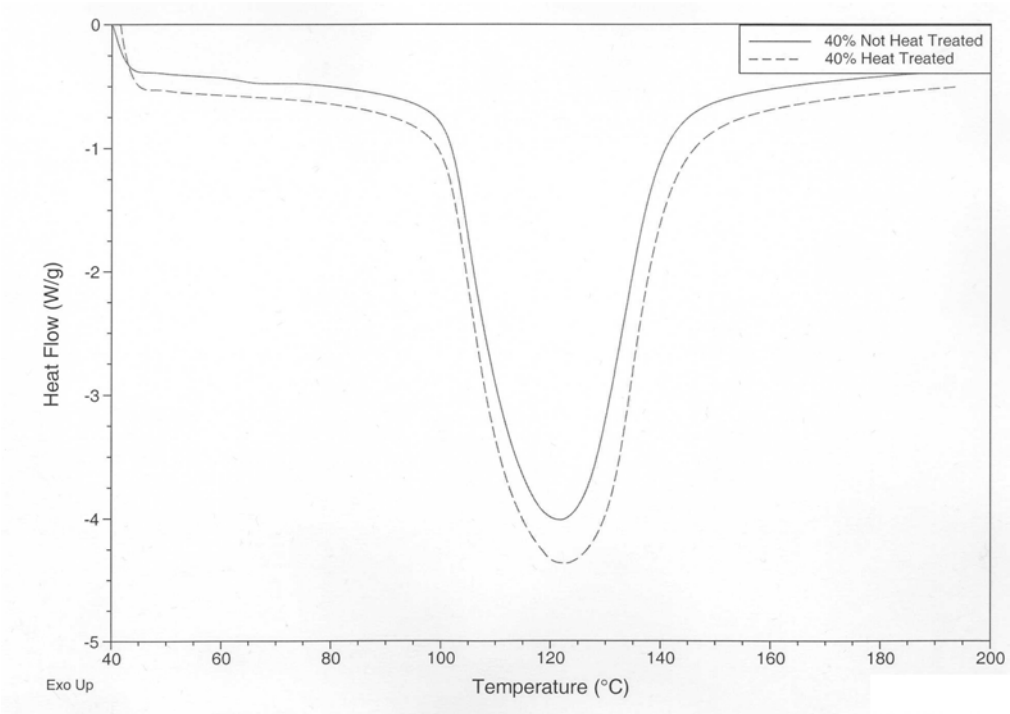

Figure 11. Representative DSC Scan of 40.0% Moisture Level Buckwheat Starch

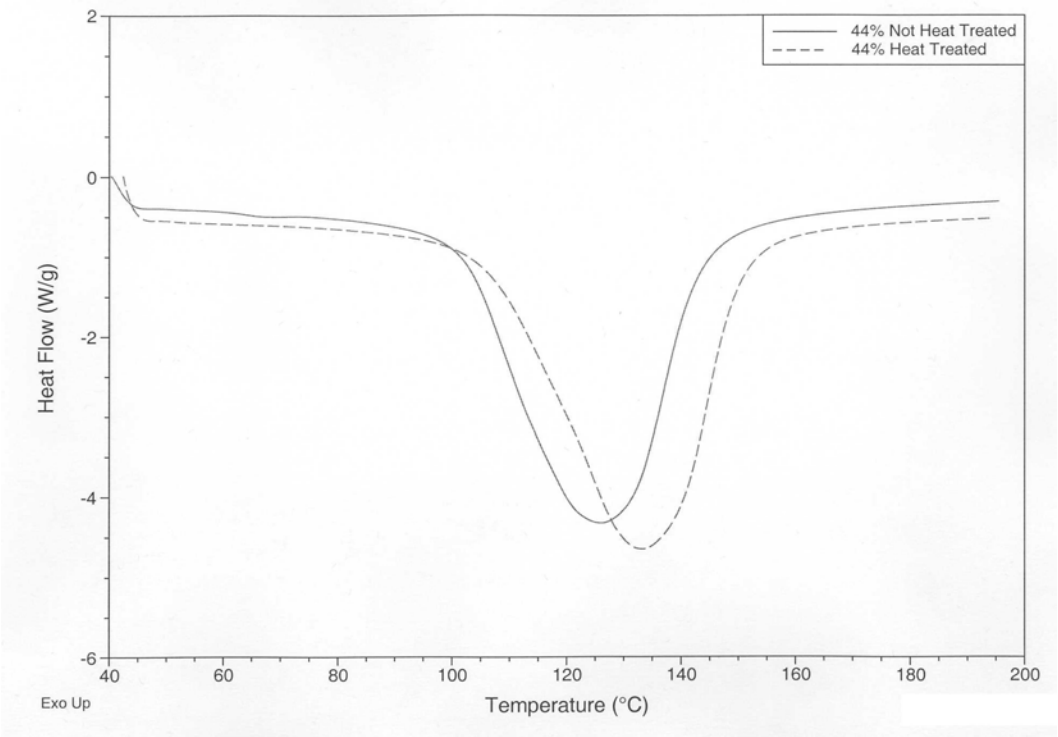

Figure 12. Representative DSC Scan of 44.4% Moisture Level Buckwheat Starch

Amylose Leaching Results

In order to determine the amylose leaching percentage 0-100% amylose standards were prepared and tested with the same procedure as the treated samples. The resulting graph is shown in Figure 13. Since there was a large deviation from 40-60%, these data points were eliminated. The resulting graph gave an equation of y = 0.573x which was used to determine the percent of amylose that leached out of the starch granules during the test using the absorbance readings from the starch-iodine test.

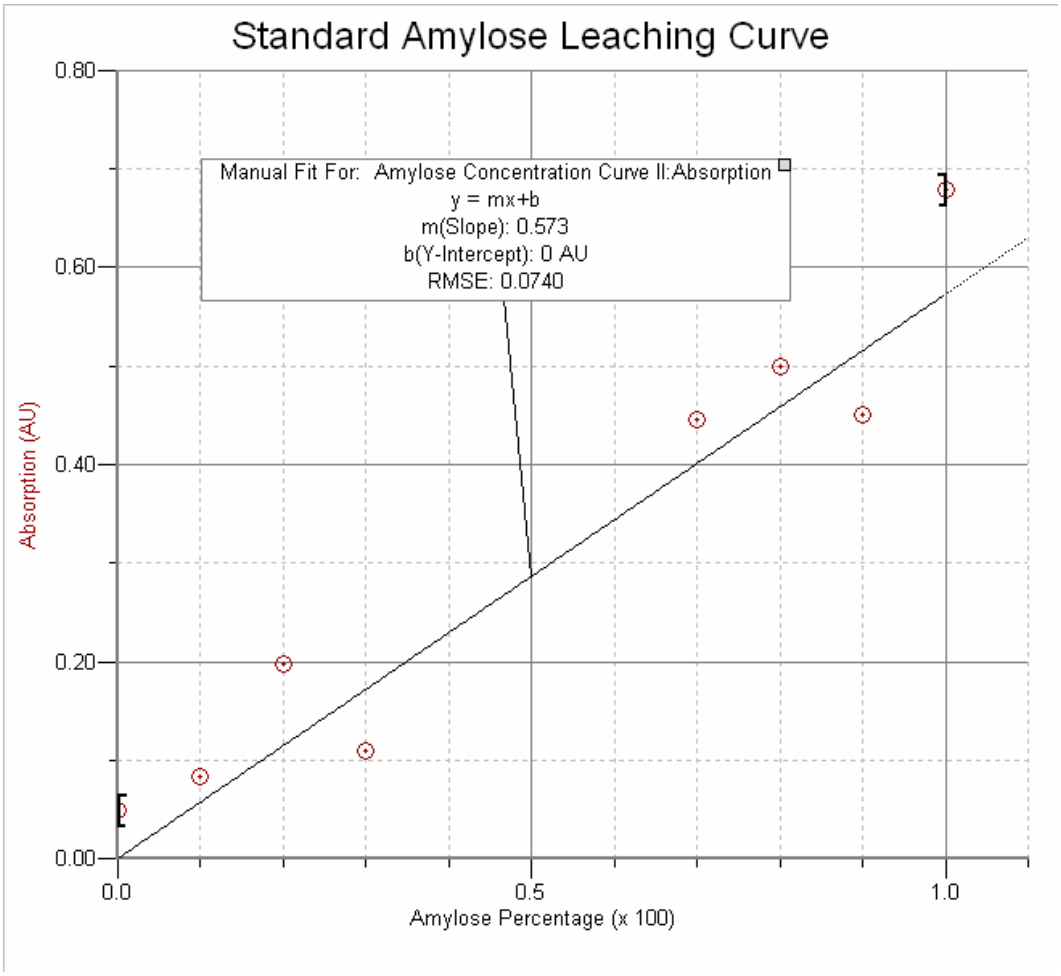

Figure 13. Standard Amylose Leaching Curve

In order to analyze the amylose leaching results two-way ANOVA and independent sample T-tests were performed at an alpha level of 0.05. Results are shown in Table 4. The two-way ANOVA indicated that microwave heating had a significant effect on mean amylose leaching readings $F(1, 54) = 10.873$, $p < 0.01$ with a large effect size (Eta = 0.185) and that the interaction between moisture level and microwave heating also had a significant effect on mean amylose readings $F(2, 54) = 4.288$, $p < 0.05$ with a large effect size (Eta = 0.152). However, moisture level alone did not have a significant effect on mean amylose readings $F(2, 54) = 1.480$, $p = 0.238$ with a medium effect size (Eta = 0.058). In

other words, moisture level alone did not affect mean amylose leaching, however it did have a combined effect with microwave heating. Levene's test of equality of error variances showed that there was no significant difference in variances among the different treatment groups $F(5, 48) = 1.314$, $p = 0.274$.

Since LSD could not be performed to determine the significance of the difference between the different treatment groups, independent sample t-tests were performed. The results of the t-tests indicated that the mean amylose leaching reading for the unheated 44.4% moisture level starch was significantly higher than the heated 44.4% moisture level starch, $p < 0.001$, and that the unheated 40.0% moisture level starch and all of the 32.3% moisture level starch were significantly higher than the heated 44.4% moisture level starch, $p < 0.01$. The unheated 44.4% moisture level starch had significantly higher mean amylose leaching than the heated 40.0% moisture level starch, $p < 0.01$. Differences among the other treatments were not significant at the selected alpha level. This means that mean amylose leaching was lowest for the heated 44.4% moisture level starch, followed by the heated 40.0% moisture level starch, the unheated 40.0% moisture level starch and both treatments of 32.3% moisture level starch, and finally the unheated 44.4% moisture level starch.

Table 4. Amylose Leaching Results

Moisture Level (%)	Microwave Time (Minutes)	Amylose Leaching (%)
32.3	0	14.25 ± 6.29^{bc}
32.3	6	14.35 ± 6.82^{bc}
40.0	0	13.66 ± 5.95^{bc}
40.0	6	9.43 ± 5.21^{ab}
44.4	0	16.89 ± 3.44^{c}
44.4	6	6.57 ± 3.51^{a}

All data is given as mean and standard deviation. Subscripts within the same column denote significant difference among data of at least $p < 0.01$. $n = 9$

CHAPTER V

DISCUSSION

In examining the results for this experiment it is important to note that, for some buckwheat starch characteristics, heat treatment or the interaction of heat treatment and moisture level had a significant effect, while for other characteristics moisture level alone had a significant effect. Three main moisture levels – 32.3%, 40.0%, and 44.4% - and two heating options – microwave heated or unheated at below the gelatinization temperature - were used to create microwave heat-moisture (32.3%, heated) and annealed (40.0%, 44.4%, heated) samples. These factors, moisture and heat, created six treatment groups which were applied to the buckwheat starch and then used to examine buckwheat starch characteristics. The three tests used in this experiment examined a characteristic which has to do with amylose interactions in the starch granule and characteristics which have to do with the crystalline region of the starch granule (concentration and stability). Results from these tests showed that buckwheat granule structures can be stabilized in some ways using microwave and moisture heat treatment to make it more resistant to breaking apart from further addition of heat and water.

X-ray diffraction results were found to be similar to previous x-ray diffraction readings of buckwheat starch (Zheng, Sosulski, & Tyler, 1998). The starch did have a cereal A-type crystallinity with two major d-spacing peaks at 5.0 Å (~17.7º) and 3.8 Å (~23.4º) and one smaller peak that was not recorded but was visible as a shoulder at about 5.7 Å (~15.4º). This did not change with percent moisture or heat treatment (see Figures 3 and 4). In general the intensity of the x-

ray diffraction readings increased as moisture level decreased. X-ray intensity also increased with microwave annealing treatment of buckwheat starch with moisture levels of 40.0% and 44.4% (see Figures 8 and 9).

Hoover and Vasanthan (1994a; 1994b) found that heat-moisture and annealing treatment of cereal did increase peak intensities without changing d-spacing. Stute (1992) found that heat-moisture treatment of B-type crystalline structures caused a change in crystalline structure to A-type and C-type whereas annealing did not cause any crystalline changes. Contrary to some of these experiments heat-moisture treatment did not result in significant changes to intensity (see Figures 5-7) while annealing did (see Figures 8 and 9). Percent moisture, particularly of unheated starch (see Figure 3), also influenced x-ray diffraction readings which could be expected since less water would mean lower swelling in amorphous regions, decreasing concentration of amorphous regions and increasing concentration of crystalline regions (Cullity, 1978). A possible explanation for the increased intensity with annealing is that the excess moisture coupled with heat may have been able to more evenly spread the amylose throughout the starch granule, allowing interaction of the amylose and amylopectin branches in the crystalline regions which would account for higher intensity readings between heated and unheated starch at higher percent moisture levels and comparable readings among several heated starches as seen in Figure 4. As suggested in Hoover and Vasanthan (1994b) interaction between amylose and amylopectin chains may also have occurred at the two moisture levels, which would also account for increased concentration of the crystalline regions. Loss of

moisture due to heating was not considered a major factor for increased x-ray diffraction readings since percent moisture level analyses of heat treated starches found little percent moisture loss (32.261% pre-treatment, 30.745% post-treatment; 40.017% pre-treatment, 38.954% post-treatment; 44.379% pre-treatment, 43.335% post-treatment). More tests, however, would need to be run to confirm these findings.

Temperature of fusion and heat of fusion results using the differential scanning calorimeter (DSC) for this experiment were higher than previous experiments which involved the use of heat and moisture treatment of cereal and buckwheat starches (Hoover & Vasanthan, 1994a; Hoover & Vasanthan, 1994b; Li, Lin, & Corke, 1997; Qian, Rayas-Duarte, & Grant, 1998). This is expected per the results of Donovan's experiment (1979) because, unlike the other experiments, this experiment did not involve the addition of water to the DSC samples prior to testing. With intermediate to low moisture levels higher endotherms could be expected since, according to Donovan's research (1979), DSC readings at lower moisture levels were due to the melting of the majority of the crystalline structure versus the small amount of crystalline structure stripping that takes place at the lower (66ºC) endotherm when excess moisture is available. In preliminary tests with buckwheat starch that had higher moisture levels and with some of the 44.4% starch samples some endotherms in the 66ºC area were visible. The lowest peak temperature for any of these readings was 67.64ºC. Heat treatment temperature was set at 65.6ºC (150ºF) in order to supply enough

heat to cause changes in the crystals without causing gelatinization which did partially occur in some samples as was noted in the results section.

DSC endothermic changes did occur, but, as stated in the results, were attributed to moisture level changes, particularly between the 32.3% and 44.4% moisture levels. The shift in higher endothermic parameters is contrary to Donovan (1979) and other researchers who have studied the effect of moisture content on DSC parameters (Rolee & LeMeste, 1999) and found DSC parameters such as onset and peak temperature to decrease with increasing moisture content. Change in enthalpy was more consistent with the findings of Donovan (1979) and Rolee and LeMeste (1999) where peaks became smaller with decreased moisture content. Although hard to conclude due to the great amount of variance in especially peak temperatures, buckwheat starch with its higher water binding capacity and higher amylose content may actually form stronger internal bonds between amylose and itself or amylose and amylopectin at higher moisture levels which would contribute to increased resistance to melting.

Amylose leaching results focused on the interaction of amylose with itself and other starch granule components. The results of this experiment found that amylose leaching was not significantly affected solely by moisture level as were DSC endotherm readings; rather the amylose leaching was affected more by the use of microwave heat treatment, and the combination of moisture and microwave treatment. This was most significant especially with the 40.0% and 44.4% moisture level annealed starch. Although the unheated 44.4% moisture level starch had the highest mean amount of amylose leaching, it was not significantly

different from the other unheated starches. The most significant finding from this test was that annealed starches had significantly lower amylose leaching. This finding is consistent with annealing treatments of different starches by Hoover and Vasanthan (1994b) but not heat-moisture treatment of different starches by Hoover and Vasanthan (1994a). The findings are also consistent with the restrictive swelling properties of buckwheat starch found by Qian, Rayas-Duarte, and Grant (1998). Lower swelling relates to lower amylose leaching in that granules that are more resistant to swelling are more resistant to leaching of their components. Higher amounts of amylose, coupled with the effects of annealing conditions, could help to form strong internal bonds between amylose and itself and amylose and other starch granules components which would make the granules more resistant to changes caused by the further addition of heat and moisture.

Overall significant changes were observed in amylose leaching and DSC endotherm parameters. Visible changes were observed in x-ray diffraction readings in heated buckwheat starch at high moisture levels and in unheated buckwheat starch at low moisture levels. The addition of moisture and in some cases heat helped to form starch granules that were resistant to the breakdown of crystalline structures and the leaching of amylose in the presence of supplemental heat and moisture. Most of these changes were attributed to changes in interactions between amylose and other components throughout the starch granule.

CHAPTER VI

CONCLUSION

The purpose of this experiment was to explore the effect of microwave heat-moisture treatment and annealing on buckwheat starch properties. The hypothesis was that both treatments would make the buckwheat starch granules more resistant to destruction by further heat and moisture application. This hypothesis was tested by isolating buckwheat starch from flour, preparing five moisture levels, and setting up three different tests which looked at the resistance of the buckwheat starch granule to melting from additional heat, the leaching of amylose, a component of starch, with application of heat and water; and the crystalline structure of the starch before and after heat treatment at the different moisture levels. High moisture levels were found to have a significant effect on melting parameters whereas annealing treatment was found to have a significant effect on amylose leaching. There were no changes in d-space angles in x-ray diffraction; however, intensities did increase with lower moisture level and annealing. These findings were attributed to interactions between amylose and other starch components throughout the starch granule.

Recommendations for Further Study

Future recommendations for studies with microwave annealing and heat-moisture treatment of buckwheat starch include.

1. Create moisture levels that are farther apart and microwave starch for longer periods of time to test the limits of microwave annealing and heat-moisture possibilities.

2. Run more x-ray diffraction analyses on treated samples to ensure reliability of results.

3. Conduct other resistance measurements such as alpha-amylase tests and acid hydrolysis tests which examine resistance of treated starches to digestion.

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