Joint Study Research

Report Summary

"Resistant Starch Formation and Functional Properties of Cooked, Extruded, and Drumdried Buckwheat and Quinoa"

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INTRODUCTION:

The purpose of this research is to investigate processing conditions and methods for increasing the resistant starch content of two underutilized pseudocereals, buckwheat and quinoa, and measure how physiochemical properties are affected. By focusing on and improving resistant starch, a type of dietary fiber and current hot health topic, these grains may play an important role in future convenience functional food products. This work hopes to lay a foundation for the expansion of buckwheat and quinoa utilization by improving the nutritional and sensorial profile of common convenience products. Other important considerations include creating dietary fiber rich healthy options for Celiac patients who often suffer dietary ailments due to limited ingredient selection and unfortified foods.

Buckwheat and Quinoa Background

Buckwheat and quinoa are grains that fall into the category of "pseudocereals". Gluten proteins (prolamins) found in commonly used cereal grains such as wheat, barley, and oats, are absent in pseudocereals and are successfully incorporated into a Celiac's patient's diet. Furthermore, a rich and bountiful profile of essential amino acids, fatty acids, minerals, and vitamins makes them an alluring alternative to traditional nutrient sparse starch based gluten-free foods (Berghofer and Schonlechner). Buckwheat and quinoa have been incorporated into a wide variety of new products including breads, pastas, cakes, and cookies.

Buckwheat (*Fagopyrum esculentum*) has its roots in southeast China. The plant grows best in cool moist climates, but is adaptable to a wide range of soil conditions including acidic or low nutrient soils. Characteristics of buckwheat include its fast growing nature and relatively high resistance to pests. The plant typically grows to five feet with broad leaves (Valenzuela and Smith 2002). China, Russia, and Ukraine are the three leading producers with 564,000, 570,000, and 188,000 tons annually respectively. The United States of America produces 86,000 tons

annually. (FAOSTAT 2009). The crop is not only used for its edible seed, but also for weed control due to the ability to outgrow weeds as a cover crop and also its ability to improve soil conditions by freeing phosphorus. Buckwheat attracts beneficial wildlife to nearby growing cash crops. The seed produced by the plant is triangular in shape resembling a pyramid with the approximate length of a grain of wheat. The seed is used for production of many cereals, breads, and traditional noodles (Thomas Jefferson Agricultural Institute). Buckwheat has a rich nutritional profile with higher protein content and amino acid diversity than common cereal crops (Berghofer and Schonlechner).

Quinoa (*Chenopodium quinoa* and others) has its roots in Pre-Columbian cultures where it was heavily cultivated and part of a staple diet. Today most quinoa is grown and imported from South America, but efforts are being made to investigate strains that will grow well in North American climates. The plant grows up to 6.5 feet with wide leaves and is drought resistant due to deep highly branched tap roots. Quinoa is especially high in fat compared to other grains and contains high levels of unsaturated fats. The quinoa grain is about 1-2 mm in diameter. Quinoa is also unique in that its starch granules are very small at 1 micrometer and tend to clump together (Tang et al. 2002) Quinoa is used in cereals, soups, salads, alcohol, or eaten whole. Currently a great market does not exist for quinoa in the United States, primarily due to its high cost, $2.40-$3.50/pound, limited availability in primarily health food stores, and unfamiliarity to consumers (Oelke and others 1992). But due to its high nutritional quality, pleasant taste, versatility, and gluten free status an opportunity exists to expand its inclusion in the average diet. The Food and Agriculture Organization claim quinoa is a crop that is destined to provide food security in the future due to its strong nutritional profile and the ability of new strains to grow in regions such as Africa, North American, and Asia (Jacobsen 2000). Because of the scarceness of the grain, much is not known about its processing capabilities. By demonstrating the health benefits, processing conditions, and improving the resistant starch content a potential exists for improved marketing and growth and sales of quinoa.

Dietary Fiber in the Human Diet

The American Association of Cereal Chemists defines dietary fiber (DF) as an edible portion of plant matter that is indigestible in the small intestine but is wholly or partially fermented in the large intestine. Beneficial physiological effects of dietary fiber include laxation, blood cholesterol attenuation, and blood glucose attenuation (AACC Report 2001). Dietary fibers can be found in high amounts in unrefined cereal grains, fruits and vegetables. Many studies have confirmed that adequate intake of dietary fiber plays a wide role in maintaining human health. Lifestyle and diet changes away from unrefined grains and vegetables has decreased the amount of dietary fiber consumed which in turn has increased the risk of many chronic health problems such as cardiovascular disease, cancer, and diabetes (Burkitt and Trowel 1977). In a 2005 study, it was found that the average American was consuming dietary fiber at a level half of the amount recommended by the Dietary Guidelines for Americans (Anderson et al 2010). Therefore a strong need to improve the dietary fiber profile of commonly eaten foods exists.

Human health is maintained through a variety of proposed mechanisms orchestrated by dietary fiber. Water absorption by dietary fibers provides a feeling of satiety and increases regularity by increasing stool firmness (Jones 2008). Increased satiety leads to decreased calorie intake which is linked to improved human health by lowering regional fat deposition (Pawlak et al 2004). Because dietary fiber is not broken down to glucose and utilized by the body, it does not provide a significant source of calories to the diet. DF also slows the digestion of other sources further reducing caloric impact and provides positive effects for stabilization of blood glucose levels. The slower transit time through the GI tract allows for increased colon fermentation producing short chain fatty acids (SCFA) that play an important role in reducing cholesterol production and increasing overall colon health. Some dietary fibers can bind bile acids which cause a reduction of blood cholesterol as it is a precursor to cholesterol (Kendall et al 2009). A large range of prospective and epidemiological studies are in agreement that dietary fiber is an essential part of the human diet. The list of benefits seems to keep growing.

Resistant Starch and Human Health

According to the AACC definition, dietary fiber includes a wide range of different compounds including cellulose, hemicellulose, lignin, pectin, gums, beta-glucans, some oligosaccharides, and resistant starch. Each dietary fiber has its own unique physical and physiological properties in the body. Of particular interest in this study is resistant starch.

Resistant starch (RS) has shown to have many benefits on human health. Previous studies have proven resistant starch fermentation by intestinal fauna in the large intestine leading to an overall increase of intestinal microbial growth and total short chain fatty acids (SCFA), a byproduct of colonic bacterial fermentation. In a 1994 study, butyrate found in fecal samples increased by 47% and acetate increased by 35% (Muster et al 1994). Other in vivo testing with rats and in vitro testing both confirm that increased RS consumption increases rates of SCFA production, fecal bulking, and anaerobic bacterial counts. Butyric acid fecal levels increased most significantly but with acetic and propionic acid also increasing (Mahadevamma et al 2004). It is well established that increased levels of RS in diet create higher fecal SCFA numbers.

Studies have shown that an increase in SCFA concentration, especially butyrate, can exhibit physiological benefits through a variety of mechanisms. SCFA lower the overall pH of the colon which inhibit the growth of potentially pathogenic bacteria that could colonize the colon (Birkett and Brown 2008). Organic acids lower colon pH reducing the growth of other non-acid tolerant potentially pathogenic bacteria and reduce secondary bile acids which are cytotoxic to cells (Topping et al. 1993 and Van Muster et al 1994). A lower pH changes the microflora makeup of the large intestine. An increase in percentage was seen in the beneficial bacteria of the group Bacteroidetes after rats were feed a high amylose maize diet (Abbel et al. 2010). Butyrate is a source of energy for colonocytes, colonic epithelial cells, that have shown to reduce risks of colon cancer by increasing apotosis of cancer cells and reducing the growth of new cancer cells in an in vivo study of rats (Hague et al. 1993, Bird et al. 2000, Smith et al. 1998). Micronutrients are also better absorbed at a lower pH due to increased solubility. Many harmful alkaline toxins are less well absorbed at a low pH (Bird et al 2000). Besides

fermentation in the colon, RS also has other traditional benefits common of other dietary fibers. RS has lower caloric content than easily digestible starch because it is not broken down to glucose in the small intestine but rather into SCFA and gases in the colon. There is also potential for RS as a delivery system for probiotics to the lower intestine as well as acting as a prebiotic. RS granules have pits and pockets allowing for the adherence and safer transportation of probiotics through the human digestive tract. Earlier studies showed that many probiotics did not survive to colonize in the large intestine due to the harsh conditions of the stomach and small intestine. Providing a delivery system could increase the amount of probiotics reaching and colonizing in the large intestine (Brown et al. 1998).

Despite the many benefits associated with resistant starch, the average American consumes less than 5 grams/day (Murphy et al 2008). Many other developed countries show similar low levels (Birkett and Brown 2008). This value is far below the 20 grams/day that have shown to give positive health effects. Therefore a strong need and market exists for creating products that contain higher levels of resistant starch and presents the opportunity of marketing a new functional food. Although resistant starch exists naturally in many food sources, the levels at which they are eaten are not adequate to reach the full health benefits (Baghurst et al 1996). Another concern of this study is the dietary fiber content of many gluten free diets. Many people affected by Celiac disease may have an especially low dietary fiber and resistant starch intake due to gluten free foods being produced from highly refined unfortified flour and starch. A need exists to create a product that is both gluten free and high in dietary fiber and specifically, resistant starch.

Besides physiological benefits, RS also can improve the overall perceived quality of a product. Increased resistant starch can increase the fiber content of a product, lower the caloric value, and impart new sensory properties such as improved crispness, color, and mouth feel (Waring 2008) Finding the best conditions for highest yield of resistant starch can be valuable in formulation of new products of potentially functional ingredients. Previous studies have shown that resistant starch can be increased through normal processing conditions. Baljeet and others

(2009) increased RS in a variety of crops including beans, peas, wheat, and potatoes after autoclaving. The RS content was further enhanced by consequent heating and cooling treatments. Gonzalez-Soto (2006) and others used extrusion cooking to improve the RS content of bananas and mangos. Extruded pastry wheat flour had elevated levels of resistant starch after extrusion cooking and cold storage (Kim et al 2006). The logical conclusion is that quinoa and buckwheat RS profile can also be increased through processing and storage conditions. Final RS is dependent on a large variety of factors including amylose/amylopectin ratio, lipid content, temperature of processing, moisture levels, storage temperature, temperature cycling, and type of processing.

Limited data exists on processing conditions and final RS content for buckwheat and quinoa. Three differing processes, extrusion (EX), drum drying (DD), and stove top cooking (ST) along with different factors such as storage time and moisture content are investigated to determine how they affect final RS content. The physical properties associated through the change are monitored through swelling power, pasting properties, water absorption index, water solubility index, freeze-thaw stability, emulsification properties, and melting temperature tests. By demonstrating the health benefits, processing conditions, and improving the resistant starch content a potential for improved marketing, growth, and sales of underutilized buckwheat and quinoa in gluten free convenience foods exists.

Resistant Starch Chemistry

Recently the acceptance of the benefits offered by resistant starch (RS) has lead researchers to investigate raw ingredients and processing conditions to increase RS content in their final product. Resistant starch is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp 1992). Resistant starch has been divided into four categories; RS1, RS2, RS3, and RS4 (Englyst et al 1992). RS1 is entrapped within the food matrix and enzymes cannot gain access to it. Disrupting the food matrix, such as milling or chewing, may decrease the level of resistance of the starch. RS2 is resistant due to conformation of the starch granule. Its high density and crystalline structure diminishes the

effect of digestive enzymes. This type of RS is most commonly found in high amounts in green bananas and raw potatoes. Resistant starch is unique in that it can be increased through processing. RS3, the focus of this study, is formed through the gelatinization and retrogradation of starch granules. The recrystallization, mainly attributed to amylose fraction of starch, creates non-digestible fraction structures (Brown et al 1995). During gelatinization, due to the forces of heat and water, the starch granule begins to absorb water and loses its original crystalline structure and begins to leach out amylose fractions. When the mixture is cooled, retrogradation begins and the amylose particles begin to aggregate forming crystal double helical structures stabilized by hydrogen bonds. Because of the dense crystal formation, it is a challenge for digestive enzymes to break down the complex allowing its transport to the colon (Eerlinger and Delcour 1994).

After gelation and during retrogradation, the unstable mixture begins to cool and aggregates/precipitates begin to form. The viscosity of the mixture increases as the aggregates form as can be seen by the Brabender graphs presented in this paper. Due to their smaller size when compared to amylopectin molecules, amylose molecules quickly align to form crystalline aggregates, a double helix with repeats every 2.1 nm or 6 glucosyl residues which is stabilized by hydrogen bonds. These crystal aggregates are thermally stable having a melting temperature of 150^{o}C. Based on X-ray diffraction patterns, amylose crystals can form either type A or B crystals depending on the cooling conditions (Eerlinger and Delcor 1994).

The amount of RS3 formed during retrogradation is dependent on a variety of factors. As RS3 is primarily composed of amylose, naturally a higher initial amylose content favors increased RS3 production. Natural as well as added lipids can reduce the formation of RS. The presence of lipids results in amylose-lipid complexes reducing the formation of amylose-amylose double helixes and overall RS3 formation. Degree of polymerization of amylose molecules also effects the rate at which retrogradation progresses (Annison and Topping 1994). Gidley and Bulpin (1989) found a variety of factors which affected the extent to which amylose precipitated.

Chain length, with shorter chains being preferred for precipitation, speed of cooling, agitation, and concentration were all factors in determining the amount of resistant starch produced.

Resistant Starch Production

Previous studies on wheat, maize, and rice have shown it is possible to increase the overall levels of RS, by increasing enzyme resistant RS3, a retrograded starch, while minimizing damage to other forms of resistant starch. RS content of pastry wheat flour was increased after extrusion cooking and storage at refrigerator temperature (Kim et al 2006). Other studies have found an increase in RS after extrusion cooking of corn, barley, and Teff flour (Huth et al. 2000, Unlu & Faller 1998, Stojceska et al 2010). A repeated cooling and autoclaving cycle increased the RS content of cereals, legumes, and tubes (Yahav et al. 2009). However, not all data is in agreement. Some studies have shown a decrease in total RS after extrusion cooking of barley, rice, and amaranth (Ostergard, Bjorck, & Vainionpaa 1989, Parchure & Kulkarni 1997, Faraj et al 2004). A confounding factor between the various studies is the storage duration, temperature of the samples after extrusion, extrusion moisture content, and shear speed. Temperature cycling and storage time effect the speed and extent of retrogradation and will affect final RS content (Yadav et al 2009, Kim et al 2006). Drum drying was also investigated as an alternative processing method versus boiling and pressuring cooking which also showed increased levels of enzyme resistant starch formation (Bjorck et al 1984). No data was found for the effect of extrusion cooking or drum drying on the underutilized pseudocereal sources such as buckwheat and quinoa investigated in this study. Based on the evidence, it seems possible to incorporate new processing methods to use healthy ingredients such as buckwheat and quinoa while further improving the resistant starch content of these products.

Cooking Methods

Three distinct cooking methods are investigated in this study, traditional stove top cooking, extrusion cooking, and drum drying. Each method has its own unique characteristics and applications. The final product from each cooking method will vary in level of starch gelatinization, moisture content, color, protein denaturation, secondary reactions, among

others. Within each cooking method, the parameters can also be manipulated to vary the nutritional and physiological properties of the cooked product.

Traditional stove top cooking has been used for centuries for the production of palatable foods. This method is suited to home use due to low equipment cost, easy versatility, and ease of use. It is more suited for batch production so is not suited for commercial use. In this study, this method is largely used as a control to compare extrusion and drum dried methods.

Extrusion cooking uses external heat, pressure, and friction to create a variety of shaped products. Twin intermeshing rotating screws fill a shotgun shaped barrel and move and mix the raw ingredients and water from one end to the other through different, increasing temperature zones, while friction and pressure from the screw begin to cook and gelatinize the product . Different screw configurations allow for increased mixing and kneading friction zones which increase the extent of gelatinization. At the end of the barrel, the cooked product is forced through a narrow opening into the open atmosphere. As the product emerges from the barrel, the pressurized water immediately flashes of and creates, depending on the raw material, different levels of puffed products. This efficient method is highly versatile with the ability to manipulate screw configuration, rotation, speed, temperature zones, moisture content, die designs, and is versatile and suited to commercial continuous production.

Drum drying uses an internally steam heated rotating drum to dry and change the physiologically properties of a raw ingredients. A layer of wet product, such as a flour slurry, tomato paste, vegetable pulp, etc. is typically applied in a 0.5 to 2 mm uniform layer across the rotating heated drum. As the drum revolves with the wet product to about a three fourth rotation, the product is dried by the heat from the steam and a static scraper removes the now dried product from the drum. The raw ingredient can be applied to the main drum using smaller applicator drums with gap control which regulates the amount of wet ingredient on the drum. More advanced applications method such as spraying atomized raw product and dipping can also be used depending on necessary outcome (Tang et al 2002). The speed, temperature,

and application thickness can be manipulated to changed level of cooking (in the case of starch product, gelatinization) and final moisture content. Drum dried products typically rehydrate well in water due to the porous nature resulting from the steam boiling away during drying. This is exhibited in the research.

Physiochemical Properties

In this study, we investigate some properties of the final samples using a variety of different tests including the water solubility index, water absorbance index, swelling power, emulsification properties, and Brabender pasting properties test. These tests are helpful in determining the quality and acceptability of the final product and are used as comparisons between samples and indicators for future potential uses. Although, it may be possible to increase the resistant starch content of the raw materials, it is also important that they meet the standards expected by consumers.

Swelling power is a test often used for noodle quality. Swelling of the starch granule occurs as heat and moisture are applied. Certain factors such as high amylose or fat content may inhibit the extent to which a starch granule will swell. Amylopectin's degree of polymerization and chain length are also factors in the extent of starch swelling. The swelling power of a sample is dependent on how well water molecules are bound to the components within the grain. Swell power of wheat is directly correlated to eating quality of noodles (Crosby 1991). Applying heat, water, and pressure to the grain will likely change the degree to which water is bound due to changing secondary structures during.

Emulsification properties are important for ingredients used in an oil and water mixture such as salad dressings. An emulsion is a mixture of oil in water or water in oil and is created by a compound with both a hydrophobic and hydrophilic moiety. Various components within the grains will determine the quality of emulsion formed. These properties are often determined by the amino acid profile, level of denaturation, and pH of the solution. (Zayas and Lin 1989). Other natural occurring compounds phospholipids such as lecithin can also act as an emulsifier.

Saponins, a natural occurring soap like chemical substance found in quinoa, may also contribute to emulsification properties of the final cooked substance. Levels of cooking and retrogradation occurring during cooling and storage may have an impact on emulsification properties due to secondary products that are formed and hydrophilic cores of proteins being exposed. Stability and ability are two emulsification properties measured that determine how well an ingredient forms an emulsion, and how well the emulsion is held.

The Brabender amylograph is another useful determinant of the gelation properties of cereal. A Brabender amylograph uses a constant speed rotor that measures viscosity of a substance during heating at from 30 to 95 ^{o}C a rate of 1.5 ^{o}C/min , with a five minute hold at 95 ^{o}C, then cooling back to 30^{o}C. This is useful in determining beginning of gelation, gelation temperature, and gelation maximum. During heating, the starch granules begin to swell increasing viscosity, as the swelling continues, the granules are completely disrupted releasing amylose components and decreasing the overall viscosity. As the mixture is cooled again, retrogradation begins and amylose components begin to crystallize, realigning and creating larger fragments and an increase in viscosity. The amylose/amylopectin, granular structure, granular size will all affect the gelation properties of each individual starch granule.

Water solubility index is a measurement of how well a substance will dissolve in water. It can affect how well a food is digested in vivo and has been used as a measure for improving digestibility of pearl millet after fermentation. (Pelembe et al. 2003). Solubility can affect the opaqueness of the final product. Endogenous enzymes breakdown low solubility starch during the heating stages increasing the total amount of soluble materials. The level and method of heating will determine final water solubility.

Statistics

Data for resistant starch analyzed using SAS (SAS Institute, Cary NC, USA) and GLM procedure with assistance from the Michigan State University Statistical Consulting Center. P-value was defined as $p < 05$. Four independent variables, raw material, moisture content, drying, and

storage time were investigated for their effect on the dependent variable, resistant starch content.

RESEARCH

Research Outline and Design

Based on the rich nutritional content, gluten free nature, possible emergence as important new crops, and relatively little research on these pseudocereals, investigating processing methods and conditions is important to more completely understand new uses of these grains.

This experiment is not only designed to investigate how different processing methods effect final resistant starch and physiochemical properties, but also how other factors such as moisture content of cooked samples, and storage temperature come into play. Based on previous studies, it is likely they will play a role in final product makeup. The experiment is designed to investigate three processing methods, with three different moisture contents of 40%, 60%, and 80%, with resistant starch readings at day 0, day 7, and day 14, in both a dried and undried form. The amylose/amylopectin content, fat content, total starch, moisture content were determined for raw ingredients prior to processing methods. After day 14, physiochemical properties of pasting, emulsification, swelling, water absorbance, and water solubility are measured. Complete data is present for traditional stove cooked samples, but due to equipment capabilities, the data for drum dried and extruded products is somewhat stunted. These will be more fully explored in the future with the necessary equipment parameters. The data presented still provides an in depth analysis of the available data.

Materials and Methods:

Quinoa (Organic White, Bolivia. Quinoa-Bol S.R.L), buckwheat (Austria, Strobl-Natiermuhle), Sodium Hydroxide, Ethanol: 99%, Mixer (Ystral D-7982 Ballrechten-Dottingen), Infrared Moisture Analyzer (Sartorius thermocontrol), grinder (IKA Werke MF 10 Basic), Acetic glacial acid (ROTIPURAN, 100% p.a. Carl Roth GmbH + Co. Kg), dimethylsulfoxide (99% pure, Merck,

Schuchardt, Germany), Brabender Viscograph E (486047, GmbH & Co. Duisburg, Germany), Cabinet Dryer (Heraeus Instruments, Kelvitron), spectrophotometer (Hitachi U-1100, Omega Metrohn Inula GmbH), pH meter (Knick Portamess), drum dryer (N.V. Goudsche Machinefabriek. Gouda, Holland), extruder (K-tron, Soder), Amylose/Amylopectin Megazyme kit, Resistant Starch Megazyme kit (Wicklow, Austria)

Sample Preparation

Whole quinoa milled to 0.5 mm using Pallman PXL18 pinmill. Buckwheat was premilled at Linz, Austria facility. Both samples tempered to 15% moisture and stored for two days at 4^{o}C to equilibrate. Stove top samples prepared by mixing water and flour in Laser induction 2000 pans and plates at 100^{o}C in the ratios of 40%, 60%, and 80% moisture. Samples cooked for 25 minutes with stirring at 10, 20, and 24 minutes to ensure even cooking. Samples cooled for 2 hours at room temperature before refrigeration at 4 C. Samples were analyzed for RS at day 0, 7, and 14 in both dried and wet states. Dried samples were left overnight at 50^{o}C with open air flow before analysis the milled to 0.5 mm. Consequent physical property analysis done after day 14.

Extruded samples passed through four temperature zonea, 80-120-140-170 ^{o}C using low shear rings and pressed through 2x4 mm die. Attempts to obtain 20%, 40%, 60% extrudate moisture level were fruitless due to challenge in simultaneously monitoring and controlling water and flour flow rates. Instead two buckwheat levels of 20.4% and 25.4%, and three levels of quinoa at 21.6%, 24.8%, and 28.7% obtained. Samples were allowed to equilibrate open air at room temperature overnight and then milled to 0.5 mm and tested for RS at day 2. Milled samples stored at 4 ^{o}C for other analysis.

Drum dried samples were prepared using the 15% tempered flour to create 65% moisture content slurries. Four applicator roll drum dryer at 150^{o}C fixed temperature used to create approximately .5 mm thickness cooked sheets by adjusting speed. Samples allowed to equilibrate open air at 4 C overnight and milled to 0.55 mm and tested for RS at day 2.

Moisture and Fat Content Determination

All samples tested done in triplicate. Moisture determined by examining difference between initial and final weight of sample left overnight in drying oven at 105 C. Samples cooled in desiccator one hour after removal from drying oven before weighing.

Fat content measured with Soxhlet procedure using petroleum ether as organic solvent. Flasks and boiling chips dried overnight at 105^{o}C and allowed to cool for one hour before use. 10 grams of each sample placed in Soxhlet inserts and heated and circulated for 3.5 hours on high temperature setting. Flasks placed in drying oven at 105^{o}C to remove residual petroleum ether and weight recorded after constant weight was obtained. Samples cooled in dessicator for 1 hour prior to weighing.

Total Starch

Total starch analyzed using Megazyme Total Starch Assay Kit and Procedure K-TSTA 04/2009c. 100 mg +/-5 placed in test tube with the addition of 0.2 mL 80% ethanol with vortexing. 2 mL 2 M KOH added with stirring by magnetic stirrer. Next 8 mL 1.2 M pH 3.8 sodium acetate buffer and 0.1 mL alpha-amylase and 0.1 mL amyloglucosidase with incubation at 50^{o}C for 30 min. Solution transferred and diluted to 100 mL using a volumetric flask. Aliquot of solution centrifuged at 1800 g for 10 minutes and then .1 mL transferred to glass test tube with addition of 3 mL glucose oxidase-aminoantipyrine for 20 minutes at 50^{o}C. Absorbance of samples, blank, and glucose standard read at 510 nm and results obtained from calculation provided in assay.

Resistant Starch Determination

Resistant starch analyzed using Megazyme Resistant Starch Assay Kit and Procedure K-RSTAR 05/2008. 100 mg +/- 5 mg sampled incubated for 16 hours with 10 mg/mL pancreatic-alpha amylase and 3 U/mL amyloglucosidase at 37^{o}C. 4 mL 95% ethanol added to stop enzyme action followed by centrifugation at 1500 g for 10 min. Two washing steps using 50% ethanol and centrifugation at 1500 g for 10 min done to remove any residues. To the pellet 2 M KOH was added with stirring using magnetic stirrer on ice for 20 minutes. After stirring, 8 mL of 1.2 M

sodium acetate buffer pH 3.8 and 1 mL 3300 U/mL amyloglucosidase added and tube incubated for 30 minutes at 50^{o}C. Samples then centrifuged for 10 minutes at 1500 g for 10 min. 0.1 mL supernatant removed from tube and incubated with 3 mL glucose oxidase-aminoantipyrine for 20 minutes at 50^{o}C. Absorbance of samples, blank (sodium acetate buffer) and glucose standard measured at 510 nm and data entered into online "MegaCalc" resource to obtain results.

Amylose/Amylopectin Determination

Amylose amount was determined using Megazyme Amylose/Amylopectin Assay Procedure K-AMYL 04/06. 100 mg samples weighed into 10 mL glass tubes. 1 mL of dimethylsulfoxide (DMSO) added to tube while vortexing. Samples were boiled for 15 minutes followed by 5 minute cool down. 2 mL 95% ethanol were added during vortexing then an additional 4 mL ethanol was added. After precipitate formation, the tube was centrifuged for 5 min at 2000g, and supernatant discarded. 2 mL DMSO added to pellet with vortexing and boiled for another 15 minutes. 4 mL of Con A buffer added and solution adjusted to 25 mL in volumetric flask with distilled water. 1 mL of this solution was pipetted into a 2 mL Eppendorf tube with the addition of 1 mL Con A solution and allowed to stand for room temperature for one hour. Eppendorf tubes centrifuged for 10 minutes at 14,000g. 1 mL of supernatant transferred to 15 mL centrifuge tube and 3 mL of sodium acetate buffer added. Tubes were boiled for 15 minutes then allowed to cool. 4 mL of GOPOD and 1 mL aliquot incubated for 20 minutes at 40^{o}C. Absorbance measured at 510 nm. Total starch absorbance determined by taking 0.5 mL aliquots from original 25 mL flask, 4 mL of sodium acetate buffer, and .1 mL amyloglucosidase/alpha-amylose solution and incubating for 10 minutes at 40^{o}C. 1 mL aliquot of this solution incubated with 4 mL GOPOD for 20 minutes at 40^{o}C. Absorbance of samples read at 510 nm. Amylose content determined according to calculation provided in assay kit.

Brabender

Pasting properties determined using Brabender Viscograph-E. Samples were heated from 30^{o}C to 95^{o}C with a 5 minute hold at peak temperature. The temperature profile was 5^{o}C per minute. Samples were diluted with distilled water to make a 90% slurry at 530 grams total.

Water Solubility Index (WSI) and Water Absorbance Index (WAI)

Water solubility and water absorbance were determined using a modified method by Anderson (1981). 0.5 gram were stirred in 50 mL centrifuge tube with a magnetic stir bar for 30 minutes at room temperature. Samples then centrifuged for 10 minutes at 3000 g. The supernatants were poured on tared aluminum evaporating dishes, weighed and heated at 105^{o}C until a constant weight was obtained. WAI was calculated as the weight of the sediment/weight of dry solids. WSI calculated as weight of dissolved solids*100/weight of dry solids.

Swelling Power

Swelling power determined using a modified method from K.M. McCormick. 0.35 mg samples and 5.0 mL distilled water vortexed for 10 seconds at high speed for 10 seconds. Tubes placed in water bath for 4 minutes at 70^{o}C with a 10 second vortex. Samples then boiled for 10 minutes then allowed to cool 5 minutes at room temperature then 5 minutes in a cold water bath. The samples centrifuged for 4 minutes at 1700 g. The supernatant was discarded and the sediment weight recorded. Swelling power was calculated as sediment weight/ dry sample weight.

Emulsification Properties

Emulsification properties were determined using a modified method of Ahn H.J. and other 1995. 20 mL of 5 % w/v suspensions were made from each flour ensuring flour was completely free of lumps. 20 mL of rapeseed oil was added to the suspensions and homogenized at 11,000 rpm for 30 seconds. Immediately 30 uL aliquots were transferred to 10 mL and diluted to 10 mL with 0.1 (w/v) sodium dodecyl sulfate. Absorbance was read at __ for time 0 minutes and time

10 minutes. Emulsification Ability (EA) and Emulsification Stability were calculated as EA = absorbance of t=0 and ES (%) = (Abs@t=10/Abs@t=0)*100.

RESULTS AND DISCUSSION

Resistant Starch Stove Cooked

Raw buckwheat and quinoa had a fat content 2.00% and 5.58%, a similar dry weight starch content of 63.89% and 62.62%, and initial moisture content of 14.65% and 7.09% respectively. The amylose content was higher in buckwheat at 19.78% versus the 7.22% amylose content in quinoa. As seen in previous studies, higher amylose starches, in this case buckwheat, have higher resistant starch content. The higher fat content in quinoa may explain the lower RS content formed during stove cooked samples due to formation of amylose-lipid complexes which reduce the amount of amylose-amylose complexes.

The initial resistant starch content of raw milled buckwheat was 0.7328 % dwb (based on grams resistant starch over total starch). In stove top cooked and dried samples, the maximum RS level was 2.2893% for the 80% moisture content sample stored for 14 days. This represents over a 3-fold increase from the raw state. RS reached the overall highest level at 2.8939% in the undried, 60% moisture, 14 day stored sample which represents almost a 4-fold increase representing a significant increase ($p<.05$). There was a strong trend for increases in RS content over the 14 day period. The one anomaly is the difference between day 0 undried and day 0 dried samples. The added heating and grinding steps for the dried samples may have disrupted some crystal formation. The added grinding may have created a greater amount of smaller particles (less than 0.5 mm) increasing surface area of the particles and increasing enzymatic destruction. It would not have been possible to analyze the dried samples without grinding them into dispersible particles.

Quinoa raw had a RS content of 0.2086 % dwb. This is significantly lower that the RS content of buckwheat at $p<.05$. A maximum RS for quinoa was reached for the 60% moisture cooked 0.2818% which does not indicate a significant increase from processing. Low amylose content

and high fat content are likely responsible for the decreased the final RS content. There was a slight increase in RS over the 14 day storage, but not as marked as that of buckwheat storage. A significant difference ($p<.05$) did not exist between dried and undried samples. S

Table 1. Resistant starch content of cooked samples % dry weight basis. A. dried samples. B undried samples.

	Day 0 (dried)	**Day 7 (dried)**	**Day 14 (dried)**
Buckwheat 40%	0.1323	0.6702	1.0802
Buckwheat 60%	0.1565	1.2326	2.1457
Buckwheat 80%	0.1641	1.5071	2.2893
Quinoa 40%	0.0160	0.0745	0.1088
Quinoa 60%	0.0193	0.0891	0.2818
Quinoa 80%	0.0151	0.0815	0.1740

	Day 0 (undried)	**Day 7 (undried)**	**Day 14 (undried)**
Buckwheat 40%	1.7376	2.2834	2.7057
Buckwheat 60%	2.1224	1.9728	2.8939
Buckwheat 80%	2.2734	2.5557	2.4429
Quinoa 40%	0.2280	0.1839	0.2043
Quinoa 60%	0.1529	0.1941	0.1641
Quinoa 80%	0.1087	0.0606	0.2178

Resistant Starch Drum Dried and Extruded

Due to equipment limitation fewer data points were available for extrusion and drum dried samples and will be repeated using differing equipment, however, the data collected still is valuable for future work. Extrusion cooked buckwheat samples had a significantly lower RS content than the raw state and stove top cooked samples. Extrusion cooked quinoa did not have a significant increase in RS content. By examining the Brabender amylograph, extruded samples were more thorough gelatinized than stove top cooked samples as a greater viscosity is interpreted as remaining intact starch granules that still swell during the heating cycles. The

added shear from the extrusion likely disrupted any RS1 and broke down amylose into smaller fragments lessening the formation of RS3 which could not make up for the total RS content. Drum dried samples were the least gelatinized. This process is the least harsh and had the lowest level of gelation. Drum dried quinoa had a higher resistant starch content than the raw and extruded samples, but the same was not true for buckwheat. Although extrusion was not important for increasing RS, it may be possible that the starch digestibility is increased making useful to incorporate in baby food products.

Table 2. Resistant starch content raw, extruded samples, and drum dried samples % dry weight basis.

	Resistant Starch (g/100g) "dwb"
Buckwheat Raw	0.7328
Buckwheat Extruded 25.43% Moisture	0.1179
Buckwheat Extruded 20.35% Moisture	0.1124
Drumdried Buckwheat	0.1422

	Resistant Starch (g/100g) "dwb"
Quinoa Raw	0.2086
Quinoa Extruded 21.62% Moisture	0.1435
Quinoa Extruded 24.77% Moisture	0.3895
Quinoa Extruded 28.67%	0.2307
Drumdried Quinoa	0.9099

Physical Properties Brabender.

Raw buckwheat had the highest peak viscosity during gelation at 851.2 Brabender Units (BU). Drum dried samples had a slightly lower peak gelation viscosity at 673.2 BU, but cooked samples had a much lower gelation viscosity at 254.2, 156.5, 40.5, for 40%, 60%, and 80% respectively. Drum dried buckwheat had the fastest time for beginning of gelation at 45 seconds and also the greatest viscosity at gelation at 260.8 BU. The next closest samples to gelation time was 960 seconds for 60%, making drum dried by far the fastest.

The greater the moisture content, the less well defined the beginning of gelation point, the lower the peak gelation viscosity, and the lower the final viscosity at the end of the cooling period. Both extruded buckwheat samples showed similar results with the lowest peak viscosity during gelation, under 100 BU, and lowest final viscosity at cooling point, under 200 BU.

The Brabender is useful to compare the degree of gelatinization of starch granules from cooking. Drum drying seems to only partially gelatinize the starch and also increase the surface area through porosity and made it ideal for quickly thickening a product (Tang et al 2002). The drum dried samples suffered the greatest decrease in viscosity during the holding period. There is less swelling of the granule, which does not increase the viscosity, therefore starch granules that have been partially gelatinized, will have broken down starch granules that will not swell with the addition of heat and moisture. The drum dried product quickly dispersed in water making it ideal as a mixed powder drink. The cooked samples were also not completely gelatinized, but to a much greater extent than the raw or drum dried samples. The extruded samples had suffered the greatest degree of gelatinization during the extrusion cooking process as demonstrated by low gelation viscosity and low final cool viscosity.

Similar trends in pasting properties were seen in quinoa. Raw quinoa showed the highest peak viscosity during gelation at 602.1 BU. Drum dried quinoa had a slightly lower peak viscosity at 506.2 BU. The final cooling viscosity between raw and drum dried quinoa was approximately

200 BU, which is much less than the almost 800 BU difference between raw and conventionally cooked samples. Cooked quinoa samples showed almost now movement in viscosity during the brabender run, which concludes that they were almost completely gelatinized. From the data we can assume that buckwheat samples have a greater gelation temperature than quinoa. Buckwheat had a greater viscosity at gelation peak and at cooling viscosity peak than quinoa (almost double). Cooked and extruded buckwheat products maintained their viscosity during holding time better than cooked and extruded quinoa samples.

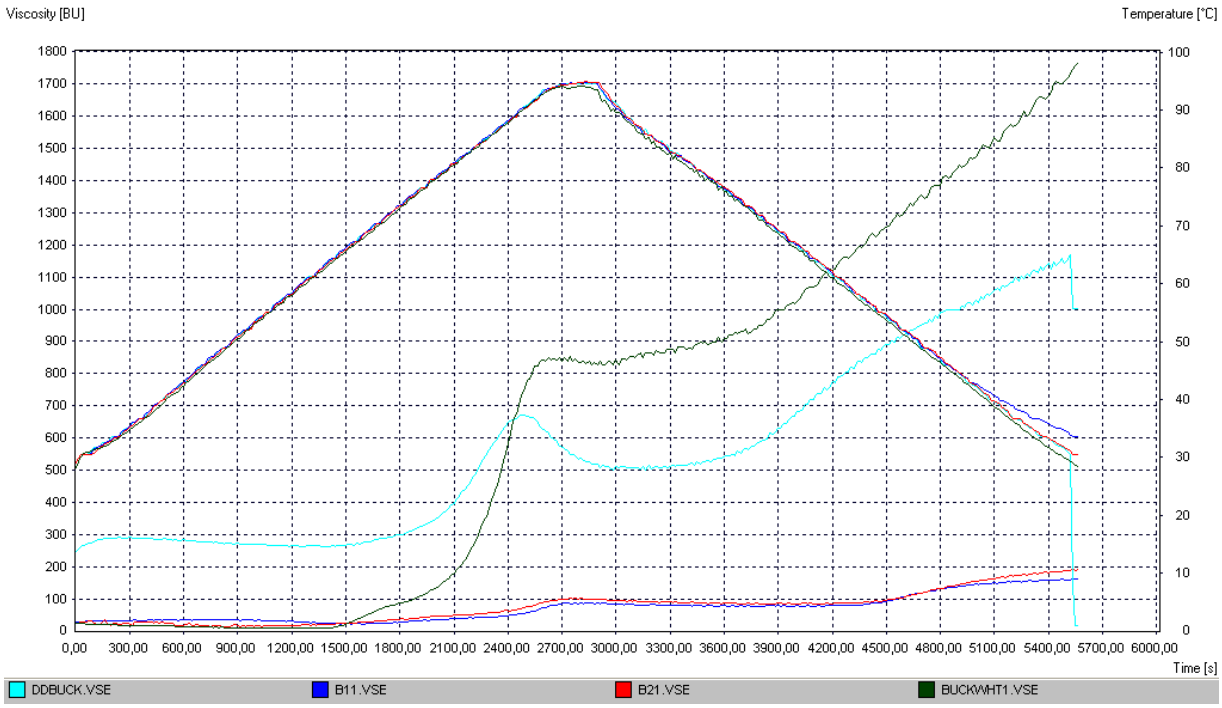
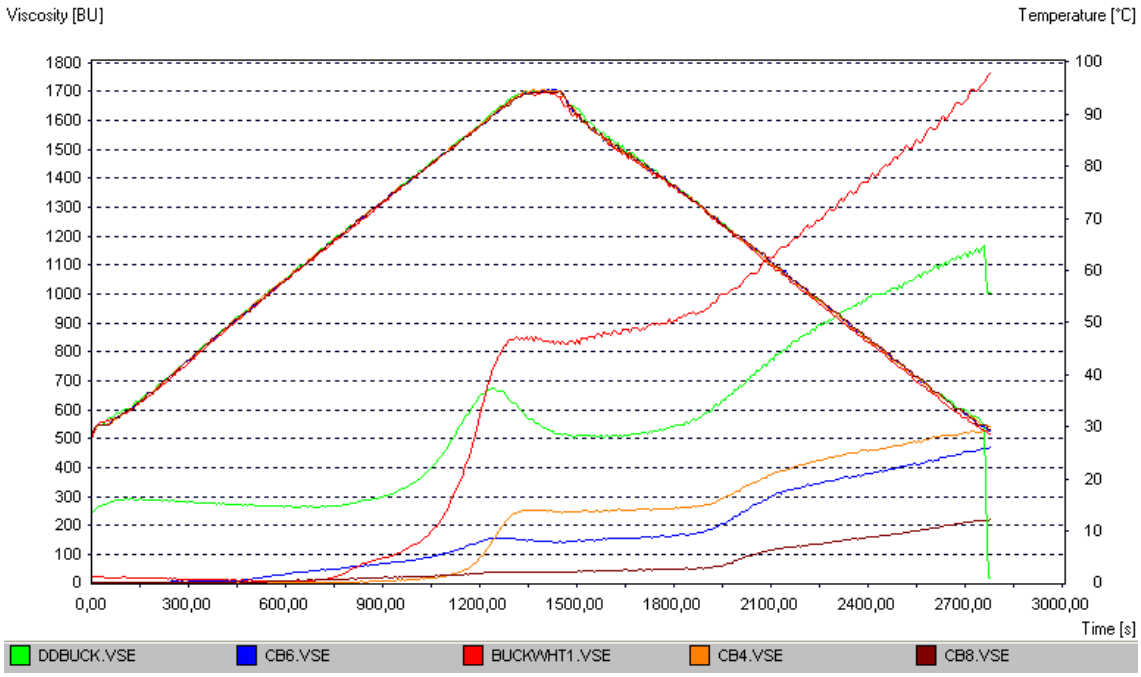


Figure 1. Brabender extruded and drum dried samples BUCKWHEAT. DDBUCK:drum dried buckwheat, B11; 25.43% moisture, B21:20.35% moisture, BUCKWHEAT1:raw buckwheat



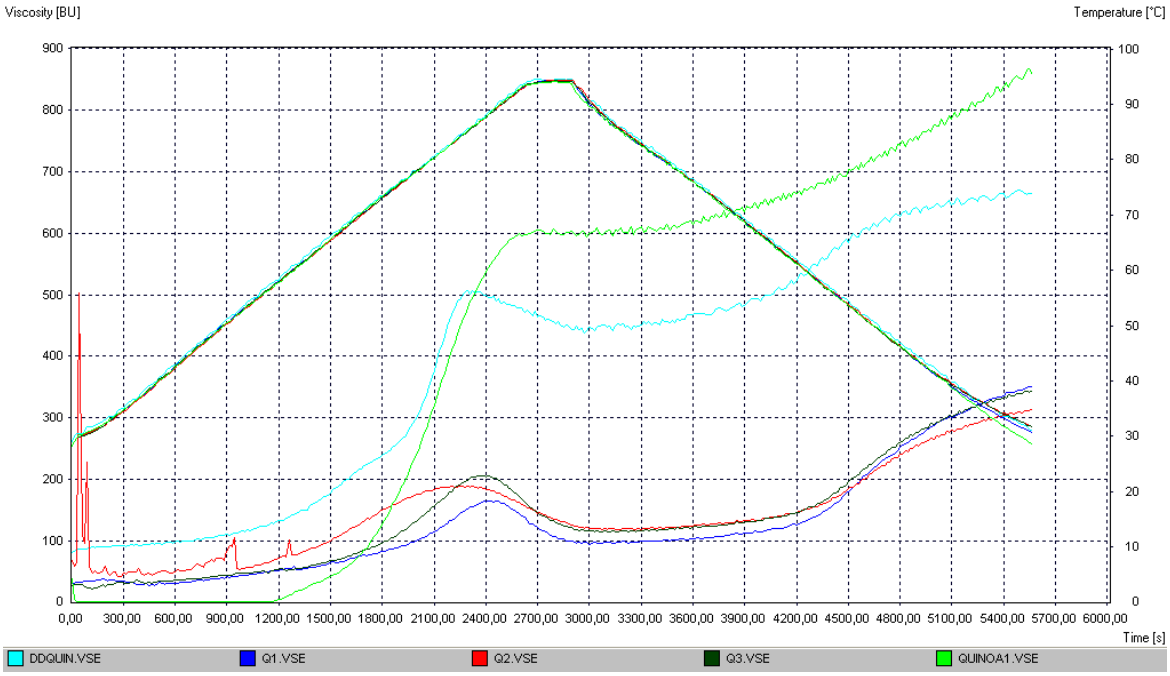


Figure 2. Brabender cooked samples BUCKWHEAT. DDBUCK: drum dried buckwheat, CB40; 40% moisture, CB6;60% moisture, CB80; 80% moisture, BUCKWHEAT1;

Figure 3. Brabender extruded samples. QUINOA. Q1; 21.62% moisture, Q2; 24.77% moisture, Q3; 28.67% moisture, DDQUIN; drum dried quinoa.

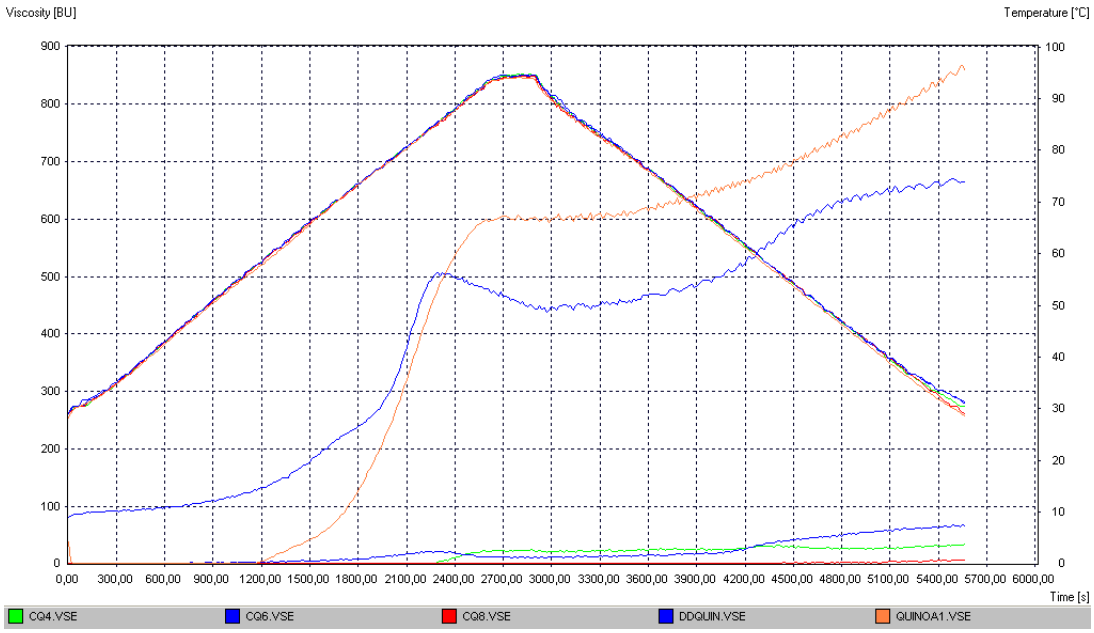


Figure 4.. Brabender stove cooked QUINOA. DDQUIN: drum dried quinoa, CQ40; 40% moisture, CQ6;60% moisture, CQ80; 80% moisture, QUINOA1; raw buckwheat

Physical properties: Swelling power

Raw buckwheat had a slightly greater swelling power number than the cooked samples at 9.6126 versus cooked samples that ranged from 6.3038 to 7.9501. Raw quinoa had a higher swelling power at 12.1624, but swelling power decreased as moisture content increased to 80% all the way down to 3.6397. This big decrease may affect the consumer likability of the product as texture is changed. Increased water content likely contributed to higher breakdown of the small clumped starch granules. The decreased swelling power could be attributed to enzyme activity during the initial heating phases that break down insoluble starch into soluble fragments that dissolved in the supernatant and did not hold any water. Hydrophobic inner portions of proteins may have become exposed as the denaturing process took place affecting the swelling power. Trapped fat involved in amylose-lipid or inside cell walls may also have become exposed as cells broke down decreasing swelling power. The rupturing and breakdown of cell walls and starch granules increased solubility and decreased swelling power.

Table 3. Swelling power of buckwheat and quinoa samples. Calculated as sediment weight/ dry sample weight.

	Swelling Power
Buckwheat Raw	9.6126
Buckwheat 40% Moisture	7.9501
Buckwheat 60% Moisture	6.3038
Buckwheat 80% Moisture	7.1966

	Swelling Power
Quinoa Raw	12.1624
Quinoa 40% Moisture	6.0312
Quinoa 60% Moisture	4.9889
Quinoa 80% Moisture	3.6397

Emulsification Properties

Raw samples had the highest emulsion ability. Stove cooking the samples decreased the emulsion ability for both quinoa and buckwheat samples. These results are counterintuitive as it seems that the denatured proteins would act as greater emulsifier with more hydrophobic areas becoming exposed. The reason again for this observation may be increased solubility. As portions of the grains are broken down, they may become soluble and no longer act as a binding agent between lipid and nonlipid portions of the emulsion. In order to better understand the factors responsible for the emulsification properties, it will be useful to separate the lipid, protein, starch, and remaining carbohydrate fractions and study their emulsification properties individually.

Table 4. Emulsification Ability and Stability for raw and stove top cooked samples.

	Emulsion Ability	**Emulsion Stability**
Buckwheat Raw	0.3417	57.6154
Buckwheat 40% Moisture	0.1640	89.5702
Buckwheat 60% Moisture	0.0693	93.4638
Buckwheat 80% Moisture	0.0823	91.3231

	Emulsion Ability	**Emulsion Stability**
Quinoa Raw	0.6170	67.9375
Quinoa 40% Moisture	0.1337	69.2641
Quinoa 60% Moisture	0.0903	76.4305
Quinoa 80% Moisture	0.1977	51.5733

Water Solubility Index (WSI) and Water Absorbance Index (WSI)

Differing trends were seen for water solubility index in buckwheat and quinoa. In buckwheat water solubility decreased from 10.4758 in raw buckwheat to 6.4895 in 80% moisture stove top cooked samples. For quinoa, the water solubility increased from 12.6615 in the raw state to 31.7980 in 80% moisture samples. Water absorbance in buckwheat and quinoa increased slightly after cooking, but remained similar between all cooked samples. A possible explanation for the larger increase in quinoa for water solubility may be that quinoa has a higher percentage of amylopectin. Amylopectin is more soluble in water than amylose due to the linear hydrogen bonded helical structure in amylose reducing the hydrogen bonding occurring with water (Green et al 1975). DSC could also be used in the future to further investigate this phenomenon. It is likely that starch from buckwheat has a higher melting temperature than quinoa starch, making quinoa more susceptible to granular structure loss and solubilization of its amylopectin fraction. This still does not explain the decrease in water solubility seen for buckwheat. It may be that chain length of amylopectin or amylose may play a role.

Table 5. Water Solubility and Water Absorbance Index

	WSI	WAI
Buckwheat Raw	10.4758	2.2756
Buckwheat 40% Moisture	7.3314	3.1212
Buckwheat 60% Moisture	8.0145	4.3713
Buckwheat 80% Moisture	6.4895	4.7782

	WSI	WAI
Quinoa Raw	12.6615	2.2832
Quinoa 40% Moisture	21.3661	3.2192
Quinoa 60% Moisture	17.5687	4.4660
Quinoa 80% Moisture	31.7980	3.6220

Future Research Direction

One of pseudocereals greatest strengths is also one of its greatest weaknesses. Being free of gluten and high in nutritional value makes it a great option from those suffering from gluten digestive problems creating a great opportunity for a developing a variety of new products. But many of the favorable eating qualities associated with the common cereal crops like wheat are directly due to the properties of gluten. Therefore it is important to find new ways to bolster pseudocereals appeal by ways such as creating a novelty by increasing resistant starch and bringing it to a new category of functional food. Investigating both new and old processing methods may improve the standing of these grains in the minds of the consumer.

Besides effecting consumers in a direct nutritional sense, the use of pseudocereals can also have an economic impact and as farmers from regions such as Eastern Europe and South America find a new growing market for their product. Genetic breeding may increase the growing regions of these pseudocereals opening the market for expanding areas like Africa and North America. Increasing the diversity of available food crops could bring food security for generations to come in case of devastation of the few monoculture crops currently in circulation. Consumer education and acceptance will play a key role in the expansion of quinoa and buckwheat.

Extrusion cooking will continue to be investigated in future studies. This process is highly versatile and adaptable to a wide array of applications and may provide new unique ways of creating pseudocereal products. The possible use of direct whole unmilled grains may be a method of increasing overall RS content by minimizing the loss of RS1 and RS2 fractions during the destructive milling or grinding process. Manipulation of storage temperatures including temperature cycling may improve final RS content. Other consideration may be defatting the grains decreasing the lipid-amylose complexes that form during retrogradation. Mixing other high amylose non-gluten contain grains like rice may be necessary to increase the overall level to a functional degree. The use of highly nutritious buckwheat with nutrient sparse rice may be the necessary compromise to create an acceptable product.

Work Cited:

AACC Report (2001). Definition of Dietary Fiber. *Cereal Foods World*, 46, 112-126.

Anderson, A.H., Levine, A.S., and Levitt, M.D. (1981). Incomplete absorption of the carbohydrate in all-purpose wheat flour. *New England Journal of Medicine*, 304, 891-892.

Anderson, J., Perryman S., Young, L., Prio, S. (2010). Dietary Fiber. Colorado State University. http://www.ext.colostate.edu/pubs/foodnut/09333.html.

Annison G., Topping D.L. (1994). Nutritional Role of Resistant Starch: Chemical Structure and Physiological Function. *Annual Review of Nutrition*. 14: 297-320.

Asp N.G. (1992). Resistant starch. Proceeding from the second plenary meeting of EURESTA: European FLAIR Concerted Action No 1.. on physiological implications of the consumption of resistant starch in man. *European Journal of Clinical Nutrition,* 46: S1

Baghurst P.A., Baghurst K.I., Record S.J. (1996). Dietary fibre, non-starch polysaccharides and resistant starch: A review. *Food Australia*, 48: S1-S36

Berghofer E and Schoenlechner R. Pseudocereals – an Overview. Department of Food Science and Technology. Vienna, Austria

Bird, A. R., Brown, I. L., Topping, D.L (2002). Starches, Resistant Starches, the Gut Microflora and Human Health. *Current Issues in Intestinal Microbiology,* 1, 25-37.

Birkett A. M. and Brown I.L. (2008), Resistant starch and health. *Technology of functional cereal products*. Ed. Bruce R. Hamaker. Woodhead Publishing Limited: Cambridge, England. 63-85.

Bjorck I., Ng. A., Birkhed D., Elia A.C. (1984). Effects of Processing on Starch Availability In Vitro and In Vivo II Drum Drying of Wheat Flour. *Journal of Cereal Science*, 2, 165-178.

Brown I.L., Wang X., Topping, D.L., Playne M.J., and Conway P.L. (1995). High-maize: new direction in starch technology and nutrition. *Food Australia*, 46, 272-275.

Brown I.L., Wang X., Topping, D.L., Playne M.J., and Conway P.L. (1998). High amylose maize starch as a versatile prebiotic for use with probiotic bacteria. *Food Australia*, 50, 602-609.

Buckwheat: A Versatile Short-Season Crop. Thomas Jefferson Agricultural Institute.

Burkitt, D.P. and Trowell H.C. (1997). Dietary fibre and western diseases. *Irish Medical Journal,* 70, 272–277.

Crosbie, G. B. (1991). The relationship between starch swelling properties, paste viscosity and boiled noodle quality in wheat flours. *Journal of Cereal Science*, 13.145-150.

Eerlingen R.C. and Delcour J.A. (1994). Formation, Analysis, Structure, and Properties of Type III Enzyme Resistant Starch. *Journal of Cereal Chemistry,* 22, 129-138.

Englyst, H.N., Kingman, S.M., and Cummings, J . H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46, S33-S50.

Faraj A., Vasanthan T. and Hoover R. (2004). The effect of extrusion on resistant starch formation in waxy and regular barley flours. *Food Research International*, 5, 517-524.

Gidley M.J., Bulpin P.V. (1989). Aggregation of Amylose in Aqueous Systems: The Effect of Chain Length on Phase Behavior and Aggregation Kinetics. *Macromolecules*, 22, 341-346.

Green, M., Blankenhorn G., and Hart, H. (1975). Which Starch Fraction is Water-Soluble, Amylose or Amylopectin? *Journal of Chemical Eduction,* 52, 729-730.

Hague A., Manning A.M., Hanlon K.A., et al. (1993) Sodium butyrate induces apoptosis in human colonic tumour cell lines in a p53-independentpathway: implications for the possible role of dietary fibre in the prevention of largebowel cancer. *International Journal of Cancer,* 55, 498–505.

Jacobsen, S.E. (2000). "Quinoa – Research and Development at the International Potato Center" *Condesan*.

Jones J.M. (2008). Fiber, whole grains, and disease prevention. *Technology of functional cereal products*. Ed. Bruce R. Hamaker. Woodhead Publishing Limited: Cambridge, England. 46-62.

Kendall, W.C., Esfahani, A., Jenkins, D. (2009). The link between dietary fiber and human health. *Food Hydrocolloids*, 24, 42-48.

Kim J.H., Tanhehco E.J, Ng P.K.W. (2006). Effect of extrusion conditions on resistant starch formation from pastry wheat flour. *Food Chemistry*, 9, 718-723.

Mahadevamma S., Shamala T.R., Tharanathan R.N. (2004). Resistant starch derived from processed legumes: in vitro and in vivo fermentation characteristics*. Journal of Food Science and Nutrition*, 5, 399-405.

Murphy, M.M., Douglass J.S., and Birkett A. (2008). Resistant Starch Intakes in the United States. Current Research. *Journal of American Diet Association,* 108, 67-78.

Oelke, E.A. Putnam D.H., Teynor, T.M., Oplinger, E.S. (1992). Quinoa. *Alternative Field Crops*. University of Wisconsin Extension.

Pawlak, D.B., Kushner J.A., and Ludwig D.S. (2004) Effects of dietary glycemic index on adiposity, glucose homeostasis, and plasma lipids in animals. *Lancet,* 364, 778-785.

Pelembe, L.A.M., Dewar, J., Taylor, J.R.N (2003). Food Products from Malted Millet. Workshop on Proteins of Sorghum and Millets: Enhancing Nutritional and Functional Properties for Africa.

Smith, J.G., Yokoyama, W.H., and German, J.B. (1998). Butyric acid from the diet: Actions at the level of gene expression. *Critical Reviews Food Science*, 38: 259-29.

Stojceska V., Ainsworth P., Plunkett A. and Ibanoglu S. (2010). The advantage of using extrusion processing for increasing dietary fibre level in gluten-free products. *Food Chemistry,* 121, 156–16.

Tang, J., Feng, H.m and Shen G.Q. (2003). Drum Drying. *Encyclopedia of Agriculture, Food, and Biological Engineering*. 211-214.

Tang H., Watanabe K., Mitsunaga T. (2002) Characterization of storage starches from quinoa, barley, and adzuki seeds. *Carbohydrate Polymers*, 49, 13-22.

Topping, D.L., Illman, R.J., Clarke, J.M., Trimble, R.P., Jackson, K.A., and Marsono, Y. (1993). Dietary fat and fiber alter large bowel and portal venous volatile fatty acids and plasma cholesterol but not biliary steroids in pigs. *Journal of Nutrition*, 123, 133-143.

Valenzuela, H. and Smith, J. (2002). Buckwheat. *Sustainable Agriculture Green Manure Crops.* College of Tropical Agriculture and Human Resources.

Van Muster, I.P., Tangerman, A., Nagengast, F.M. (1994). Effects of Resistant Starch on Colonic Fermentation, Bile Acid Metabolism and Mucosal Proliferation. *Digestive Diseases and Sciences*, 39, 823-842.

Waring, S. (2008) Resistant Starch in Food Applications. *Cereal Foods World.*

Yahav, S.B., Sharma A., Yadav R.B. (2009). Studies of effect of multiple heating/cooling cycles on the resistant starch formation in cereals, legumes, and tubers. *International Journal of Food Sciences and Nutrition*, 60, 258-272.

Zayas, J.F., and Lin C.S. (1989). Emulsifying Properties of Corn Germ Proteins. *Cereal Chemistry,* 66(4), 263-267.