THESIS

COMPARISON AND ACCEPTABILTY OF GLUTEN-FREE YEAST BREADS MADE WITH QUINOA FLOUR

Submitted by

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ABSTRACT

COMPARISON AND ACCEPTABILTY OF GLUTEN-FREE YEAST BREADS MADE WITH QUINOA FLOUR

Quinoa (*Chenopodium quinoa Willd)* is a plant that is native to South America and is grown in the Andean mountains. The quinoa plant is resistant to harsh weather conditions and drought. It is a gluten free (GF) grain and has significance in making a GF yeast bread for people who cannot safely consume gluten proteins due to celiac disease, gluten allergies, or other issues. Most GF yeast breads on the market are made with a large portion of white or brown rice flour, which is neutral in taste, easily digestible, but low in vitamins and nutrients compared to quinoa flour. Quinoa flour is more expensive than rice flour and can impart negative aftertastes. The objectives of this study were to develop GF yeast bread formulas incorporating quinoa flour for rice flour and potato starch at 0, 36, 72, or 100% and obtain sensory and instrumental data on the breads.

Specific gravity was calculated on the batter before baking with significant ($p<0.05$) differences existing among all batters. The 100% GF quinoa yeast bread was ($p<0.05$) smaller in volume than the other breads. The GF bread made with 100% rice flour and potato starch was significantly ($p<0.05$) softer (less hardness or firmness values) than the GF breads containing quinoa flour, while the 100% GF quinoa flour bread was firmest and least tender. Crust and crumb color did not ($p>0.05$) differ among any of the breads. The 100% GF quinoa yeast bread had the lowest water activity ($p<0.05$). Sensory analysis showed that for tenderness, flavor, and overall acceptability the 100% GF quinoa yeast bread was liked less ($p<0.05$) compared to the

other breads. Based on the instrumental and sensory data collected, both the 36 and 72% QF yeast breads are acceptable GF yeast bread options containing QF.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

It is estimated that 3 million people in the United States have been diagnosed with celiac sprue disease and must follow a gluten-free (GF) diet (celiaccentral, 2014). In August 2013, the Food and Drug Administration (FDA) formally decided on a definition for what the term "gluten-free" means on food labels, in accordance with the Food Allergen Labeling and Consumer Protection Act (FALCPA) that was passed in 2004 (FDA, 2013). According to the FALCPA, all foods labeled as GF must contain < 20 ppm gluten. It is no surprise that food producers and retailers have been meeting the demand for commercially available GF foods for their customers. Since 2004, annual sales of GF products has jumped 78% with annual sales predicted to reach $6.6 billion by 2017 (Packaged Facts, 2014). In order to produce GF products that mimic their gluten-containing counterparts, much research has been performed on how to bake with grain flours other than wheat. This paper will focus on the various aspects of GF baking such as different flour and starch sources, their properties, and use of other ingredients, for instance, hydrocolloids and stabilizers.

Celiac disease

Celiac disease (CD) is an autoimmune disease characterized by intestinal damage resulting from the ingestion of products containing gluten, or other prolamin (storage) proteins found in cereal grains (Alvarez-Jubete, 2010). The autoimmune response leads to inflammation in the digestive tract, damages the microvilli, and inhibits nutrient absorption. Gluten is the main protein complex formed from wheat flour, and it gives breads and other baked products their porous, spongy texture. In addition to being found in wheat, prolamins that can trigger the autoimmune response are found in rye, barley, and possibly oats (Kusunose, 2009).

The term “celiac” can be traced back to 250 A.D. when a Greek physician described bowel irritation and bloating in his patients (Celiac Support Association, 2013). He referred to them as "koiliakos," which means “suffering in the bowels” (Celiac Support Association, 2013). Later, when being translated into English, the name celiac, or coeliac, was given to sufferers of the disease (Celiac Support Association, 2013). Diagnoses of celiac disease are growing in number as more people are becoming aware of symptoms and due to improvements in testing sensitivity. Originally thought to be a disease suffered only by children, one physician prescribed that those with “chronic indigestion” should avoid milk, and foods with a high amount of starch, such as rice, as well as fruits and vegetables. Instead he said, they should stick to eating only raw meat and toast (Gee, 1888). In 1924 those recommendations were slightly improved upon by a diet consisting of bananas, rice, and chicken (Hass, 1924). Following this diet for a few years was thought to cure people of the disease, after which time they could reintroduce gluten back into their diets without any negative digestive issues. While this treatment theory has been disproven, it should be noted that Gee and Haas observed that the disease could only be regulated through the diet.

It was not until the 1950’s that the prolamins causing the disease were examined to determine what was causing the reaction (Gallagher, 2009). Van de Kramer et al. (1953) set up a clinical trial in which persons suffering from the digestive disorder were fed different fractions of wheat, and reported their symptoms. The samples that contained gliadin, the prolamin in wheat, elicited a strong reaction (Van de Kramer et al., 1953). The researchers gradually added gliadin back into the subject’s diet and measured the amount of fat that was passed in their stool. High levels of fat in the stool indicated that the subjects were not absorbing fat, which meant there was a problem with their digestive functions. This study helped to negate the myth that starches in

general were the cause of the symptoms, as well as to identify the specific protein fraction which was actually responsible (Van de Kramer et al., 1953).

CHAPTER 2: REVIEW OF THE LITERATURE

Gluten is classified as a prolamin, meaning it is alcohol-soluble, according to the Osborne classification of proteins, and is a storage protein in plants (Niewinski, 2008). The problematic protein fraction in wheat gluten is gliadin, and it elicits the autoimmune response. In rye and barley the prolamins that cause the response are secalin and hordein, respectively (Niewinski, 2008). In wheat, gluten is formed from both gliadin and glutenin. Even though rye and barley do not contain gliadin, they are still classified as sources of gluten, in the terms of initiating a celiac response, which is not always made clear in the literature. It was not until 2003 that the other offending proteins were identified. Vader et al. (1953) sought to identify protein fractions that could also increase the symptoms of celiac disease. The researchers were looking for a mediated T-cell response in the presence of prolamin fractions from rye and barley (secalin and hordein, respectively) (Vader et al., 2003). They included avenin from oats too, since it was observed that oats also seemed to produce symptoms matching gluten consumption. Gluten-specific T-cells were stimulated in the presence of hordein and secalin, but not of avenin (Vader et al., 2003). The significance of avenin not eliciting a response is that oats can be contaminated post-harvest by gluten, a fact that is important when trying to avoid all sources of gluten. This study was key in increasing the knowledge of what celiac sufferer's should avoid in their diets in order to remain healthy and avoid uncomfortable digestive issues.

Gluten forming proteins

People trying to avoid gluten must retain a constant vigilance, as some processed foods and medications use wheat for fillers. Gluten can also be used in meat fillers, as in hot dogs and sausages, salad dressings, and also in seasoning packets (Vader et al., 2003; Alvarez-Jubete, 2010). As mentioned above, even oats can be problematic if they were processed in a facility that

also processes wheat, rye or barley, as the oats can be cross-contaminated. Triticale, a cross-breed between wheat and rye, should also be avoided by those with celiac disease (CD) (Yazynina et al., 2008). Total avoidance of any foods that may be a trigger for the disease increases the quality of health for celiac sprue patients. Symptoms are avoided which in turn leads to healing of the microvilli, ultimately resulting in a normal functioning digestive tract (Yazynina et al., 2008). It is estimated that one in 133 people in the Unites States has been diagnosed with celiac disease, and the Celiac Sprue Foundation estimates there may be over one million people who are still undiagnosed (CeliacCentral, 2013). Researchers have shown that CD stems from the combination of genetics, environmental factors and inflammation from immune responses (Murray, 1999).

Diagnosis of celiac disease

There are many symptoms of celiac disease which can make it difficult to diagnose. For example, the classic hallmarks are bloating and abdominal pain, diarrhea, and constipation (Celiac.org, 2014). The lesser known symptoms are migraines, canker sores inside the mouth, irritability, and discolored teeth (Celiac.org, 2014). There is also asymptomatic CD, in which the microvilli in the small intestine are not too badly damaged, and can still absorb some nutrients (celiaccentral.org). In terms of diagnosis, the preferred method is comparing two biopsies of the small intestine, first while a patient is consuming gluten, and then when not consuming gluten (Niewinski et al., 2009). However, the state of the microvilli can be nursed back to health eventually with the adherence to a GF diet.

Nutrient deficiencies associated with celiac disease

Some nutrient deficiencies associated with celiac disease include iron, calcium, vitamin D, and B vitamins like B6, B12, and B9 folate. Sometimes celiac patients may need to take a multivitamin in order to make up for lost nutrients (Celiac.org, 2014). The deficiencies are the result of damage to the microvilli of the small intestine, which hamper absorption of those nutrients. Figure 1 illustrates the decreased state of the villi after someone who is gluten intolerant consumes gluten over a period of years (Maki et al., 1990). The microvilli are fingerlike projections which function in vitamin and mineral absorption. The subsequent damage to them that is followed by eating gluten flattens them so there is decreased surface area for nutrient absorption (Maki et al., 1990). Figure 1 illustrates both healthy microvilli and the decreased state of the villi after someone who is gluten intolerant consumes gluten over a period of years (Maki et al., 1990).

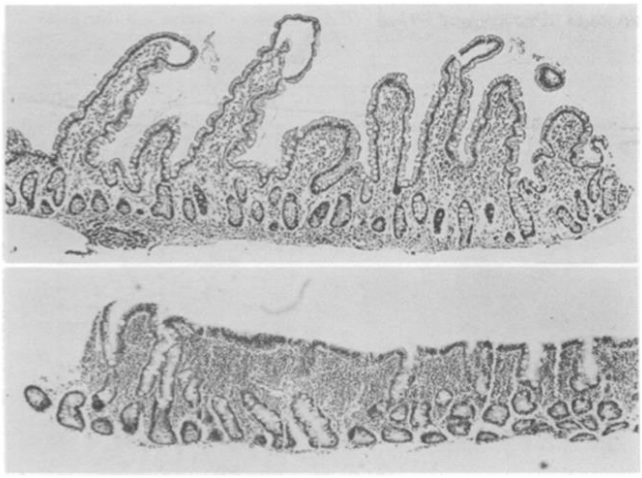

Figure 2.1. Intestinal biopsies of healthy (upper) and blunted microvilli (lower) (Maki et al., 1990)

Following adherence to a GF diet, the patient's microvilli should heal and be able to absorb nutrients normally. However, many GF products on the market are made with white rice flour to better mimic white wheat flour. While the color and bland flavor of rice flour allow it to resemble wheat flour better, it is relatively low in vitamins, nutrients, and protein than other GF grains (Nascimento, 2014).

Other reasons to follow a GF diet

Even for those who do not suffer from any gluten digestion problems, consuming gluten-free grains can have additional nutritional benefits from those found in wheat flour. One example is the protein content and value found in the quinoa seed. The total protein content can be up to 15%, and it is high in lysine, which is the limiting amino acid in most other cereals (Belton, 2002). This can have significance for vegetarians and especially vegans, who do not consume animal products and must obtain protein from plant sources. A deterrent for non-gluten sufferers to purchase GF products is the price. In 2007 Cureton found that the average cost of a loaf of GF bread was around $6.00 compared to $1.09 (for 453g of white wheat bread). According to the 2014 US Department of Labor consumer price index, a loaf of wheat bread has increased to $1.47, and presumably the cost of a loaf of GF bread had increased as well (Cureton, 2007; United States Department of Labor).

While the increased cost of a GF lifestyle is not unknown, it does make a difference in a family's budget. The U.S. government offers tax deductions for those with physician-diagnosed celiac's disease (celiaccentral, 2014). The government requires consumers to keep a receipt of all the GF purchases made, and the price difference between the non-gluten item and the GF item is the reported deduction. The example used on Celiaccentral.org, 2013 is the difference in cost

between white wheat flour and rice flour per pound. Items such as xanthan gum that do not have a direct gluten-containing counterpart are counted entirely as the deduction (celiaccentral, 2014).

Federal agencies role in GF foods

In 2003 a multicenter study published in the United States reported that one in 133 people (1% of the population) suffer from celiac disease and federal government agencies began to have a role in the increased diagnoses (Fasano et al., 2003; Gallagher, 2009). In 2004 the National Institute of Health convened in order to determine how to proceed with Fasano et al. findings (Gallagher, 2009). The panel resolved that celiac disease was a bigger issue than previously thought, and that physicians and other healthcare workers needed to be educated on the disease (NIH Consensus Statement on Celiac Disease, 2004). Other results from this meeting were that there should be standardized serological and pathologic tests in order to accurately identify celiac disease in a patient, procedures needed to be developed for testing for the presence of gluten in foods, as well as minimum acceptable amounts determined for gluten in foods. The panel also recommended the formation of groups and organizations to focus on CD to educate and bring awareness to the general population about CD (NIH Consensus Statement on Celiac Disease, 2004).

In addition to paying more per year for GF foods, it is estimated that families shopping for GF foods spend 10-20 hours more per month than average consumers (Gluten Intolerance Group, 2005). This is because of all the reading and research that must be done in order to verify that the foods one is purchasing are truly GF, and have not been contaminated. In 2004 the Food Allergen Labeling and Consumer Protection Act (FALCPA) was signed into law (FDA, 2004). Under this law, all major food allergens must be clearly stated on food packages. Foods that are considered major allergens and are required to appear on labels in the United States as food

allergens are milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, and soybeans (Food Allergen Labeling and Consumer Protection Act, 2004). This labeling greatly simplifies the shopping time that GF consumers spend on searching for safe foods. It should be noted that the FALPCA does not include rye or barley, making it necessary for GF consumers to do the research themselves if they want to know whether products contain those grains.

On August 2, 2013, the FDA finally settled on an established definition of the term "gluten free." A product that is GF contains less than 20 ppm of gluten, does not have an ingredient that is any type of wheat, rye, barley, or crossbreeds of these grains, and does not have an ingredient derived from these grains and that has been processed to remove gluten, if it results in the food containing 20 or more parts per million (ppm) gluten (Food Labeling; Gluten-Free Labeling of Foods, FDA, 2013; federalregistrar.gov, 2013). These standards match the guidelines established by CODEX, which are established by the World Health Organization (codexalimentarius.org, 2013).

GF foods on the market

Food companies have been trying to fill the gap for GF foods in the marketplace. Since the publication of the multicenter study and the FDA's contributions through set laws and regulations on how to handle GF products, GF foods became more widely available on the market. To put it in perspective, in 2001 market sales of GF products in the United States reached $210 million (Gallagher, 2009), and Packaged Facts reports that sales reached $2.6 billion in 2010, and are predicted to reach $6.6 billion by 2017 (Packaged Facts, 2012).

Since companies have recognized the value of producing GF products, much research has gone into producing GF alternatives for pastas, breads, and other baked products. Advances have been made with hydrocolloids and gums, proteins, and even enzymes, and the literature shows

many examples of each. In August 2013, shortly after the FDA released a clear definition of what they deem to be "gluten-free", Pillsbury announced its new GF chocolate chip cookie dough, thin crust pizza dough, as well as a GF pie and pastry crust dough (Pillsbury, 2013). Udi's, a company that produced its first GF loaf of bread for retail sale in Colorado in 2008 now sells breads in major grocery stores, and even sports stadiums, all over the country (Udis, 2013). Even the Girl Scouts have taken notice of the value of offering consumers a GF option, and in 2014 offered a GF chocolate shortbread cookie (celiaccentral, 2014). The average price of a box of Girl Scout cookies is $3.50 (sizes range from 6 oz.- 9oz), but the GF cookies came in only 5 oz. packages and were $5.00 per box (girlscouts, 2013). With about 83% of Americans with celiac disease going undiagnosed, the demand for more GF foods available on the market can continue to rise, especially as testing for gluten intolerance becomes more sensitive (celiaccentral.org, 2013).

The National Foundation for Celiac Awareness (NFCA) offers training online for food companies and their employees (celiaccentral, 2013). In February 2014 the University of Nebraska in Lincoln announced its plans to offer a wide array of GF options to its students in the dining halls on campus (celiaccentral, 2013). The NFCA's training program, GREAT (Gluten Free Resource and Education Awareness Training), offers separate programs for dining halls/cafeterias and professional restaurant kitchens too. The training programs incorporate the guest, the kitchen staff, and the front of the house staff and includes ingredient information, which allows for a full GF implementation into the dining program (celiaccentral, 2013).

Wheat

Wheat kernel

There are two main varieties of wheat available, *Triticum aestivum* and *Triticum durum* (Schofield, 2010). *T. aestivum* is the variety used most commonly for baking applications. Wheat can be divided into hard and soft wheat, and winter and spring wheat. They may also be described in terms of color, such as red and white wheat, although other colors exist (Kulp, 2000). Wheat is referred to as hard wheat because a higher force is required to crush the kernel. It not only has thicker cells walls, but it was also found to have less airspace in the protein matrix of the endosperm (Kulp, 2000). This has also been shown to make the resulting milled flour denser. Hard wheat tends to have a higher protein percentage and is used in the production of breads (Kulp, 2000). Soft wheat needs less force to crush the kernels in contrast to hard wheat kernels. Soft wheat has more airspace in the matrix of the endosperm and makes the flour chalkier. It tends to have less protein and is better suited for cookies and cakes, or other baked goods where a tender crumb is desired (specifically due to the reduced amount of gluten). Wheat with differing protein contents can be mixed in order to form all-purpose flour, cake flour, and pastry flour, depending on the intended application (Bennison, 2000).

The main parts of the wheat kernel are identified as the bran, endosperm, and germ (Figure 2.2). The bran refers to the outer layers of the kernel: the pericarp, seed coat, nucellar epidermis, and the aleurone (Schofield, 2010). These layers surround the endosperm and the germ, protecting them from weather, pests, mold and bacteria (Cargillfoods.com, 2013). The bran has about 6% protein, 2% ash, 20% fiber, and 0.5% fat. The aleurone layer makes up the largest portion of the bran at 75% (Cargillfoods.com, 2013) and has the highest amounts of niacin, thiamin, and riboflavin, which is why white flour, in which the bran has been removed,

must be enriched with those nutrients after the milling process (Delcour, 2010). It should be noted that the aleurone layer is technically considered part of the endosperm, but is difficult to separate from the bran during milling and is thus separated from the endosperm (Kulp, 2000).

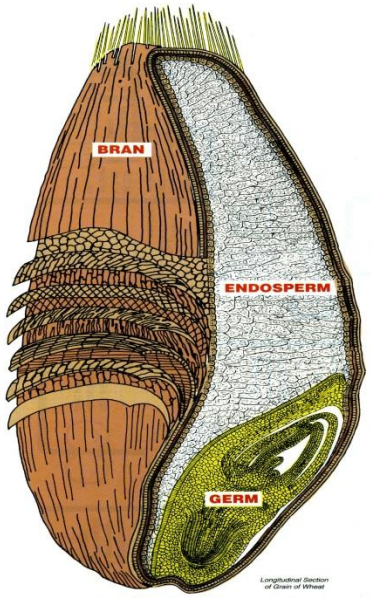

Figure 2.2. Wheat kernel. North American Miller's Association, 2014.

The endosperm is the main storage of energy for the wheat kernel and is the center of the grain. About 50-75% of the endosperm is starch, with about 8-18% of protein with few vitamins or minerals (Cargillfoods, 2013). There are three cell types that have identified in the endosperm: peripheral, prismatic, and central (Delcour, 2010). Peripheral cells are formed as an outer layer and are uniform in size. The prismatic cells are clustered around the center of the endosperm. Finally, in the center of the endosperm, are the central starchy endosperm cells. These cells are mainly composed of B-glucans and hemicelluloses. These cells contain starch granules which are suspended in a protein matrix (Delcour, 2010).

The germ contains the plant embryo and accounts for 2.5-3.5% of the kernel (Delcour, 2010). It contains a high amount of fat (48%) and is high in protein (25%). The germ

also contains 18% sugar (mostly sucrose and raffinose), as well as most of the kernel's vitamin E (Delcour, 2010).

Wheat carbohydrates

Starch makes up about 80% of the total weight of whole meal wheat flour (Hager et al., 2012). The starch granules of wheat are held in amyloplasts which can be either lenticular or sphere shapes. The lens shaped plasts tend to be larger (25-40um) than the spheres (5-um in diameter). These two shapes have a difference in digestibility, and the lenticular shaped (commonly referred to as Type-A) are more readily digested than the sphere (Type-B) (Hager et al., 2012). The two main types of starches found in wheat are amylose (25%) and amylopectin (75%) (Kulp, 2000). Amylose is the linear starch consisting of α-1,4 glycosidic linkages of glucose. Amylopectin consists of linear α-1,4 bonds with the inclusion of glucose branches held by α-1,6 bonds. Starch is important in baking because it helps enforce structure of the baked product by gelatinization of the starch after it has been treated with water. As the amount of free water is reduced, the product gelatinizes and thus increases in rigidity (McWilliams, 2005).

In terms of fiber, whole wheat flour, which contains the bran, has a higher amount than white flour (11.4g/100g and 3.4g/100g respectively) (Hager et al., 2012). About 1.6g/100g of whole wheat flour is soluble, while the rest is considered insoluble (Hager et al., 2012). Phytate averages to about 0.77g/100g of whole wheat flour and is also found in the bran (Hager et al., 2012). Although it is identified as chelating iron and zinc, it also stores phosphorous. Some research has been done to try to increase the amount of phosphorous in the endosperm so that it can be retained during white wheat milling, as well as be unbound to phytates in whole wheat, thus potentially limiting its chelating effects (Soils, 2013).

Wheat proteins

The amount of protein found in wheat varies in percentages based on the type of wheat used (soft or hard, spring or winter). Most of the proteins in wheat can be divided into soluble proteins (albumins and globulins) which make up 15% gluten protein (glutenin and gliadin), as well as enzymes (Delcour, 2010). While protein is essential for giving baked products their structure, they are found in relatively small amounts when compared to starches (McWilliams, 1995). The storage protein in wheat is gluten (Figure 4), which is made up of glutenin and gliadin, found in the endosperm. Glutenin is the alcohol-insoluble protein. It is fibrous and elongated, and is what gives elasticity to gluten, thought to be determined by its intra-chain disulfide bonds (Belton, 2002). Gliadin is soluble in alcohol, compact in shape, and is sticky due to its intra-chain disulfide bonds (Belton, 2002). Gliadin and glutenin make up about 85% of the protein found in wheat, and are attributed to dough formation. These two proteins form disulfide bonds as they are mixed and stretched, which can trap gas formed either by yeast or baking powder (or baking soda plus an acid reactant). As the product is baked, the gluten stretches under the pressure from the heated gas and, and ultimately determines the crumb of the finished product (Figure 4) (McWilliams, 1995).

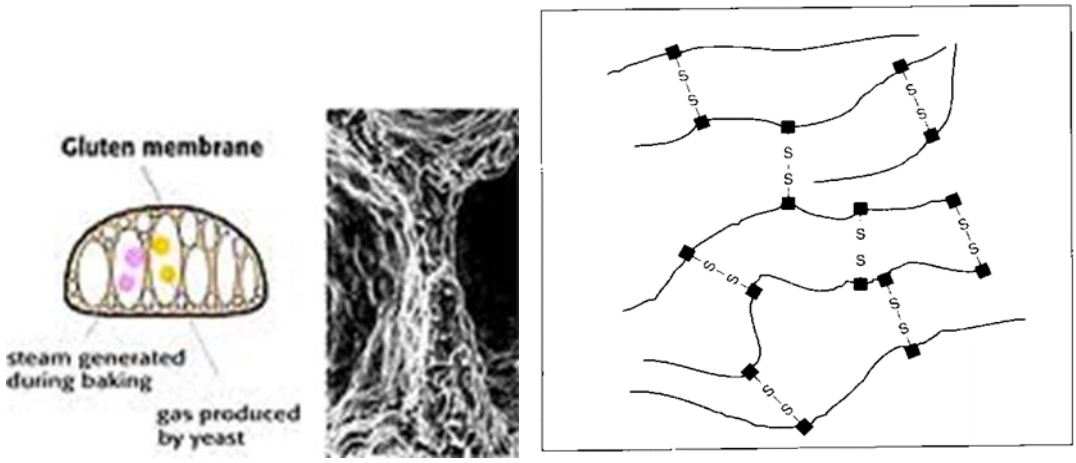

Figure 2.3. Functionality of gluten matrix in baking. (Rikenvitamin.jp; emeraldinsight.com)

Gliadin can be broken down into four subfractions: alpha, beta, gamma, and omega (Kulp, 2000). Under the Osborne classification it is a prolamin and is soluble in 70% ethyl alcohol. Glutenin is classified as glutelin and is soluble in weak acids or bases. These classifications are used to extract the respective protein fractions from a sample (Kulp, 2000). HMW (high molecular weight) fractions of glutenin form disulfide bonds and are thought to give the elasticity. The HMW fractions are also comprised of glutamin sequences that form hydrogen bonds, which also contribute to the elasticity of the dough (Shewry, 2002). Albumins and globulins are also found in wheat, but in low levels (10% of the total protein) (Delcour, 2010).

The gluten matrix has starch granules embedded in it. Upon baking, those starches gelatinize within the gluten network, forming the chewy, springy mouth feel we associate with bread, or other baked products made with wheat. Without the gluten, the protein matrix isn't formed, and the bread ends up with a crumbly texture (Zannini, 2012).

Wheat enzymes

Many different enzymes (proteinases and glycosidases) are found in wheat, although they are found in low quantities (Lasztity, 1996). Alpha-amylase, an enzyme classified in the glycoside hydrolase Family 13 enzyme, hydrolyses the α-1,4 linkages of starch, breaking it down during germination in preparation of sprouting. Alpha amylase can also be added to a starch that has been gelatinized and pasted, decreasing its viscosity (Lasztity, 1996). Another enzyme that is part of Family 13 is called pullulanese, which hydrolyzes the α-1,6 bonds in starches, removing side chains from the α-1,4 body (Delcour, 2010).

An enzyme from the Family14 is beta amylase and it produces maltose by breaking every second α-1,4 linkage and stops at the α-1,6 branch (Kulp, 2000). Beta amylase only works on non-reducing ends, so it works well with α-amylase, especially in beer making, which produces

non glycoside hydrolase -reducing ends as it works. It is difficult to measure beta amylase without α-amylase for this reason. Together these two enzymes can convert 85% of starch to sugar (Delcour, 2010).

The third family of glycoside hydrolases is Family 15 which includes glucoamylase. Like β-amylase it works on the non-reducing ends of starch, but has the ability to continue and hydrolyze the α-1,6 linkages as well. While neither α-amylase or β-amylase can totally convert starch to sugar, glucoamylase can because it works on both the α-1,4 and the α-1,6 linkages that form starch (Delcour, 2010).

Wheat lipids

Lipid content in wheat can range from about 2-2.9% (Delcour, 2010). The oil is found primarily in the germ, while the endosperm contains the least, and the bran has an intermediate level (Kulp, 2000). This is why whole wheat flour typically has a shorter shelf life than white wheat flour, and it also explains why wheat germ should be stored in the refrigerator. Also, since the oil from the germ is highly unsaturated, it is more susceptible to rancidity. The oil has a high percent of unsaturated fatty acids. They are characterized by 70% nonpolar lipids, 20% glycolipids, and 10% phospholipids (Delcour, 2010). Vitamin E is found in amounts of 3.9mg/100 g in whole wheat flour, and the total tocopherol count is about 200mg/100 g of wheat oil. The lipids found in the endosperm can be bound to starch. The form in which the lipid is found in the wheat is important for selection of extractability method. Wheat flour contains two types of lipids: starch lipids and non-starch lipids (Hebeder, 1996). The non-starch lipids that can be extracted using petroleum ether are deemed “free lipids” and can be found as either polar or non-polar. On the other hand, the fraction that cannot be removed with petroleum ether is considered to be “bound lipids” (Hebeder, 1996). The state in which it is bound will determine

which solvents should be used to extract which type of fat (Delcour, 2010). Since the oil content in wheat is so low it isn't specifically produced on its own, but rather is a co-product of the other wheat processing methods (Delcour, 2010).

One effect of wheat lipids in concern to baking are that they have the ability to increase volume by stabilizing the lipoprotein films that are formed in conjunction with the viscoelasticity from the gluten (Schofield, 1995). The lipoprotein films stabilize the foams produced during the proofing stages, giving extra support to the gluten network. The main lipids in wheat flour that are identified as contributing to this lipoprotein film are the polar lipids which act as emulsifiers and decrease surface tension within the foams (Hebeder, 1996). In studies that compared the bread loaf volume of whole wheat flour and defatted wheat flour, the whole wheat flour was reported to increase volume (Kulp, 2000). Another effect of lipids on bread dough is the formation of a tender crumb which is related to the increase in volume, making the bread less dense (Hebeder, 1996). Tenderness is also seen in cake batters, where a higher amount of fat leads to an increase of shortening power (McWilliams, 2005).

Wheat vitamins and minerals

The majority of vitamins and minerals are found in the aleurone layer, although there are varying amounts found in the endosperm, germ, and bran (Delcour, 2010). The content of minerals found in whole wheat flour are 30.7mg/100g of calcium, 0.27mg/100g of iron, 0.18mg/100 g of zinc, 400mg/100g of potassium, and 78mg/100g of magnesium (Hager, 2012).

Vitamins found in whole wheat flour include folate, which is essential for growth and metabolic processes and is found in wheat at levels of 34ug/100g (Bennion, 2010). Bran contains the highest amount of niacin and riboflavin, 86% and 42%, and the germ has higher amounts of thiamin at 64%, and both the endosperm and the germ have similar amounts of riboflavin with

32% and 26% (Bennion, 2010). Since the bran contains mostly cellulose, some of its nutrients may not be well absorbed, so enriched white flour may actually be a better source for those nutrients. The amount of vitamins added back (per pound) for enrichment include 2.9 mg of thiamin, 1.8 mg of riboflavin, 24 mg of niacin, 0.7 mg of folic acid, and 20 mg of iron. Calcium also may be added at a minimum of 960mg per pound (FDA.gov, 2013). The A, D, E and K fat soluble vitamins, found in the germ, are in low amounts in wheat because it is low in lipids itself. The amount of vitamin E is about 0.5mg/100g and while it can act as an antioxidant, is not very stable when exposed to oxygen, leaving lipids susceptible to oxidative rancidity (Kulp, 2000).

Quinoa

The United Nations declared that 2013 was the "International Year of Quinoa" (Nascimenta, 2014). The U.N. has high hopes that due to its excellent nutritional profile quinoa (*Chenopodium quinoa)* will stave off food poverty and generally increase nutrition of people around the world. The pseudocereal has an appealing nutty flavor, good protein profile (12g/100g compared to 7g/100g in rice), and is significantly higher in copper, iron, calcium, and potassium than rice (Alvarez, 2010; Nascimenta et al., 2014). However, due to an added step of washing the seeds to remove bitter saponins on the outer layer, the mineral content might be decreased. Nascimenta et al., 2014 shows that quinoa just about reaches 50% of the DRI's for iron, calcium, manganese, magnesium, and phosphorous. Further studies would be required to determine the bioavailability although small amounts of starch in the quinoa seed are found in the seed coat and embryo, the bulk of it is stored in the perisperm. The granules can be found in a mix of individual (polygonal shape) and compound (oval shape) granules, and are smaller than many other grains (6.4-32um) (Belton, 2002). The amylose content is low, around 11%, and the

main form of the starch in quinoa is amylopectin. The functional impact of this is that quinoa gelatinizes at a lower temperature and has a higher pasting viscosity when compared to wheat flour.

Quinoa seed

Quinoa is referred to as a pseudocereal since it is not a grass, and is often compared to amaranth and buckwheat, as they are also classified as pseudocereals (Figure 2.4).

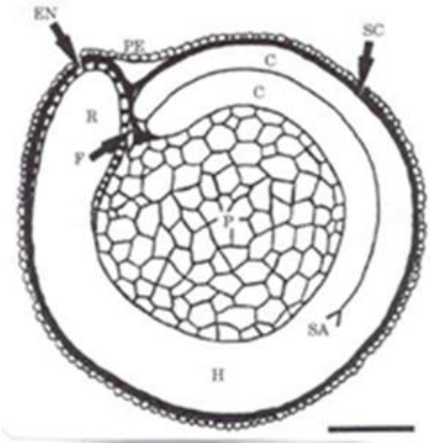

Figure 2.4. Quinoa Grain PE: Pericarp, SC: Seed cover, EN: Endosperm; C: Cotyledons, H: Hypocotyl; SA: Apical meristem; R: Radicle, P: Perisperm; F: Funiculus, chemical composition. (www.leader-trade.com)

The outer layer of the seed is made up of a two-layered pericarp, with the seed coat below that. The outer layer of the pericarp is not smooth, and contains many cone-shaped protuberances (Belton, 2002). The pericarp contains saponins, which are glycosylated secondary metabolites made up of oleanic acid and hederagenin, bitter tasting compounds. The saponins are thought to be a natural deterrent to birds and pests. However, they are washed off during processing so quinoa is palatable (Vega-Galvez et al., 2010). The seed coat contains the pigments that determine what color the seed will be (red, white, brown, etc.), depending on the presence of the pigments betacyanins (Belton, 2002). Quinoa differs from wheat in that the

endosperm does not contain the main amount starch, but rather acts as another layer that surrounds the seed. The starchy inside of the quinoa is called the perisperm and it contains 48% of the starch (Hager, 2012). The embryo sits above the perisperm and contains lipids and proteins that have phytates holding phosphorous, potassium and magnesium (Belton, 2002). The growing season usually takes 5-7 months, although experiments using hydroponics have produced mature quinoa plants in as little as one month. Quinoa has been cultivated in the Andean region of South America since as far back as 7,000 years ago during the Incan civilization. It grows well at altitudes 2,000-4,000 m, and is frost and drought resistant (Belton, 2002).

Quinoa carbohydrates

Although small amounts of starch in the quinoa seed are found in the seed coat and embryo, the bulk of it is stored in the perisperm. The granules can be found in a mix of individual (polygonal shape) and compound (oval shape) granules, and are smaller than many other grains (6.4-32um). The amylose content is low, around 11%, and the main form of the starch in quinoa is amylopectin. The functional impact of this is that quinoa gelatinizes at a lower temperature and has a higher pasting viscosity when compared to wheat starch. Compared to a control bread of rice flour, bread dough made with quinoa flour showed greater loaf volume, due to its amylose: amylopectin ratio as well as its high peak viscosity before baking (Alvarez, 2010). The high amount of amylopectin also allows quinoa flour to have excellent freeze-thaw stability, making it a good candidate for frozen products, as well as increased potential as a thickener in pie fillings (Vega-Galvez et al., 2010). The small size of the starch granules can be valuable when used in reduced fat products because it provides a mouth-feel similar to fats (Belton, 2002). Quinoa also has a high content of fiber (7.4g/100g of flour), if milled whole (Hager, 2012).

Quinoa protein

Although protein in quinoa can be found in many different parts of the seed, the bulk of it is located in the embryo, the major storage protein being an 11 S globulin called chenopodin (Alvarez, 2010). It also contains 2 S albumins which consist of small and large subunits joined with two inter-chain disulfide bonds and rich in methionine (Belton, 2002). Quinoa has been valued as a grain because of its high amount of protein (~15%), and because of its protein quality, which includes all of the essential amino acids, making it complete (Hager, 2012). The proteins are mainly globulin and albumin in classification and the content differs from true cereals in that it is rich in lysine, which is usually the limiting amino acid in cereals. Like quinoa fiber, the protein is not detrimentally affected by the milling process. The Protein Efficiency Ratio (PER) of quinoa has been compared to that of soy beans and milk, although it is lower in phenylalanine and tyrosine (Belton, 2002).

Due to its complete protein content quinoa proteins follow the FAO's guidelines for being an ideal protein source for children. In developing countries where potato porridge is used for feeding infants, quinoa porridge has been developed as an alternative. When compared to quinoa, potatoes are a lower source for energy as well as nutrients, so the substitution of quinoa in infant diets is ideal (Belton, 2002).

Quinoa enzymes

Quinoa, like wheat, contains α-amylases, found in significantly high amounts in grains that have not yet germinated. The amounts are increased after milling and are thought to be mainly contained in the germ. Other enzymes present in quinoa are proteinases, hemicellulases,

and cellulase (Belton, 2002). The cellulases and hemicellulases hydrolyze cellulose, while the proteinases hydrolyze the proteins present in the grain (Belton, 2002).

Quinoa lipids

Quinoa contains about 6% lipids mostly found in the embryo, and about 70% are unsaturated triglycerides. It is a rich source for the essential fatty acid oleic acid (24%) and linoleic acid (50%) and has about 4.5% linolenic acid. Like wheat, quinoa has natural antioxidants (alpha and gamma tocopherols) that help protect against oxidative rancidity but they are in higher amounts than wheat (Belton, 2002). Oil from quinoa has been proposed as a new oil crop. Quinoa contains more oil than corn, and has a similar fat profile to corn oil which the added bonus of the essential fatty acids previously discussed. The natural fat-soluble antioxidants found in quinoa oil also nominate it as an alternative oil source (Koziol, 1993). A by-product from quinoa oil production (and quinoa processing and milling in general) is saponin, which has been shown to be useful surfactant and can be used in detergents, cosmetics, and fire extinguishers. There has also been some work done on the cholesterol-lowering potential of saponins and on their potential as antibacterials due to lysing properties. However, due to its low yield, the implementation of quinoa oil may not feasible (Belton, 2002).

Quinoa vitamins and minerals

The minerals in quinoa are mostly found in the outer layers. It is rich in calcium (110mg/100g), magnesium (500mg/100g), and phosphorus (360mg/100g) (Belton, 2002). Iron, copper, and zinc are also found in good amounts (9mg/100g, 1mg/100, 3mg/100g) when compared to other grains (Belton, 2002). However, like in wheat, the bioavailability of these minerals can be hindered by the phytates present which chelate to these minerals and block

absorption by the body. Compared to cereals, quinoa has a higher average content of phytic acid at about 1% (Belton, 2002).

Quinoa is a rich source for B vitamins niacin, riboflavin, and thiamin (1.5mg/100g, 0.4mg/100g, and 0.4mg/100g respectively) (Belton, 2002). As was previously discussed in the lipids section, quinoa has high levels of vitamin E (alpha-tocopherol) at 5.3mg/100g. Vitamin A is present at around 18mg/100g, and unlike most cereals, quinoa has a significant of vitamin C at levels of 16mg/100g (Belton, 2002).

Quinoa can be seen as nutritionally equivalent to wheat in fiber, protein content, and some vitamins and minerals. However, wheat is the clear winner in baking applications. This is because of the unique proteins, glutenin and gliadin, which are found in wheat and produce bread with a quality crumb texture that is difficult to find with other gluten-free breads, as previously discussed. However, due to the increase in diagnoses of people with celiac sprue disease and gluten allergies, much research has been going into suitable food alternatives for wheat products. Quinoa flour was found to be the most acceptable GF flour substitute in GF breads compared to rice and the other pseudocereals amaranth and buckwheat (Alvarez et al., 2010). Searches on the internet do not reveal any gluten-free breads commercially available on the market which have a high percentage of quinoa flour. The drawback to using quinoa flour, as discussed above, is that it is sometimes milled whole, so it is always associated with mineral-binding phytates, as well as fiber (cellulose) that can also bind minerals. With quinoa increasing in popularity (due to increased consumer awareness and company marketing), even amongst people who are not GF, further research into a gluten-free bread containing quinoa may be beneficial.

Additional ingredients used in gluten-free baking

Rice

As stated above, rice flour is most commonly used as the basis for GF baking, and as a result there is a wealth of information on GF baking with rice flour in the literature. This is due to its white color, neutral taste, and easy digestibility of its carbohydrates (Hager et al., 2012). The starch granules of rice are also small (2-5um) and are found as aggregates in the rice grain (Delcour, 2010). They are primarily polygonal in shape, and the gelatinization temperature of rice starch granules is about 50% at 70˚C (Jubete et al., 2009). Rice flour, in comparison to quinoa flour, exhibited a higher peak and final viscosity than quinoa flour in a study comparing GF breads made with rice flour and the pseudocereals quinoa, buckwheat, and amaranth (Jubete et al., 2009). In addition, rice flour also had the highest setback even though its breakdown was higher than quinoa, buckwheat and amaranth (Jubete et al., 2009). Even though rice flour appeared to have the least resistance to shear stress, its high rate of setback and its high final viscosity produced a bread that was 0.30ml/g less in volume than the buckwheat bread, and 0.10 ml/g less than the quinoa loaf in volume.

De la Hera et al., 2013 compared flour made from long grain white rice (LGR) and short grain white rice (SGR), and some differences came to light. It was shown that both the variety (LGR or SGR), and size of the starch granules had an effect on bread baking. In general, the LGR had a higher amylose content than amylopectin. The significance was that more retrogradation was observed, indicating a shorter shelf life (De la Hera et al., 2013). The smaller starch granules in both rice varieties tested showed a higher water-holding capacity, which the researchers surmised was due to an increased surface area. The granules in SGR were less compact and smooth on the surface than the LGR meaning that can overall hydrate and interact

more easily with other ingredients in the recipe (De la Hera et al., 2013). However, in both LGR and SGR, the larger starch fractions had greater dough development, resulting loaves with higher volume due to increased gas retention. At the end of the fermentation time, the bread dough made with the smaller fraction of starch granules had a weak structure that broke, which allowed for gas to escape, leading to a decreased loaf volume. Adding hydroxypropyl methocellulose (HPMC) helped the doughs made with the small fraction to retain volume and gas during fermentation. Overall, the researchers found that a higher bread volume was achieved with LGR than the SGR, especially when the particle size was 90-120 um. Finer particle fractions had lower gas retention (De la Hera et al., 2013). And while the LGR, as stated above, had a higher retrogradation amount due to high amylose, some amylose is necessary for crumb setting (too much makes the crumb too hard), while the amylopectin helps soften the crumb texture. This study was interesting in that it showed in order to maintain consistency in a baked, GF product, the characteristics of flour should be known, especially in the case of starch granule size and shape (De la Hera et al., 2013). Zannini et al. 2012, preferred SG rice flour over LG rice flour due to softer crumb production that was observed in the SG, from a higher amount of amylopectin.

Roles of different starches and flours

The majority of GF products on the market today are made with a high amount of starch and little protein (Huttner, 2010). The proteins in GF flours do not have the same characteristics as gluten proteins in wheat, and therefore, do not result in the same crumb and texture normally attributed to a baked good. However, in terms of protein quality, many GF flours are better than wheat. For example, quinoa flour is a complete source of protein, and is rich in lysine, an amino acid that is usually lacking in cereal grains (Belton, 2002). This may not be true for all GF free

flours though, as rice flour, which is commonly used in GF baking, has one of the lowest protein contents amongst the different cereal grain (Delcour, 2010). Usually, rice flour is used for the preparation of GF baked goods, but using other GF flours increases the nutrition and palatability, and also variety, of the baked product (Zannini et al., 2012).

Hydrocolloids/gums

Some examples of gums most frequently used in GF baking are hydroxypropyl methocellulose (HPMC), guar gum, locust bean gum, and xanthan gum (Gallagher, 2009). Gums used in GF baking are usually hydrophilic, long chain polysaccharides that can form gels when combined with water (Gallagher, 2009). Gums can come from plants, plant exudates, and also from microbiological sources. They help create a viscoelastic dough in GF bread. Gums are used to increase the viscosity, which in the case of bread baking, turns a batter into more of cohesive, doughy mass (Zannini et al., 2012). Gums help GF breads by improving loaf volume, overall loaf appearance, and it helps the texture by limiting water loss in the baked bread (Zannini et al., 2012).

Since any baked good is a complex food system, there are many aspects to consider when choosing a food polymer. Things like the source, manufacturing process, chemical make-up and shape, and amount needed in a recipe. In terms of consistency, it has been shown that HPMC and xanthan gum are the most reliable hydrocolloids in GF baking, and therefore are the most widely used (Zannini et al., 2012). Research is also being done with enzymes, such as transglutamase (TGase), which help with protein and lipid cross-linking (Zannini et al., 2012). This helps to mimic the viscoelastic property of gluten, by forming a protein matrix, producing a more cohesive product (Zannini et al., 2012).

Hydrocolloids can also increase the palatability of GF breads and bakery products by increasing specific volume (Matos et al., 2013). The gums compete with the other ingredients, namely starch, for water absorption, with help delay gelatinization during baking, allowing the loaf to get a better oven spring (Matos et al., 2013). If starch gelatinization happens at lower temperatures, the structure of the bread/baked product is set, and there is no room for further expansion, which produces a denser baked product. Also, by absorbing more water, hydrocolloids like HPMC and xanthan decrease the water activity in the finished product which has implications for increasing shelf life (Matos et al., 2013).

Demirksen et al., (2010) looked at the interaction of emulsifiers and other hydrocolloids on the rheology of a GF bread made primarily with rice flour. Those researchers found that while xanthan gum did increase viscosity of the dough, even at low concentrations (1%), and attributed to the formation of aggregates with the other ingredients, it also produced a bread with a lower specific volume when compared to a bread made with HPMC. The theory for this is that the high thickening ability of xanthan gum makes the system unyielding, preventing it from expanding during baking (Demirksen, 2010). The emulsifiers used were diacetyltartaric ester of monoglycerides (DATEM) and Purawave™, both used with the gums and also without. All breads made with the emulsifier DATEM did have higher specific volumes compared to the use of Purawave™, as well as when only gums were used (Demirksen, 2010; Sciarini et al., 2012). DATEM is known to encourage protein binding in order to form a gluten-like complex (Ribotta, 2003). This helps entrap the gases produced, and lead to a bread with a higher specific volume. DATEM has also been shown to delay starch gelatinization which delays the formation of the bread structure, and also encourages a higher specific volume. While DATEM is used commercially in wheat based breads to help soften the crumb, this study found that it was

necessary to add both a hydrocolloid and an emulsifier in order to achieve the desired crumb in the final product (Demirksen, 2010). However it should be noted that the use of hydrocolloids and emulsifiers is complex, due to the types of flours and starches used, and the protein levels in the system.

Other gums that can be used in GF baking are extracted from cereal grains, like pentosans from rye and B-glucans from oats. Not only are these associated with increasing viscosity in a batter/dough, but they also provide additional health benefits like increasing dietary fiber and reducing cholesterol, respectively. Pentosans have been shown to increase water holding capacity of a dough by enforcing the air pockets formed by gas in fermenting dough (Gallagher, 2009). They provide the extra strength by forming a film around the inside of the gas pocket, making it less susceptible to rupture and a loss of loaf volume (Gallagher, 2009). Beta glucans are most closely associated with oats, but can also be extracted from barley (Gallagher, 2009).

Egg and milk proteins:

Adding proteins from non-grain sources has also been shown to improve dough functionality. Matos et al., (2013) found that when nonfat dry milk powder and whole egg powder were added to GF breads made with primarily rice flour, the proteins helped the mix transform from a batter to a dough. At the end of the mixing stage the researchers observed that the dough formed a more cohesive mass in a shorter amount of time than the bread doughs formed without the added proteins.

Proteins, like hydrocolloids, absorb more water. This in turn produces a GF baked product with a lower water activity, helping to increase the storage time. It was also observed that when compared to a GF bread that had only added gums, a bread with added milk and egg proteins had a softer crumb (Matos et al, 2013).

High altitude GF baking

Baking over 3,000 feet above sea level or more is considered high altitude baking. Ingredients and baking times and temperatures may need to be adjusted, and baking GF products may require different changes than what is commonly used for non-GF baking at high altitude. Kell (2012) found that in a survey conducted on people who live at high altitude and follow GF diets, the most requested recipe was for a GF yeast bread. The formula used in this study for GF yeast bread was developed at a high altitude, and may help fit a niche, especially for those living at a high altitude, who are following a GF diet.

Objectives

The review of the literature revealed a gap of information related to GF yeast bread made with quinoa flour. While there are researchers who have used QF when making GF breads, they have used whole milled QF (which has off-flavors), and efforts have not been geared towards formulating an appealing product for consumers. The aim of this study was to develop a GF yeast bread that contained a level of QF acceptable to consumers. GF yeast breads containing QF were compared to a GF yeast bread made with brown rice flour. The samples were tested both quantitatively and qualitatively using instruments and sensory panelists in order to test the differences between the bread made with brown rice flour, and the bread made with QF. The breads were tested at high altitude conditions.

CHAPTER 3: MATERIALS AND METHODS

Preliminary work

Preliminary work was performed based on various formulas for GF yeast breads containing quinoa. The original recipe was based on a recipe from *How to Cook Gluten-Free* (Barbone, 2012) for a GF sandwich bread made with brown rice flour. Formula ingredients and amounts were changed and standardized into gram measurements. The baking powder was omitted and several ingredients (vegetable oil, water, yeast, salt, and sugar) were increased. Some test loaves contained varying amounts of quinoa flour (QF). Samples were evaluated by faculty and the students of the Department of Food Science and Human Nutrition at Colorado State University, Gifford Building, before the final treatments were chosen. The first proposed formula for the GF yeast bread contained brown rice flour and potato starch exclusively. Several trial GF breads were made incorporating various amounts of QF. Half of the brown rice flour was substituted with QF (36% QF) for one loaf. Another loaf had the QF replacing all of the brown rice flour (72% QF), and a final loaf omitted both the brown rice flour and potato starch. Those were replaced with 100% QF (Table 3.1). All percentages were based on baker's percentages where the total flour amount of a recipe is 100% (Healea, 2007).

Product development

All procedures were performed in Colorado State University's food laboratories in the Gifford Building. Feedback based on the aforementioned informal evaluation of the GF quinoa bread was used to eventually determine a final batter weight and the final amounts of sugar, salt, yeast, vegetable oil and water. The final formulas for all the treatments were composed of both liquid and dry phases, and the consistency can be described as a batter (flour to water ratio of

about 1.5:1 cups) more than a dough. The liquid phase contained eggs, vegetable oil, and water. The dry phase consisted of the flours (brown rice, potato starch, and/or quinoa), xanthan gum, sugar, salt, and yeast. Xanthan gum was used to improve the texture of the bread (Zannini et al., 2012).

Table 3.1. Baker's percentage formulas for GF yeast breads.

Ingredient	0% Quinoa (g)	36% Quinoa (g)	72% Quinoa (g)	100% Quinoa (g)
Brown Rice Flour	489	244.5	-	-
Potato Starch	191	191	191	-
Quinoa Flour	-	244.5	489	680
Xanthan Gum	10	10	10	10
Sugar	20	20	20	20
Salt	12	12	12	12
Yeast	11	11	11	11
Water	483	483	483	483
Eggs (Large)	100	100	100	100
Vegetable Oil	56	56	56	56
Total Weight	1372	1372	1372	1372

Procedure

The GF flours (brown rice, potato starch, and/or quinoa), xanthan gum, sugar, salt, and yeast were combined using a KitchenAid^{TM} mixer (KitchenAid Inc., St. Joseph, Michigan) fitted with a paddle attachment. In a separate mixing bowl the water, eggs, and vegetable oil were whisked by hand until combined, about 15 seconds, and added to the dry ingredients in the stand mixer. The batter was mixed on medium speed (speed 5 on the mixer) for two minutes, forming a loose batter (Barbone, 2012). The mixture was then transferred into a 22.86 x 12.7 (9 x 5") cm loaf pan generously greased with spray vegetable oil, and a flat-edged spatula dipped in hot water was used to even the surface. The top of the batter was coated with a light spray of vegetable oil, then lightly covered with plastic wrap. The loaves were placed on an unlined sheet tray and allowed to rise on top of a pre-heated oven 177˚C (350˚F) for an hour. The loaves were

then placed on the middle rack inside the oven and the timer was set for thirty minutes. After thirty minutes the loaves were turned180˚ and a thermometer probe was put into the center of the loaf. The loaves were baked to an internal temperature of 93˚C (200˚F). Once they reached 93˚C (200˚F), they were taken out of the oven to cool for five minutes before being inverted onto a cooling rack and cooled to 26˚C (78˚F). The loaves were then double-wrapped in plastic wrap overnight. Breads were analyzed the day after baking.

Materials

The ingredients used in this study were brown rice flour (Sprouts Supermarket, Fort Collins, CO), potato starch (Bob's Red Mill Natural Foods Inc., Milwaukie, Oregon), quinoa flour (Keen Ingredients INC., Denver, CO), xanthan gum (Bob's Red Mill Natural Foods Inc., Milwaukie, Oregon), granulated white sugar (Sprouts Supermarket, Fort Collins, CO), salt (Morton Salt Inc., Chicago, IL), active dry yeast (Red Star Yeast, Milwaukee, WI), large, grade A eggs, and vegetable oil (Great Value soybean oil, Wal-Mart Stores, Inc., Bentonville, AZ).

Specific gravity

Specific gravity was used to test the density, or air incorporation, in the batter. Specific gravity was determined using a specific gravity cup. Ambient temperature of 20˚C (68˚ F) and batter temperature of 185˚C (73˚F) were noted. The weight of the cup was recorded with and without water at 185˚C (73˚F). A batch of each of the four bread batters was made, and panned up following the same procedures for baking. Once the loaves had risen for an hour, nine 100 g samples were scooped out and used to test for specific gravity. The sample was placed into the empty specific gravity cup, and a flat-edged spatula was used to level the batter at the top of the cup, and then weighed. The formula used was as follows (Stone, 2013):

$$\text{Specific gravity} = \frac{\text{(weight of cup with batter)} - \text{(weight of cup)}}{\text{(weight of cup with water)} - \text{(weight of cup)}}$$

Image analysis

A center slice of bread, approximately 8 x 10 cm, 1.27 cm thick (1/2 in.), from each loaf replicate was used in order to examine the crumb structure. Each slice was set on the photocopier (bizhub C454s, Konica Minolta Business Solutions, USA, Ramsey, NJ 07446) separately. The resulting copies were used to visually compare the internal crumb structure of the different loaves.

Bread loaf volume

A polar planimeter was used to measure the volume of the bread. The same center slice of bread (1.27 cm thick) used for image analysis from each loaf replication was traced onto a piece of paper nine times. An "X" was drawn on the outline to mark a start-stop place. Moving in a clockwise direction, the tracer arm of the polar planimeter was used to follow the outline back to the original pint. The area of the slice in cm^2 was recorded and used as an index to volume of the bread (Stone, 2013).

Hardness

Each test run was completed using the TA-XT2 texture analyzer on the sample surface. Nine samples from each treatment (0, 36, 72 and 100% QF) were analyzed for hardness with a P/36R aluminum cylindrical probe (36mm) attached on the TA-XT2 Texture Analyzer version 5.16 1994 with a 5Kg load cell (Texture Technologies, Scarsdale, NY). Each sample was placed on the texture analyzer base platform and the probe was centered perpendicularly then lowered to five centimeters above the sample (Sadeh, 2004). The crust was cut from the center slice of the

loaf 1.27 cm (1/2 in. thick) and cut into 9 pieces, approximately 1.27 cm thick (1/2 in.”). The cylindrical probe compressed the slice to 50% of its original height at a pre-test speed of 2 mm/s, 1 mm/s test speed, and 5 mm/s post-test speed (Hager and Wolter, 2012). Grams of force were measured which indicated the hardness (firmness) of the samples, and the results were measured in grams.

Water activity

Nine samples of quinoa bread from each treatment (0, 36, 72 and 100% QF) were used in each replication in order to determine water activity (A_w). All samples were taken one day after the baking day, from the same center slice of bread used in previous tests. Before using, the AquaLab water activity meter (AquaLab Series 3 Quick Start, Pullman, WA) was allowed to run for the recommended thirty minutes. Then the machine was standardized using verification standard cups (Decagon Devices, Inc). Each bread sample was crumbled into three separate plastic cups, not exceeding half the volume of the container. Water activity was measured nine times for each sample by placing the plastic cup with the sample into the AquaLab chamber and turning the chamber knob from OPEN/LOAD to READ to start the measurement. Once the measurement was completed, the AquaLab beeped and blinked, indicating that the measurement has been completed, and the water activity and the temperature were recorded.

Color analysis

Nine samples of bread from each treatment (0, 36, 72 and 100% QF) were analyzed for color using the HunterFlex^{TM} machine (Hunter Associates Laboratory, Inc., Fairfax, VA). The ColorFlex^{TM} was standardized before reading the samples with both the black plate and the white plate provided by the manufacturer. The standardization L* a* b* values for the black tile were

0.20, 0.26, and 0.17, respectively. The standardization values for the white tile were 93.13, -0.87, and 0.33, respectively. Once standardized, nine bread samples from the center slice of each loaf were tested by placing a one inch piece of bread into the sample cup and the L* a* b* values were recorded where L*= lightness, a*= red/green, and b*= yellow/blue. For the L* value, the closer the value is to 100, the whiter (lighter) it is, and a value closer to 0 indicates black (darkness). The scale for the a* is -100 to 100+ where -100 is green, and 100+ is red. The scale for b* is the same as a*, except -100 is blue, and 100+ is yellow.

Sensory analysis

Sensory analysis was performed on the campus of Colorado State University in Fort Collins, CO in the Gifford Building. Student and faculty volunteers (n=92) were recruited to taste the GF yeast breads. Samples were served on plastic plates coded with 3-digit numbers with a glass of water, a plain, unsalted cracker, the scorecard and a pencil (Appendix 2.1). All panelists were told that the samples were GF yeast bread, and that they were to score the samples based on how the they related to each other, and to not compare them to wheat bread. Sensory testing was based on a 7-point hedonic scale (7= "Extremely like" and 1= "Extremely dislike"). The four characteristics that tasters were asked to evaluate were crust color, tenderness, flavor, and overall acceptability. Panelists were also asked to rank the samples by preference where 1= liked most and 4= like least.

Statistical analysis

Statistical analyses for all data was performed with SAS/STAT™ 9.4 software (Copyright 2002-2012, SAS Institute Inc., Cary, NC, USA) using analysis of variance (ANOVA). PROC MIXED was used to block panelists for sensory data, and to block on

replications for instrumental test data. Tukey's adjusted p-values were used to test for differences among means at a significance level of 5%.

CHAPTER 4: RESULTS AND DISCUSSION

Four GF breads were prepared from formulas containing 0, 36, 72 and 100% quinoa flour (QF). Samples from each bread were evaluated for specific gravity, image analysis, volume, hardness, crust and crumb color, and water activity. Sensory analysis was obtained from 92 consumer panelists from CSU students and faculty in the Gifford building. In addition to scoring the GF breads for crust color, tenderness, flavor, and overall acceptability, panelists were also asked to rank the four breads based on preference with 1 indicating the most preferred, and 4 indicating the least preferred.

Instrumental Analysis

Specific gravity

The specific gravity of a batter indicates the volume of air incorporated into the system during mixing (Chunli et al, 2014). All GF breads were significantly ($p < 0.05$) different from each other. The means were 0.67, 0.69, 0.75, and 0.80 for the 0, 36, 72 or 100% QF breads, respectively. The batter made from the 0% QF had the lowest specific gravity and the 100% QF batter had the highest, indicating that the 100% QF was denser and had less air incorporation. This can be explained by the fact that the QF used in this study did not contain the bran portion of the quinoa seed, resulting in a greater proportion of protein (Scanlin, 2014). Specific gravity was also related to the volume of the loaves. The 100% QF bread was significantly ($p < 0.05$) smaller in volume (72.69 cm^2) than the other loaves, whose volumes ranged from 82.06 cm^2 – 87.33 cm^2. A lower specific gravity means that there is more air incorporation that would lead to greater air expansion during baking, thus resulting in a higher volume. In Table 4.1, the 100% QF batter/bread had the highest specific gravity, lowest volume, and the highest hardness. In the

photocopy image (A2.3), the 100% QF bread appeared to have a more compact crumb compared to the other breads, which have an open crumb.

Table 4.1. Least square means[A] for specific gravity, hardness, volume and water activity by percentage of quinoa flour[B]

Quinoa Flour (%)	Specific Gravity g/cm^2	Hardness (g)	Volume cm^2	Water Activity
0	0.67 ± 0.01^{a}	55.70 ± 363.64^{a}	87.33 ± 4.32^{a}	0.98 ± 0.0029^{a}
36	0.70 ± 0.01^{b}	$710.58 \pm 363.64^{a,b}$	82.60 ± 4.32^{a}	$0.98 \pm 0.0029^{a,b}$
72	0.76 ± 0.01^{c}	$1346.14 \pm 363.64^{b,c}$	84.02 ± 4.32^{a}	$0.97 \pm 0.0029^{b,c}$
100	0.80 ± 0.01^{d}	1770.89 ± 363.64^{c}	72.69 ± 4.32^{b}	0.97 ± 0.0029^{c}

[A] Each value is a mean of 27 determinations
[B] Means within a column with a common letter are not significantly different ($p > 0.05$)

Image analysis

The inner crumb structure of a slice of a baked product is helpful in order to visually depict results and explain volume. Some vocabulary used to describe crumb are uniformity and fineness (closed vs. open) (Scanlon, 2002). Each center slice of the loaves were photo copied on a Xerox machine one day after baking. The images are shown in Appendix 2.3. The 0% QF appears to have an open, uniform crumb structure, with a large number of irregular holes, both small and large. The crumb structures for the 36% QF and 72% QF appeared less open, but still contained both small and large irregular holes. The 72% QF had fewer small holes than the 36% QF. The greatest visual difference was that the 100% QF bread had a closed structure (when compared to the other samples), with less uniformity.

Volume

A compensating polar planimeter was used on the center slice of each of the breads to determine its area. Area is indicative of volume because the total weight of each batter was the

same, as were the dimensions of the baking pans. The difference was the amount each batter increased in volume in the pans during baking, and the higher the batter was increased, the greater the volume. It was found that the 100% QF loaf had a significantly ($p < 0.05$) smaller volume than the other breads (Table 4.1) (Appendix 2.4). This loaf did not rise as much as the others in the time (one hour) allotted for rising, and also the batter appeared stiffer when mixed (Appendix 2.2). Alvarez-Jubete and Arendt (2010) indicated pseudocereal flours, including quinoa flour, have a higher water binding capacity than wheat flours, so more water may be needed to add to the batter to improve crumb structure and volume. Rosell and Rojas (2001) found that xanthan gum increased the water binding capacity of a wheat batter due to increased hydrogen bonding between hydroxyl groups, allowing for more contact with water. Hager et al. (2012) found that in order to have their GF breads containing pseudocereals, including quinoa, perform like a wheat batter, they had to increase the amount of water added to the system to around 95% of the weight of the flours (compared to 71% used in this study). The 100% QF batter appeared stiffer, indicating increased/efficient water absorption, which may have led to less water available for reactions with yeast. Increased water absorption could mean less steam formation, which also aids in leavening. Based on the images of the slices, the 100% seems denser than the other loaves (Appendix 2.3). Increased viscosity may also mean that the batter will not stretch as well with the carbon dioxide being formed.

Hardness

Textural hardness (firmness) was determined on all GF breads using a TXTi2 texture analysis (TA). TA values were 555.7, 710.58, 1346.14, and 1770.89g for 0, 36, 72 and/or 100% QF, respectively (Table 4.1). The 100% QF bread was significantly ($p < 0.05$) harder in texture than the other three breads. Bread made with 100% QF had the smallest volume (72.69cm^2),

whereas the volume for the other loaves ranged from 84.02 cm2 – 87.33 cm^2 with no differences. Correspondingly, the 100% QF batter also had the highest specific gravity (0.80 g/cm^2). A higher specific gravity indicates that a smaller amount of air pockets (increased mass), which could lead to a lower rise and expansion in the oven. During the mixing stage, the increase in QF in led to the batter becoming stiffer (Appendix 2.2), which might also explain why the 100% QF had a high specific gravity and low volume. Platt (2008) used Keen Ingredients, Inc. QF as well, and found that the QF had a higher water holding capacity (0.8 ml/g) than soft wheat flour (0.75 ml/g). However, the QF used by Platt contained the bran. A higher water holding capacity has practical meaning for commercial food production because a higher amount of water can be used in formulations, which increases yield while reducing costs. While some researchers reported lower averages for GF quinoa bread hardness, (Wolter and Hager, 2013), and some reported higher (Alvarez-Jubete, 2009), they used different bread formulas with different percentages of QF, baking conditions, and evaluation methods.

Water activity

Values for water activity were similar to another studies that reported the water activity (Aw) in GF breads to be between 0.97-0.99 (Lazaridou et al., 2012; Hager et al., 2012). Statistical differences ($p < 0.05$) for Aw appeared among some of the breads. However, since the total range for Aw values was 0.97 - 0.98, these differences have little relevance to practical applications in the food industry.

Color

L*, a*, and b* measurements were taken on both the crust and the crumb color of the breads after baking. These values are reported in Tables 4.2 and 4.3 respectively. For crumb color, the L* and b* values were not significantly different ($p < 0.05$) among the treatments. For

a* the 72% treatment significantly differed from the control. For L* the means ranged from 71.11 to 72.97 indicating that the crumb of the breads tended to be on the lighter side of the spectrum, while for b* the values indicated that it was yellow with means from 21.59 to 22.63. For a* the control, 36%, and 100% were more towards the red end, while the 72% was slightly greener. However, since the scale ranges from -100 (green) to +100 (red), and the means ranged from 0.49 to 2.00, all samples can be described as being brown with hues of red, green, and yellow mixed in.

Differences between L*, a*, and b* were not significant ($p > 0.05$) for crust color. The L* estimates for crust color ranged from 44.90 – 46.63, which are darker than the aforementioned L* estimates for crumb color, as expected. Higher a* values were seen in the crust versus the crumb, with means ranging from 8.75- 9.72, indicating a redder hue.

Table 4.2. Least square means[A] for crumb color by percentage of quinoa flour[B]

Quinoa Flour (%)	L*	a*	b*
0	71.12 ± 3.77[a]	2.00 ± 0.67[a]	22.63± 1.21[a]
36	72.97 ± 3.77[a]	1.45 ± 0.67[a]	21.59± 1.21[a]
72	71.22 ± 3.77[a]	0.49 ± 0.67[b]	21.96± 1.21[a]
100	72.59 ± 3.77[a]	1.35 ± 0.67[a]	22.46 ± 1.21[a]

[A] Each value is a mean of 27 determinations
[B] Means within a column with a common letter are not significantly different ($p > 0.05$)

Table 4.3. Least square means[A] for crust color by percentage of quinoa flour[B]

Quinoa Flour (%)	L*	a*	b*
0	45.53 ± 0.94[a]	9.41 ± 0.31[a]	25.57 ± 0.29 [a]
36	45.40± 0.94 [a]	9.30 ± 0.31[a]	25.18 ± 0.29 [a]
72	45.69 ± 0.94 [a]	9.06 ± 0.31[a]	25.16 ± 0.29 [a]
100	45.84 ± 0.94 [a]	9.32 ± 0.31[a]	25.32 ± 0.29 [a]

[A] Each value is a mean of 27 determinations
[B] Means within a column with a common letter are not significantly different ($p > 0.05$)

Sensory Evaluation

Sensory testing on the GF bread samples was based on a 7-point hedonic scale (7= "Extremely like" and 1= "Extremely dislike") (Table 4.4). The four characteristics that tasters were asked to evaluate were crust color, tenderness, flavor, and overall acceptability. Panelists were also asked to rank the samples by preference where 1= "most preferred" and 4= "least preferred" (Table 4.4 and Table 4.5).

No significant differences were found between the treatments for crust color of the breads by sensory panelists. However, this is not surprising since no differences were found instrumentally for crust color either. Panelists determined tenderness, flavor, and overall acceptability of the 100% QF bread were significantly ($p < 0.05$) different and lower than the other treatments. For tenderness the 100% QF bread received an average score of 4.42, indicating that it was the least tender of all the samples. This parallels tests for specific gravity, inner crumb, volume, and hardness. The 100% QF bread had the highest specific gravity (0.80) and the lowest volume, and appeared to be the densest bread based on the PhotoCopy images of

the inner crumb. The 100% QF bread also was the hardest of all the samples when texture was analyzed, potentially explaining why it scored lowest for tenderness in sensory testing.

Flavor was scored highest in breads made with no QF or lower amounts. The mean score for flavor for the 100% QF bread was 4.2, which was a lower flavor score than the other breads. There were no follow-up questions for the panelists asking them to indicate why they preferred the other breads over the 100% QF bread. Brown rice flour was possibly preferred because it has a milder flavor. Hager et al., (2012) found that quinoa bread scored a low acceptance due to strong aromas of pea, cooked potato, and mold. They reported that an undesirable aftertaste was associated with increasing amounts of quinoa flour, leading to a decrease in flavor scores (Lorenz, 1991). Platt (2008) found that in follow-up questions in sensory testing for a cookie make with QF versus wheat flour, tasters commented that the 100% QF cookie was bitter and had an unpleasant aftertaste. Bitter saponins not entirely removed from the quinoa seed during processing could negatively impact the flavor in products with high amounts of QF because bran was contained in the QF used by Platt. However, there was no bran in the QF used in the current study.

The bread made with brown rice flour and potato starch bread (contained no QF) was ranked as being significantly ($p < 0.05$) preferred over the other breads. The 100% QF bread was not only significantly ($p < 0.05$) least preferred for overall acceptability, but was also ranked last. Table 4.5 shows that 34% of panelists ranked the brown rice flour and potato starch bread that contained no QF as most preferred. This was followed by 25% and 24% for the 72% QF bread and the 36% QF bread, respectively. The bread that contained QF as the only flour had 14% of panelists rank it as most preferred. While there were no follow-up questions asked as to what in

particular the taster's found unacceptable from the 100% QF bread, it can be assumed that the tenderness and the flavor were negatively impacted by increasing amounts of quinoa flour.

Table 4.4. Least square means[A] of 7-point hedonic scale measurements (7=like extremely, 4= neither like nor dislike, 1=dislike extremely) and rank (1 = liked most, 4 = liked least) by percentage of quinoa flour[B]

Quinoa Flour (%)	Crust Color	Tenderness	Flavor	Overall Acceptability	Rank
0	5.14 ± 0.27^{a}	5.30 ± 0.28^{a}	4.83 ± 0.30^{a}	4.92 ± 0.29^{a}	2.11 ± 0.22^{a}
36	5.34 ± 0.27^{a}	5.45 ± 0.28^{a}	5.03 ± 0.30^{a}	5.23 ± 0.29^{a}	$2.35 \pm 0.22^{a,b}$
72	5.12 ± 0.27^{a}	5.03 ± 0.28^{a}	4.74 ± 0.30^{a}	4.79 ± 0.29^{a}	2.55 ± 0.22^{b}
100	5.30 ± 0.27^{a}	4.42 ± 0.28^{b}	4.20 ± 0.30^{b}	4.04 ± 0.29^{b}	2.99 ± 0.22^{c}

[A] Each value is a mean of 92 determinations using confidence interval
[B] Means within a column with a common letter are not significantly different ($p > 0.05$)

Table 4.5. Percentages of each GF bread ranked 1 = most preferred

Quinoa Flour (%)	Ranked #1 (%)
0	34
36	24
72	25
100	14

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS FOR FUTURE STUDIES

The current study focused on determining the instrumental differences and acceptability between GF bread made with rice flour and quinoa flour. In all instrumental tests, the 100% quinoa flour yeast bread was different from the 0% QF bread. The 100% QF bread had a higher specific gravity, a lower volume, and a denser crumb (as demonstrated by the photocopy image) than the other breads tested. In terms of hardness (firmness), the 100% QF bread was firmer than the 0 and 36% QF breads, showing that as the proportion of QF was increased in the GF yeast bread recipe, the firmer the bread became. No differences were found in crust and crumb color using the colorimeter. For sensory analysis, the 100% QF yeast bread was scored significantly lower in likability than the 0, 36, and 72% QF breads for tenderness, flavor, and overall acceptability. The 100% QF bread was only ranked as most preferred by 14% of panelists. No differences were found for tenderness, flavor, and overall acceptability for the 0, 36, and 72% QF breads. The 0% QF bread was ranked as most preferred by 34% of panelists, followed by the 72 and 36% QF breads with 25% and 24% as most preferred, respectively. Based on the instrumental and sensory data collected, both the 36 and 72% QF yeast breads are acceptable GF, yeast bread options containing QF. The 100% QF bread was the least liked and least preferred compared to other breads.

A limitation of this study was that people with celiac disease, or others adhering to a GF diet, were not specifically used for sensory testing. These people are the target audience and the ones most familiar with GF products. Their opinions for the GF quinoa yeast breads would have been valuable. Also, no information was gathered as to whether the panelists used were familiar with GF yeast bread, or any other GF products. Tasters were asked to rate the samples as they related to one another and to not compare them to wheat bread. However, their familiarity with

GF breads, or lack of it, may have influenced how they rated the samples. The reference frame for people who regularly consume GF products versus those who do not varies greatly. Also, as previously pointed out, no questions were asked about the panelists' familiarity with quinoa. Some may have been tasting quinoa for the first time and were unfamiliar with quinoa's flavor profile. Descriptive questionnaires could also be incorporated into a similar study in order to reflect why tasters scored the samples the way they did.

In addition to expanding the scope of sensory testing, instrumental tests can be added as well. One test would be to determine how much extra water should be added to the quinoa batters by measuring the water holding capacity as described by Matos and Rosell (2012). Adding more water to the system can lead to expanded volume and improved tenderness, which increases yield and keeps cost low. This might be ideal for the 72% treatment, which tasters scored high. Another valuable test would be determining the shelf life and staling rate of the quinoa bread. Shelf stability and staling rate tests should also be conducted if this product were to be sold to consumers so that it can be stored properly. This product could be packaged and sold as a GF yeast bread mix. The flour blend could be in a package with separate packages of yeast and sugar included.

In terms of a different preparation technique, more research can be done with incorporating a sourdough starter into the GF quinoa yeast bread batter. There are many published studies showing positive improvements with GF breads made with a sourdough starter, based on various microbial strains. Relating to ingredients, further experimentation could be done with different hydrocolloids and gums to try to improve the texture.

For creating a marketable product to be sold to consumers, nutritional information should be calculated. This entails deciding on what the serving size is and what the total amount of

servings would be. Including this information is not only necessary, but important so that consumers can be educated on what it is that they are buying. Mixing and baking instructions would also be printed on the package. Package directions would include detailing the stages of mixing and the amount of water, eggs, and oil to use, the size of the pan, and baking time and temperature.

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APPENDIX I: STATISTICAL TABLES

Table A1.1. Tests of fixed effects crumb color for L* value

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	0.38	0.77

Table A1.2. Least square means for L* value

Quinoa Flour (%)	Estimate	Standard Error
0	71.12	1.54
36	72.97	1.54
72	71.22	1.54
100	72.59	1.54

Table A1.3. Differences of least squares means for L* value

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.83
0	72	1.00
0	100	0.90
36	72	0.85
36	100	1.00
72	100	0.92

Table A1.4. Tests of fixed effects crumb color for a* value

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	5.12	0.04

Table A1.5. Least square means for a* value

Quinoa Flour (%)	Estimate	Standard Error
0	2.00	0.28
36	1.45	0.28
72	0.49	0.28
100	1.35	0.28

Table A1.6. Differences of least squares means for a* value

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.53
0	72	0.03
0	100	0.41
36	72	0.17
36	100	0.99
72	100	0.22

Table A1.7. Tests of fixed effects crumb color for b* value

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	1.09	0.42

Table A1.8. Least square means for b* value

Quinoa Flour (%)	Estimate	Standard Error
0	22.63	0.50
36	21.59	0.50
72	21.96	0.50
100	22.46	0.50

Table A1.9. Differences of least squares means for b* value

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.44
0	72	0.74
0	100	0.99
36	72	0.93
36	100	0.56
72	100	0.86

Table A1.10. Tests of fixed effects for hardness

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	19.06	0.00

Table A1.11. Least square means for hardness

Quinoa Flour (%)	Estimate	Standard Error
0	555.70	148.61
36	710.58	148.61
72	1346.14	148.61
100	1770.89	148.61

Table A1.12. Differences of least squares means for hardness

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.83
0	72	0.02
0	100	0.00
36	72	0.05
36	100	0.00
72	100	0.20

Table A1.13. Tests of fixed effects for hardness log10 transformation

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	35.23	0.00

Table A1.14. Least square means for hardness log10 transformation

Quinoa Flour (%)	Estimate	Standard Error
0	2.74	0.05
36	2.85	0.05
72	3.13	0.05
100	3.24	0.05

Table A1.15. Differences of least squares means for hardness log10 transformation

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.34
0	72	0.00
0	100	0.00
36	72	0.01
36	100	0.00
72	100	0.29

Table A1.16. Tests of fixed effects for volume

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	14.41	0.00

Table A1.17. Least square means for volume

Quinoa Flour (%)	Estimate	Standard Error
0	87.33	1.77
36	82.60	1.77
72	84.02	1.77
100	72.69	1.77

Table A1.18. Differences of least squares means for volume

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.28
0	72	0.54
0	100	0.00
36	72	0.93
36	100	0.02
72	100	0.01

Table A1.19. Tests of fixed effects for water activity

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	15.60	0.00

Table A1.20. Least square means for water activity

Quinoa Flour (%)	Estimate	Standard Error
0	0.98	0.00
36	0.98	0.00
72	0.97	0.00
100	0.97	0.00

Table A1.21. Differences of least squares means for water activity

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.21
0	72	0.02
0	100	0.00
36	72	0.21
36	100	0.02
72	100	0.31

Table A1.22. Tests of fixed effects for water activity

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	15.60	0.00

Table A1.23. Least square means for water activity

Quinoa Flour (%)	Estimate	Standard Error
0	0.98	0.00
36	0.98	0.00
72	0.97	0.00
100	0.97	0.00

Table A1.24. Differences of least squares means for water activity

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.21
0	72	0.02
0	100	0.00
36	72	0.21
36	100	0.02
72	100	0.31

Table A125. Tests of fixed effects crust color for L* value

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	0.85	0.52

Table A1.26. Least square means crust color for L* value

Quinoa Flour (%)	Estimate	Standard Error
0	45.53	0.38
36	45.40	0.38
72	45.69	0.38
100	45.84	0.38

Table A1.27. Differences of least squares means crust color for L* value

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.97
0	72	0.94
0	100	0.73
36	72	0.76
36	100	0.49
72	100	0.96

Table A1.28. Tests of fixed effects crust color for a* value

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	3.79	0.08

Table A1.29. Least square means crust color for a* value

Quinoa Flour (%)	Estimate	Standard Error
0	9.41	0.13
36	9.30	0.13
72	9.06	0.13
100	9.31	0.13

Table A1.30. Differences of least squares means crust color for a* value

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.71
0	72	0.06
0	100	0.81
36	72	0.24
36	100	1.00
72	100	0.19

Table A1.31. Tests of fixed effects crust color for b* value

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	3	6	2.68	0.14

Table A1.32. Least square means crust color for b* value

Quinoa Flour (%)	Estimate	Standard Error
0	25.57	0.12
36	25.18	0.12
72	25.16	0.12
100	25.32	0.12

Table A1.33. Differences of least squares means crust color for b* value

Quinoa Flour (%)	Quinoa Flour (%)	Adj P
0	36	0.18
0	72	0.15
0	100	0.48
36	72	1.00
36	100	0.83
72	100	0.76

APPENDIX II: SENSORY SCORECARDS AND IMAGES

A2.1. Sensory Scorecard

Panelist No. 001

SCORECARD FOR QUINOA BREAD

Please taste each of the following samples from left to right. Check the box to the right based on how you feel about each sample, for CRUST COLOR, TENDERNESS, FLAVOR, and OVERALL ACCECPTABILITY.

CRUST COLOR					TENDERNESS				
	Sample Numbers					Sample Numbers			
	421	755	391	643		421	755	391	643
Like extremely	☐	☐	☐	☐	Extremely like	☐	☐	☐	☐
Like moderately	☐	☐	☐	☐	Like moderately	☐	☐	☐	☐
Like slightly	☐	☐	☐	☐	Like slightly	☐	☐	☐	☐
Neither like or dislike	☐	☐	☐	☐	Neither like or dislike	☐	☐	☐	☐
Dislike slightly	☐	☐	☐	☐	Dislike slightly	☐	☐	☐	☐
Dislike moderately	☐	☐	☐	☐	Dislike moderately	☐	☐	☐	☐
Extremely dislike	☐	☐	☐	☐	Extremely dislike	☐	☐	☐	☐

FLAVOR					OVERALL ACCEPTABILITY				
	Sample Numbers					Sample Numbers			
	421	755	391	643		421	755	391	643
Extremely like	☐	☐	☐	☐	Extremely like	☐	☐	☐	☐
Like moderately	☐	☐	☐	☐	Like moderately	☐	☐	☐	☐
Like slightly	☐	☐	☐	☐	Like slightly	☐	☐	☐	☐
Neither like or dislike	☐	☐	☐	☐	Neither like or dislike	☐	☐	☐	☐
Dislike slightly	☐	☐	☐	☐	Dislike slightly	☐	☐	☐	☐
Dislike moderately	☐	☐	☐	☐	Dislike moderately	☐	☐	☐	☐
Extremely dislike	☐	☐	☐	☐	Extremely dislike	☐	☐	☐	☐

Please write in the sample number in the space provided by ranking the samples in order of **your preference** **(1 = Liked most; 4 = Liked least): 1) _____ 2) _____ 3) _____ 4) _____**

A2.2. Visual comparison of dough viscosity after mixing.

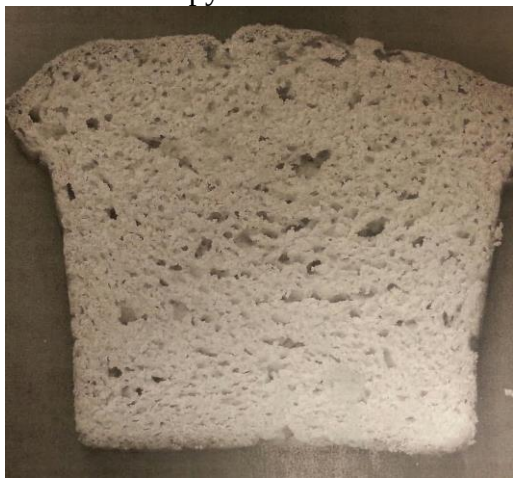
0% Quinoa flour

36% Quinoa flour

72% Quinoa Flour

100% Quinoa flour

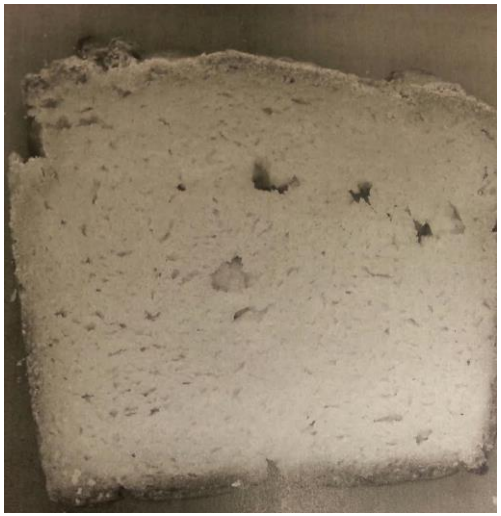
A2.3. Photocopy of bread slices.



0% Quinoa flour

36% Quinoa flour

72% Quinoa flour



100% Quinoa flour

A2.4. Images of bread loaves.

0% Quinoa flour

36% Quinoa flour

72% Quinoa flour

100% Quinoa flour

0, 36, 72 and 100% Quinoa flour loaves, respectively