

DISSERTATION

**PRODUCTION AND FUNCTIONALITY OF QUINOA (*CHENOPODIUM
QUINOA*) PROTEIN CONCENTRATE**

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

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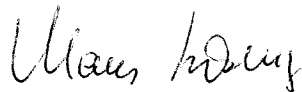
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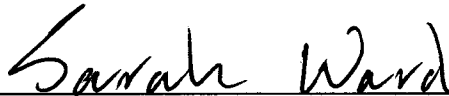
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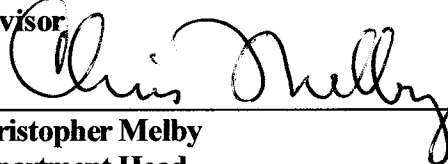


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ABSTRACT OF DISSERTATION

PRODUCTION AND FUNCTIONALITY OF QUINOA (*CHENOPODIUM QUINOA*) PROTEIN CONCENTRATE

Four whole grain quinoa [two commercial genotypes from Colorado (black and white) and two abrasion conditions (polished and unpolished)] were analyzed for saponin, ash, fat, moisture, nitrogen, 1000-grain weight, and yield loss. Whole grain materials were defatted under controlled conditions and analyzed for nitrogen content. Quinoa protein concentrate was prepared from defatted flour by a modified isoelectric precipitation method and analyzed for nitrogen content and nitrogen recovery. Quinoa protein was characterized by amino acid analyses, chemical score, SDS-PAGE, and isoelectric focusing. Wheat pasta was fortified with quinoa protein concentrate at two levels, 3.5% or 7.0% and effects of formulation modification and cooking times on cooking characteristics were evaluated. Effects of formulation modification on color of dry pastas and sensory characteristics of cooked pastas were also evaluated. A consumer sensory panel rated pasta treatments for sensory characteristics using 9-point hedonic scales.

When black and white quinoa genotypes were polished saponin was reduced, but the magnitude of the reduction was less in black quinoa compared to white quinoa. An 11% loss in fat was found in the white quinoa after polishing. Nitrogen increased after polishing, but the magnitude was greater in white quinoa compared to the black quinoa. Yield loss due to polishing was 13% in black and white quinoa. Defatted quinoa was

2.3% lower in nitrogen content than whole quinoa. Nitrogen content of quinoa protein concentrates increased with polishing, but the magnitude was higher in the white quinoa than the black quinoa. Nitrogen recoveries were highest in white quinoa. Mean nitrogen values for quinoa protein concentrate ranged from 11.23 to 14.83% (dwb). Amino acid analyses were comparable to reported values for quinoa. Quinoa protein concentrate received a chemical score of 91. Major quinoa proteins with molecular weights of approximately 11 and 30 kDa and isoelectric points of 4.4, 5.4, 5.6, and 5.8 were identified.

Differences in cooking characteristics and color of dry pastas were found among pasta treatments. Lysine content was increased by 40% or 80% by formulation modification of semolina pasta with quinoa protein concentrate at levels of 3.5% or 7.0%, respectively, without affecting sensory characteristics.

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CHAPTER 1

INTRODUCTION

As world food demands steadily increase, it becomes obvious that production of protein has to be maximized as well as augmented. New protein resources must be developed through chemistry and technological advances. Protein-rich crops, such as quinoa, that give comparable yields in underutilized growing regions to traditional cereal crops, are of paramount value. In order to increase commercial potential of quinoa, research efforts into value-added processing need to occur.

Vegetable protein from cereals and legumes represent the main protein and energy supply for both human and animal nutrition. Animal protein, on the other hand, contributes less protein to human and animal nutrition globally, but requires a much higher energy demand for production. In order to produce 1 kg of animal protein, 3-20 kg of vegetable protein is needed depending on the animal species (Cheftel et al 1985). Consequently, as demands for animal protein increase, the need for vegetable protein increases drastically. Clearly, the focus of vegetable protein production has been on major cereals and legumes, however, concerns with mono-cropping and continuous cultivation and their effects on genetic erosion have escalated (Fleming and Galwey 1995). This concern has led to research efforts and commercial interests in the production of vegetable protein from alternate cereals and pseudocereals.

Protein concentrates, such as those extracted and concentrated from soybean, milk, egg, and wheat, are profitable ingredients in consumer products and pet foods. The

Food Allergen Labeling and Consumer Protection Act, passed by the House of Representatives, July 20, 2004, became law on August 2, 2004 (FAAN 2004, FDA 2005). The new law requires labeling of FDA-regulated foods with respect to the top eight food allergens: milk, eggs, fish, crustacean shellfish, peanuts, tree nuts, soybeans, and wheat, by January 1, 2006. Proteins in these eight foods are responsible for 90 percent of food allergies. Prior to this act, FDA regulations have allowed food manufacturers flexibility with ingredient declarations in order to protect proprietary information. This is problematic for individuals who have food allergies and this new regulation addresses previously undisclosed allergens. By January, 2006, allergen declaration must be present in terms commonly understood by consumers, for example, “milk” for whey or casein, “soybean” for hydrolyzed vegetable protein, or “eggs” for albumin.

This new law has led to industry efforts to replace some allergenic ingredients as an option to declare, what has been coined, “warning label” on products. Quinoa is considered hypo-allergenic by the scientific community and has future potential for use as a commercial vegetable protein, either as an alternative to allergenic proteins, or to augment current demands for protein. Furthermore, the high quality of quinoa protein would be well-suited as protein concentrate ingredients used to fortify nutritional and vegetarian products, as well as, animal feeds.

The goal in concentrating protein is a product of high protein content as well as high protein recovery. Isoelectric precipitation extraction methods that can be adapted to knowledge of grain structure and physico-chemical properties inherent to quinoa are required to produce quinoa protein concentrate. In-depth review articles on quinoa have

been reported (Risi and Galwey 1984; Koziol 1992; Fleming and Galwey 1995; Taylor and Parker 2002).

Quinoa proteins have been studied in the literature (Fairbanks et al 1990; Prakash and Pal 1998a, 1998b; Aluko and Monu 2003; Lindeboom 2005) but quinoa protein concentrate for use as a food ingredient has yet to be commercially developed. Once a quinoa protein concentrate is produced, the protein can be characterized through molecular and chemical evaluation. For example, isoelectric focusing is a tool that can be used to investigate surface properties of proteins and has not been used to investigate quinoa protein. Understanding the physical and chemical properties of a protein concentrate can be helpful in determining functionality in suitable food applications.

Although quinoa has gained interest in North America and Europe and demand is on the rise, current production is too low to compete economically with traditional grains and legumes. Continued efforts in breeding, genotype selection, and yield research of quinoa, such as significant contributions made by Risi and Galwey (1991), Ward (2000, 2001), and Jacobsen (2003) are of value in advancing quinoa crop production. However, consumer demands for quinoa need to continue and coincide with improved cultivation.

The majority of quinoa is produced in Bolivia, Peru, and Ecuador. Most of the South American quinoa is sold as whole grain for consumer markets in South America, however, exports to North America and Europe have increased since 1975 (Watson 2005). Although products exist in South America, North America, and Europe that are made from quinoa flour and flakes, most of the quinoa is sold as whole grain. Quinoa is commercially produced in Colorado on a small scale and is also sold as whole grain for niche, organic markets and gourmet restaurants in the southwestern United States. There

is a limited market and limited value received for whole quinoa, flour, and flakes.

Developing value-added ingredients, such as protein concentrates, yields economic value above the whole grain and opens up a vast product market. Review articles regarding the potential of quinoa as a significant food crop have been published (Cusack 1984; Galwey 1989; Coulter and Lorenz 1990; Galwey et al 1990; Johnson and Ward 1993). Yet, in comparison to traditional crops, value-added ingredient processing and understanding ingredient functionality is relatively unexplored and is essential before marketing quinoa ingredients to industry manufacturers, thus, increasing quinoa potential and demand for crop production.

CHAPTER 2

LITERATURE REVIEW

History

Quinoa (*Chenopodium quinoa* Willd.) is cultivated as a cereal crop, yet unlike most cereal grains that are seeds of a grass, quinoa is a fruit of a broad leaf plant. For this reason, it is has been classified as a “pseudocereal”. Quinoa is a member of the same family (Chenopodiaceae) as sugar beets, beetroot, mangold, and spinach (Fleming and Galwey 1995). The genus *Chenopodium* contains about 250 species such as *Chenopodium quinoa* Willd. (quinoa) and *Chenopodium pallidicaule* Aellen. (cañihua) that are cultivated and used for grain in South America (Giusti 1970).

Quinoa fruits are disc-shaped and range in diameter from 1-3 mm. Scanning electron microscopy shows the major anatomical parts of quinoa fruit are the pericarp, the perisperm, and the embryo (Varriano-Marston and DeFrancisco 1984; Prego et al 1998). The cells of the perisperm are filled with starch granules that occur either singly or as compound structures consisting of spherical or oblong aggregates. The granules are surrounded by matrix protein. However, most of the protein is located in protein bodies in the embryo, unlike cereal grains that store protein in endosperm.

Quinoa has a long history in South America and has been utilized in the Andean highlands since 5,000 BC (Tapia 1979). In the Quechua language of the Incas, quinoa is named *chisiya mama* or “mother grain” due to its esteemed position in their culture as a

strong food staple (National Research Council 1989). In the time of the Incas, quinoa sustained armies that frequently marched for days and would eat a mixture of quinoa and fat known as “war balls” (Johnson and Ward 1993). Spanish conquerors replaced quinoa with wheat and barley because they believed quinoa was a source of great strength for the Incas.

Although historically, there has been a decline in cultivation, several million Andean people still grow quinoa on subsistence farms in the Andes mountain region (McGill 1986). Today it is produced in southern Peru and Bolivia as a sole crop. To a smaller extent, it is grown in central Peru, Ecuador, southern Columbia, Argentina, and Chile. Current world production figures are 45,898 metric tons grown annually in Peru, Bolivia, and Ecuador (FAO 1999). It is also commercially produced outside of South America in sparse locations in Europe, Asia, southern Mexico, US, and Canada. In the US, commercial production exists in southern Colorado and research plots to adapt quinoa to Maine and Montana show promise (Ward 2003).

Quinoa’s popularity originated from its known value as a nutritionally sustainable food of the Incas. Until 1975, cultivation was limited to subsistence farms in South America where it grows well at altitudes 2,000-4,000 meters above sea level. However, cultivation has expanded outside of South America due to a number of factors. Quinoa is recognized to have an exceptional protein value (Mahoney et al 1975), desirable for both human consumption and animal feed, and contains high levels of calcium, phosphorus, and iron. Because it has been cultivated for thousands of years, it has developed genetic diversity that enables the crop to adapt to environmental extremes such as high altitudes, soil pH extremes, and frost and drought resistance with a capacity for high yields (Risi

and Galwey 1984). Adaptation to climates that are unfavorable to major cereals and legumes make quinoa a crop of potential agricultural value.

Interest in quinoa has come from US health food markets. Since production in the North America is too minimal to supply the US demand, sole crop production facilities were developed in Ecuador to supply export markets. In the early 1980's, Sierra Blanca and Associates initiated breeding programs to adapt quinoa to the Rocky Mountain region (Cusack 1984). In 1987, White Mountain Farm, located in Mosca, Colorado, became the first large scale producer in North America (New 2002).

Due to its tolerance to environmental extremes and ability to grow in marginal soils, quinoa is recommended for conditions such as those found in the Rocky Mountain region (Risi and Galwey 1984). Quinoa has significant potential as a profitable enterprise for farmers in areas with marginal soils, especially in areas where traditional grains and legumes fair poorly. Quinoa has been shown to be well-suited for agricultural communities of Colorado because of high yield prospective at elevations ranging from 7,000 feet to 10,000 feet (Johnson and Croissant 1985).

Nutritional Value

Quinoa is superior in nutrient content compared to traditional cereals like barley, buckwheat, corn, millet, oat, rice, rye, or wheat and has been found to be superior to milk solids in feeding trials (White et al 1955). The Food and Agriculture Organization of the United Nations compares the nutritional quality of quinoa to that of dried whole milk (Oelke et al 1992). DeBruin (1964) was the first to evaluate the chemical composition of quinoa. Quinoa contains approximately 15% protein, 63% carbohydrate, and 6% fat (Table 1.1).

TABLE 1.1
Proximate Composition of Quinoa Compared with that of Cereals

Material	Proximate Composition (g/100g dwb)				
	Protein	Fat	Carbohydrate	Fiber	Ash
Wheat ^a	14.3	2.3	78.4	2.8	2.2
Barley ^b	11.8	1.8	78.1	5.3	3.1
Oats ^b	11.6	5.3	69.8	10.4	2.9
Rye ^b	13.8	1.4	79.7	2.6	2.2
Maize ^b	10.6	4.6	81.0	2.2	1.6
Sorghum ^b	12.4	3.6	79.7	2.7	1.7
Rice, Paddy ^b	9.1	2.2	71.3	10.2	7.2
Brown ^b	11.0	2.7	83.2	1.3	1.8
Polished ^b	9.8	0.5	88.9	0.3	0.6
Quinoa ^b	14.8	6.0	63.3	5.6	3.4

^aMeans of values for *Triticum aestivum* and *Triticum durum* reported by Koziol (1992).

^bAdapted from Galwey et al (1990).

Dehulled quinoa flour was found to contain over 58% starch, 15.6% protein (N X 6.25), 2.7% sugars, 8.9% total dietary fiber, and nearly 7% lipids and ash (Ranhotra et al 1993).

The caloric value of quinoa was shown to have 435.5 kcal per 100g (González et al 1989).

Quinoa is a good source of vitamins and minerals. Specifically, it is rich in Vitamins E, B₂, B₆, folic acid and biotin, calcium, potassium, iron, copper, magnesium, manganese, and chloride (Fleming and Galwey 1995). Additional mineral analyses show quinoa to be a rich source of calcium, phosphorus, and iron (Chauhan et al 1992; Oshodi et al 1999). Calcium and iron contents are higher in quinoa than in barley, rice, maize, wheat, or oat (White et al 1955; DeBruin 1964; González et al 1989). Quinoa is lower in sodium and higher in calcium, phosphorus, magnesium, potassium, iron, copper, manganese, and zinc than barley, corn, or wheat, as shown in Table 1.2. Consequently, high ash contents of 4.2% have been reported for quinoa (DeBruin 1964).

TABLE 1.2
Comparison of Mineral Contents in Quinoa with Barley, Yellow Corn, and Wheat

Crop	%				PPM				
	Ca	P	Mg	K	Na	Fe	Cu	Mn	Zn
Quinoa ^a	0.19	0.47	0.26	0.87	115	205	67	128	50
Barley	0.08	0.42	0.12	0.56	200	50	8	16	15
Maize	0.07	0.36	0.14	0.39	900	21	-	-	-
Wheat	0.05	0.36	0.16	0.52	900	50	7		14

^aData based on the average of 15 cultivars

Source: Reported by Oelke et al (1992).

Protein Value and Amino Acid Composition

Koziol (1990) found average protein contents of 14.6% and certain varieties of quinoa contained protein levels as high as 21.9%. The amino acid composition is of higher quality than that of wheat, barley, or soybean and is comparable to casein protein found in milk (Fleming and Galwey 1995). Quinoa protein is particularly high in histidine and lysine (3.2% and 6.1% of protein composition, respectively) and also contains more isoleucine, and methionine+cysteine than traditional cereals (Table 1.3). Authors Ruales and Nair (1992) also found that it contains comparatively high levels of lysine and methionine, amino acids limiting in grains and legumes, respectively, and gave the protein quality of quinoa a chemical score of 85. However, high protein content or favorable amino acid does not in itself determine protein quality, more important is its bioavailability.

Mahoney et al (1975) were forerunners in discovering the bioavailability of quinoa proteins. The authors evaluated the quality of quinoa protein in terms of rat response and amino acid composition. Cooking of quinoa improved nitrogen efficiency for growth (NEG) by 40%, weight gain by 100%, and protein efficiency ratio (PER) by 29%. Furthermore, mixing 20% quinoa with 80% wheat flour improved NEG by 43%,

TABLE 1.3
Essential Amino Acid Composition of Quinoa Compared with that of Cereals and Recommended Values

Protein Source	Essential Amino Acid								
	HIS	ILE	LEU	LYS	M+C	P+T	THR	TYR	VAL
Quinoa ^a	3.2	4.9	6.6	6.0	5.3	6.9	3.7	0.9	4.5
Quinoa ^b	3.1	3.8	6.5	6.1	4.2	7.6	3.9	1.3	4.5
Mean	3.2	4.4	6.6	6.1	4.8	7.3	3.8	1.1	4.5
Rice ^c	2.1	4.1	8.2	3.8	3.6	10.5	3.8	1.1	6.1
Maize ^d	2.6	4.0	12.5	2.9	4.0	8.6	3.8	0.7	5.0
Wheat ^d	2.0	4.2	6.8	2.6	3.7	8.2	2.8	1.2	4.4
Recommendations^e									
Infants	2.6	4.6	9.3	6.6	4.2	7.2	4.3	1.7	5.5
Pre-school	1.9	2.8	6.6	5.8	2.5	6.3	3.4	1.1	3.5
School children	1.9	2.8	4.4	4.4	2.2	2.2	2.8	0.9	2.5
Adults	1.6	1.3	1.9	1.6	1.7	1.9	0.9	0.5	1.3

^aMeans of values reported by Cardozo and Tapia (1979), Mahoney et al. (1975), Risi and Galwey (1984), Romero (1981) and Sánchez-Marroquín (1983).

^bMeans of values reported by Koziol (1992).

^cMeans of values reported by Lásztity (1984) and Romero (1981).

^dMeans of values reported by Lásztity (1984), Risi and Galwey (1984) and Romero (1981).

^eRecommendations of WHO/FAO/UNU (1985).

weight gain by 11%, and PER by 72% over wheat flour alone.

Other authors have studied *in vitro* and *in vivo* bioavailability of quinoa proteins. *In vitro* protein digestibility of quinoa was found to be higher than that of rye or wheat and comparable to corn, millet, and sorghum (Dahlin 1991). In animal studies based on PER, protein digestibility, and nitrogen balance, the quality of protein in quinoa equaled that of casein (Ranhotra et al 1993). White et al (1955) found when quinoa was used as the sole source of protein in otherwise balanced rat diet, *in vivo* digestibility was determined to produce rates of growth greater than that obtained with whole dried milk. Gross et al (1989) found that rat diets based on quinoa were equivalent to those of casein and serum urea levels were 11.3 ± 3.4 and 25.5 ± 3.8 , for quinoa and casein, respectively,

indicative of a high quality protein. Furthermore, Hurrell (1988, cited by Koziol 1992) found cooked quinoa had higher PER than casein, and more than three times that of cooked wheat flour. Similar results have been obtained when quinoa is used as a protein supplement for pigs, cattle (Cardozo and Bateman 1961), and chicks (Negrón et al 1976; Weber 1978).

Oil and Antioxidants

High lipid content compared with traditional cereals and essential fatty acid profiles make quinoa a potential valuable oil crop. Quinoa oil is a rich source of essential fatty acids linoleic and linolenic, which constitute approximately 55-63% of the oil (Ruales and Nair 1993; Fleming and Galwey 1995), and make it similar to that of soya oil. In a comparison of fatty acids and triacylglycerol compositions of seed oils, quinoa oil had the lowest saturate to unsaturate ratio compared with oils from five *Amaranthus* accessions, buckwheat, corn, ricebran, sesame, soybean, and cottonseed (Jahaniaval et al 2000). In addition, quinoa and soybean oils had an optimal linoleic to linolenic acid ratio compared to the preceding oils.

Quinoa oil has potential to be a greater nutritional source than oil produced from cereals crops (Fleming and Galwey 1995). The oil content of quinoa is about 5.6%, with some varieties having lipid contents up to 9.5%. The yield of extractable vegetable oil per hectare could easily exceed that obtained from maize (80-400 kg/ha and 20-50 kg/ha, quinoa and maize, respectively) making quinoa a valuable new oil crop (Koziol 1990). In spite of high levels of unsaturated fatty acids, quinoa oil is quite stable to oxidation. This is attributed to high levels of natural antioxidant vitamin E, 690-740 ppm α -tocopherol and 790-930 ppm γ -tocopherol. Although Koziol (1990) found concentrations fall after

refining to 450 ppm α -tocopherol and 230 ppm γ -tocopherol, 100-200 ppm is sufficient for optimal antioxidant activity of these isomers (Hudson and Ghavami 1984). In addition, flavonoid glycosides with antioxidant activity have been identified (De Simone et al 1990; Zhu et al 2001). Zhu et al (2001) has shown certain saponin molecules have antioxidant activity.

Starch and Other Carbohydrates

Average carbohydrate and crude fiber content of quinoa is 55.7% and 4.9%, respectively (Galwey et al 1990). Dahlin (1991) reported in vitro carbohydrate digestibility was higher in quinoa than six other unprocessed cereals. Germinating quinoa has been shown to increase palatability of starchy foods by decreasing the viscosity of starch through increased α -amylase activity (Atwell et al 1988). Upon 12 hours of germination, α -amylase activity raised four fold in quinoa. The authors added germinated quinoa flour to hard red spring, wheat flour and found lower amylograph peak viscosities compared to wheat flour alone.

Germinated sorghum and maize, investigated as supplements to starchy foods have beneficial effects on the nutrient density of weaning diets (Mosha and Svanberg 1983). Consequently, germinated quinoa could be included in foods at weaning to increase nutritional qualities, caloric density, and palatability of diets high in starch, such as potato-rich diets of the Andean people.

Studies on the physico-chemical characteristics of quinoa starch have been reported (Wolf et al 1950; Scarpati de Briceño and Biceño 1982; Atwell et al 1983; Varriano-Marston and de Francisco 1984; and Lorenz 1990; Ahamed et al 1996a; Lindeboom et al 2005). The size of the starch granule and its amylose content are

important factors in determining functional properties in food systems. Quinoa starch granules occur in the perisperm cells as compound granular aggregates and the individual starch granule, with an average particle size of 1-2 μm (Atwell et al 1983), is small and uniform compared with that of maize and wheat, 1-23 μm and 2-40 μm , respectively (Wolf et al 1950; Swinkels 1985). Although small starch granules have been shown to have reduced baking potential (Kulp 1973), the small size and uniformity of quinoa starch granules impart a smooth texture and mouthfeel. This attribute has gained considerable interest from food, paper, and cosmetic industries. For example, The NutraSweet Company filed a patent for the manufacture of a carbohydrate-based cream substitute from quinoa starch (Singer et al 1992). Small quinoa starch granules also have application as inexpensive fillers in low density polyethylene films (Ahamed et al 1996b).

Atwell et al (1983) performed an in-depth characterization of quinoa starch. Analysis indicates 11% amylose content, which is low in comparison to most cereal starches. It is comparable, however, to some varieties of rice, as reported by Williams et al (1958). Lorenz (1990) found that quinoa starch performs poorly in cake and bread baking due to its low amylose content and small starch granule. This researcher also found a higher swelling power for quinoa starch than that of barley, wheat, rice, amaranth, or potato starch, and thus, performs well as a thickening agent in fillings.

Free sugars in quinoa were evaluated to contain 4.55%, 2.41%, and 2.39%, glucose, fructose, and sucrose, respectively (González et al 1989). In the same study, the starch level was much lower than that reported by other authors (Ranhotra et al 1993).

Saponins and Saponin Removal

Saponins are a large group of bitter-tasting compounds located in the pericarp or outer hull (Villacorta and Talauera 1976) that must be removed prior to consumption as human food or animal feed. Koziol (1992) estimated saponin content of quinoa to range from 0.01-4.65%. Although high levels of saponins are found in unpolished quinoa, levels in polished quinoa are minimal when compared with some common foods (Table 1.4).

TABLE 1.4
Comparison of Saponin Contents of Quinoa and Common Foods

Food	Saponins (%)
Chickpeas	5.00
Kidney beans	1.40
Spinach	0.55
Quinoa (polished)	0.01
Quinoa (unpolished)	4.65

Source: Galwey et al (1990).

Saponins are known to have anti-nutritional properties (Reichert et al 1986). In a review article by Oakenfull and Sidhu (1990), the authors critically evaluated the literature and made firm conclusions as to the potential usefulness of saponins in cholesterol-lowering diets. They concluded that a substantial body of evidence supports the theory that dietary saponins can lower plasma cholesterol levels.

The basic structure of a saponin molecule is a glycoside with a triterpenoid ring to which a number of sugar moieties are attached. Further research is underway to separate and isolate saponins into those that may have pharmaceutical applications, and those that may have antimicrobial or pesticide applications (Becker and Hanners 1990; Fleming and Galwey 1995).

Bitterness associated with saponins reduces the palatability of quinoa and creates significant problems in processing (Atwell 1988; Koziol 1990). Saponins are removed from quinoa in several ways that make use of solubility properties and the location of saponins being primarily in the outer layer of the grain. Traditional methods of saponin removal involve hand or sand abrasion followed by rinsing the seeds in water. Mechanical abrasion, also called polishing, removes the outer pericarp of quinoa. Abrasive-type dehullers have been designed for effective saponin removal for commercial processing of quinoa (Reichert 1982; Reichert et al 1984). Abrasive dehulling to a flour extraction ranging from 85.2 to 98.9% reduced the saponin content to a low level (Reichert et al 1986).

General research methods to reduce or remove saponins include genetic selection for reduced grain saponin content (Ward 2000) or solvent extraction. Mohamed et al (2000) used ethanol to reduce high saponin levels found in seed kernels of *Balanites aegyptiaca*, a small tree native to the Sudano-Sahelian region with several commercial uses for the seed kernels, wood, and fruit, whose common names include: hingota, soapberry tree, thorn tree, and desert date (CABI 2006). Methanol is also used to extract saponins for study (Koziol 1991; Chauhan et al 1999; Zhu et al 2001).

Protein Characteristics and Enzymatic Activity

Osborne solubility classifies protein into water soluble albumins, salt soluble globulins, ethanol soluble prolamins, and dilute acid and base soluble glutelins. Quinoa proteins are primarily albumins and globulins (44-77% of total protein), whereas, prolamins and glutelins are present in rather insignificant amounts (Fairbanks et al 1990; Koziol 1992). Albumins and globulins are reported to be as high as 74.1-79.9% of total

seed protein (Gorinstein et al 1996). Concentrations of albumins and globulins are greater than those found in maize, rice, or wheat (Ríos et al 1978; Scarpati de Briceño and Briceño 1980; Romero 1981) and these proteins account for the favorable amino acid profile of quinoa.

Electrophoretic characterization of quinoa seed protein indicated an insignificant level of prolamins (Fairbanks et al 1990); therefore, quinoa may be considered gluten free. Most prolamins, like gliadins found in wheat, ignite immune responses in patients with gluten-induced enteropathy, also known as celiac disease.

Nitrogen solubility, evaluated over a range of pH values, is a guide to protein functionality. Coulter and Lorenz (1991a) found quinoa protein to be more soluble than that of corn grits, specifically, nitrogen solubility increased with increasing quinoa in extruded quinoa to corn grit blends. A shift in the isoelectric point (the pH of minimum protein solubility) of the material toward the isoelectric point of quinoa protein (pH 4.0), was also observed. Oshodi et al (1999) found maximum solubility of quinoa protein occurs at pH 10.0.

A majority of the proteins of quinoa have metabolic or enzymatic activity. Enzyme activity is important for use of grain or its milled products in baking or brewing applications. Lorenz and Nyanzi (1989) studied enzyme activity of quinoa and found optimal pH for protease activity to be 4.2. Total amylase, cellulase and hemicellulase activity was highest in the unprocessed seeds and activities were found to decrease after mechanical abrasion. As expected, heat treatment (toasting at 170°C) after saponin removal caused a further decrease in activity of these enzymes (Lorenz and Nyanzi 1989). Authors suggest that cellulase and hemicellulase activities can be used to indicate

effectiveness of mechanical abrasion to remove saponins. In a paper by Becker and Hanners (1990), stone milling removed 33-40% of the seed as the bran fraction. This fraction contained most of the protein, fat, fiber, ash, and saponins. However, they found this fraction turned rancid within a few days due to enzymatic lipid oxidation.

Allergenicity of Quinoa Proteins

True food allergies include: (1) immunoglobulin E (IgE)-mediated reactions (e.g., oral allergy syndrome, anaphylaxis), (2) cell-mediated reactions [e.g., celiac disease, food protein-induced enterocolitis], and (3) combined Ig-E- and cell-mediated immunity (e.g., eosinophilic gastroenteritis, atopic dermatitis) (FDA 2005). True food allergies are abnormal responses of the immune system to naturally occurring proteins, the antigen or allergen, of certain foods that can lead to serious manifestations. An estimated 30,000 emergency room visits and 150-200 deaths occur annually from allergic reactions to foods (Bock et al 2001; FDA 2005). The common allergens are from milk, eggs, fish, crustacean shellfish, peanuts, tree nuts, soybeans, and wheat. These eight food allergens account for more than 90% of all food allergic reactions and all but wheat have elicited well documented fatal reactions (Taylor and Hefle 2001). Evidence indicates that only 1-3 mg of peanut, milk, and egg can elicit mild allergic reactions in the most sensitive individuals (Taylor et al 2002).

Current estimates are 10-12 million (3.5-4% of the US population) have a true food allergy, compared with estimates of 1-2% ten years ago, and even a larger percentage 10% or more believe they have a food allergy (Sicherer et al 2004). One fourth of the population suffering from a true food allergy, or 1% of Americans have

celiac disease (Aubrey 2005), a disease that only 5 yrs ago was considered rare (<20,000 Americans).

The mechanism of an allergic reaction occurs in two phases, an initial sensitization and the reaction. Sensitization occurs when a susceptible individual produces IgE antibodies against specific proteins in a food upon initial exposure. These allergen specific IgE antibodies combine with mast cells and basophils to produce sensitized cells. Upon subsequent exposure to the same food allergen, the reaction occurs when sensitized cells combine with the allergenic protein, resulting in degranulation and release of mediators (inflammatory molecules), such as histamine, leukotrienes, prostaglandins, substances that elicit allergic symptoms (FDA 2005; Taylor and Hefle 2005).

Grain proteins of concern in celiac disease are of the prolamin fraction and include gliadin in wheat, secalin in rye, hordein in barley and possibly avenin in oats. Wheat gliadin subtypes alpha, gamma, and omega, have been shown to affect individuals with celiac disease. Prolamins of rye, barley and triticale (a cross-bred hybrid of wheat and rye) are also considered to affect individuals with celiac disease because they are taxonomically related to wheat (i.e. express peptides structurally similar to those of wheat) and have been shown to affect individuals with celiac disease.

Although quinoa is a minor world crop, it has been cultivated and consumed in South America for thousands of years. Over the past 25 years, quinoa has been cultivated on each continent and consumed by a diverse genetic population. There are no known allergic reactions to quinoa proteins according to the Food Allergy & Anaphylaxis Network (FAAN 2003). The Celiac Disease Foundation recommends quinoa as a food

included in a gluten-free diet (Lee 1999); however, the Celiac Sprue Association does not (Inman-Felton and Rottman 1999), most likely due to contamination of quinoa from other grains. Quinoa is evolutionarily very distant from true cereals of the family *Graminaceae* that contain gluten fractions toxic for those with celiac disease (Berti et al 2004). Quinoa is a dicot (two seed leaf) and very distantly related to grains in the monocot (one seed leaf) subclass. As a result, quinoa would not contain the same harmful amino acid sequences found in wheat (Thompson 2001). Albumins (31%) and globulins (37%) are predominant in quinoa, whereas, prolamins (0.8%) are found at low levels (Fairbanks et al 1990). Furthermore, the prolamins of quinoa are unable to agglutinate K562(s) cells (De Vincenzi et al 1999). De Vincenzi et al (1999) separated A and B fractions of quinoa prolamins and found fraction A contains protective peptides that interfere with agglutinating activity of toxic peptides found in fraction B. Upon immunochemical and molecular evaluation of quinoa proteins, Berti et al (2002, 2004), concluded that quinoa could be a safe choice for the production of gluten-free products. The immunoreactivity of the extracted quinoa proteins was low compared with either commercial anti-gliadin antibody or serum of a celiac disease subject and was comparable to that of proteins in gluten-free flour. Although from a technological standpoint the low content of interprotein disulfide interactions, quinoa protein would not be able to form the stable network structure typical of the highly structured gluten found in bread.

CHAPTER 3

RECOVERY AND CHARACTERIZATION OF QUINOA PROTEINS

ABSTRACT

Four whole grain quinoa [two commercial genotypes from Colorado (black and white) and two abrasion conditions (polished and unpolished)] were analyzed for proximate composition (saponin, ash, fat, moisture, and nitrogen), 1000-grain weight and yield loss. Whole grain materials were defatted under controlled conditions and analyzed for nitrogen content (dwb). Quinoa protein concentrate was prepared from defatted flour by a modified isoelectric precipitation method and analyzed for nitrogen content (dwb) and nitrogen recovery. Quinoa protein was characterized by amino acid analysis, chemical score, SDS-PAGE, and isoelectric focusing. With the exception of ash content, proximate composition values of whole grain materials depended on genotype and abrasion condition. There was no difference in saponin content in the black or white genotype in the unpolished quinoa. When black and white quinoa genotypes were polished saponin was reduced, but the magnitude of the reduction was much less in black quinoa (a 53% decrease) compared to white quinoa (a 94% decrease). The fat content was higher in the white quinoa than the black quinoa genotype before polishing. No effect of polishing was found on the fat content of the black quinoa genotype, but an 11% loss in fat was found in the white quinoa after polishing. The nitrogen content was higher in the black quinoa than the white genotype before polishing. Nitrogen increased after

polishing, but the magnitude was much greater in the white quinoa (an 18% increase) compared to the black quinoa (a 5% increase). Main effects were found for ash content. White quinoa was higher in ash (3.9%) than black quinoa (3.3%) and polished quinoa was higher in ash (3.9%) than unpolished quinoa (3.3%). Main effects were found for 1000-grain weight. Black quinoa had a higher 1000-grain weight (3.8 g) than white quinoa (3.2 g) and unpolished quinoa was higher (3.8 g) than polished quinoa (3.3 g). Yield loss due to polishing was 13% in black or white quinoa. Defatted quinoa was lower in nitrogen content (2.3%) than whole quinoa, regardless of genotype or abrasion condition. Nitrogen content and recovery of prepared protein concentrate was dependent on genotype and abrasion condition. Nitrogen content of quinoa protein concentrates increased with polishing, but the magnitude was much higher in the white quinoa (a 32% increase) than the black quinoa (a 5% increase). Nitrogen recoveries were highest in white quinoa genotype. Nitrogen recoveries were increased with polishing in black quinoa, but nitrogen recoveries decreased with polishing in the white quinoa. Mean nitrogen values for quinoa protein concentrate ranged from 11.23 to 14.83% (dwb). Amino acid analyses were comparable to reported values for quinoa. The first limiting amino acids in comparison to the corresponding reference amino acids were threonine and isoleucine in quinoa protein concentrate and whole quinoa, respectively; giving a chemical score of 91 for quinoa protein concentrate and 80 for whole quinoa. Major quinoa proteins with molecular weights approximately 11 and 30 kDa were resolved with electrophoresis and are consistent with 11S globulins and 2S albumins. Major proteins with isoelectric points of 4.4, 5.4, 5.6, and 5.8 were determined using isoelectric focusing.

INTRODUCTION

Quinoa is commercially produced in Colorado in the San Luis Valley, but on a relatively small scale. Protein-rich crops have increased value, especially in agricultural regions that are considered marginal (Cheftel et al 1985). Johnson and Croissant (1985) have outlined the potential for quinoa to improve Colorado agriculture. Developments are needed in the following areas in order to increase the agricultural value of quinoa in Colorado: saponin removal, value-added ingredient processing, ingredient functionality, and selection of genotypes suitable for value-added ingredient processing.

Saponins must be removed prior to value-added ingredient processing. Saponins are a group of bitter triterpenoid aglycones found in the pericarp (outer hull) of quinoa. Commercial quinoa is either mechanically abraded to remove the saponin-rich pericarp or rinsed to remove saponins which are water-soluble, or a combination of both methods (Villacorta and Talauera 1976; Reichert et al 1986; Atwell 1988; Koziol 1990). Rinsing is costly, due to the expense of expedient drying in order to prevent germination and up to 6% of the water soluble proteins are lost in the rinse water (Koziol 1991). Abrasion has been shown to reduce yields up to 18% and may remove a part of the embryo (Lorenz and Nyanzi 1989), which contains valuable nutrients that may be lost during abrasion. Ethanol has been used to extract saponins (Carlsson 1980; Mohamed 2000) and is also a common industrial solvent used to extract oil, an initial step in concentrating plant proteins in order to produce value-added protein ingredients used throughout the food industry. The goal in producing a protein concentrate ingredient is to achieve high protein content and high protein recovery from the starting material. Quinoa contains insignificant levels of prolamins (Fairbanks et al 1990; Koziol 1992), ethanol-soluble

protein; therefore, minimal protein loss should occur with use of ethanol to extract saponins and lipids. Solvent extraction using ethanol may be a viable alternative to mechanical abrasion and/or rinsing to remove saponins.

The objectives of this study were to 1) evaluate effects of quinoa genotype and abrasion condition on yield loss, proximate composition (including nitrogen and saponin content), nitrogen content of prepared protein concentrate, and nitrogen recovery; 2) evaluate effects of quinoa genotype, abrasion condition, and material stage on nitrogen content; and 3) study quinoa protein through amino acid analysis, chemical score, molecular weight, and isoelectric point characterization.

MATERIALS AND METHODS

Materials

Two commercial grain genotypes, white quinoa genotype (Colorado D407, a Chilean landrace of *Chenopodium quinoa*, Willd.) and black quinoa genotype (result of outcrossing Colorado D407 and *Chenopodium berlandieri*, a weedy relative of *Chenopodium quinoa*, Willd.), were purchased from White Mountain Farm (Mosca, Colorado) from the 2001 harvest.

Sample Preparation

Whole Quinoa

Half of the quinoa material of each genotype was mechanically abraded (polished) at White Mountain Farm using a combination barley pearling machine, modified for on-farm use, and rice polisher (Satake Rice Milling Machine, Satake USA, Inc, Stafford, TX), for pericarp and saponin removal as described by New (2002). The other half of the material of each genotype remained unpolished (pericarp intact).

Samples of four whole grain quinoa materials (black unpolished, black polished, white unpolished, and white polished) were retained for proximate composition (including saponin and nitrogen content) and yield loss analyses.

Defatted Flour

Four whole grain quinoa materials were defatted under the following controlled conditions. Grains were flaked at ambient temperature (25°C) with Flaker Series No. 2188 size 18 x 12 HD (Ross Machine & Mill Supply, Oklahoma City, OK) with a roll gap of 0.051mm. Flakes were immediately extracted with 95% ethanol 1:1 w/v with lab Model IV Extractor size 0.25 cu ft (Crown Iron Works, Roseville, MN) and micelle and solvent mixture (containing saponins and lipids) were separated from the oilcake. The lipid micelle and solvent mixture were discarded. The oilcake was desolventized and air-dried overnight at ambient temperature to produce defatted quinoa flour. Defatted flour was stored at 0°C until further analysis. Samples of four defatted quinoa flours were retained for nitrogen and moisture analysis.

Quinoa Protein Concentrate

Quinoa protein concentrate was prepared from four defatted quinoa flours with a modified isoelectric precipitation method (Mohamed et al, 2000). A diagram of the protein concentrate preparation is shown in Figure 3.1. A 20 g portion of defatted flour was fine milled to less than 105 micron (thru US No. 140) with Bel-Art Products Micro-Mill (Pequannock, NJ). The fine flour was suspended in 1:10 w/v of alkaline solution (0.03 mol/L NaOH) and mechanically stirred at ambient temperature for 4 hr. The suspension was centrifuged for 30 min at 6,000 g at 0°C. The pellet residue was discarded. The supernatant was adjusted to pH 4.25 with concentrated HCl and

centrifuged for 30 min at 13,000 g at 0°C. The supernatant was discarded. The protein pellet was resuspended in 20 mL deionized water, neutralized (pH 7.0) with concentrated NaOH, and the resulting protein curd was freeze-dried at 25°C, 2 mbar for 72 h. The quinoa protein concentrate was stored at 0°C until analyzed.

Analyses of Whole Quinoa

Proximate Composition

Whole quinoa was analyzed for proximate composition for percent saponins (Afrosimetric Method, Koziol 1991), percent ash (Method 08-01, AACC 2000), percent fat (Method 30-20, AACC 2000), percent moisture (Method 44-15A, AACC, 2000), and percent nitrogen (Dumas Combustion Method 46-13, AACC 2000). Moisture content was measured and used to express nitrogen content on a dry weight basis. Nitrogen content was converted to percent crude protein by multiplying by a factor of 6.25.

1,000-Grain Weight and Yield Loss

The 1,000-grain weight was gravimetrically measured after counting 1,000 individual whole quinoa grains. Yield loss was a calculated value obtained by dividing the difference of the 1000-grain weights of unpolished whole quinoa and polished whole quinoa from the 1000-grain weight the unpolished whole quinoa and multiplying by a factor of 100.

Analyses of Defatted Flour

Defatted flour was analyzed for proximate composition for percent moisture (Method 44-15A, AACC, 2000) and percent nitrogen (Dumas Combustion Method 46-13, AACC 2000) and expressed on a dry weight basis.

Analyses of Quinoa Protein Concentrate

Quinoa protein concentrate was analyzed for proximate composition for percent moisture (Method 44-15A, AACC, 2000) and percent nitrogen (Dumas Combustion Method 46-13, AACC 2000 and micro-Kjeldahl Method 12.1.7, AOAC 1990) and expressed on a dry weight basis.

Characterization of Quinoa Proteins

Amino Acid Analysis and Chemical Score

Amino acid analysis was determined by acid hydrolysis and HPLC using an internal method (detection level 0.005%) of Medallion Labs (Minneapolis, MN). Amino acid analysis of whole quinoa (white polished) and quinoa protein concentrate (white polished) were measured in duplicate. Chemical score was calculated by dividing the limiting amino acid in the test protein (mg/g protein) by the corresponding amino acid in a reference protein (mg/g protein) and multiplying by 100 (Ruales and Nair 1992).

Molecular Weight Characterization by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Whole quinoa and quinoa protein concentrate were prepared from white polished quinoa for gel electrophoresis in denaturing-reducing buffer of 62.5 mM Tris (pH 6.8), 2% (w/v) sodium dodecyl sulfate (SDS), 5% β mercaptoethanol (β -ME), 10% (v/v) glycerol, and denatured by boiling for 5 min. The profile of quinoa proteins was analyzed by SDS-PAGE on a 12% polyacrylamide gel. Resolving gel size was approximately 10 cm x 8 cm x 1.5 mm. Kaleidoscope Prestained Standards (10 μ l) molecular weight markers (MWM) (Bio Rad Cat #161-0375) were included. Sample amounts for whole quinoa and quinoa protein concentrate ranged from 2-20 μ l per well.

Samples were electrophoresed at 90 V (constant) for approximately 90 min. Gels were stained with Coomassie blue, destained and imaged on a Bio-Rad ChemiDoc® XRS system. Subsequent image manipulations were performed using Bio-Rad Quantity One® software.

Isoelectric Point Characterization by Polyacrylamide Gel Isoelectric Focusing (IEF)

Whole quinoa and quinoa protein concentrate prepared from white polished quinoa protein were diluted 1 part sample with 2 parts sample buffer (50% glycerol). The profile of quinoa proteins in native conditions was analyzed by isoelectric focusing with Ready Gel IEF, pH range 3-10, 15 wells (Bio Rad, Cat No. 161-1129) and IEF MIX, pH range 3.5-9.3 (Sigma I-3018), isoelectric point markers (pI M). A 5 µl (18µg total protein) aliquot was loaded in wells containing standards. Running buffers consisted of 20mM lysine and 20 mM arginine (cathode) and 7mM phosphoric acid (anode). Sample amounts ranged from 2.5-10 µl per well. Samples and standards were electrophoresed at 100 V constant for 60 min, 250 V constant for 60 min, and 500 V constant for 30 min for a total run time of 150 min. Gels were stained in IEF Gel Staining Solution (Bio Rad Cat No. 161-0434) for 45 min and subsequently destained in 40% methanol, 10% acetic acid solution for 2-3 hours. Gels were photographed on a Photodyne Variquest 100 transilluminator (Photodyne Technologies Inc) using a yellow filter.

Statistical analysis

Whole Quinoa

A two-factor 2 x 2 factorial design was used with factor genotype at two levels (white and black quinoa) and factor abrasion condition at two levels (unpolished and polished). Response variables measured on the four treatments were proximate

composition (saponin, ash, fat, moisture, nitrogen) and 1000-grain weights. Data were analyzed by two-way analysis of variance (ANOVA) at a 95% confidence level using SAS Mixed procedures (v. 9.1 for Windows, SAS Institute, Cary, NC). Least significant difference (LSD) tests at $P < 0.05$ were used to separate means when significant differences were found.

Whole and Defatted Quinoa

In order to determine the effects of defatting, a procedure required for protein concentrate, a three-factor $2 \times 2 \times 2$ factorial design was used. The design contained factor genotype at two levels (white and black quinoa), factor abrasion condition at two levels (unpolished and polished), and factor material stage at two levels (whole and defatted quinoa). Response variables measured on the eight treatments were moisture and nitrogen content. Data were analyzed by three-way analysis of variance (ANOVA) at a 95% confidence level using SAS Mixed procedures (v. 9.1 for Windows, SAS Institute, Cary, NC). Least significant difference (LSD) tests at $P < 0.05$ were used to separate means when significant differences were found.

Quinoa Protein Concentrate

A two-factor 2×2 factorial design was used with factor genotype at two levels (white and black quinoa) and factor abrasion condition at two levels (unpolished and polished). Response variables measured on the four treatments were moisture, nitrogen content, and nitrogen recovery. Data were analyzed by two-way analysis of variance (ANOVA) at a 95% confidence level using SAS Mixed procedures (v. 9.1 for Windows, SAS Institute, Cary, NC). Least significant difference (LSD) tests at $P < 0.05$ were used to separate means when significant differences were found.

Characterization of Quinoa Proteins

Separation of proteins by denaturing gel electrophoresis and isoelectric focusing were repeated numerous times until conditions were appropriate to produce photographic quality gels and identify molecular weights and isoelectric points of the major quinoa proteins. Means of duplicate samples and standard deviations were calculated for amino acid analysis. Chemical scores were calculated from mean data from amino acid analysis.

RESULTS AND DISCUSSION

Analysis of Whole Quinoa

Proximate Composition

The two-way analysis of variance for the significance of genotype and abrasion condition on proximate composition values for whole quinoa show significant main effects for ash content and two-way interactions for saponin, fat, moisture, and nitrogen (Table 3.1). The significant main effects for ash content are discussed later.

Saponin, fat, moisture, and nitrogen content depended on both genotype and abrasion condition as manifested by significant two-way interactions (Table 3.1). Mean saponin, fat, moisture, and nitrogen values are reported in Table 3.2. There was no difference in saponin content in the black or white genotype in the unpolished quinoa (Table 3.2).

TABLE 3.1
Analysis of Variance for the Significance of Genotype x Abrasion Condition on
Proximate Composition for Whole Quinoa

Response Variable	Effect	DF	F value	Prob > F
Saponin	Genotype	1	4.90	0.0577
	Abrasion Condition	1	83.74	<0 0001
	Genotype x Abrasion Condition	1	7.26	0.0273
	Corrected Total	11		
Ash	Genotype	1	15.65	0.0042
	Abrasion Condition	1	14.68	0.0050
	Genotype x Abrasion Condition	1	1.18	0.3094
	Corrected Total	11		
Fat	Genotype	1	29.07	0.0007
	Abrasion Condition	1	26.32	0.0009
	Genotype x Abrasion Condition	1	20.05	0.0021
	Corrected Total	11		
Moisture	Genotype	1	9.00	0.0050
	Abrasion Condition	1	24.48	< 0.0001
	Genotype x Abrasion Condition	1	16.21	< 0.0001
	Corrected Total	39		
Nitrogen	Genotype	1	30.58	< 0.0001
	Abrasion Condition	1	204.99	< 0.0001
	Genotype x Abrasion Condition	1	80.33	< 0.0001
	Corrected Total	39		

TABLE 3.2

Mean Proximate Composition (%) for Whole Quinoa^a

Response Variable (%)	Genotype x Abrasion Condition			
	Black Unpolished	Black Polished	White Unpolished	White Polished
Saponin ^b	0.49 ± 0.11a	0.23 ± 0.05b	0.50 ± 0.06a	0.03 ± 0.01c
Fat ^b	5.57 ± 0.13b	5.52 ± 0.07b	6.32 ± 0.20a	5.59 ± 0.10b
Moisture ^c	9.31 ± 0.37a	8.54 ± 0.32b	9.22 ± 0.21a	9.14 ± 0.12a
Nitrogen (dwb) ^c	2.58 ± 0.14c	2.67 ± 0.09b	2.32 ± 0.05d	2.73 ± 0.07a

^aValues followed by the same letter in the same row are not significantly different ($P < 0.05$).

^bValues reported as mean of three samples ± one standard deviation.

^cValues reported as mean of ten samples ± one standard deviation.

The objective of mechanical abrasion is to remove the outer hull of quinoa whole grain in order to reduce saponin to a level for quinoa to be considered non-bitter. Values equal to or greater than 0.11% saponin in quinoa are considered bitter and values less than 0.11% saponin are considered non-bitter (Ward 2003). When black and white quinoa genotypes were polished, saponin was reduced as expected, but the magnitude of the reduction was much less in black quinoa (a 53% decrease) compared to white quinoa (a 94% decrease). A level of 0.23% saponin was found in black polished quinoa, indicative of black polished quinoa being bitter; therefore, mechanical abrasion was not effective in reducing saponin to a level considered to be non-bitter in the black quinoa genotype. On the other hand, a level of 0.03% saponin was found in white polished quinoa, indicative of white polished quinoa being non-bitter; therefore, mechanical abrasion was effective in reducing saponin to a level considered to be non-bitter in the white quinoa genotype.

The fat content was higher in the white quinoa than the black quinoa genotype before polishing. No effect of polishing was found on the fat content of the black quinoa genotype ($P > 0.05$), but an 11% loss in fat was found in the white quinoa after polishing. A loss in fat in the white quinoa after polishing could indicate the embryo was affected by mechanical abrasion and lipid bodies could have exuded from the white polished quinoa.

Percent moisture was determined and used to adjust the nitrogen values to a dry weight basis (dwb). The nitrogen content was higher in the black quinoa than the white genotype before polishing. Nitrogen increased after polishing, as expected, due to a concentration effect of removing the pericarp (made primarily of cellulose and saponin),

but the magnitude was much greater in the white quinoa (an 18% increase) compared to the black quinoa (a 5% increase). A larger treatment effect for polishing on nitrogen content in white quinoa as compared to black is logical, because mechanical abrasion was considered effective in the white quinoa but not effective in the black quinoa genotype. Crude protein was a calculated value obtained by multiplying the nitrogen by a factor of 6.25.

Treatment effects were the same for crude protein as for nitrogen. Average crude protein values for quinoa reported in the literature were 14.6% (Koziol 1992). Mean crude protein values in the current study ranged from 15.0 to 17.5% for quinoa commercially grown in Colorado. It is interesting to note that mean crude protein values for Colorado quinoa in this study are comparable or higher than average crude protein values reported in the literature.

Ash content is the inorganic residue from the incineration of organic matter. Ash content reflects the total mineral content and is used extensively for quality control and proximate composition of a food product or food ingredient. Mean ash values for the four treatments are shown in Table 3.3 and were less than the ash value reported for quinoa (4.2%) by DeBruin (1964). White quinoa was higher in ash content (3.9%) than black quinoa (3.3%) regardless of abrasion condition and polished quinoa was higher in ash content (3.9%) than unpolished quinoa (3.3%) regardless of genotype (Table 3.3).

1,000-Grain Weight and Yield Loss

The two way analysis of variance for the significance of genotype and abrasion condition on 1000-grain weights of whole quinoa showed significant main effects, but no

TABLE 3.3**Mean Ash (%) for Whole Quinoa^{a,b}**

Genotype	
Black	White
3.27 ± 0.51 b	3.88 ± 0.27 a
Abrasion Condition	
Unpolished	Polished
3.28 ± 0.44 b	3.87 ± 0.40 a

^aValues followed by the same letter in the same row are not significantly different ($P < 0.05$).

^bValues reported as mean of six samples ± one standard deviation.

interaction (Table 3.4). Mean 1000-grain weights for the four treatments are shown in Table 3.5 and were within the range reported in the literature for quinoa (1.9 - 4.3 g) by Koziol (1993). Values reported in the literature and in this study for 1000-grain weights for quinoa are lower in comparison to that of traditional grains. Black quinoa had a higher 1000-grain weight (3.8 g) than white quinoa (3.2 g) regardless of abrasion condition and unpolished quinoa was higher (3.8 g) than polished quinoa (3.3 g) regardless of genotype (Table 3.5).

TABLE 3.4**Analysis of Variance for the Significance of Genotype x Abrasion Condition on 1000-Grain Weights for Whole Quinoa**

Effect	DF	F value	Prob > F
Genotype	1	583.50	<0.0001
Abrasion Condition	1	396.78	<0.0001
Genotype x Abrasion Condition	1	0.89	0.3645

Yield loss is an indirect measure of the efficiency of mechanical abrasion to remove the saponin-rich pericarp. Polishing reduced 1000-grain weights by 13% in both the black or white quinoa for a yield loss of 13%. Loss in yield due to mechanical abrasion at White Mountain Farms was estimated to range from 5-15% (New 2002) and

TABLE 3.5

Mean 1000-Grain Weights (g) for Whole Quinoa^{a,b}

Genotype	
Black	White
3.84 ± 0.28 a	3.23 ± 0.26 b
Abrasion Condition	
Unpolished	Polished
3.79 ± 0.34 a	3.29 ± 0.32 b

^aValues followed by the same letter in the same row are not significantly different ($P < 0.05$).

^bValues reported as mean of eight samples ± one standard deviation.

values reported in the literature are as high as 18% (Lorenz and Nyanzi 1989). As previously noted, high saponin values for black polished quinoa shown in Table 3.2 indicated ineffective polishing. Black quinoa had a thicker pericarp than white quinoa. This needs to be taken into consideration during polishing to effectively remove the saponins. Lipids were lost in the white polished quinoa which indicated excessive polishing.

Values of 0.5% saponin, as found in unpolished black and white quinoa, are considered high levels and are difficult to quantify using the Afrosimetric method. This method tends to underestimate true saponin content above 0.5% (Ward 2003), but this method has been determined suitable for monitoring the efficacy of mechanical abrasion on saponin removal (Koziol 1991). However, the Afrosimetric method does not indicate if mechanical abrasion is too excessive or if yield loss is too high. On the other hand, the Afrosimetric method used in combination with 1,000-grain weights could monitor effects of mechanical abrasion more accurately. In addition, these combined methods are quick, inexpensive, and could be used on-site of mechanical abrasion processing as quality control tools to assess polishing efficiency.

Analysis of Whole and Defatted Quinoa

Nitrogen Content

Material stage (whole or defatted quinoa) was introduced as a third factor in order to evaluate effects of defatting on nitrogen content. The three-way analysis of variance for the significance of genotype, abrasion condition, and material stage on nitrogen content of whole and defatted quinoa had a significant main effect for material stage and no three-way interaction (Table 3.6).

TABLE 3.6
Analysis of Variance for the Significance of Genotype x Abrasion Condition x Material Stage on Nitrogen Content for Whole and Defatted Quinoa

Effect	DF	F value	Prob > F
Genotype	1	30.6	<0.0001
Abrasion Condition	1	205.0	<0.0001
Genotype x Abrasion Condition	1	80.3	<0.0001
Material Stage	1	11.4	0.0012
Genotype x Material Stage	1	0.0	0.9543
Abrasion Condition x Material Stage	1	0.0	0.9625
Genotype x Abrasion Condition x Material Stage	1	0.1	0.7624
Corrected Total	79		

There was a two-way interaction between genotype and abrasion condition for nitrogen content, but it was the same as described previously for Table 3.1. Defatted quinoa was lower in nitrogen content than whole quinoa, regardless of genotype or abrasion condition (Table 3.7).

TABLE 3.7
Mean Nitrogen (%) for Whole and Defatted Quinoa^{a,b}

Material Stage	
Whole	Defatted
2.61 ± 0.18 a	2.55 ± 0.17 b

^aValues followed by the same letter are not significantly different ($P < 0.05$).

^bValues reported as mean of forty samples ± one standard deviation.

There was a 2.3% loss in nitrogen for defatted quinoa. This was unexpected since ethanol was used for fat extraction and there is an insignificant level of ethanol-soluble prolamin proteins in quinoa (Fairbanks et al 1990). This discovery presented a new problem which necessitated evaluation in addition to the original experimental design.

Whole quinoa, regardless of grain color, contains red betalain pigments (Taylor 2002). Betalains differ in structure from anthocyanins, the major category of red pigments found in plants, because betalains contain nitrogen. Betalains are ethanol soluble and a source of non-protein nitrogen. Therefore, a loss of nitrogen during defatting using ethanol could have resulted from a loss of betalain pigments rather than a loss of nitrogen from protein. Color scores are typical quality control measurements used to assess the color of grain, as well as numerous food products. Color scores were determined using HunterLab Colorimeter (Reston, VA) and measured in triplicate. Positive a-values represent redness with higher values representative of greater intensity. The three-way ANOVA for whole and defatted quinoa had a significant three-way interaction (Table 3.8).

TABLE 3.8
Analysis of Variance for the Significance of Genotype x Abrasion Condition x Material Stage on Hunter Color a-values (Redness) for Whole and Defatted Quinoa

Effect	DF	F value	Prob > F
Genotype	1	446.55	<0.0001
Abrasion Condition	1	232.85	<0.0001
Genotype x Abrasion Condition	1	3.64	0.0747
Material Stage	1	301.61	<0.0001
Genotype x Material Stage	1	0.72	0.4096
Abrasion Condition x Material Stage	1	128.79	<0.0001
Genotype x Abrasion Condition x Material Stage	1	88.12	<0.0001
Corrected Total	23		

Effect of the material stage depended on the level of the other two factors, genotype or abrasion condition. There was a 35% reduction in redness as a result of defatting in the unpolished white quinoa (Table 3.9). In black quinoa, fat removal decreased redness by approximately 5 a-value units in polished but had no effect in unpolished (Table 3.9). In white quinoa, fat removal decreased redness by approximately 2 a-value units in unpolished and approximately 3 a-value units in polished quinoa (Table 3.9). The difference in the magnitude of the fat removal effect is reflected by the 3-way interaction.

Although a-values are not quantitative for betalain pigments, the reduced a-values found in the defatted materials of black polished, white unpolished, and white polished, as compared to corresponding a-values for the whole materials help explain the slight loss of nitrogen with defatting. Betalain pigments are used in the food industry as natural food dyes.

Analysis of Quinoa Protein Concentrate

The two-way analysis of variance for the significance of genotype and abrasion condition on nitrogen content and nitrogen recovery of quinoa protein concentrate had significant two-way interactions for both nitrogen content and recovery (Table 3.10). Nitrogen content and recovery of protein concentrate have practical significance and means are presented in Table 3.11.

TABLE 3.9
Mean Hunter Color a-values (Redness) for Whole and Defatted Quinoa^{a,b}

Genotype x Abrasion Condition x Material Stage							
Black				White			
Unpolished		Polished		Unpolished		Polished	
Whole	Defatted	Whole	Defatted	Whole	Defatted	Whole	Defatted
7.5 ± 0.4 b	8.0 ± 0.2 a,b	8.5 ± 0.7 a	3.4 ± 0.1 e	6.2 ± 0.4 c	4.0 ± 0.2 d	4.2 ± 0.1 d	1.4 ± 0.1 f

^aValues followed by the same letter are not significantly different ($P < 0.05$).

^bValues reported as mean of three samples ± one standard deviation.

TABLE 3.10
Analysis of Variance for the Significance of Genotype x Abrasion Condition on Nitrogen Content and Nitrogen Recovery for Quinoa Protein Concentrate

Response Variable	Effect	DF	F value	Prob > F
Nitrogen	Genotype	1	396.91	<0.0001
	Abrasion Condition	1	1493.20	<0.0001
	Genotype x Abrasion Condition	1	647.07	<0.0001
	Corrected Total	39		
Nitrogen Recovery	Genotype	1	235.26	<0.0001
	Abrasion Condition	1	3.48	0.0731
	Genotype x Abrasion Condition	1	59.50	<0.0001
	Corrected Total	39		

TABLE 3.11
Mean Nitrogen (%) and Nitrogen Recovery (%) for Quinoa Protein Concentrate^{a,b}

	Genotype x Abrasion Condition			
	Black Unpolished	Black Polished	White Unpolished	White Polished
Nitrogen (dwb)	13.78 ± 0.18 c	14.52 ± 0.15 b	11.23 ± 0.24 d	14.83 ± 0.29 a
Nitrogen recovery	36.33 ± 2.13 d	40.24 ± 2.84 c	51.76 ± 3.42 a	45.35 ± 1.41 b

^aValues followed by the same letter in the same row are not significantly different ($P < 0.05$).

^bValues reported as mean of ten samples ± one standard deviation.

Nitrogen content of quinoa protein concentrate increased with polishing, but the magnitude was much higher in the white quinoa (a 32% increase) than the black quinoa (a 5% increase). Values for nitrogen content of quinoa protein concentrate were confirmed by micro-Kjeldahl analysis. This analysis was conducted to validate methods for nitrogen determinations. No significant differences in methods were found between micro-Kjeldahl and Dumas combustion (Table 3.12). There was a two-way interaction between genotype and abrasion condition for nitrogen content, but it was the same as described previously for Table 3.1.

TABLE 3.12
Analysis of Variance for the Significance of Genotype x Abrasion Condition x
Method of Analyses (Dumas vs micro-Kjeldahl) on Nitrogen Content of Quinoa
Protein Concentrate

Effect	DF	F value	Prob > F
Genotype	1	90.90	<0.0001
Abrasion Condition	1	353.89	<0.0001
Genotype x Abrasion Condition	1	139.64	<0.0001
Method	1	0.44	0.5185
Genotype x Method	1	2.33	0.1529
Abrasion Condition x Method	1	0.26	0.6202
Genotype x Abrasion Condition x Method	1	2.10	0.1733
Corrected Total	31		

Nitrogen recoveries were highest in white quinoa genotype. Nitrogen recoveries were increased with polishing in black quinoa, but nitrogen recoveries decreased with polishing in the white quinoa.

Crude protein was a calculated value obtained by multiplying the nitrogen value by a factor of 6.25. Treatment effects were the same for crude protein as for nitrogen. Mean crude protein values for quinoa protein concentrate ranged from 70.2 to 92.7%. In selecting quinoa suitable for making a value-added protein ingredient, a 93% protein concentrate is more valuable per pound than a 70% protein. Although white polished quinoa had a lower recovery than white unpolished, the recovery values of the white quinoa are typical of industrial recovery values for cereal proteins (Hahn 1999). Therefore, the white polished quinoa was selected as the focus for the remainder of this research project.

Characterization of Quinoa Proteins

Amino Acid Analysis and Chemical Score

Amino acid analysis for whole quinoa and quinoa protein concentrate prepared from white polished quinoa are presented in Table 3.13.

TABLE 3.13
Amino Acid Content (mg/g Protein) for Quinoa

Amino Acid	Reference^a	Quinoa Protein Concentrate^b	Whole Quinoa^b	Whole Quinoa USDA^c
Aspartic Acid		90.3 ± 1.0	76.3 ± 1.1	73.0
Threonine ^d	35	31.8 ± 0.2	31.4 ± 0.1	35.0
Serine		45.6 ± 0.8	39.6 ± 0.8	37.0
Glutamic Acid		166.0 ± 2.1	140.7 ± 3.2	119.0
Proline		36.9 ± 0.0	32.9 ± 0.5	31.1
Glycine		46.8 ± 0.9	52.4 ± 0.8	52.0
Alanine		60.3 ± 1.2	44.1 ± 0.9	47.0
Cystine ^d		nd	nd	18.0
b-Valine ^d	48	44.4 ± 0.1	40.4 ± 0.3	45.0
Methionine ^d	26 ^e	21.3 ± 0.3	21.0 ± 0.2	20.0
b-Isoleucine ^d	42	41.6 ± 0.1	33.7 ± 0.2	36.0
b-Leucine ^d	70	67.9 ± 0.5	59.9 ± 0.7	60.0
Tyrosine		33.9 ± 0.4	29.9 ± 0.4	28.0
Phenylalanine ^d	73 ^f	42.9 ± 0.4	37.4 ± 0.3	41.0
Histidine ^d	17	31.9 ± 0.0	27.7 ± 0.1	24.0
Lysine ^d	51	52.0 ± 0.3	56.1 ± 0.2	56.0
Arginine		98.7 ± 1.5	80.8 ± 0.9	70.0
Chemical Score	100	91	80	86

^aReference amino acid value used to calculate chemical score (WHO/FAO/UNU 1985; FNB 1990; Ruales and Nair 1992).

^bValues reported as mean of two samples ± one standard deviation from white polished quinoa (White Mountain Farm, Mosca CO).

^cUSDA database (2002).

^dEssential amino acid.

^eMethionine + cystine.

^fPhenylalanine + tyrosine.

Data for amino acid content of whole quinoa in this study (Table 3.13) are similar to USDA values for whole quinoa. A perfect chemical score for amino acid content of a protein is 100. The first limiting amino acids in comparison to the corresponding reference amino acids were threonine and isoleucine in quinoa protein concentrate and whole quinoa, respectively; giving a chemical score of 91 for quinoa protein concentrate and 80 for whole quinoa. The chemical scores in this study are similar to reported chemical scores for whole quinoa and milk protein; in comparison, chemical scores for

wheat and corn protein are lower at 51 and 57, respectively (Ruales and Nair 1992; Fleming and Galwey 1995).

Molecular Weight Characterization

Several proteins ranging from approximately 10 to 50 kDa in size and numerous minor polypeptides were resolved electrophoretically from whole quinoa and quinoa protein concentrate prepared from white polished quinoa. Based on SDS-PAGE results, the major quinoa proteins consisted of molecular weights of approximately 10, 30, and 50 kDa (Figures 3.2 and 3.3). Major quinoa proteins of whole quinoa and quinoa protein concentrate had similar electrophoretic patterns (Figure 3.4). These data were in agreement with reported values of 8-9 kDa, 22-39 kDa, and 50 kDa by Gorinstein et al (1996). The major fraction at approximately 30 kDa was comparable to the globulin polypeptides at 34-36 kDa, identified by Fairbanks et al (1990), and the 11S-type globulin storage protein called Chenopodin (Brinegar and Goundan, 1993), with two classes of polypeptides with reported molecular weights of 22-23 kDa and 32-39 kDa. Brinegar and Goudan (1993) reported native gel electrophoretic migration values for Chenopodin at 320,000 kDa. The major fractions at approximately 10 kDa are consistent with high-cysteine 2S-type storage proteins of quinoa reported by Brinegar et al (1996) with MW of 8-9 kDa. The 2S and 11S polypeptides comprise approximately 35% and 37% of the total protein, respectively (Brinegar et al 1996). Albumins and globulins represent 74.1-79.9% of the total seed protein for quinoa (Gorinstein et al 1996). Small proteins (< 7100 kDa) were lost due to an extended resolution time to allow improved separation of larger proteins.

Isoelectric Point Characterization

Isoelectric focusing differs from denaturing gel electrophoresis by the following: focusing is performed under native rather than denaturing-reducing conditions; proteins are placed into a pH gradient rather than a fixed pH; proteins are separated on the basis of surface charge rather than molecular size. Isoelectric focusing gels are cast with ampholytes, amphoteric molecules that set up a pH gradient across the gel. Proteins migrate in gels to their neutral isoelectric point, where the protein has zero net charge.

Based on the isoelectric focusing conditions of this study, major quinoa proteins of whole quinoa and quinoa protein concentrate prepared from white polished quinoa were found to have isoelectric point of approximately 4.4, 5.4, 5.6, and 5.8 (Figure 3.5). Determining the isoelectric point of quinoa proteins is important in determining functionality in product application and value-added processing. For example, a protein is least soluble and most readily coagulated at its isoelectric point.

PERSPECTIVE

Quinoa protein concentrate for use as a value-added food ingredient has yet to be commercially developed. Understanding value-added ingredient processing of quinoa, as well as, the functional properties of quinoa proteins are necessary steps in determining suitable food product applications for quinoa protein concentrate. Extraction and concentration of quinoa protein and evaluation of its functional properties were the primary focus of the current study.

Methods of removing quinoa saponins that complement efforts in value-added ingredient processing need to be considered. Saponins need to be completely removed, but the embryo needs to remain intact so that protein and lipid bodies are not disturbed.

In addition, value-added ingredient processing of quinoa needs to be considered in its entirety. Every step must consider effects on other components, such as starch, oil, and fiber. For example, increasing protein recoveries, by damaging starch granules is senseless. Quinoa lipids are a valuable source of edible oil and need to be removed in the initial processing in order to minimize interference with remaining extraction and improve the quality of starch and protein ingredients. Yet selection of oil extraction and drying methods that minimize protein denaturation are also factors. Betalain pigments are a source of non-protein nitrogen in quinoa and need to be considered in protein recovery studies, as well as, included in value-added processing. Betalain pigments are more stable than anthocyanins and are used in the industry as natural food dyes.

Protein concentrates are highly valued ingredients added to thousands of food products. Protein concentrates in this study were made from Colorado white, polished quinoa that had protein contents of 93%. This is comparable to protein isolate percentages that bring higher value in the marketplace than concentrates.

Selection of genotypes suitable for ingredient processing is necessary in order to maximize efficiency of ingredient production. Understanding functional properties of quinoa ingredients, such as protein concentrate, will allow for proper selection of food product application. The unique attributes of quinoa grain, such as high protein content and high protein quality, are best utilized with value-added processing and will increase the potential for quinoa to become a valued agricultural crop.

CONCLUSIONS

Based on the conditions of this study the following conclusions can be made:

- With the exception of ash content, proximate composition values of whole grain materials depended on genotype and abrasion condition.
- There was no difference in saponin content in the black or white genotype in the unpolished quinoa. When black and white quinoa genotypes were polished saponin was reduced, but the magnitude of the reduction was less in black quinoa compared to white quinoa.
- The fat content was higher in the white quinoa than the black quinoa genotype before polishing. No effect of polishing was found on the fat content of the black quinoa genotype, but an 11% loss in fat was found in the white quinoa after polishing.
- The nitrogen content was higher in the black quinoa than the white genotype before polishing. Nitrogen increased after polishing, but the magnitude was greater in the white quinoa compared to the black quinoa.
- Main effects were found for ash content. White quinoa was higher in ash than black quinoa and polished quinoa was higher in ash than unpolished quinoa.
- Main effects were found for 1000-grain weight. Black quinoa had a higher 1000-grain weight than white quinoa and unpolished quinoa was higher than polished quinoa.
- Yield loss due to polishing was 13% in black or white quinoa.
- Defatted quinoa was lower in nitrogen content (2.3%) than whole quinoa, regardless of genotype or abrasion condition.

- Nitrogen content and recovery of prepared protein concentrate was dependent on genotype and abrasion condition. Nitrogen content of quinoa protein concentrates increased with polishing, but the magnitude was higher in the white quinoa than the black quinoa.
- Nitrogen recoveries were highest in white quinoa genotype. Nitrogen recoveries were increased with polishing in black quinoa, but nitrogen recoveries decreased with polishing in the white quinoa.
- Mean nitrogen values for quinoa protein concentrate ranged from 11.23 to 14.83% (dwb).
- Amino acid analyses were comparable to reported values for quinoa.
- Chemical scores were 91 for quinoa protein concentrate and 80 for whole quinoa.
- Major quinoa proteins with molecular weights approximately 11 and 30 kDa were resolved with electrophoresis and are consistent with 11S globulins and 2S albumins.
- Major proteins with isoelectric points of 4.4, 5.4, 5.6, and 5.8 were determined using isoelectric focusing.

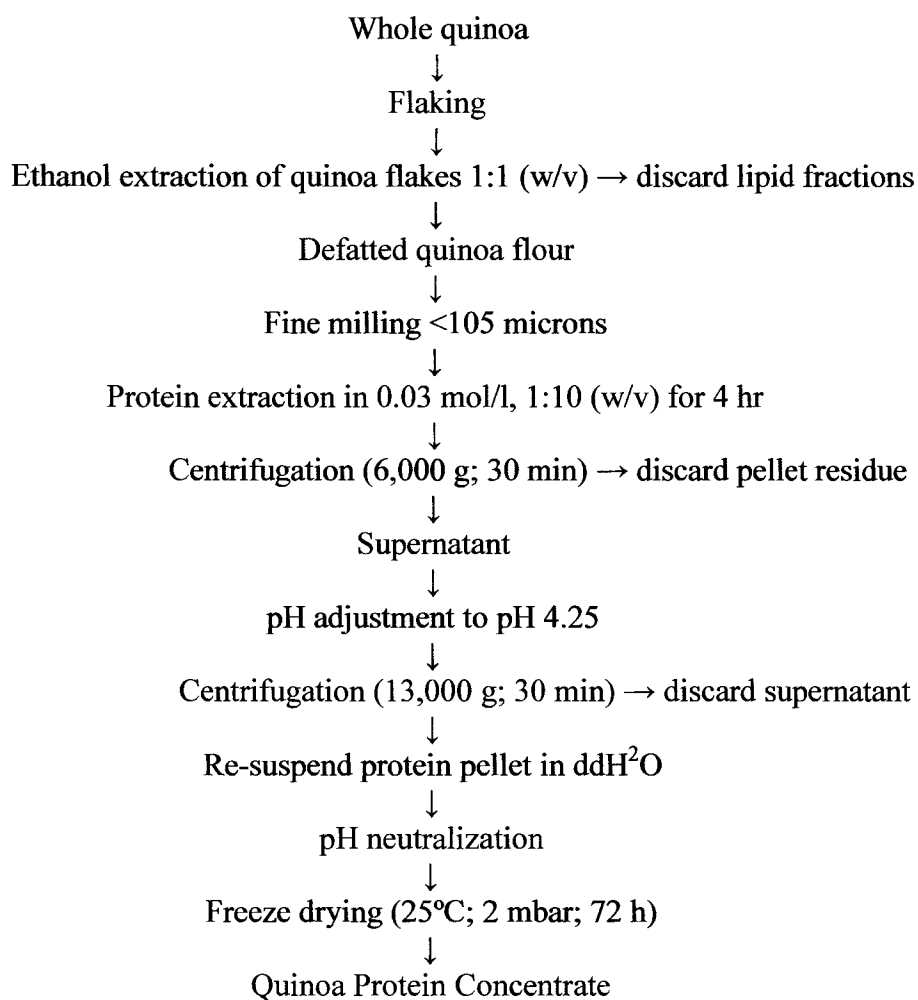


FIGURE. 3.1. Protein Concentrate Preparation of Quinoa (*Chenopodium quinoa*).

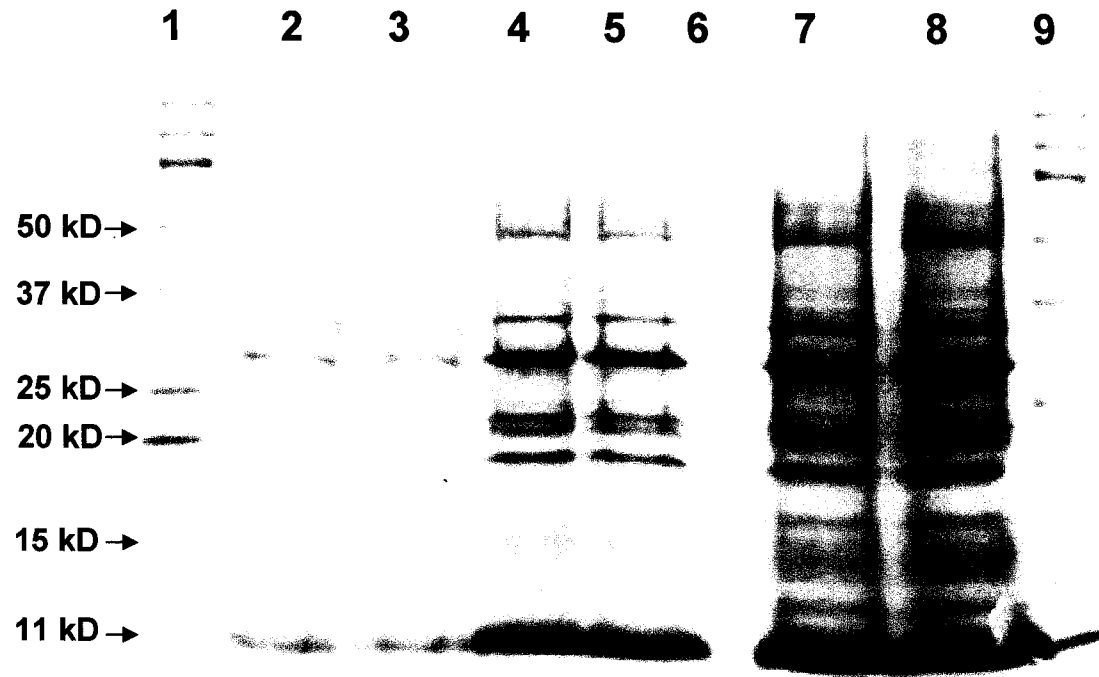


FIGURE 3.2. SDS-PAGE on a 12% polyacrylamide gel under denaturing-reducing conditions. Evaluation of whole quinoa (prepared from white polished quinoa). Lane 1, Kaleidoscope MWM (Bio-Rad catalog # 161-0375); Lane 2 and 3, whole quinoa 2 μ l; Lane 4 and 5, whole quinoa 5 μ l; Lane 6, empty; Lane 7 and 8, whole quinoa 10 μ l; Lane 9, Kaleidoscope MWM (Bio-Rad catalog # 161-0375). Gel was stained with Coomassie Blue, destained and imaged on a Bio-Rad ChemiDoc® XRS system.

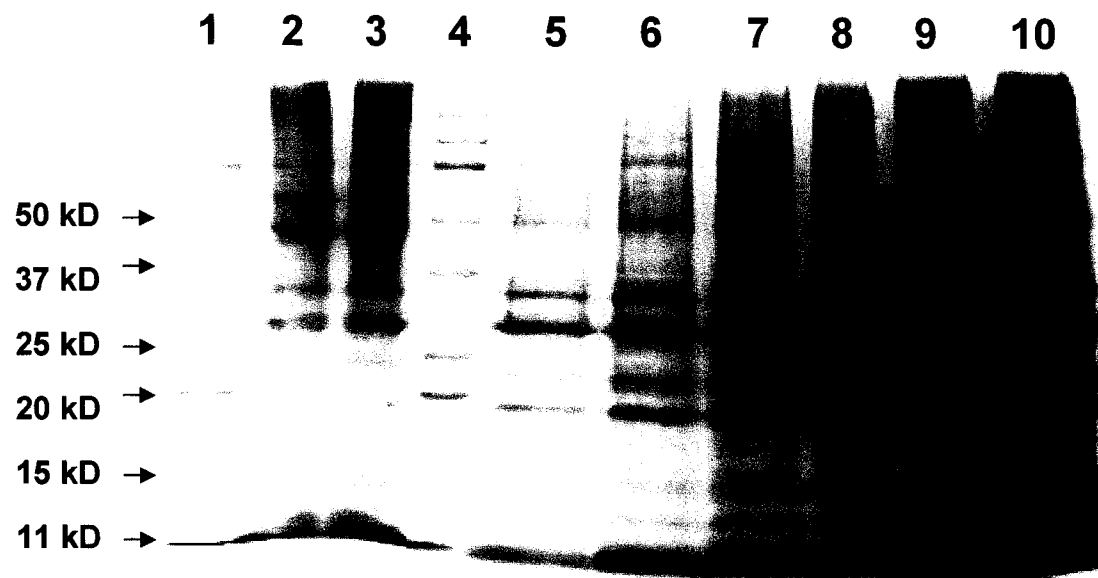


FIGURE 3.3. SDS-PAGE on a 12% polyacrylamide gel under denaturing-reducing conditions. Evaluation of quinoa protein concentrate (prepared from white polished quinoa). Lane 1, Kaleidoscope MWM (Bio-Rad catalog # 161-0375); Lane 2, 3, 5, and 6 quinoa protein concentrate 2 µl; Lane 4, Kaleidoscope MWM (Bio-Rad catalog # 161-0375); Lane 7 and 8, quinoa protein concentrate 5 µl; Lane 9 and 10, quinoa protein concentrate 10 µl. Gel was stained with Coomassie Blue, destained and imaged on a Bio-Rad ChemiDoc® XRS system.

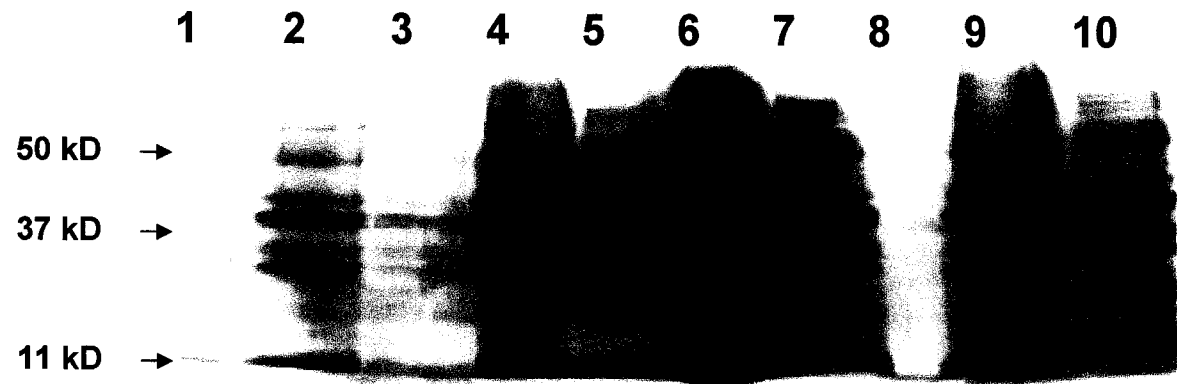


FIGURE 3.4. SDS-PAGE on a 12% polyacrylamide gel under denaturing-reducing conditions. Evaluation of whole quinoa and quinoa protein concentrate (prepared from white polished quinoa). Lane 1, Kaleidoscope MWM (Bio-Rad catalog # 161-0375); Lane 2, quinoa protein concentrate 2 µl; Lane 3, whole quinoa 2 µl; Lane 4 and 9, quinoa protein concentrate 10 µl; Lane 5 and 10, whole quinoa 10 µl; Lane 6, quinoa protein concentrate 20 µl; Lane 7, whole quinoa 20 µl; Lane 8, empty. Gel was stained with Coomassie Blue, destained and imaged on a Bio-Rad ChemiDoc® XRS system.

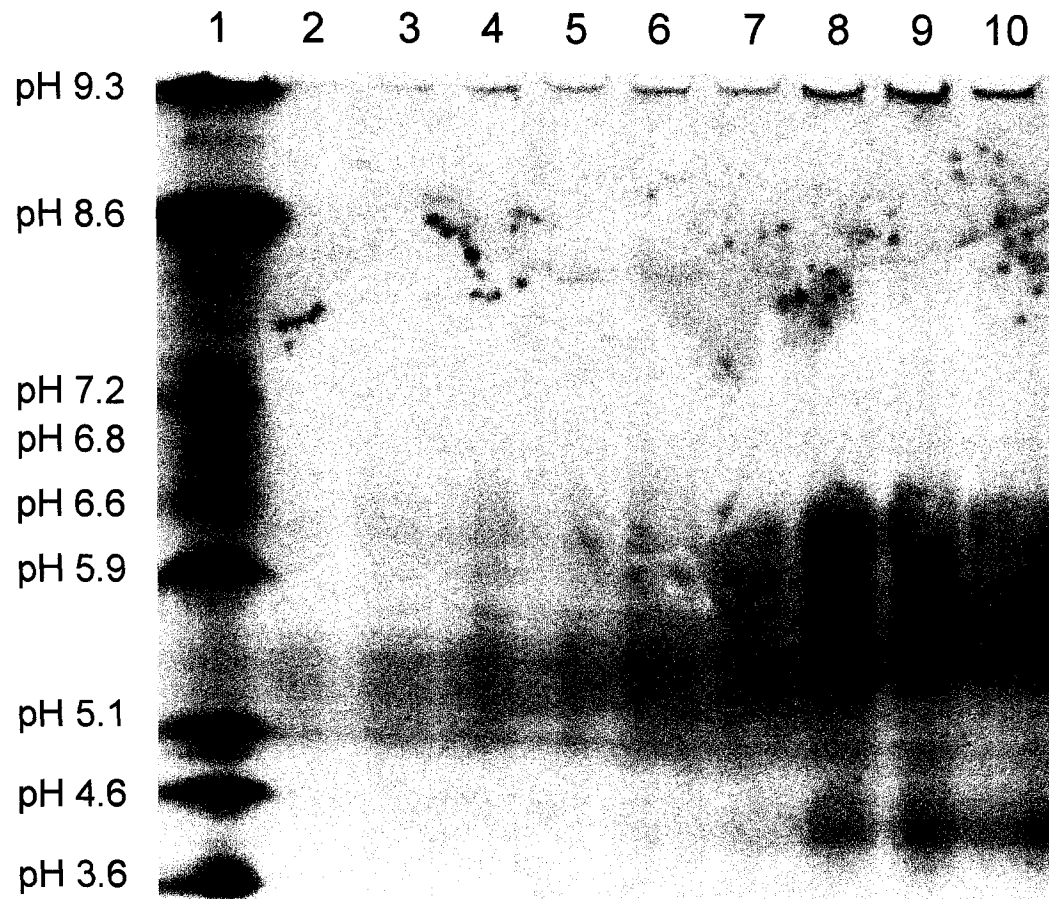


FIGURE 3.5. IEF on Ready Gel IEF, pH range 3-10 under native conditions. Evaluation of whole quinoa and quinoa protein concentrate (prepared from white polished quinoa). Lane 1, IEF pI markers (Sigma I-3018); Lane 2, 3, and 4, quinoa protein concentrate 2.5, 5, and 10 μ l; Lane 5, 6, and 7, quinoa protein concentrate 2.5, 5, and 10 μ l; Lane 8, 9, and 10, whole quinoa 2.5, 5, and 10 μ l. Gel was stained with Coomassie Blue, destained and imaged on a Photodyne Variquest 100 transilluminator (Photodyne Technologies Inc) using a yellow filter.

CHAPTER 4

PERFORMANCE OF QUINOA PROTEIN CONCENTRATE IN FORTIFIED WHEAT PASTA

ABSTRACT

Wheat pasta was fortified with quinoa protein concentrate at two levels, 3.5% or 7.0% (fwb) and effects of formulation modification and cooking times on cooking characteristics were evaluated. Effects of formulation modification on color of dry pastas and sensory characteristics of cooked pastas were also evaluated. A consumer sensory panel rated pasta treatments for sensory characteristics using 9-point hedonic scales. No differences were found in sensory scores when pasta was fortified with quinoa concentrate. Differences in cooking characteristics and color of dry pastas were found among pasta treatments. There was an increase in cooked weights as cooking time increased from 12 to 16 min and an increase in loss of solids as cooking time increased from 12 to 14 min. Loss of solids was comparable for control pasta and pasta fortified with quinoa protein concentrate at level of 3.5% and increased for pastas fortified at levels of 3.5% to 7.0%. Hunter Color L-, a-, and b-values were reduced with formulation modification as compared to control pasta. Pastas fortified with quinoa protein concentrate at levels of 3.5% or 7.0% were darker, less red, and less yellow than that of pasta control. Lysine content was increased by 40% or 80% by formulation modification

of semolina pasta with quinoa protein concentrate at levels of 3.5% or 7.0%, respectively, without affecting sensory characteristics.

INTRODUCTION

Traditional cereals are limiting in lysine which result in poor protein quality. Several authors have incorporated whole quinoa flour into cereal flours in order to increase lysine content in attempts to improve protein quality of cereal-based products. Tapia (1979) proposed incorporating quinoa at concentrations above 12% would improve protein quality of various cereal-based foods, but found that levels greater than 20% give unacceptable products. Coulter and Lorenz (1991a) extruded quinoa at levels of 10, 20, and 30% in combination with corn grits to determine various nutritional and functional properties. At 30% quinoa addition, lysine content was increased 50% above that of corn grits alone and lysine was not degraded at the extrusion temperature of 146°C. However, frying the extrudates at 190°C caused a loss in lysine content, as expected. Nevertheless, the highest quinoa level (30%) had the poorest extrusion properties (Coulter and Lorenz 1991b). Caperuto et al (2000) evaluated corn pasta fortified with quinoa flour and found that even 10% addition of quinoa flour to corn flour was sufficient to improve the lysine content of the pasta product by a factor of almost three. In order to make the two flours compatible in terms of particle size and cohesiveness to produce a quality pasta product, it was necessary for the authors to mill the quinoa grain, resulting in 72% decrease in protein content compared to that of whole quinoa flour. At 1:9 ratio, milled quinoa flour to corn flour, the contribution of lysine from quinoa flour and corn flour was 0.022% and 0.085%, respectively, with a total lysine content of 0.11% in the blended flour used to make pasta, in comparison to 0.09% lysine content of pasta made from 100% corn flour.

Although the lysine content was improved with addition of quinoa flour, the improvement was at most 20% above the level of pasta made with 100% corn flour, not a 3-fold increase as the authors had concluded. Vela and Cabrera (1984) showed that substitution of whole quinoa flour at amounts greater than 15% resulted in aesthetically unacceptable wheat pasta and corn arepas (cakes). The accepted level of fortification of cereal flours with whole quinoa flour is 15% and lysine contents of wheat, barley, and corn flours can be improved by 18%, 24%, and 35%, respectively (Koziol 1992).

In either case, flours made from whole or milled quinoa used to fortify cereal flours, have a lower protein content compared to a protein concentrate made from quinoa. By using quinoa protein concentrate, instead of whole or milled quinoa flour, it may be possible to increase lysine content of cereal-based products, without negatively effecting product quality by minimizing the percent modification of cereal flours.

Pastas, made from durum wheat semolina, are the most frequently consumed pasta product, sensory attributes are well-recognized and easily assessed by un-trained consumer sensory panelists. Protein quality of wheat pasta could be improved with fortification of quinoa protein concentrate. Pasta cooking temperatures are considered mild compared to the high-heat of frying therefore lysine degradation should not occur. Effects of product modification on sensory and quality attributes must be considered in the development of fortified products (Setser and Racette 1992) and is necessary in determining suitable applications for quinoa protein concentrate.

The objectives of this study were to 1) evaluate effects of three wheat pasta formulations (control pasta with no fortification, pasta fortified with quinoa protein concentrate at levels of 3.5%, or 7.0%) and four cooking times (12, 14, 16, or 18 min) on

cooking characteristics (cooked weight increase and loss of solids), and 2) evaluate effects of three wheat pasta formulations (control pasta with no fortification, pasta fortified with quinoa protein concentrate at levels of 3.5%, or 7.0%) on pasta color (Hunter Color L-, a-, and b-values) and sensory characteristics (appearance, texture, flavor, and overall acceptability).

MATERIALS AND METHODS

Materials

Commercial white quinoa, mechanically abraded (polished) to remove the saponin-rich pericarp, was purchased from White Mountain Farm (Mosca, Colorado). Quinoa protein concentrate was prepared from white, polished quinoa using an isoelectric precipitation method as described in Chapter 3. Durum wheat semolina (UNF1 CO 2693849088) was purchased from Wild Oats Market (Ft Collins, CO).

Pasta Formulations and Preparation

Wheat pasta, or control pasta, prepared without addition of quinoa protein concentrate, is typically 50% water on a flour (semolina) weight basis (fwb). Control pasta and pastas fortified with quinoa protein concentrate were prepared using the formulations shown in Table 4.1.

TABLE 4.1
Pasta Formulations (fwb) for Control and Pasta Fortified with Quinoa Protein Concentrate

Ingredients	Control	Fortification (fwb)	
		3.5%	7.0%
Durum wheat semolina	100	100	100
Quinoa protein concentrate	0	3.6	7.2
Distilled water	50	50	50

Durum wheat semolina and quinoa protein concentrate contained 12.9% and 89.8% protein (as is basis), respectively. Quinoa protein concentrate was added to durum semolina to obtain fortification levels of 3.5% or 7.0% (fwb), which gave 15.5% total protein (20% increase in protein) and 18.1% total protein (40% increase in protein), respectively. Dry ingredients and water were blended by hand until a cohesive-dough was formed. Dough was extruded into pasta using a manual extruder Model 150 mm Series No. 1048534 (Marcato Atlas, Padova, Italy). Pastas were air-dried overnight and stored at 0°C until analyzed.

Cooking Characteristics of Pasta

Cooked weight increase and loss of solids are standard measurements of pasta used to assess cooking characteristics and to determine optimal cooking time (end point of boiling). Cooked weight increase and loss of solids of pasta formulations were determined by AACC method 16-50 (1995) with some modifications. A 10 g portion of uncooked pasta was placed in 100 ml boiling distilled water and cooked to 12, 14, 16, or 18 min. Cooked weight increase (presented as cooked weight) was calculated by subtracting the weight of uncooked pasta (10 g) from that of cooked and drained pasta. Loss of solids was calculated from the residue obtained by evaporating the cooking water in an oven at 55°C to constant weight.

Amino Acid Analysis

Amino acid analysis of cooked pasta formulations was determined by acid hydrolysis and HPLC using an internal method (detection level 0.005%) of Medallion Labs (Minneapolis, MN). Pasta formulations were cooked to 16 min, drained, and stored

at 0°C until analyzed. Amino acid analysis for three cooked pastas was measured in duplicate.

Sensory Analysis

Sensory research was approved by the Human Research Committee (Office of Regulatory Compliance, CSU, Ft Collins, CO) and a consent form was signed by each panelist (Figure 1, Appendices). Sensory characteristics were determined for cooked pastas. Pastas were cooked to 16 min, drained, and portioned into 25-30 g (approximately 1 oz.) samples. Samples were immediately evaluated by 45 consumer panelists, both male and female, aged 18-30 years. The panelists evaluated the samples for four sensory characteristics (appearance, texture, flavor, and overall acceptability) using a nine-point, hedonic scale ranging from “like extremely” to “dislike extremely” (Figure 2, Appendices).

Color Analysis

Color scores are typical quality control measurements used to assess the color of intact dry pasta. Color scores L-, a-, and b-values for dry pasta formulations were determined using HunterLab Colorimeter (Reston, VA) with calibration standards of L = 78.2, a = -2.4, and b = 21.9 and measured in triplicate. Positive values for L-, a-, and b-values represent brightness, redness, and yellowness, respectively, and negative values for L-, a-, and b-values represent darkness, greenness, and blueness, respectively.

Statistical Analysis

Cooking Characteristics of Pasta

A two-factor 3 x 4 factorial split plot design with the whole plots arranged as a randomized block design was replicated 4 times (replications = blocks). The whole plot

effect was the level of pasta formulation and the split plot effect was the cooking time. The factor of pasta formulation had three levels (control pasta with no fortification, pasta fortified with quinoa protein concentrate at levels of 3.5%, or 7.0%). The factor of cooking time had four levels (12, 14, 16, or 18 min). Response variables measured on the twelve treatments were cooked weights and loss of solids. Data were analyzed by two-way analysis of variance (ANOVA) at 95% confidence level using SAS GLM procedures (v. 8.1 for Windows, SAS Institute, Cary, NC). Least significant difference (LSD) tests at $P < 0.05$ were used to separate means when significant differences were found.

Amino Acid Analysis

Means of duplicate samples and standard deviations were calculated for amino acid analysis of cooked pastas.

Sensory Analysis

The factor of pasta formulation had three levels (control pasta with no fortification, pasta fortified with quinoa protein concentrate at levels of 3.5%, or 7.0%). Response variables measured on the three treatments were the sensory characteristics appearance, texture, flavor, and overall acceptability. Sensory data were decoded using a nine-point, hedonic scale and analyzed by one-way analysis of variance (ANOVA) at 95% confidence level using SAS GLM procedures (v. 8.1 for Windows, SAS Institute, Cary, NC). Least significant difference (LSD) tests at $P < 0.05$ were used to separate means when significant differences were found.

Color Analysis

The factor of pasta formulation had three levels (control pasta with no fortification, pasta fortified with quinoa protein concentrate at levels of 3.5%, or 7.0%).

Response variables measured on the dry pasta from three treatments were color L-, a-, and b-values. Data were analyzed by one-way analysis of variance (ANOVA) at 95% confidence level using SAS GLM procedures (v. 8.1 for Windows, SAS Institute, Cary, NC). Least significant difference (LSD) tests at $P < 0.05$ were used to separate means when significant differences were found.

RESULTS AND DISCUSSION

Cooking Characteristics of Pasta

The ANOVA for the significance of pasta formulations and cooking times on cooked weight (Table 4.2) indicated that the model was significant for cooking times but not significant for pasta formulations. Mean cooked weights increased from 12 to 16 min (Table 4.3).

TABLE 4.2

Analysis of Variance for the Significance of Pasta Formulations x Cooking Times on Cooked Weights

Effect	Num df	Den df	F value	Prob > F
Pasta formulations	2	36	1.54	0.2290
Cooking times	3	36	40.23	< 0.0001
Pasta formulations x Cooking times	6	36	0.14	0.9902

TABLE 4.3

Mean Cooked Weights (g) for Cooking Times: 12, 14, 16, or 18 min^{a,b}

Cooking Times (min)	Cooked Weights (g)
12	10.69 ± 1.03 a
14	12.09 ± 0.86 b
16	13.60 ± 0.74 c
18	14.21 ± 0.60 c

^aValues followed by the same letter are not significantly different ($P < 0.05$).

^bValues reported as mean of twelve samples ± one standard deviation.

Although cooking times of 16 or 18 min were not significantly different, there was a trend for mean cooked weights to increase from 12 to 18 min. Pasta formulations by cooking times interaction was not significant for cooked weights.

The ANOVA for the significance of pasta formulations and cooking times on loss of solids (Table 4.4) indicated that the model was significant for both cooking times and

TABLE 4.4
Analysis of Variance for the Significance of Pasta Formulations x Cooking Times on Loss of Solids

Effect	Num df	Den df	F value	Prob > F
Pasta formulations	2	36	4.83	0.0139
Cooking times	3	36	4.88	0.0060
Pasta formulations x Cooking times	6	36	0.21	0.9710

pasta formulations. Loss of solids increased from fortification 3.5% to fortification 7.0% and from control to fortification 7.0% (Table 4.5).

TABLE 4.5
Mean Loss of Solids (g) for Pasta Formulations^{a,b}

Pasta Formulations	Loss of Solids (g)
Control	0.38 ± 0.10 a
Fortification at 3.5%	0.40 ± 0.09 a
Fortification at 7.0%	0.47 ± 0.08 b

^aValues followed by the same letter are not significantly different ($P < 0.05$).

^bValues reported as mean of sixteen samples ± one standard deviation.

Although control wheat pasta or pasta with fortification of quinoa protein concentrate at 3.5% were not significantly different, there was a trend for mean loss of solids to increase with quinoa protein fortification of wheat pasta. Loss of solids increased from cooking time 12 to 14 min (Table 4.6). Although times 14, 16, or 18 min were not significantly different, there was a trend for mean loss of solids to increase with cooking time. Pasta formulations by cooking times interaction was not significant for loss of solids.

TABLE 4.6
Mean Loss of Solids (g) for Cooking Times: 12, 14, 16, or 18 min^{a,b}

Cooking Times (min)	Loss of Solids (g)
12	0.34 ± 0.10 a
14	0.42 ± 0.09 b
16	0.44 ± 0.08 b
18	0.46 ± 0.08 b

^aValues followed by the same letter are not significantly different ($P < 0.05$).

^bValues reported as mean of twelve samples ± one standard deviation.

There was an increase in cooked weights as cooking time increased from 12 to 16 min and an increase in loss of solids as cooking time increased from 12 to 14 min. Cooked weights at 16 or 18 min and loss of solids at 14, 16, or 18 min were statistically equal. In addition, the cooking time at which there was no visible center core (a criterion of the condition of the central hylum to determine the optimum cooking point) was observed at 16 or 18 min, but not at 12 or 14 min. Since 16 min or 18 min were equivalent, the shorter cooking time of 16 min was selected to be the optimal cooking time or cooking end point.

Amino Acid Analysis

Amino acid values for cooked pasta formulations are presented in Table 4.7. Quinoa protein fortification levels of 3.5% or 7.0% were calculated to achieve a 40% or 80% increase in lysine, respectively, above control wheat pasta. Lysine contents for cooked pasta (Table 4.7) reflected these amounts when compared to the control.

Sensory Analysis

The ANOVA for the significance of pasta formulations on sensory characteristics (Table 4.8) indicated that the model was not significant for appearance, texture, flavor, or overall acceptability. Formulation modification of semolina control pasta with quinoa protein concentrate at levels of 3.5% or 7.0% was achieved without affecting sensory

TABLE 4.7

Amino Acid Content (%) for Cooked Pasta Formulations ^{a,b}

Amino Acid	Control	Fortification (fwb)	
		3.5%	7.0%
Aspartic Acid	0.27 ± 0.01	0.36 ± 0.03	0.52 ± 0.03
Threonine ^c	0.17 ± 0.00	0.21 ± 0.00	0.24 ± 0.01
Serine	0.35 ± 0.03	0.40 ± 0.01	0.45 ± 0.01
Glutamic Acid	1.89 ± 0.06	2.08 ± 0.07	2.31 ± 0.07
Proline	0.54 ± 0.01	0.57 ± 0.01	0.65 ± 0.01
Glycine	0.21 ± 0.00	0.25 ± 0.01	0.30 ± 0.00
Alanine	0.35 ± 0.01	0.43 ± 0.01	0.51 ± 0.01
Cystine ^c	nd	nd	nd
b-Valine ^c	0.24 ± 0.01	0.28 ± 0.00	0.37 ± 0.03
Methionine ^c	0.11 ± 0.00	0.14 ± 0.00	0.16 ± 0.01
b-Isoleucine ^c	0.21 ± 0.01	0.25 ± 0.01	0.30 ± 0.03
b-Leucine ^c	0.42 ± 0.00	0.48 ± 0.04	0.61 ± 0.06
Tyrosine	0.19 ± 0.01	0.24 ± 0.00	0.31 ± 0.01
Phenylalanine ^c	0.28 ± 0.03	0.34 ± 0.01	0.42 ± 0.03
Histidine ^c	0.14 ± 0.01	0.19 ± 0.00	0.21 ± 0.00
Lysine^c	0.13 ± 0.00	0.19 ± 0.01	0.25 ± 0.01
Arginine	0.24 ± 0.00	0.40 ± 0.01	0.51 ± 0.00

^aMean of 2 samples.

^bValues reported as mean of two samples ± one standard deviation.

^cEssential amino acids.

nd, not determined.

TABLE 4.8

Analysis of Variance for the Significance of Cooked Pasta Formulations on Sensory Characteristics: Appearance, Texture, Flavor, or Overall Acceptability

Sensory Characteristics	Effect	Num df	Den df	F value	Prob > F
Appearance	Pasta Formulation	2	131	2.80	0.0645
Texture	Pasta Formulation	2	131	0.62	0.5400
Flavor	Pasta Formulation	2	131	1.67	0.1922
Overall Acceptability	Pasta Formulation	2	130	0.71	0.4923

characteristics. All pasta formulations received sensory scores ranging from 3.3 to 4.3, which corresponded to “like moderately” to “like slightly” (Table 4.9).

TABLE 4.9
Mean Sensory Scores for Cooked Pasta Formulations^{a,b,c}

Sensory Characteristics	Control	Fortification (fwb)	
		3.5%	7.0%
Appearance	3.5 ± 1.5	3.7 ± 1.4	4.3 ± 1.8
Texture	3.6 ± 1.6	4.0 ± 1.7	3.7 ± 1.6
Flavor	3.3 ± 1.5	3.9 ± 1.6	3.4 ± 1.8
Overall Acceptability	3.3 ± 1.5	3.7 ± 1.3	3.5 ± 1.5

^aScores were not significantly different ($P < 0.05$).

^bValues reported as mean of forty five samples ± one standard deviation.

^cScores were based on the following hedonic scale:

1 = like extremely	4 = like slightly	7 = dislike moderately
2 = like very much	5 = neither like nor dislike	8 = dislike very much
3 = like moderately	6 = dislike slightly	9 = dislike extremely

Color Analysis

The ANOVA for the significance of dry pasta formulations on color (Table 4.10) indicated that the model was significant for all color scores: L-, a-, and b-values.

TABLE 4.10
Analysis of Variance for the Significance of Dry Pasta Formulations on Hunter Color L-, a-, and b-values

Hunter Color Score	Effect	Num df	Den df	F value	Prob > F
L-value	Pasta Formulation	2	6	63.11	<0.0001
a-value	Pasta Formulation	2	6	11.37	0.0091
b-value	Pasta Formulation	2	6	25.10	0.0012

Mean color scores are given in (Table 4.11).

TABLE 4.11
Mean Hunter Color L-, a-, and b-values for Dry Pasta Formulations^{a,b}

Color Score	Control	Fortification (fwb)	
		3.5%	7.0%
L-value	68.53 ± 0.87 a	61.73 ± 0.72 b	60.89 ± 1.11 b
a-value	5.18 ± 0.27 a	4.69 ± 0.13 b	4.54 ± 0.03 b
b-value	32.64 ± 0.86 a	29.65 ± 0.59 b	29.41 ± 0.29 b

^aValues followed by the same letter in the same row are not significantly different ($P < 0.05$).

^bValues reported as mean of three samples ± one standard deviation.

All color values for dry pasta formulations were on the positive spectrum, which indicated brightness, redness, and yellowness. Wheat pasta fortified with quinoa protein concentrate at levels of 3.5% or 7.0% was significantly darker, less red, and less yellow than wheat pasta control.

Change in the color of wheat pasta with formulation modification is unfavorable and could possibly affect consumer acceptance at time of purchase. The difference in color found in this study was slight, yet apparent upon visual observations of the dry pasta. Even so, there were no significant differences in appearance scores of the cooked pasta formulations evaluated by consumer panelists and scores were on the favorable side of the scale (Table 4.9).

PERSPECTIVE

Protein fortification of cereal-based products is important, particularly in diets that are nutritionally inferior. This study indicated that it was possible to fortify pasta with quinoa protein concentrate at levels well below accepted maximum levels of whole or milled quinoa flour without negatively affecting sensory characteristics. It is suggested that quinoa protein concentrate can be used to fortify wheat pasta in order to increase lysine content and improve protein quality, however, additional amino acid and protein bioavailability studies should be conducted. Effects of product modification of cereal-based foods on sensory and quality attributes must be considered in determining suitable applications for quinoa protein concentrate, as well as processing conditions that would negate effects of fortification.

CONCLUSIONS

Based on the conditions of this study the following conclusions can be made:

- No differences were found in sensory scores when pasta was fortified with quinoa concentrate. Lysine content was increased by 40% or 80% by formulation modification of semolina pasta with quinoa protein concentrate at levels of 3.5% or 7.0%, respectively, without affecting sensory characteristics.
- Differences in cooking characteristics were found among pasta treatments. There was an increase in cooked weights as cooking time increased from 12 to 16 min and an increase in loss of solids as cooking time increased from 12 to 14 min. Loss of solids was comparable for control pasta and pasta fortified with quinoa protein concentrate at level of 3.5% and increased for pastas fortified at levels of 3.5% to 7.0%.
- Differences in color of dry pastas were found among pasta treatments. Hunter Color L-, a-, and b-values were reduced with formulation modification as compared to control pasta. Pastas fortified with quinoa protein concentrate at levels of 3.5% or 7.0% were darker, less red, and less yellow than that of pasta control.

CHAPTER 5

IMPLICATIONS

Protein concentrates are high-value ingredients added to thousands of food products. A quinoa protein concentrate of high protein content and favorable protein recovery was achieved in this study and research efforts made significant contribution to the understanding of quinoa grain fractionation, processing, and production of value-added ingredients. Though, processing of quinoa is in its infancy in comparison to that of traditional cereals and legumes and research efforts need to continue in this area. Understanding the physical and chemical properties of quinoa ingredients, such as protein concentrate, is needed to determine proper food applications. Effects of product modification of cereal-based foods on sensory and quality attributes must be considered in determining quinoa protein concentrate functionality. Extreme processing conditions of finished products that may negate effects of quinoa protein fortification also need to be considered. Continued research in breeding and yield of quinoa is necessary, as well as, selection of genotypes suitable for ingredient processing in order to maximize efficiency of ingredient production.

Quinoa has a future for use as a commercial vegetable protein, either as an alternative to allergenic proteins or to augment current demands for nutritious protein. As most of the protein consumed worldwide comes from a narrow selection of crops, new protein resources must be developed from alternate cereals and pseudocereals.

Protein-rich crops, like quinoa, that give equitable yields in underutilized growing regions are of paramount value. Quinoa is adapted to environmental extremes such as high altitudes, harsh climates and sandy soils that are unfavorable to major cereals and legumes.

The superior nutritional quality and hypo-allergenic status of quinoa protein is well-suited as a value-added ingredient that has use in fortification of cereal-based products, nutritional and vegetarian products, as well as, animal feeds and supplements. The amino acid composition is of higher quality than that of wheat, barley, and soybean. Quinoa protein is particularly high in lysine and methionine+cysteine, amino acids that are limiting in cereals and legumes, respectively. Quinoa is also a good source of histidine, coupled with the high bioavailability and hypo-allergenic status, makes it suitable for infant products. The quality of quinoa protein is comparable to milk protein, which is rare for a plant protein to resemble that of an animal protein.

Value-added ingredient processing of quinoa needs to be considered in its entirety. Every step must considered effects on other components, such as starch, oil, and fiber. Quinoa contains many valuable components, including saponins and betalain pigments, that have pharmaceutical and food-dye applications, respectively. Processing steps that minimize damage to other components that maximize yields of each component need to be considered with value-added ingredient processing.

Quinoa has been investigated by North American and European researchers and industrialist since the early 1980's but has failed to reach its commercial potential. Interest in quinoa is on the rise, from the scientific community and the health food industries as indicated by a rise in imports from South America and consumer recognition

through recent news articles. Analogous to the production and processing of commodity crops, quinoa will not see its potential without value-added ingredient processing. The new FDA regulation mandating that allergens need to be clearly labeled by food manufacturers, opens up an immediate opportunity for quinoa ingredients. Developing value-added ingredients such as protein concentrate, not only bring economic value above whole grain, but open up a vast product market. The unique attributes of quinoa grain, such as high protein content and high protein quality, are best utilized with value-added processing and will increase the potential for quinoa to become a valued agricultural crop.

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APPENDICES

COLORADO STATE UNIVERSITY
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Sensory evaluation of pasta fortified with quinoa protein concentrate

NAME OF PRINCIPAL INVESTIGATOR: Martha B. Stone

NAME OF CO-INVESTIGATOR: Laurie A. Scanlin

CONTACT NAME AND PHONE NUMBER FOR QUESTIONS/PROBLEMS: Laurie Scanlin
(970) 491-3874

PURPOSE OF THE RESEARCH:

You will evaluate the quality of pasta containing quinoa. You will be asked to state your degree of "liking" of the appearance, texture, flavor and overall acceptability of the pasta. The purpose of the project is to add quinoa protein to wheat pasta in order to improve the nutritional quality and maintain pasta quality.

PROCEDURES/METHODS TO BE USED:

You will receive 3 samples of pasta, a control and two samples of pasta fortified with quinoa protein concentrate (less than 50 grams of pasta total). You will be asked to evaluate each sample based on the following sensory characteristics: color, texture, flavor and overall acceptability. This will be a one-time evaluation for each product and will take less than 15 minutes. No training is needed for your participation.

RISKS INHERENT IN THE PROCEDURES:

No known risks. Individuals allergic to milk, wheat, quinoa or cereal grains may not participate. Ingredients in the pasta are: wheat semolina and quinoa protein. Only federally approved ingredients will be used in this study. It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known and potential, but unknown, risks.

BENEFITS:

You will gain experience in taste testing of food and could potentially impact increased use of quinoa by the food industry.

CONFIDENTIALITY:

No identifying data will be collected. Data will be stored in a locked file cabinet retained by the principal investigator for three years after the study is completed, and then destroyed. Publication of findings will be presented as group results.

LIABILITY:

The Colorado Governmental Immunity Act determines and may limit Colorado State University's legal responsibility if an injury happens because of this study. Claims against the University must be filed within 180 days of the injury.

Questions about participants' rights may be directed to Celia S. Walker at (970) 491-1563.

Page 1 of 2 Participant's initials _____ Date _____

PARTICIPATION:

Your participation in this research is voluntary. If you decide to participate in the study, you may withdraw your consent and stop participating at any time without penalty or loss of benefits to which you are otherwise entitled.

Your signature acknowledges that you have read the information stated and willingly sign this consent form. Your signature also acknowledges that you have received, on the date signed, a copy of this document containing 2 pages.

Participant name (printed)

Participant signature

Date

Witness to signature (project staff)

Date

Obtain your parent's permission ONLY if you are under 18 years of age:

PARENTAL SIGNATURE FOR MINOR

As parent or guardian you authorize _____ (print name) to become a participant for the described research. The nature and general purpose of the project have been satisfactorily explained to you by _____ and you are satisfied that proper precautions will be observed.

Minor's date of birth

Parent/Guardian name (printed)

Parent/Guardian signature

Date

Page 2 of 2 Participant's initials _____ Date _____

FIGURE 1. Informed Consent Form for Sensory Panelists

SENSORY EVALUATION OF QUINOA PROTEIN FORTIFIED PASTA

Please rinse your mouth with water before starting and between samples. Evaluation of the pasta samples must be on an individual basis. Evaluate the three samples in the order presented, from left to right. Evaluate each sample for the following characteristics by placing the numerical score in the box below using the scoring system indicated:

Characteristic	Sample No.		
Appearance: Look at your sample and evaluate its appearance: 1=like extremely 4=like slightly 7=dislike moderately 2=like very much 5=neither like nor dislike 8=dislike very much 3=like moderately 6=dislike slightly 9=dislike extremely			
Texture: Take a bite of the sample and evaluate its texture: 1=like extremely 4=like slightly 7=dislike moderately 2=like very much 5=neither like nor dislike 8=dislike very much 3=like moderately 6=dislike slightly 9=dislike extremely			
Flavor: Take another bite of the sample and evaluate its flavor: 1=like extremely 4=like slightly 7=dislike moderately 2=like very much 5=neither like nor dislike 8=dislike very much 3=like moderately 6=dislike slightly 9=dislike extremely			
Overall acceptability: How well do you like the sample overall? 1=like extremely 4=like slightly 7=dislike moderately 2=like very much 5=neither like nor dislike 8=dislike very much 3=like moderately 6=dislike slightly 9=dislike extremely			

Comments:

Thank you for your participation!

FIGURE 2. Sensory Panelist Scorecard