STUDIES ON THE CHARACTERIZATION, BIOSYNTHESIS AND ISOLATION OF STARCH AND PROTEIN FROM QUINOA (*CHENOPODIUM QUINOA* WILLD.)

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ABSTRACT

Starches isolated from sixteen quinoa lines ranged in amylose content from 3 to 20%. With the exception of pasting temperature, large variations in pasting characteristics were found among starches and were correlated with amylose content. The gelatinization onset (44.7-53.7 ºC) and peak (50.5-61.7 ºC) temperatures and retrogradation tendencies (19.6-40.8%) were positively correlated with amylose content. No significant variation in gelatinization enthalpy was observed. Swelling, solubility, freeze-thaw stability and water-binding capacity also differed among starches and were correlated with amylose content. The wide variation in amylose content and physicochemical characteristics among quinoa starches suggests applications in a variety of food and non-food products.

Two major polypeptides with apparent molecular masses of 56 and 62 kDa were present in quinoa starch and were identified as isoforms of Granule Bound Starch Synthase I (GBSSI). The content of the two isoforms was positively correlated with the concentration of amylose in starch. Starch synthase activity in developing seed was positively correlated with the amylose concentration in starch during seed development.

An integrated process was developed for the fractionation of quinoa into starch, protein, oil and saponins. Seed was first roller milled, yielding a coarse bran fraction (48% of the seed weight) that was high in protein (22.9%, db), oil (8.8%, db), and saponins (7.4%, db), and a fine, starch-rich fraction [52% of the seed weight containing 77.2% (db) starch]. Protein, oil and saponins were extracted from the bran under optimized conditions. The protein extracts were concentrated and purified using isoelectric precipitation or ultrafiltration. The means of concentration as well as the presence of saponins strongly affected protein recovery and functionality. Starch was recovered using aqueous alkali (pH 9) to solubilize the protein followed

by centrifugation, after which the starch-rich pellet was washed and the sediment which accumulated on top of the pellet was removed. The end-products of the integrated extraction process were a crude saponin extract, a crude oil product, and several protein and starch products. Forty-one percent of the protein present in the seed was recovered as a protein product that contained over 77% (db) protein. Sixty-eight percent of the starch was recovered as a starch product that contained 97% (db) starch and 1.2% (db) protein.

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ABBREVIATIONS

AACC	American Association of Cereal Chemists
AAFC-1	Quinoa breeding line from Agriculture and Agri-food Canada
AAFC-2	Quinoa breeding line from Agriculture and Agri-food Canada
ADP	Adenosine-5'-diphosphate
ADP-Glc PPase	ADP-glucose pyrophosphorylase
ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
CELLS	Controlled ecological life support system
cpm	Counts per minute
CPV	Cold paste viscosity (RVU)
d	Particle diameter (cm)
db	Dry weight basis
dm	Dry matter
DBE	Debranching enzyme
D [4,3]	Volume mean diameter of particles as determined by low-angle light scattering
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
ds	Dehulled seed
dp	Degree of polymerization
DTT	Dithiothreitol
η	Viscosity (dyne s cm^{-1})
EDTA	Ethylenediaminetetraacetic acid
ELSD	Evaporative light scattering detector
F	Indication of the fit of a statistical model. Mean square divided by the error mean square.
FAO	Food and Agriculture Organization of the United Nations
g	Gravity
G	Centrifugal force (cm s^{-2})

GBq	Giga Becquerel (1 Bq = 1 disintegration s^{-1})
GBSSI	Granule bound starch synthase I
Glc	Glucose
Glc-1-P	Glucose-1-phosphate
GLM	General linear model
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
HPV	Hot paste viscosity (RVU)
ΔH	Gelatinization enthalpy (J g^{-1})
IEP	Isoelectric precipitation
kDa	Kilodalton
LDPE	Low density polyethylene
MWCO	Molecular weight cut-off
μ	Settling velocity (cm s^{-1})
μm	Micrometer; one millionth of a meter
NCBI	National Center for Biotechnology Information
NQC	Quinoa seed obtained from Northern Quinoa Corporation (Kamsack, SK)
ns	Not significant
NSI	Nitrogen solubility index
ω	Radial velocity of a centrifuge (cm s^{-1})
PBS	Phosphate buffered saline
PER	Protein efficiency ratio
ppm	Parts per million
ρ_f	Fluid density (g cm^{-3})
ρ_s	Particle density (g cm^{-3})
PV	Peak viscosity (RVU)
PVDF	Polyvinylidene fluoride
QC	Quinoa seed obtained from Quinoa Corporation (Gardena, CA)
r	Radius of centrifuge (cm)

R^2	Coefficient of variation (model sum of squares/ total sum of squares)
RVA	Rapid viscosity analysis
RVU	Rapid viscosity units
SBE	Starch branching enzyme
SD	Standard deviation
SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SGP	Starch granule associated protein
SS	Starch synthase
TADD	Tangential abrasive dehulling device
T_c	Gelatinization conclusion temperature (ºC)
TDF	Total dietary fibre
TFA	Trifluoroacetic acid
T_o	Gelatinization onset temperature (ºC)
T_p	Gelatinization peak temperature (ºC)
TRIS	Tris(hydroxymethyl)aminomethane
UF	Ultrafiltration
UNU	United Nations University
V_0	Initial volume of the extract (mL)
V_p	Volume of the permeate (mL)
V_r	Volume of the retentate (mL)
VCR	Volume concentration ratio
WHO	World Health Organization
WMF	Quinoa obtained from White Mountain Farm (Morca, CO)
ws	Whole seed
X1	Mean particle size of bran (μm)
X2	Liquid-to-bran ratio
X3	Centrifugal force (*g*)
Y1	Protein recovery (%)
Y2	Protein content (% db)

1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal native to South America. It was an important staple food in the Incan civilization and has been cultivated in the Andean highlands since 3,000 BC. In the Quechua language of the Incas, quinoa is the *chisya mama* or "mother grain" and is nowadays also called Incan rice. However, following the Spanish conquest, quinoa cultivation was discouraged (National Research Council 1989).

In recent years, there has been renewed interest in quinoa because of its unique and interesting properties. Its starch exists as very small granules and is reported to be low in amylose (Lorenz 1990). Its protein content and quality, with an amino acid profile similar to that of casein, are high compared to those of true cereals. Quinoa is not genetically modified and is rarely allergenic because of the absence of gluten (Berti et al. 2004). Hence, it could be used in foods designed to reduce allergies in sensitive individuals, such as celiac disease patients, and it seems ideal for specialty foods such as infant formulae (Coulter and Lorenz 1990, Javaid 1997, Morita et al. 2001). Moreover, within many South American countries there is economic pressure to reduce food imports, which would encourage local production and consumption of quinoa.

Currently, agronomists, nutritionists and the food industry are evaluating quinoa in terms of genotype improvement, agronomics and processing to encourage its further cultivation as a specialty crop in other parts of the world, especially in Western Canada. In order to be used regularly by the food industry, production has to meet the quantities and qualities required by industrial food manufacturers. It is expected that when quinoa is grown in areas to which it has adapted, it could compete with cereals in both human and animal diets. The genetic variability of quinoa is believed to be high, with cultivars being adapted to growth from sea level

to 4000 m above sea level, from 40ºS to 2ºN, and from cold, highland climates to subtropical conditions. Additionally, it is frost-resistant and can be grown under conditions of drought (Coulter and Lorenz 1990). Quinoa has been selected by the FAO as one of the crops destined to offer food security in the next century.

The major quinoa producing countries are Bolivia, Peru and Ecuador. In 2003, these three countries produced 53,000 tonnes, which was up from 19,000 tons in 1973 (FAO, www.fao.org, Dec 2004). Outside South America, quinoa is grown in the USA (Colorado and California) and in Canada. It is also cultivated experimentally in Finland and the UK.

Starch is the major component of quinoa, comprising approximately 55% of the seed. It is present in the form of small granules about 1.5 μm in diameter (Chauhan et al. 1992). Several reports have been published on the characteristics of quinoa starch (Atwell et al. 1983, Lorenz 1990, Inouchi et al. 1999, Tang et al. 2002). Other cereals and pseudocereals, such as oat, rice and amaranth, also contain small granule starches, with granules typically smaller than 5 μm in diameter. One of the consequences of small granule size is that separation of starch and protein is more difficult to achieve industrially using conventional technology. High cost as well as poor recovery and quality limit the commercial opportunities for small granule starch. At the present time, rice is the only significant commercial source. Several existing and potential uses for small granule starches have been described in the literature (Lindeboom et al. 2004).

A comparison of quinoa starches from different genotypes or cultivars has not been reported. Information in the literature suggests that considerable variability exists in the amylose content of quinoa starch (7-20%) (Lorenz 1990, Praznik et al. 1999, Tang et al. 2002). Amylose content is a very important factor affecting starch functionality, but it is not the only factor. It is not clear if the variation that exists in the reported amylose contents is truly a reflection of genetic variability or due to variations in cultural practices or environment, or both, or attributable to differences in the methods employed for amylose measurement. Amylose content affects the functional and physicochemical properties of starch, including its pasting, gelatinization, retrogradation and swelling characteristics (Li et al. 1994, Wootton

and Panozzo 1998, Baldwin 2001, Bao et al. 2001, Grant et al. 2001, Svegmark et al. 2002). No known reports exist in which the physicochemical and functional properties of quinoa starches differing in amylose content are compared. Relating the characteristics of starch from different quinoa lines to amylose content may provide insight into possible industrial or food-related applications of quinoa starch.

Starch in plants is synthesized using two different groups of enzymes, starch synthases and starch branching enzymes. Different isomers of these enzymes are present at various times during starch synthesis. Each isomer has a specific role in the formation of amylose and amylopectin. The major enzyme involved in the biosynthesis of amylose is Granule Bound Starch Synthase I (GBSSI). During the last decade, GBSSI was identified in a large variety of crops, but not in quinoa. By identifying GBSSI and measuring its content and activity in different lines, a connection will be sought between starch biosynthesis in quinoa and its amylose content.

Most studies on quinoa starch have been focused on obtaining a pure fraction in order to study its characteristics (Atwell et al. 1983, Lorenz 1990, Qian and Kuhn 1999). The only study on quinoa starch separation and purification for more commercial purposes was performed by Wilhelm et al. (1998). These studies, however, did not pay much attention to the other components of the seed. The same can be said for studies done on quinoa protein (Brinegar and Goundan 1993, Chauhan et al. 1999a and b, Aluko and Monu 2003), where the main objective was obtaining a highly purified protein for characterization purposes. No integrated process for separating quinoa into its different components has been reported. Therefore, apart from focussing on purifying quinoa starch, an integrated process will be designed whereby the protein and other non-starch components can also be recovered.

The principal objectives of this study are as follows:

1. to evaluate the physicochemical characteristics of starch isolated from several quinoa lines, thereby documenting the degree of variability in amylose content among quinoa lines and the relationship between amylose content and functionality;

2. to determine the content and activity of GBSSI in quinoa lines and its relationship to amylose content;
3. to extract starch from quinoa;
4. to extract and concentrate protein from quinoa;
5. to develop an integrated process for the fractionation of quinoa seed.

Several questions arose during the development of an integrated process for quinoa fractionation, which led to separate projects with the following objectives:

1. to clarify the complexity and uniqueness of quinoa fractionation in comparison to that of other grains (i.e., barley, rice, buckwheat, corn);
2. to evaluate differences in composition and α-amylase activity among three quinoa lines that were obtained in bulk, and the effect of limited dehulling on seed composition;
3. to investigate the possibility of taking advantage of the quinoa seed structure in the separation of quinoa into its components;
4. to compare two methods for concentrating and purifying the extracted protein; i.e., isoelectric precipitation and ultrafiltration, and to determine their effect on protein composition and functionality.

2. LITERATURE REVIEW

2.1 Quinoa

2.1.1 Structure

Quinoa seed ranges in colour from white and yellow to red, brown and black. The seed is approximately 2.5 mm in length and 1.0 mm in diameter. The weight of 1000 seeds can vary from 1.9 to 4.3 g. Large differences in appearance of the seed are found among varieties (Koziol 1993). Quinoa seed is actually a fruit and its major anatomical parts are the outer covering (pericarp and seed coat), the perisperm, and the embryo (radicle and cotelydons) (Prego et al. 1998) (Figure 2.1). Quinoa differs from cereals in that the storage reserves for the developing embryo are found in the perisperm rather than in the endosperm. The embryo that surrounds the perisperm is dicotyledonous and is part of the bran fraction of the seed; it is high in protein and lipid and contains most of the ash, fibre and saponins (Varriano-Marston and DeFrancisco 1984, Becker and Hanners 1990) (Table 2.1).

2.1.2 Composition

The composition of the whole seed is summarized in Table 2.2. For comparison purposes, the composition of corn, wheat, soybean and rice are provided in Table 2.3.

2.1.2.1 Protein

Quinoa has a high protein content compared to most cereals, but is lower in protein than oilseeds and legumes (Mazza et al. 1992). The protein quality has been shown to be very good by biological assay. A wide range of protein efficiency ratios (PER, 1.95-3.10) has been reported (Guzmán-Maldonado and Paredes-López 1998, Gross et al. 1989). Raw, debittered quinoa had a PER that was slightly lower than

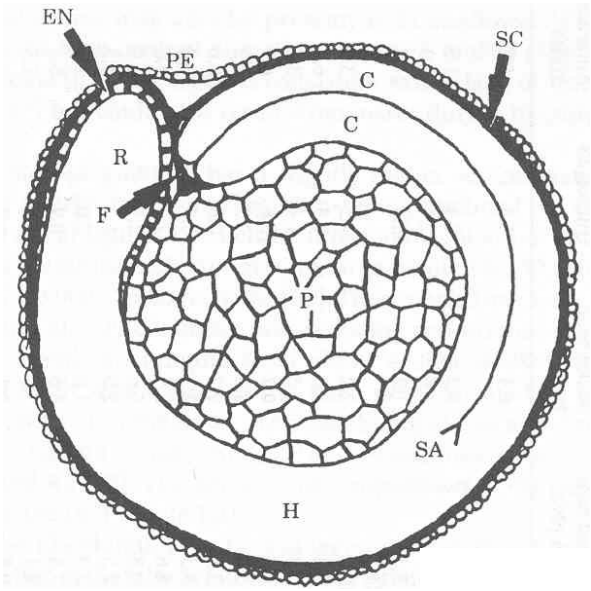


Figure 2.1 Medial longitudinal section of quinoa seed showing the pericarp (PE), seed coat (SC), hypocotyl-radical axis (H), cotelydons (C), endosperm (EN) (in the micropylar region only), radicle (R), funicle (F), shoot appendix (SA) and perisperm (P) (Prego et al. 1998).

Table 2.1 Composition of the anatomical parts of the quinoa seed (Becker and Hanners 1990).

	Crude Protein (%, db)	Lipid (%, db)	TDF[a] (%, db)	Ash (%, db)
Whole	11.0-13.7	6.0-6.6	1.1-1.8	2.6-3.0
Bran	22.3-32.2	14.2-17.8	1.2-1.7	5.7-6.8
Perisperm	4.8-7.4	1.0-2.8	0.6-1.0	0.8-1.4

[a] Total dietary fibre.

Table 2.2 Composition of whole quinoa seed.

Chemical components, %db		Minerals, mg/100g (db)	
Protein (N×6.25)	10-18[a]	Calcium	86-141[gh]
Fat	4.4-8.8[a]	Phosphorus	22-449[gh]
Ash	2.4-3.7[a]	Potassium	714-1040[ag]
Total dietary fibre	1.1-13.4[bc]	Magnesium	232-310[ag]
Starch	32.6-61.5[de]	Iron	2.6-9.1[ag]
Saponins	0.01-4.7[f]	Manganese	4.3[a]
		Copper	0.6-7.6[ag]
		Zinc	3.8[g]
		Sodium	93[b]
Vitamins, mg/100g (db)			
Thiamine	0.24[a]	γ-tocopherol	5.3[c]
Niacin	1.17[a]	δ-tocopherol	0.3[c]
Riboflavin (B2)	0.22[a]	Vitamin C	16.4[c]
Vitamin E	0.46-0.59[a]	Vitamin A	0.2[c]
α-tocopherol	2.6[g]	Folic acid	0.08[c]
β-tocopherol	0.2[c]		

[a] Coulter and Lorenz 1990, [b] Becker and Hanners 1990, [c] Ruales and Nair 1993a, [d] González et al. 1989, [e] Wolf et al. 1950, [f] Koziol 1990, [g] Oshodi et al. 1999, [h] Theurer-Wood 1985.

Table 2.3 Composition of wheat, corn, rice and soybean (http:/www.usaid.gov).

Crop	Protein (%, db)	Fat (%, db)	Carbohydrate (%, db)	Fibre (%, db)
Corn	9.4	4.7	74.3	7.4
Rice	7.1	0.7	73.3	1.3
Soybean	21.3	5.9	46.9	13.2
Wheat	11.7	1.8	80.0	12.5

that of casein, but after cooking it increased to a level similar to that of casein (Koziol 1992). More than 37% of the protein in quinoa comprises essential amino acids like that of the milk protein, casein (Koziol 1992). Viewing amino acid composition and animal studies together, the protein quality of quinoa equals that of casein (Ranhotra et al. 1993), which is unusual for protein from a plant source (Ruales and Nair 1992).

Quinoa protein is particularly rich in histidine, isoleucine, methionine and lysine (Table 2.4) compared to cereals, which are generally limiting in lysine. The high lysine content of quinoa is attributable to its high contents of albumins and globulins (44-77% of the total protein) (Fairbanks et al. 1990). Its high methionine and cysteine contents make quinoa a good complement to legumes, which are limiting in these amino acids (Theurer-Wood 1985). Quinoa protein is low in prolamins (0.5-7.0%), which indicates that quinoa is free of gluten and, therefore, non-allergenic (Galwey 1993, Berti et al. 2004). It is suitable and desirable for use in foods designed to reduce allergies in sensitive individuals such as celiac disease patients, and in specialty foods such as infant formulae (Coulter and Lorenz 1990, Javaid 1997, Morita et al. 2001).

Quinoa protein consists of two major protein fractions: one is an 11S-type globulin, termed chenopodin, which has been characterized by Brinegar and Goundan (1993). It accounts for 37% of the total protein and contains polypeptides having molecular masses of 22-23 kDa and 32-39 kDa. This protein is relatively low in sulfur-containing amino acids (methionine and cysteine) when compared to the amino acid composition of the total protein (Brinegar and Goundan 1993). The other major protein, which accounts for 35% of the total protein in quinoa, is a 2S-type protein with a molecular mass of 9 kDa. This protein is high in cysteine, arginine and histidine, but relatively poor in methionine (Brinegar et al. 1996). The two major classes of quinoa proteins differ particularly in their solubilities at pH 5, where most of the 11S is precipitated while the 2S protein remains soluble (Brinegar et al. 1996).

Table 2.4 Amino acid composition of quinoa protein compared to the FAO/WHO/ UNU reference pattern (g/ 100 g protein)[a].

Amino acid	**Quinoa**	**FAO/WHO/UNU reference patterns**	
Essential		*Adult*	*Child*
Histidine	2.4[b]- 3.2[c]	1.6	1.9
Isoleucine	3.6[a]- 4.4[c]	1.3	2.8
Leucine	5.8[d]- 6.9[e]	1.9	6.6
Lysine	5.1[b]- 6.1[c]	1.6	5.8
Methionine+Cysteine	3.8[d]- 4.4[f]	1.7	2.5
Phenylalanine+Tyrosine	6.6[d]- 8.4[f]	1.9	6.3
Threonine	3.5[d]- 4.4[e]	0.9	3.4
Tryptophan	1.2[b]	0.5	1.1
Valine	3.7[e]- 4.9[d]	1.3	3.5
Non-essential			
Alanine	4.1[d]- 5.5[e]		
Arginine	7.0[b]- 7.5[d]		
Aspartic acid	7.3[b]- 10.5[e]		
Glutamic acid	11.9[b]- 17.3[e]		
Glycine	5.2[b]- 6.3[e]		
Proline	3.1[b]- 3.5[e]		
Serine	3.7[b]- 5.6[e]		

[a] WHO 1985, [b] Coulter and Lorenz 1990, [c] Atwell et al. 1983, [d] Becker and Hanners 1990, [e] Ranhotra et al. 1993, [f] Theurer -Wood 1985.

2.1.2.2 Lipid

Quinoa contains 4.4-8.8% crude fat, with the essential fatty acids linoleic and linolenic acid accounting for 55 to 63% of the total fatty acids (Table 2.5). Quinoa oil is high in polyunsaturated fatty acids and has a composition similar to that of soybean oil (Wood et al. 1990). The oil is particularly stable due to the presence of high amounts of natural antioxidants, namely 69-75 mg of α-tocopherol and 76-93 mg of γ-tocopherol in 100 g of crude oil. These numbers fall to 45 and 23 mg, respectively, in 100 g of refined oil (Koziol 1992). Given the high quality of its oil and the fact that some varieties exhibit a crude fat concentration up to 8.8%, quinoa is sometimes termed a pseudo-oilseed and has been considered as a crop to be grown for its oil content (Koziol 1993).

2.1.2.3 Starch

Starch is the major component of quinoa, comprising approximately 55% of the seed and is present in the form of small granules 1.5 μm in diameter (Chauhan et al. 1992). The granules can be found in the perisperm as single entities or as aggregated, compound structures (Lorenz 1990). The starch is embedded in a matrix of protein which reduces the enzymatic hydrolysis of the starch, thereby decreasing its digestibility and extractability (Ruales and Nair 1994). Several reports have been published on the characteristics of quinoa starch (Atwell et al. 1983; Lorenz 1990; Ahamed et al. 1996a; Praznik et al. 1999; Tang et al. 2002). However, a comparison of starches from different genotypes or cultivars has not been reported.

Starch consists only of glucose residues, which are linked together by α-1,4 bonds and branched via α-1,6 bonds to form amylose and amylopectin. Amylose is mainly linear with very few branches, while amylopectin is highly branched. In general, the branches in amylopectin do not occur randomly; rather, they are arranged in clusters thereby allowing the formation of double helices. These helices can pack together in organized crystalline lamellae, which are separated by amorphous regions that are primarily composed of amylose. This organization of amylopectin and amylose is the basis for the semi-crystalline structure of the starch granule (Ball et al. 1998). The ratio of amylose to amylopectin is one of the key

Table 2.5 Fatty acid composition of crude fat from quinoa seed (Ruales and Nair 1993a).

Fatty acid			**Quantity (g/100 g fat)**
Myristic acid	C 14:0		0.1
Palmitic acid	C 16:0		9.7
Palmitoleic acid	C 16:1	(n-7)	0.2
Stearic acid	C 18:0		0.6
Oleic acid	C 18:1	(n-9)	24.8
Linoleic acid	C 18:2	(n-6)	52.3
α-Linolenic acid	C 18:3	(n-3)	3.9
Arachidic acid	C 20:0		0.4
Eicosenic acid	C 20:1		1.4
Eicosadienoic acid	C 20:2	(n-4)	0.2
Behenic acid	C 22:0		0.5
Erucic acid	C 22:1	(n-9)	1.4
Lignoceric acid	C 24:0		0.2
Nervonic acid	C 24:1	(n-9)	0.4

factors determining industrially important properties. In most plants, starch consists of 20-30% amylose and 70-80% amylopectin. Reports in the literature suggest that considerable variability exists in the amylose content of quinoa starch (7-27%) (Lorenz 1990, Inouchi et al. 1999, Praznik et al. 1999, Tang et al. 2002).

Quinoa starch has an average molar mass of 11.3×10^6 g/mol, which is comparable to that of amaranth (11.8×10^6 g/mol) starch, higher than that of wheat starch (5.5×10^6 g/mol), but lower than that of waxy maize starch (17.4×10^6 g/mol). The starch is highly branched, with a minimum degree of polymerization (dp) of 4,600 glucan units, a maximum dp of 161,000 and a weighted average dp of 70,000 (Praznik et al. 1999). Tang et al. (2002) reported a dp of 6,700 glucan units for the amylopectin fraction of quinoa starch. Quinoa amylopectin, like amaranth and buckwheat amylopectins, contains a large number of short chains with a dp from 8 to 12, and a small number of larger chains of a dp 13 to 20, as compared to the endosperm starches of other cereals (Noda et al. 1998, Inouchi et al. 1999). Quinoa starch has been shown (Inouchi et al. 1999) to exhibit the typical A-type X-ray diffraction pattern (reflections at 15.3º, 17.0º, 18.0º, 20.0º and 23.4º 2θ angles) characteristic of cereal starches (Zobel 1988), and a relative crystallinity of 35.0% (Tang et al. 2002).

The gelatinization properties of starch are related to a variety of factors including the size, proportion and kind of crystalline organization, and the ultra-structure of the starch granule. Quinoa starch gelatinizes at a relatively low temperature (T_o = 46.1-57.4ºC, T_p = 54.2-61.9ºC, T_c = 66.2-68.5ºC) which is similar to the gelatinization temperatures of wheat and potato starch, but lower than that of corn starch (Inouchi et al. 1999). Goering and DeHaas (1972) reported that small granule starch had, in general, a lower gelatinization temperature than did large granule starch. On the other hand, Lorenz (1990) showed that monomodal small granule starches had higher gelatinization temperatures than did large granule starches. Additionally, in wheat and barley starches, the smaller B-granules gelatinize at a higher temperature and over a wider temperature range than do the larger A-granules (Eliasson and Larsson, 1983, MacGregor and Bhatty 1996, Myllärinen et al. 1998, Chiotelli and LeMeste 2002). Quinoa starch has a gelatinization enthalpy (ΔH) of 7.3-10.5 J/g (Inouchi et

al. 1999), compared to 17.2-20.5 J/g for corn starch, 12.1 J/g for wheat starch, 14.2-16.3 J/g for rice starch and 18.8 J/g for potato starch (Zobel 1984). Although quinoa starch gelatinizes at similar temperatures, its pasting behaviour is considerably different from that of wheat starch. At equal starch concentrations, quinoa starch exhibited a higher viscosity when measured with a Brabender amylograph (Atwell et al. 1983).

Quinoa starch was found to have a higher water-binding capacity and higher swelling power than did wheat or barley starch. Furthermore, it is highly freeze-thaw stable and shows little retrogradation, which was thought to be due to its low amylose content (Lorenz 1990, Ahamed et al. 1996a). However, Praznik et al. (1999) reported low freeze-thaw stability for quinoa starch gels compared to amaranth, buckwheat and even wheat starch gels.

Starch from quinoa showed to be a better thickener for fillings than did wheat, potato, barley and amaranth starch (Lorenz 1990). The overall performance of quinoa starch in leavened baked goods was similar to that of other non-cereal starches like amaranth and potato starch, but poor compared to barley and wheat starch (Lorenz 1990).

Ruales et al. (1993b) studied the nature and extent of modification of quinoa starch caused by various processes such as cooking, autoclaving, drum drying and extrusion, by measuring its physicochemical properties. All processes modified the physicochemical properties of quinoa starch to varying degrees. A drum-drying process that included pre-cooking was chosen to produce an infant food from quinoa flour. This process resulted in the highest degree of starch gelatinization of the unit operations examined.

2.1.2.4 Fibre

Although crude fibre percentages as low as 1.1% have been reported (Becker and Hanners 1990), quinoa is generally considered to be high in fibre (Ranhotra et al. 1993, Ruales and Nair 1994). According to Ranhotra et al. (1993), quinoa contained 8.9% total dietary fibre, of which more than 80% was insoluble. Ruales and Nair (1994) reported a total dietary fibre content of 13.4% in quinoa consisting of 11.0%

insoluble fibre and 2.4% soluble fibre. The insoluble fibre content was slightly lower than, and the soluble fibre content was similar to, that of rye, and both were higher than that of wheat. Removal of the outer layers of the seed by scrubbing and washing, with the purpose of removing saponins, did not affect its dietary fibre content (Ruales and Nair 1994).

2.1.2.5 Secondary plant metabolites and antinutrients

The concentration of saponins in quinoa varies with variety and environmental conditions and ranges from 0.01% to 4.65% of dry matter (Koziol 1992). Saponins are water- and methanol-soluble, detergent-like molecules that consist of hydrophilic sugar chains attached to lipophilic triterpenoid aglycones. The saponins in quinoa are generally derivatives of three main triterpenes or sterols termed sapogenins: phytoaccagenic acid, hederagenin and oleanolic acid (Figure 2.2) (Mizui et al. 1988 and 1990, Ridout et al. 1991). The saponins are mainly located in the outer layers of the quinoa seed. Chauhan et al. (1992) reported that 34% of the saponins were present in the bran and that the amount was twice as high as that in the perisperm.

Saponins taste bitter, foam in water and have been demonstrated to damage intestinal mucosal cells by altering cell membrane permeability and interfering with active transport (Gee et al. 1989). The level of toxicity of saponins depends on their structure as well as the organism exposed and the means of exposure. The consequences of prolonged consumption of saponins are unknown, but it is possible that the membranolytic activity might increase the uptake of antigens by the small intestine, which is undesirable, particularly in infants (Koziol 1992). Although saponins are generally seen as antinutritional compounds that distract from the utility of quinoa, once extracted they could be used in a variety of applications. For example, there is pharmacological interest in saponins because of their ability to aid in the absorption of certain drugs (Basu and Rastogi 1967) and their hypocholesterolemic effects (Oakenfull and Sidhu 1990). Furthermore, saponins protect the crop against attack by birds and, probably, other pests (Risi and Galwey 1984, Dutcheshen and Danyluk 2002).

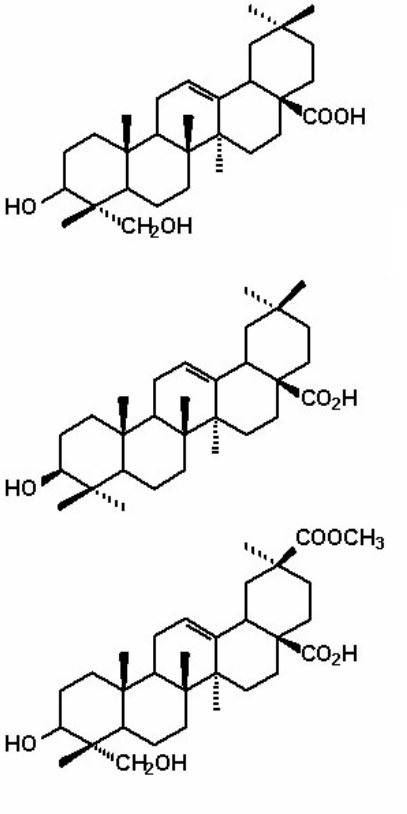


Figure 2.2 The structures of the aglycones hederagenin (a) oleanolic acid (b) and phytoaccagenic acid (c) as they are present in quinoa saponins.

Low saponin quinoa varieties are available. These varieties are sometimes called 'sweet' and have saponin contents approximately one-tenth those of normal varieties (Gee et al. 1993). Although these varieties are preferable from the consumers' perspective, they are grown in small quantities because of their susceptibility to bird attack, pests and disease. Production for domestic or commercial use is generally of the bitter, high-saponin varieties (Fleming and Galwey 1995). The quinoa produced in North America is almost exclusively high in saponins.

Quinoa contains 0.7 to 1.2% phytate (Koziol 1992; Ruales and Nair 1993a) which is comparable to whole grain wheat, lentil, fababean or rye (Ruales and Nair 1993a). Phytates can form insoluble complexes with multivariate cations such as Fe^{3+}, Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+} in the gastrointestinal tract, thereby reducing their bioavailability (Serraino et al. 1985). Quinoa is especially high in iron compared to other cereals; however, the phytates could markedly decrease the bioavailability of this iron. Valencia et al. (1999) reported that soaking, germination and lactic fermentation of quinoa resulted in improved iron solubility and reduced phytate content. The most effective treatment for reducing phytate was fermentation of germinated quinoa flour, whereby phytate was almost completely hydrolyzed and the iron solubility increased five to eight times compared to its unfermented counterpart. Quinoa contains very little or no tannin (Chauhan et al. 1992, Ruales and Nair 1993c) or trypsin inhibitors (Chauhan et al. 1992, Ruales and Nair 1993c).

The main flavanoids in quinoa are kaempferol and quercetin. Both are strong antioxidants and free-radical scavengers (Zhu et al. 2001). Along with the saponins, the flavanoids may contribute to the bitterness/astringency of quinoa as well as the colour of the seed.

2.2 Physicochemical characteristics of starch

Knowledge of the physicochemical characteristics of a starch is important to be able to select it in particular applications. Starches from different sources range widely in their characteristics. This is due to the genetic, environmental, and agronomic background of the material.

Starch gelatinization is a phenomenon involving the disruption of the molecular order within the starch granule by heating it above its gelatinization temperature in an excess of water. Apart from heat, high concentrations of an alkaline agent may cause starch gelatinization (Lai et al. 2002, Roberts and Cameron 2002). Gelatinization is accompanied by an increase in the viscosity of the starch slurry. Concurrent with swelling, linear amylose molecules are disentangled, leave the granule, and become solubilized in the surrounding medium. The temperature range and increase in viscosity over which starch swells and disrupts are specific for each starch (Bean and Setser 1992). Upon continued heating, most unmodified starch pastes exhibit a decrease in viscosity after the maximum, so-called peak viscosity has been reached. This decrease is known as breakdown of the starch paste. This results from extensive solubilization and fragmentation of granule structures such that they can no longer hold onto a large volume of water (Bean and Setser 1992).

As a starch paste is cooled, retrogradation begins. Free molecules of amylose realign through hydrogen bonds. This realignment causes the viscosity of the paste to increase and its clarity to diminish, which affects the appearance and palatability of the end product. The increase in viscosity on cooling is known as setback of the starch paste. Subsequent cooling and storage of cooked pastes results in additional realignment of amylose as well as alignment of the longer chains of amylopectin. If the starch-to-water concentration is sufficiently high (3-7%), a gel structure may be formed on cooling, whereby the characteristics of this gel depend on the starch employed (Bean and Setser 1992). Starch retrogradation is a problem when the gel is frozen and thawed, as happens with many food products. On thawing, water is rejected from the gel due to realignment of the molecules. This process is termed syneresis. When the amylose content of the starch is low, the extent of syneresis is reduced and the starch paste is more freeze-thaw stable. Therefore, starch with low amylose content is preferred in certain products (Bean and Setser 1992).

Apart from its influence on retrogradation, there are other ways in which the amylose content influences the structural, functional and technological properties of starch. Starch with lower amylose content (e.g. high in amylopectin) is generally associated with a higher peak viscosity and greater breakdown, as well as a lower

final viscosity and less setback (Baldwin 2001, Bao et al. 2001, Grant et al. 2001). Furthermore, such a starch has less amorphous and more crystalline regions, which raises the gelatinization temperature.

Apart from the amylose content, other factors such as the molecular weight of amylose and amylopectin, the degree of branching of the molecules and their molecular fine structure, lipid content and granule size, impact the characteristics of starch. In cereals, however, starch properties seem to be dominated by variability in amylose content (Zobel 1988).

Additionally, a large amount of the commercially available starch is not used in its native form, but rather is chemically or physically modified to improve its functionality for use in modern food formulations. In general, the improvement is directed towards paste stability at high temperature, shear stability, paste clarity and freeze-thaw stability. Most native starches are lacking, to different extents, in most of these aspects. Crosslinking is the most important chemical modification in the starch industry (Taggart 2004). The hydrogen bonding between starch chains is replaced by more permanent, covalent bonds. Starch esters are commonly produced by phosphatising the starch and can be characterized by their higher paste viscosities, especially if a high degree of cosslinking is achieved (Cornell 2004). Stabilisation, mainly to prevent retrogradation, is the second most important modification. Bulky groups are substituted onto the –OH groups of amylose and amylopectin, whereby the degree of substitution of these groups is an important characteristic of the modification. These groups take up space and hinder (steric hindrance) the realignment of the dispersed (cooked), linear fragments (Taggart 2004). An example is the production of starch ethers by the reaction of ethylene oxide with starch that improves gel clarity by preventing retrogradation. Another means of starch modification is conversion, whereby the amylose and amylopectin chains are cleaved by acid hydrolysis, oxidization, dextrinisation or enzyme hydrolysis (Taggart 2004). Starch can also be physically modified by pregelatinisation whereby the starch is pre-cooked or ‘instantised’ by simultaneously cooking and drying using drum drying, extrusion or spray-drying (Taggart 2004).

2.3 Starch biosynthesis

During photosynthesis, transitory starch is synthesized in the chloroplasts. At night, this starch is degraded and transported as sucrose to the amyloplasts of the storage organs where it is incorporated as storage starch. By means of studies in *Chlamydomonas reinhardtii* and higher plants, the involvement of four groups of enzymes (ADP-glucose phosphorylase, starch synthases, branching enzymes and debranching enzymes) in the biosynthesis of starch has been documented (Buléon et al. 1998, Myers et al. 2000, Denyer et al. 2001) (Figure 2.3). Of these enzymes, different isoforms are present during starch synthesis, all of which have specific roles in the formation of amylose and amylopectin. Some of these enzymes are granule bound, in the interior as well as on the surface of the developing granule. They are the so-called starch granule associated proteins (SGP) (Smith 2001), which are compositionally and functionally distinct from the plant storage proteins and contain basic as well as hydrophobic amino acids in high quantities. This might explain the strong binding of SGP to the hydrophobic starch molecules (Baldwin 2001). The major class of SGP is granule bound starch synthase I (GBSSI) (Smith 2001).

The synthesis of starch in storage organs consists of three steps: the synthesis of ADP-glucose (ADP-Glc), the transfer of a glucose residue from ADP-Glc to the growing α-1,4 linked polysaccharide, and the formation of α-1,6 branch points. ADP-Glc is the building block for amylose and amylopectin and the substrate for the starch synthesizing enzymes. It is formed from glucose-1-phosphate (Glc-1-P) and adenosine triphosphate (ATP) catalyzed by ADP-glucose pyrophosphorylase (ADP-Glc PPase). In potato, legumes and cereals, the conversion of Glc-1-P into ADP-glucose is the rate limiting step in overall starch biosynthesis (Buléon et al. 1998).

Subsequent steps in starch synthesis are all catalyzed by starch synthases or starch branching enzymes, or both (Wal 2000, Smith 2001). After ADP-Glc enters the amyloplast, starch synthase (SS) adds a glucose unit from ADP-Glc to the non-reducing end of a glucose chain via an α-1,4-linkage. Once a linear glucose chain of a certain length has formed, starch branching enzyme (SBE) cleaves the chain. The cleaved portion gets transferred to a glucose residue within an acceptor chain to form a branch. Starch organs seem to contain at least two different isoforms of this SBE

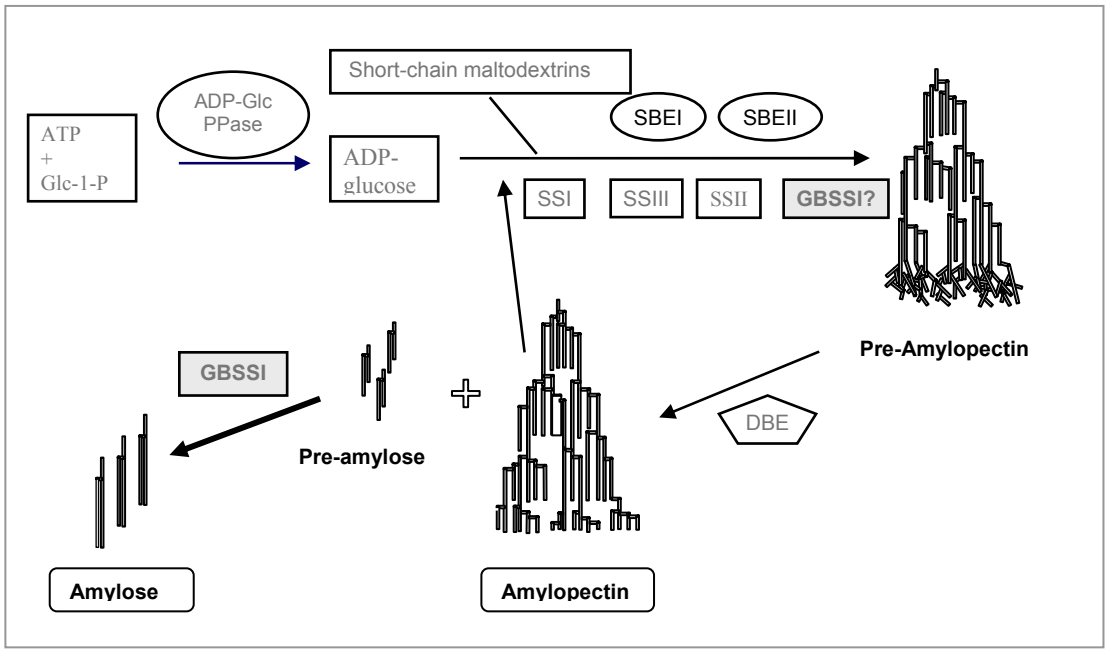


Figure 2.3 Schematic model of starch biosynthesis in plants, known as the pre-amylopectin trimming model. The following abbreviations are used for substrate and enzymes: Glc-1-P = Glucose-1-phosphate, ADP-Glc = ADP-glucose, ADP-Glc PPase = ADP-glucose pyrophosphorylase, SBE = starch branching enzyme, SS = starch synthase, GBSS = granule bound starch synthase, and DBE = debranching enzyme (Båga et al. 1999).

and five isoforms of SS. However, the precise route and enzymes involved in starch synthesis differ from crop to crop and has not always been totally clarified (Smith 2001).

It has been known for sometime that the synthesis of amylose is a function of a particular class of isoforms of starch synthase known as granule bound starch synthase I (GBSSI). This approximately 60 kDa enzyme has been identified as an ADP-Glc: α-1,4-D-glucan-4-α-glucosyl transferase. GBSSI was first revealed because of its absence in several crops. Mutations leading to defects in GBSSI have been isolated in *waxy (wx)* maize (Weatherwax 1922), *wx* rice (Murata et al. 1965), *wx* barley (Eriksson 1969), *wx* wheat (Nakamura et al. 1995), amylose-free (*amf)* potato (Hovenkamp-Hermelink 1987), low amylose (*lam)* pea (Denyer et al. 1995), and *wx* amaranth (Konishi et al. 1985), and have resulted in the biosynthesis of starch granules that contain little or no amylose. The precise mechanism by which GBSSI synthesizes amylose is not clear. Synthesis of amylose has been postulated to proceed via elongation of amylopectin chains followed by cleavage or elongation of malto-oligosaccharides (Ball et al. 1996, Zeeman et al. 1998, Wal et al. 1998, Båga et al. 1999). The model for starch biosynthesis depicted in Figure 2.3 is known as the pre-amylopectin trimming model.

2.4 Isolation of starch and protein

2.4.1 Commercial starch

The industrial process of starch production consists mainly of the separation of starch from protein, fibre and oil. Important considerations thereby are avoidance of amylolytic or mechanical damage to the starch granules, effective deproteinization of the starch, minimization the loss of the small granules, and avoidance of starch gelatinization (Schulman and Kammiovirta 1991). The major sources from which starch is refined are corn, wheat and potato.

Most of the commercial fractionation of corn is based on a wet milling process. The grains are steeped in sulphur dioxide and lactic acid followed by a grinding step prior to separation of the oil-rich germ using hydrocyclones. The starch and protein are separated using settling, centrifugation or hydrocycloning. All of these processes

are based on the difference in density between the starch and hydrated protein particles. In most applications, total protein and soluble protein levels of 0.30-0.35% (db) and 0.010-0.015% (db), respectively, in the final starch product is acceptable (Blanchard 1992).

Most processes of wheat starch production are unique, because they make use of the capability of wheat protein (i.e. gluten) to form a tight network from which the starch can be washed quite easily. There are a variety of processes for producing wheat starch, of which the so-called Martin or "dough ball process", in which starch is washed out of the dough with water, is most commonly used (Knight and Olson 1984). The so called Fresca process for wheat fractionation does not take advantage of the gluten network. Instead, flour is dispersed in water by shearing to prevent gluten formation followed by centrifugation of this dispersion resulting in a starch-rich pellet and a supernatant containing soluble proteins (Knight and Olson 1984). One of the shortcomings of this process is that the decanters and hydrocyclones used for the centrifugation are not very efficient in separating particles smaller than 5 μm. Wheat starch has a bimodal starch granule size distribution, whereby 70% (by weight) of the starch granules have a diameter of 10-35 μm and approximately 30% are smaller than 10 μm (Lindeboom et al. 2004). These small granules, together with a portion of the damaged starch granules, appear in the overflow of the centrifuge and are lost (Esch 1991). Furthermore, there is a tendency for the small granules to associate with the protein fraction which is formed on top of the starch cake during centrifugation. Scraping of this sediment, as is sometimes done to further purify starch after centrifugation, removes small and damaged starch granules. The loss of starch in this matter is even more drastic than that occuring during washing in hydrocyclones (Esch 1991, Andersson et al. 2001).

Potato starch production consists of disintegration of the tuber by rasping or milling, mixing the pulp with water and screening the obtained slurry to separate the free starch from the pulp (Mitch 1984).

2.4.2 Small granule starch

Several issues are associated with the production of small granule starch, which result in small granule starch that has often a high protein content and a process that exhibit low starch yields. A major problem encountered is the entrapment of small granules in the protein and fine fibre sediments generated during centrifugation (McDonald et al. 1991, Schulman and Kammiovirta 1991, Lim et al. 1999, Andersson et al. 2001, Xie and Seib 2002). When these sediments are scraped off and discarded, which is common in laboratory purification methods and in some industrial processes, a severe loss of small granules occurs (Szczodrak and Pomeranz 1991). A similar phenomenon was discussed in the production of wheat starch (Section 2.4.1). To reduce the entrapment of small granules in the protein layer, researchers have degraded the protein enzymatically, followed by separation of the peptides and starch using centrifugation (Radosavljevic et al. 1998; Wang and Wang 2001). These protein digestion methods produced starches with higher or comparable yields and reduced starch damage. However, these processes require chromatography to purify the protease free of any amylase activity (Radosavljevic et al. 1998). Enzymes like hemicellulase and xylanase have also been used to degrade the polysaccharides present in the sediments entrapping the starch granules (Wilhelm et al. 1998).

Currently, rice starch is the only commercially-available small granule starch. The process employed for its separation consists of steeping rice in a dilute sodium hydroxide solution, milling the slurry, removing the cell wall (fibre) by screening, extracting the protein with sodium hydroxide solution, and recovering the starch by centrifugation followed by washing and drying (Juliano 1984).

2.4.3 Protein production

Protein is generally recovered from plant material by extraction of the raw material with a suitable solvent (usually aqueous) with the aim of producing an enriched protein product. Based on the type of proteins present in the material, the protein is best extracted in water (albumins), aqueous salt solution (globulins), 70-80% ethanol (prolamins) or alkali/acid (glutelins). However, prior to protein

extraction, raw material that contains high levels of oil must be defatted. This is to prevent emulsion formation during protein extraction and to produce oil-free protein products. Mechanical pressing (Shrestha et al. 2002) as well as solvents such as hexane (Abbott et al. 1991) and petroleum ether (Sathe et al. 2002) are used for fat removal. Furthermore, seeds generally contain high levels of phytate, phenolic compounds and other phytochemicals, like saponins, that interfere with protein isolation (i.e., reduce yield) or contribute to discoloration, off-flavour or reduced functionally of the final protein product (Aluko 2004). Often, these compounds are removed prior to protein extraction. For example, phenolic compounds have been extracted with 80% aqueous methanol before isolation of sunflower seed protein (Gonzalez-Perez et al. 2002). Blaicher et al. (1983) produced a low-phytate rapeseed protein isolate by employing an initial extraction of the meal at pH 4.0 whereby 70% of the phytate in the meal was removed.

Protein extraction is most of the time followed by centrifugation to separate the soluble proteins (i.e. supernatant) from the insoluble material (Aluko 2004). After purification, the extracted proteins are referred to as protein concentrates or protein isolates. Concentrates contain a minimum of 65% (db) protein, whereas isolates have a minimum protein content of 90% (db) (Uzzan 1988). Protein concentrates and isolates can be further fractionated or purified based on protein molecular properties such as size, hydrophobicity, ionic properties and affinity for certain ligands (Aluko 2004).

The result of protein extraction is an intermediate that is much diluted with the extraction medium. Therefore, the next step in protein production is concentration. In recent years, a large number of articles have been published on the use of ultrafiltration (UF) (Diosady et al. 1984, Tzeng et al. 1990, Hamada 2000, Moure et al. 2001, Xu et al. 2003) for protein concentration. During ultrafiltration, water and small molecules, such as glucosinolates, phytates and phenolics, selectively pass through a membrane while larger molecules, like proteins, remain in the retentate and, therefore, are concentrated. Depending on the pore size and nature of the membrane employed, some proteins might pass through the membrane as well (Tzeng et al. 1990).

Proteins can also be concentrated by precipitation. During precipitation, soluble proteins are converted to insoluble ones by altering their structure (surface characteristics) or changing the environment (Raphael 1997). This leads to supersaturation which in turn results in nucleation followed by aggregation. Several strong acids, such as HCl, H_2SO_4 and H_3PO_4, have been used for isoelectric precipitation (Bell et al. 1983). At the isoelectric point, solubility is minimal because dipole-dipole and electrostatic attraction between neighbouring protein molecules increases, allowing the molecules to pack together (Raphael 1997). After precipitation, the proteins can be recovered by means of centrifugation, settling or filtration. Apart from changing the pH of the protein solution to the isoelectric point, proteins can also be precipitated using heat (>45ºC), neutral salts (NaCl, $(NH_4)_2SO_4$, Na_3PO_4, Na_2SO_4 and K_2SO_4), metal ions (Ca^{2+}, Ba^{2+} and Zn^{2+}), ionic polymers, polyelectrolytes and organic solvents (ethanol, acetone and ether) (Bell et al. 1983).

Ultrafiltration preserves the native properties of the protein to a greater extent than does precipitation (Fuhrmeister and Meuser 2003), because no chemicals are employed that can result in protein denaturation. Moreover, UF recovers more protein, and the nutritional and the functional properties of UF isolates are often superior to those of protein obtained by isoelectric precipitation (Moure et al. 2001). Additionally, ultrafiltration does not need to be followed by a dialysis step, which is often used after precipitation to remove salts or other substances utilized or formed during precipitation. After either ultrafiltration or precipitation, protein is typically dried to a powder by spray or freeze drying.

The functional characteristics of a protein product, along with its colour and purity, influence its utility. The functional properties of protein products are affected by the size of the protein bodies, the molecular mass, the amino acid profiles of the constituent proteins, the molecular conformation (secondary and tertiary structures), their interactions with other constituents (lipid, carbohydrates, salt, saponins) in the system, and the isolation and concentration methods used (Damodaran and Paraf 1997, Chauhan et al. 1999b). Protein composition could be changed during the extraction and concentration as compared to the original seed. This could result in a

different amino acid profile in the final product, which in turn could affect the functionality or the nutritional quality of the recovered protein (Tzeng et al. 1988).

2.4.4 Protein and starch separation using sedimentation

The separation of starch and protein often consists of the solubilization of the protein and the sedimentation of starch granules out of a slurry. The latter is based on the average density of starch granules (1.5 g cm^{-3}) being greater than that of the protein particles (1.1 g cm^{-3}) (Gausman et al. 1952, Biss and Cogan 1988, Steinke and Johnson 1991). This is described in Stokes' law, which relates particle density to sedimentation.

$$d = \sqrt{\frac{18\,\eta\,\mu}{(\rho_s - \rho_f)\,g}} \qquad \text{(Equation 2.1)}$$

where d = particle diameter (cm), η = viscosity (Poise), μ = particle settling velocity under gravity (cm s^{-1}), ρ_s = particle density (g cm^{-1}), ρ_f = fluid density (g cm^{-3}) and *g* = acceleration due to gravity (cm s^{-2}).

When sedimentation under a centrifugal field is used, instead of settling under the earth's gravitational field, Stokes' law is as follows:

$$\mu = \frac{d^2 G\,(\rho_s - \rho_f)}{18\,\eta} \qquad \text{(Equation 2.2)}$$

where μ = settling velocity (cm s^{-1}), d = particle diameter (cm), G = centrifugal gravity ($\omega^2 r$) (cm s^{-2}), ρ_s = particle density (g cm^{-1}), ρ_f = fluid density (g cm^{-3}), η = viscosity (dyne s cm^{-1}), r = measured radius of centrifuge (cm) and ω = radial velocity of the centrifuge (cm sec^{-1}).

Density data previously reported for different starches are 1.48-1.60 g cm^{-3} for wheat starch (Berry et al. 1971, Dengate et al. 1978), 1.446-1.495 g cm^{-3} for rye starch (Berry et al. 1971, Patek et al. 1978), and 1.5 g cm^{-3} for corn starch (Gausman et al. 1952, Biss and Cogan 1988, Steinke and Johnson 1991). Apart from particle density, the sedimentation of starch depends on the viscosity of the slurry and the

size and shape (e.g. spherical, disk, cylindrical, etc.) of the starch granules (Equation 2.2, Snow et al. 1997, Tilton 1997). Starch particles generally are ~10-30 μm in diameter. However, small granule starches, such as quinoa, may have diameters as small as 1 μm. Protein particles are typically 5-10 μm in diameter (Singh 1994).

Some protein remains soluble and appears in the supernatant, other protein settles. Because this non-soluble protein has a lower density than does the starch, it settles on top of the starch layer and could be scraped off (Ji et al. 2004).

2.4.5 Quinoa fractionation

There are different means by which quinoa has been fractionated on a laboratory scale. Most protein (Brinegar and Goundan 1993, Chauhan et al. 1999a and b, Aluko and Monu 2003) or starch (Atwell et al. 1983, Lorenz 1990, Wilhelm et al. 1998, Qian and Kuhn 1999) was recovered for further characterization rather than for industrial processing. No integrated process for the recovery of both starch and protein has been described. Ideally, a process for fractionation of quinoa should include high starch and protein recovery, high starch and protein purity, recovery of lipids and saponins, low energy costs, a minimal waste stream and economic feasibility.

2.4.5.1 Starch production

Atwell et al. (1983) and Lorenz (1990) extracted starch from quinoa by wet-milling using a Waring blender after soaking the seed in acetate buffer at pH 6.5. The protein and starch were separated by centrifugation. Qian and Kuhn (1999) used a similar method but soaked the seed in 0.3% (w/v) NaOH.

Wilhelm et al. (1998) optimized a small-scale extraction of quinoa starch using basic technology, machinery and enzymes (i.e., xylanase, cellulase and hemicellulase). The basic processes examined were dry milling as well as soaking the seed prior to wet milling. Both processes were followed by starch extraction using a variety of media (water, 0.25% NaOH or 0.05% sodium metabisulfite). The advantage of the dry milling method in this research was the shorter steeping time. The dry milling process, however, resulted in a higher degree of starch granule

damage than did wet milling. Furthermore, the alkaline conditions used in some of the extraction processes were shown to change the starch properties. Therefore, the starch obtained under alkaline conditions was not applicable in specific end uses, e.g. biodegradable thermoplastics, as was the objective of the study.

2.4.5.2 Protein production

Brinegar and Goundan (1993) extracted quinoa protein to study its composition. Flour was stirred in 0.5 N NaCl/Tris HCl, 10% (w/v), pH 8, for one hour at room temperature. A protein recovery of 65 mg/g of defatted flour was achieved.

Chauhan et al. (1999a, b) and Aluko and Monu (2003) extracted protein from defatted flour by stirring it in 0.015N NaOH, for two hours at 25ºC, after which the slurry was filtered through cheesecloth. Protein was precipitated from the supernatant by adjusting the pH to 4.7. The protein precipitate was recovered by centrifugation and than neutralized.

During protein extraction from quinoa, the saponins tend to be co-extracted with the protein fraction. Saponins can be removed from the quinoa seed/flour prior to protein extraction or from the protein product obtained. Their removal affects the characteristics and functionality of the resultant protein product. Saponin removal from protein products has been reported to increase the protein efficiency ratio (Chauhan et al. 1999a), reduce nitrogen solubility, and reduce emulsifying and foaming properties (Chauhan et al. 1999b). Because of this loss of functionality of quinoa protein, Aluko and Monu (2003) investigated the use of enzymatic hydrolysis to improve protein functionality. Quinoa protein was hydrolyzed with protease and the hydrolysate was fractionated by ultrafiltration (UF) using a 10,000 or a 5,000 molecular weight cut-off (MWCO) membrane. An increase in protein solubility and foaming capacity was found, along with a decrease in the emulsifying capacity of the hydrolyzed protein compared to the non-hydrolyzed protein.

2.4.5.3 Saponin removal

If quinoa is intended for food use, the saponins need to be removed prior to consumption to reduce bitterness and astringency as well as to avoid adverse effects

on the intestinal mucosa (Koziol 1992). Traditionally, the South American Indians removed saponins by steeping or washing the seed in cold water or aqueous alkali, followed by laboriously hand scrubbing the seeds (Simmonds 1965). Another means of removing saponins is by abrasive dehulling of the seed (Reichert et al. 1986, Ridout et al. 1991, Chauhan et al. 1992). Reichert et al. (1986) used a tangential abrasive dehulling device (TADD) to remove the outer layers of the seed. They found that the amount of seed that had to be removed to obtain an acceptable level of saponins varied from 1.2% to 14.8% depending on the original saponin content of the seed. Mechanical abrasion has been found to significantly increase α-amylase activity in quinoa seed (Lorenz and Nyanzi 1989). This is due to the removal of the pericarp (relatively low in α-amylase) during abrasion milling. Additionally, quinoa has significantly higher α-amylase content than most cereals to start with (Lorenz and Nyanzi 1989). To reduce the amount of saponins, a combination of abrasive milling and washing seemed to be most effective. Some of the saponin-rich pericarp material would be physically removed, while the losses of nutrients concentrated in the hull would be minimized (Taylor and Parker 2002).

2.5 Uses of quinoa and quinoa products

2.5.1 Whole seed and flour

Traditionally, quinoa has been used in a wide variety of foods. Whole seed is utilized in broths, soups, stews and rice-like products. Flour is made into porridge and coarse bread. Quinoa can also be fermented to make beer called *chichi* (Taylor and Parker 2002). The main uses of quinoa at present are for cooking, baking, animal feed and processed food products such as breakfast cereals, pasta and cookies. In these products, quinoa is largely employed as a supplement to wheat flour because of its high protein quality and non-allergenicity (Chauhan et al. 1992, Jacobsen 2003).

Dogan and Karwe (2003) optimized the extrusion of quinoa flour. They demonstrated that quinoa can be used in novel, healthy, snack-type food products. Because of its high lipid and low amylose contents, however, extrusion cooking of quinoa required very high shear to disrupt the starch granules.

Lorenz and Coulter (1991) studied the use of quinoa flour in baked products as an additive to wheat flour, and concluded that addition of 5 to 10% was acceptable in breads, cakes and cookies. Lorenz et al. (1993) added quinoa to pasta products whereby different ratios of durum semolina and quinoa flour were employed. However, noodles made with quinoa were inferior in colour, flavour, texture and overall acceptability compared to noodles prepared only from durum semolina.

An infant food product was manufactured by drum drying a slurry of quinoa flour. It was shown that the product was a potential source of valuable nutrients such as protein, vitamin E, thiamine, iron, zinc and magnesium for pre-school children (five years of age) (Ruales et al. 2002).

Quinoa has been considered as a potential crop for NASA's Controlled Ecological Life Support System (CELLS). The CELLS concept will utilize plants to remove carbon dioxide from the atmosphere, thereby generating food, oxygen and water for crews on long-term human space missions. Quinoa was selected for its high productivity and desirable nutritional characteristics, especially its high protein and mineral concentrations and superior amino acid profile. Typically, CELLS has had to combine the nutritional values of several crops to obtain the right amino acid balance; quinoa may supply this on its own (Schlick and Bubenheim 1996).

2.5.2 Starch

Quinoa starch has a small-sized granule with a narrow granule size distribution. This makes it applicable in fine printing paper (Jane et al. 1994, Wilhelm et al. 1998), as a binder with orally active ingredients, and as a carrier material in the cosmetics (Whistler 1995), and in textile and photographic industries (Biliaderis et al. 1993). Another application of quinoa starch is as a filler in biodegradable films. A small granule size can substantially increase the level of starch that can be incorporated into these films while maintaining film quality (Lim et al. 1992). Commercial applications of biodegradable films include garbage bags, composting yardwaste bags, grocery bags and agricultural mulches. Commercial biodegradable films are generally manufactured from low-density polyethylene (LDPE) containing degradative additives such as starch and pro-oxidants. Starch incorporation results in

a plastic film with a porous structure, which enhances the accessibility of the plastic molecules to oxygen and microorganisms (Lim et al. 1992, Ahamed et al. 1996b). Because of its extremely small granule size and resultant good dispersion properties in films, quinoa starch has been used as a biodegradable filler in LDPE films. At a given loading, films filled with quinoa starch showed better mechanical properties than did films filled with corn starch (Ahamed et al. 1996b).

In food applications, microgranular and uniform granule size starches produce a creamy mouthfeel, which is desirable in low-fat and fat-free food formulations. The Nutrasweet Company (Chicago, IL) was awarded a patent in 1992 for making a carbohydrate cream substitute from quinoa starch (Singer et al. 1992). Quinoa starch was extracted and then cross-linked. The cross-linked starch was mixed with carboxymethyl cellulose and heated to 95ºC. After cooling, a pourable white fluid that exhibited a creamy texture remained. Whistler (1997) also patented the production of a fat substitute from quinoa. The starch was partially (5%) hydrolyzed with α-amylase and amyloglucosidase below its gelatinization temperature, after which the starch was recovered by filtration or centrifugation and then dried.

Ahamed et al. (1996a) found unusual freeze-thaw stability for quinoa starch pastes due to their resistance to retrogradation. They suggested applying quinoa starch in frozen food products and in emulsion-type food products such as salad dressings.

2.5.3 Protein, oil and saponins

Until now, no specific products are documented in the literature where quinoa oil or protein is applied. Nevertheless, quinoa protein, due to its complete amino acid profile, could be used to supplement other plant based proteins from cereals and legumes in both food and feed. The oil, which is rich in essential fatty acids and also quite stable, could be employed as an alternative to other vegetable oils like olive, canola and corn oil.

Quinoa saponins, due to their foaming capabilities, may have application in soaps, detergents, shampoos, cosmetics, beer production and fire extinguishers (Johnson and Ward 1993). The saponins might have a cholesterol lowering effect

(Oakenfull and Sidhu 1990) and could possibly be used as a nutraceutical. Saponins could be raw materials for the chemical or pharmaceutical industry (Fleming and Galwey 1995). Additionally, saponins might find application as antibacterial and antifungal agents (Koziol 1992). Dutcheshes and Danyluk (2002) controlled and/or prevented plant diseases, especially fungal diseases, with saponins from quinoa. Saponins cause cell lysis and could possibly be applied as moluscicides (Fleming and Galwey 1995). However, the specific effects attributable to quinoa saponins have not been studied extensively; hence few specific commercial uses have been identified.

3. MATERIAL AND METHODS

3.1 Characterizing starch from eight quinoa lines

3.1.1 Samples

Five quinoa lines from the collection of Agriculture and Agri-Food Canada (Saskatoon, SK) were grown in the summer of 2002 at the Agriculture and Agri-Food Canada experimental farm at Beaverlodge, AB (119 deg 23′06′′ W, 55 deg 11′57′′ N). The lines were Ames 22155 (Chile), Ames 13745 (USA), Ames 21926 (Bolivia), and two breeding lines (AAFC-1 and AAFC-2) developed by Agriculture and Agri-Food Canada. The seeds were harvested by hand and air dried at room temperature. Commercial seed was purchased from Northern Quinoa Corporation (NQC) (Kamsack, SK), White Mountain Farm (WMF) (Morca, CO) and Quinoa Corporation (QC) (Gardena, CA). The characteristics of starch isolated from these eight quinoa lines were compared to those of normal and waxy corn starch [Staley® Pure Food Powdered Starch and Staley® 7350 Waxy No.1 Starch, respectively (Staley, Decator, IL)].

3.1.2 Starch isolation

Quinoa seed was steeped in deionized water (1:5 w/v) at room temperature for 16 hr and then ground in a Waring blender for 1 min. The slurry was stirred for 1 hr, screened over 200-mesh (W.S. Tyler, Mentor, OH), and centrifuged for 20 min at 4,300 × *g*. The brown/grey sediment which accumulated on top of the white starch pellet during centrifugation was carefully scraped off and discarded. The pellet was then dispersed in deionized water (1:5) and centrifuged for 20 min at 4,300 × *g*, with the brown/grey sediment scraped off after each wash. The process was repeated three times in total, then the starch pellet was washed with 95% ethanol and with acetone, after which the starch was air-dried at room temperature.

3.1.3 Amylose

Two assays were used to determine the amylose content of quinoa starch. The colorimetric assay was as described by Martinez and Prodolliet (1996). Forty milligrams of starch was dispersed in 5.0 mL urea:DMSO (1:9 w/v), boiled for 15 min with stirring, followed by incubation at 100ºC for 1 hr. After cooling the sample to room temperature, a 1.0 mL aliquot was added to 9.0 mL ethanol (95%) and kept at room temperature for 15 min while vortexing every 5 min. The sample was centrifuged at 3000 × *g* for 15 min and the pellet was dried in the oven at 130ºC for 20 min. The dried starch was redissolved in 1.0 mL urea: DMSO (1:9 w/v) and this sample was transferred to a 100 mL volumetric flask containing 95 mL water. Two millilitres of iodine solution was added (0.2 g I_2 + 2.0 g KI in 100 mL water) as well as water up to 100 mL in total volume. The sample was kept at room temperature for 30 min and its absorbance was measured at 635 nm. The amylose concentration was calculated using the Blue Value as defined by Morrison and Laignelet (1983).

Amylose content was also determined by high-performance size-exclusion chromatography (HPSEC) using the method described by Demeke et al. (1999). A 5-mg starch sample was suspended in 5 mL of double-distilled water in a glass tube and incubated at 130°C for 30 min. To 1 mL of the vortexed starch solution, 55 mL of 1*M* sodium acetate, pH 4.0, was added. The solution was vigorously mixed, and four units of isoamylase (200 units/mL of stock solution, Megazyme) were added to debranch the starch. After 4 hr of incubation at 40°C, the reaction mixture was boiled for 20 min to inactivate the isoamylase, following which the starch solution was freeze-dried. The debranched starch was dissolved in 200 mL of DMSO solution (99% DMSO and 1% nano pure water) and centrifuged at 15,000 × *g* for 10 min. Supernatant (40 mL) was injected into a PLgel 5 m*M* MiniMix-C guard column attached to a PLgel MiniMix 4.6-mm i.d. column (Polymer Laboratories, Inc., Amherst, MA) to separate amylose and amylopectin using an HPLC system (Waters 600 controller, Waters 610 fluid unit, Waters 717 plus autosampler, Waters 410 differential refractometer). The data were collected and analyzed using Millenium 2010 chromatography software. Starch samples, column, and detector were maintained at 40, 100, and 45°C, respectively. DMSO (99%) was used as an eluent at

a flow rate of 0.2 mL/min. The amylose concentration of the starch samples was calculated by integration of the peak area corresponding to amylose to that of the peak area corresponding to both amylose and amylopectin.

3.1.4 Protein content

The protein contents of the starch samples were determined using a FP-528 protein/nitrogen analyzer (LECO, St. Joseph, MI) according to AACC method 46-30 (AACC 2000), which is based on the Dumas method for protein analysis whereby the sample is combusted followed by the measurement of the amount of nitrogen released. Protein content was calculated as N x 6.25.

3.1.5 Granule size

The volume mean diameter (D[4,3]) and granule size distributions of the isolated starches were determined by low-angle light scattering using a Malvern Mastersizer (Model 2000SM, Malvern Instruments Ltd., Malvern, UK). Deionized water was used as the dispersant at a starch concentration of 10% (w/v).

3.1.6 Thermal properties and retrogradation

The thermal characteristics of the starches were measured using a TA 2010 Differential Scanning Calorimeter (DSC) (TA Instruments Inc., New Castle, DE) according to the method described by Paton (1987) with 50% (w/v) starch in water samples. The starch in water samples were equilibrated for 12 hr. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures were recorded. Enthalpy of gelatinization (ΔH) was expressed as J/g of dry starch and calculated as the area under the endotherm when it deviated from the constant temperature increase during heating of the filled DSC pan.

Retrogradation was assessed by analyzing the samples a second time by DSC, after storage at 4ºC for 4 d. Percent retrogradation was calculated as the enthalpy of gelatinization after storage divided by the enthalpy determined in the initial analysis (Paton 1987).

3.1.7 Pasting properties

A Rapid Visco-Analyzer (RVA) (Newport Scientific Pty Ltd., Narrabeen, Australia) was used to determine the pasting properties of starch samples according to AACC method 61-02 (AACC 2000). A 5% (w/v) starch slurry was made in the RVA canister which was kept at 50ºC for 1 min then heated to 95ºC in 3.8 min, held at 95ºC for 1 min, and cooled to 50ºC within 3.8 min where it was held for 1.4 min. For the first 10 s of the test, the slurry was stirred at a speed of 960 rpm, and at 160 rpm for the remainder of the test.

3.1.8 Swelling power and solubility, freeze-thaw stability, water-binding capacity and shear stability

Swelling power and solubility were determined over a temperature range of 65-95ºC according to the method of Leach and McCowen (1959). In a graduated tube (15 mL), 0.35 g of starch was added to 12.5 mL water followed by vortexing for 1 min. The tube was placed in a waterbath of desired temperature for 15 min whereby the tube was mixed thoroughly by vortexing every 5 min for 20 s. After 15 min, the tube was cooled in an ice bath to 25ºC after which it was centrifuged at 2000× *g* for 20 min. The supernatant was carefully removed using a pipette and its amount as well as its dry matter content were determined. Starch solubility was calculated as the amount of dry matter present in the supernatant divided by the initial starch weight. The weight of the sedimented paste was also recorded and the swelling power was calculated as the weight of the sediment divided by the weight of the original sample.

The freeze-thaw stability of quinoa starch was assessed by subjecting 5% (w/v) starch pastes to repeated cycles of freezing and thawing and measuring the amount of water separated on centrifuging the thawed pastes. The pastes used for these analyses were prepared with the RVA according to AACC method 61-02 (AACC 2000). The pastes were stored at –18ºC for 18 hr, thawed at room temperature for 6 hr, and then centrifuged at 4,300 × *g* for 10 min. This cycle of freezing and thawing was repeated five times. Freeze-thaw stability was expressed as the percentage of water separated from the paste after each freeze-thaw cycle. Water-binding capacity was determined

according to the method of Medcalf and Gilles (1965). Shear stability was determined for 5% (w/v) aqueous starch pastes as described by Praznik et al. (1999). The pastes used for the analyses were prepared with the RVA according to AACC method 61-02 (AACC 2000).

3.1.9 Scanning electron microscopy (SEM)

Starch was sprinkled onto double-adhesive tape attached to aluminum studs. Samples were coated with gold using a sputter coater (Model S150 B, Edwards, Crawley, UK) and examined in a Philips 505 SEM (Philips, Eindhoven, NL) at 30 kV and a 10,000 × magnification.

3.1.10 Statistical analysis

All measurements were replicated a minimum of three times. Data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was used to determine if starch characteristics differed among lines. Least significant differences were calculated using the General Linear Model (GLM) procedure. Linear correlation coefficients between starch characteristics and amylose content were also calculated.

3.2 Granule bound starch synthase I (GBSSI) in quinoa and its relationship to amylose concentration

3.2.1 Samples

Quinoa seed was collected from sixteen lines (Table 1) that were grown in a greenhouse (supplemented light intensity to a minimum of 230 μmol m^{-2} s^{-1}; photoperiod of 16 hr light at 20±1ºC). Once the plants reached physiological maturity in the greenhouse, the watering regime was reduced to approximately field conditions. Physiological maturity was defined by the following parameters: the leaves senesced, seeds were well formed (plump), and the panicles were well formed.

To determine the starch synthase activity in developing seed, immature seeds from three lines were harvested at 4, 6 and 10 weeks after flowering. The plants were

harvested without reduction in the watering regime. The panicles were directly frozen in liquid nitrogen after harvesting and they were stored at -70ºC.

3.2.2 Starch isolation

Starch was isolated from mature seed as described by Zhao and Sharp (1996). Seed (500 mg) was soaked overnight in 40 mL of deionized water, drained, ground and resuspended in 30 mL of water. The suspension was layered on 30 mL of an 80% (w/v) cesium chloride solution and than centrifuged at 6,000 × *g* for 15 min. The pellet was resuspended and layered on more cesium chloride solution and centrifuged as before. The pellet was washed twice with 9.5 mL of buffer [55m*M* Tris-HCl, pH 6.8; 2.3% (w/v) SDS; 10% (v/v) glycerol; 5% (v/v) β-mercaptoethanol], three times with 20 mL of deionized water, and once with 20 mL of acetone. The pellet then was dried at room temperature under vacuum.

3.2.3 Total starch determination

Total starch content was determined using AACC method 76-13 (AACC 2000). The analyses were done in triplicate.

3.2.5 Isolation of starch granule bound proteins

Starch granule bound proteins (SGP) were isolated according to Demeke et al. (1999). Briefly, starch (4 mg) was dispersed in 600 μL of extraction buffer [62.5 m*M* Tris-HCl, pH 6.8; 2.3% (w/v) SDS; 5% (v/v) β-mercaptoethanol; 10% (v/v) glycerol; 0.0005% (w/v) bromophenol blue], boiled for 5 min, and cooled on ice. The suspension was centrifuged at 13,000 × *g* for 20 min at 4°C. The supernatant containing the SGP was decanted from the gelatinized starch pellet.

3.2.6 Immunoblot analysis

Starch granule bound proteins were separated by SDS-PAGE [10% (w/v) polyacrylamide; 30:0.135 acrylamide/bisacrylamide] and visualized by silver staining (Bio-Rad Laboratories, Hercules, CA). The polypeptides were electrophoretically transferred at 4°C onto a PVDF membrane (Immobolin™-P

Transfer Membrane, Millipore, Billerica, MA) using transfer buffer [40m*M* Tris-HCl, pH 7.4; 20 m*M* $NaAc{\cdot}3H_2O$; 2 m*M* EDTA; 20% (v/v) CH_3OH; 0.05% (w/v) SDS] and the membrane was then incubated for 2 hr in blocking buffer [5% (w/v) skim milk; 1 × PBS; 0.1% (v/v) Tween) followed by incubation with primary antibodies [rabbit serum against Starch Synthase I (SSI) (Peng et al. 2001) (1:8000 dilution); Starch Synthase II (SSII) (Gao and Chibbar 2000) (1:2000 dilution); Starch Branching Enzyme I (SBEI) (Båga et al. 2000) (1:5000 dilution); Starch Branching Enzyme II (SBEII) (Nair et al. 1997) (1:5000 dilution); and GBSSI (Matus-Cadiz 2000) (1:8000 dilution) from wheat]. The excess of primary antibody was removed by four washes of 15 min each with the blocking buffer. The washed membrane was incubated for 1 hr with a secondary antibody [phosphatase-conjugated goat anti-rabbit serum, 1:5000 dilution (Sigma-Aldrich, St. Louis, MO)]. The excess secondary antibody was removed by three washes of 10 min each with the blocking buffer and three washes of 10 min each with the Tris-sodium chloride buffer (50 mM Tris-HCl, pH 7.5; 150 mM NaCl). The immuno reactive polypeptides were detected as blue bands with 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphatase (Stratagene, La Jolla, CA).

3.2.7 Quantification of GBSSI

Starch granule bound proteins, separated and visualized using the immunoblot analysis as described in section 3.2.6, were quantified in duplicate with a Chem-Doc using Quantity I software (Bio-Rad Laboratories, Hercules, CA).

3.2.8 Peptide sequencing

Starch granule bound proteins were extracted as described in section 3.2.5 from 10 mg of quinoa starch and resolved on a preparative SDS-PAGE gel [10% (w/v) polyacrylamide; 30:0.135 acrylamide/bisacrylamide]. The migration of the two different GBSSI polypeptides was determined by Coomassie blue staining. The stained polypeptides were separated from the rest of the gel and subjected to internal peptide sequencing after trypsin digestion at the Genome BC Proteomics Centre, University of Victoria, Victoria, BC. The digests were compared to the NCBI

database in a MS/MS Ion Search using the following as search parameters: carbamidomethyl as a fixed modification; oxidation as a variable modification; monoiotopic mass values; unrestricted protein mass; ±0.1 Da peptide as the mass tolerance; ±0.1 Da as the fragment mass tolerance; and 1 as the maximum number of mixed cleavages (Mascot, Matrix Science, Version 4.1).

3.2.9 Starch synthase activity

Starch synthase (SS) activity was determined in quinoa using incorporation of (U-^{14}C)glucose according to Smith (1990) and Vos-Scheperkeuter et al. (1986). The incubation conditions were optimized for substrate concentration (1.2 to 5.2 m*M* ADP-glucose), time (10 to 40 min), temperature (25 to 40ºC) and pH (6.5 to 8) to reach maximum starch synthase activity. To avoid problems related to inactivation of enzymes during extraction of the starch, cell lysates from whole seeds at different stages of maturation were used instead. The cell lysates were prepared by means similar to those described by Smith (1990) for the solubilization of SGP from starch. The assay mixture contained 20 µL cell lysate (0.125 g ml^{-1}) in protein extraction buffer [100 m*M* Tris-acetate, pH 7.7; 100 m*M* *N,N*-bis-(2-hydroxyethyl)glycine; 0.5 *M* Na-citrate, pH 7.5; 2.6 m*M* ADP(U-^{14}C)glucose (Amersham, Little Chalfont, UK) at 2 GBq·mol^{-1}; 1.5 mg amylopectin (potato); 100 mM KCl; 1 mM DTT; 1 mM EDTA; 5% (v/v) glycerol] and was incubated for 30 min at 32ºC. The enzyme reaction was stopped by heating the assay mixture at 90ºC for 2 min. The heat-inactivated reaction mixture (100 µL) was spotted on a membrane (943AH, 2.1 cm, Whatman, Brentford, UK) and dried. The unbound ADP-glucose was removed by washing the membranes four times for 30 min each time with 75% (v/v) methanol containing 1% (w/v) KCl. The washed membranes were dried at room temperature and mixed with scintillation fluid in a scintillation vial. The radioactivity incorporated into the amylopectin was counted with a 1219 Rackbeta liquid scintillation counter (Perkin Elmer, Boston, MA). The enzyme activity was determined in triplicate and expressed as the amount of (U-^{14}C) glucose incorporated into amylopectin in 30 min and expressed as counts per minute (cpm) per µL of cell lysate.

3.3 Development of an integrated process for purification of protein and starch from quinoa

3.3.1 Wet fractionation of flours from a variety of crops

Quinoa (WMF) was dehulled using a Satake abrasive mill (Model TM 05, Satake Corporation, Hiroshima, Japan) equipped with a 40 grit stone. One hundred and fifty gram batches of seed were milled for 5 min at 1518 rpm. The fines and abraded seed were separated using a 16 mesh (Tyler) screen. Flour was prepared from whole or dehulled quinoa by milling in a UDY Cyclone Sample Mill (UDY Lab Equipment and Supplies, Ft. Collins, CO) equipped with a 0.5 mm screen. Barley flour, white rice flour, buckwheat flour and corn flour were purchased at Herbs and Health Foods (Saskatoon, SK), Mom's Health Foods (Saskatoon, SK) and Real Canadian Superstore (Saskatoon, SK), respectively.

Flour and water (1:5 w/v) were mixed thoroughly using an Ultra-Turrax T25 (IKA Works, Wilmington, NC) at a shear speed of 13,500 rpm for 3 min. The slurry was centrifuged at 3,500 × *g*, 20ºC for 20 min. The viscosity of the initial flour-in-water slurry and that of the supernatant after centrifugation were measured using an Ubbelohde viscometer. Photographs were taken following centrifugation to document differences among flours in the appearance of the pellets, e.g. number of layers, colour of layers, etc.

The layers comprising the pellets were separated by scraping them off one by one. These layers were quantified, freeze-dried and analyzed for protein and starch content according to AACC methods 76-13 and 46-30, respectively (AACC 2000).

Starch was isolated from the pellet by taking the white coloured layer and homogenizing it in 100 mL water and 20 mL toluene. The mixture was left to settle overnight at room temperature, with subsequent removal of the toluene layer with a pipette. The wet starch fraction was centrifuged at 3,500 × *g* for 20 min. After discarding the supernatant, the sediment on top of the white pellet was removed with a spatula. The pellet was than washed with 95% ethanol, followed by acetone, after which the sample was air-dried overnight at room temperature. The starches obtained were ground with a mortar and pestle to pass through a 100 mesh (Tyler) screen.

The sedimentation velocity of the starches was determined using the Andreasen pipette method, which has been used to study sedimentation of particles in liquid media (DallaValle 1948, Tyler 1982). The Andreasen apparatus (Figure 3.1) consists of a half-litre cylindrical vessel equipped with a stopper carrying a pipette with a two-way stopcock and a 10 mL reservoir. The dip-tube of the pipette projects downward a certain distance beneath the surface of the starch in water suspension (Tyler 1982). A 500-mL sample of starch in water suspension [0.35% (w/v)] was homogenized for 10 s in an Ultra-Turrax T25 (IKA Works) at a shear speed of 13,500 rpm, followed by shaking the suspension for 2 min in the Andreasen pipette. Every 10 min, 1 mL of slurry was taken out of the pipette and its dry matter content determined according to AACC method 44-40 (AACC 2000). Sedimentation was visualized as the amount of dry material still present in the suspension after a certain amount of time.

The particle size of the starches was determined by low-angle light scattering using a Mastersizer (Model 2000SM, Malvern Instruments Ltd., Malvern, UK). Water was used as the dispersant with a starch concentration of 10% (w/v).

3.3.2 Comparison of the composition, α-amylase activity and pasting profiles of whole and dehulled seed from three quinoa lines

3.3.2.1 Sample preparation

Seed was partially dehulled as described in section 3.3.1 whereby 9.7, 12.1 and 13.6% of the seed was removed as hull from NQC, QC and WMF, respectively. The appropriate degree of dehulling was based on the visual appearance of the dehulled product.

3.3.2.2 Proximate analysis, amino acids and fatty acids

Seed (whole or dehulled) was ground in a UDY Cyclone Sample Mill (UDY Lab Equipment and Supplies, Ft. Collins, CO) equipped with a 0.5 mm screen. Analysis of moisture, crude fat, dietary fibre, starch, protein and ash was carried out according to the AACC methods 44-40, 32-05, 76-13, 46-30 and 08-01, respectively (AACC 2000). All analyses were performed in triplicate. Amino acid composition was

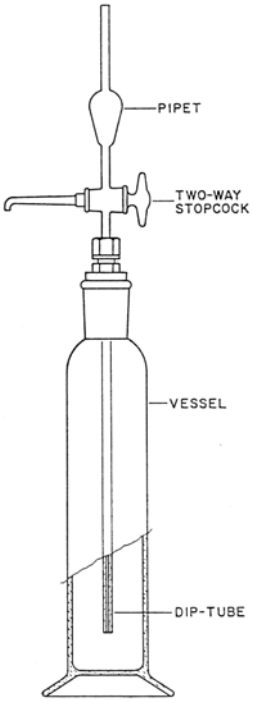


Figure 3.1 Line drawing of an Andreasen sedimentation pipette (Tyler 1982).

determined using a Pico-Tag analyzer (Waters Corporation, Milford, MA). Fatty acid composition was determined according to AOCS method 1e-91 (AOCS 2001). Mineral, fatty acids and amino acids analyses were performed by POS Pilot Plant Corp. (Saskatoon, SK).

3.3.2.3 Saponin content

Saponin content was determined according to the method described by Muir et al. (2000, 2002). Saponins were extracted from quinoa flour by dispersing 0.5 g of flour in 5 mL of 50% (v/v) aqueous ethanol, followed by extraction for 4 hr at 55ºC with vortexing every 30 min. The extract was filtered using a 3 mL syringe equipped with a 0.45 μm pore-size nylon syringe tip filter (Chromatographic Specialties Inc., Brockville, ON) and analyzed by HPLC using the following: model 2690 separation module (Waters, Milford, MA), Symmetry® C_{18} 5 μm 3.0 mm × 150 mm column (Waters) and model PL-AMP 960 ELSD detector (Polymer Laboratories Inc., Amherst, MA)]. To obtain optimal peak separation, the mobile phase comprised 0.05% trifluoroaceticacid (TFA) in water and 0.05% TFA in acetonitrile at a flow rate of 0.4 mL/min. Standard curves for each of the sapongenins (*phytoaccagenic acid, hederagenin* and *oleanolic acid*) were generated. These sapogenins were purified from quinoa by Dr. A. D. Muir (Agriculture and Agri-Food Canada, Saskatoon, SK).

3.3.2.4 Alpha-amylase activity and pasting profile

The α-amylase activity of flour was determined by AACC method 22-02 (AACC 2000). The analyses were performed in triplicate.

Pasting profiles were generated by rapid viscosity analysis of whole and dehulled quinoa flour in water (5.5% w/v) and in 1m*M* $AgNO_3$ (5.5%, w/v) as described in section 3.1.6. Silver nitrate was used to eliminate α-amylase activity (Bhattacharya and Corke 1996). The heating cycle used for the pasting profiles was according to AACC method 61-02 (AACC 2000).

3.3.2.5 Statistical analysis

All measurements were replicated a minimum of three times. Data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was used to determine if differences in composition and α-amylase activity existed among lines. Least significant differences were calculated using the General Linear Model (GLM) procedure.

3.3.3 Processing of quinoa seed by means of abrasive milling or roller milling

3.3.3.1 Abrasive milling

A tangential abrasive dehulling device (TADD), as developed by Reichert et al. (1986), was used to study the dehulling of quinoa seed (line WMF). The device was equipped with an 8-cup plate and a stone of grit 85. Five grams of seed per cup were dehulled over periods of 2 to 10 min at 1,750 rpm, which resulted in different degrees of dehulling. Dehulled seed was weighed and the yield expressed as a percentage of the initial seed weight. The effect of dehulling on the protein and crude fat contents of the seed was determined after grinding the seed in a coffee grinder.

A Satake abrasive mill (Model TM 05, Satake Corporation) equipped with a 40-grit stone was used to scale up the dehulling process. One hundred and fifty gram batches of seed were milled for 21 min at 1518 rpm. The fines and abraded seed were separated using a 16 mesh (Tyler) screen, whereby 55% of the seed was removed as fines.

3.3.3.2 Roller milling

Quinoa seed (line WMF) was tempered for 16 hr at room temperature to a final moisture content of 15.5%, which was according to Chauhan et al. (1992). Tempered and non-tempered seed was milled using a Quadrumat Junior Mill (Brabender, Duisburg, Germany). The mill was equipped with four rolls with corrugations of 7, 9, 9 and 10 flutes/cm and gaps between the rolls of 0.2 mm. The flour was separated into fractions of varying particle size using a Ro-Tap sieve shaker (W.S. Tyler, Mentor, OH). The shaker was equipped with screens of 35, 40, 50, 60, 80 and 200 (Tyler) mesh, which corresponded to openings of 500, 425, 300, 250, 187 and 75

μm. The protein and starch contents of the fractions were determined according to AACC methods 46-30 and 76-13, respectively (AACC 2000).

3.3.4 Protein extraction from quinoa bran

3.3.4.1 Samples

A bran fraction was produced from tempered WMF quinoa using roller milling as described in section 3.3.3.2, followed by screening the bran obtained (35 mesh, Tyler). The bran was defatted with hexane in a Soxhlet apparatus. Starch, protein (%N×6.25), moisture and residual oil in the defatted bran were determined according to AACC methods 76-13, 46-30, 44-40 and 30-25, respectively (AACC 2000). The defatted bran contained 23.4% protein, 0.9% fat and 32.1% starch (db). The bran was ground to different degrees of fineness using a UDY Cyclone Sample Mill (UDY Lab Equipment and Supplies, Ft. Collins, CO) equipped with a 0.5-mm screen, a Wiley (Brabender, Duisburg, Germany) mill equipped with 0.5-mm and 1.0-mm screens, or a hammer mill (Culatti AG, Zürich) equipped with a 2-mm screen. The mean bran particle size was determined using an Allen-Bradley sonic sifter (Rockwell Interational, Milwaukee, WI) equipped with 40, 60, 100, 140, 200 and 325 mesh (Tyler) screens and defined as the maximum point in the weight distribution of the ground bran.

3.3.4.2 Protein extraction

Protein was extracted from defatted bran by blending bran-solvent (sodium hydroxide solution, pH 9) mixtures for 8 min at room temperature in an Osterizer blender (Sunbeam Products Inc., Boca Raton, FL) at high speed. Five liquid:bran ratios (expressed in L/kg) were used: 5, 10, 15, 20 or 25. Brans differing in fineness (mean particle sizes of 40, 150, 230, 375 or >500 μm) were extracted. The bran having a mean particle size larger than 500 μm consisted of the coarse bran fraction resulting from roller milling without further grinding. The slurry of bran in alkali was centrifuged at five centrifugal forces (1,000; 3,500; 6,000; 8,500 or 11,000 × *g*) for 20 min at 20ºC. The mass of the supernatant was noted, as well as its dry matter and protein content determined according to the AACC methods 44-40 and 46-30,

respectively (AACC 2000). Protein recovery was calculated as the amount of protein extracted expressed as a percentage of the total amount of protein present in the original bran. Each extraction was performed in triplicate.

3.3.4.3 Experimental design

Response surface methodology was used to determine the influence of three independent variables on protein recovery in (Y_1), and the protein content (Y_2) of, the extract. The three independent variables were mean particle size of the bran (X_1), liquid-to-bran ratio (X_2) and centrifugal force (X_3). They were determined to be the most influential factors affecting protein extraction from quinoa bran in preliminary studies. The experimental design adopted was a modification of Box's central composite design for three factors each at five levels, as described by Haaland (1989) and as used by Oomah et al. (1994) for the optimization of protein extraction from flaxseed meal. The coded values of the independent variables were -1.68 (lowest level), -1, 0 (middle level), 1 and 1.68 (highest level). The correspondence between these coded values and the actual values is given in Table 3.1. The experimental design consisted of twenty points, including six replications at the central point, and was carried out in random order.

3.3.4.4 Statistical analysis

Data were analyzed using the RSREG (Response Surface REGression) procedure of SAS (1990) to fit the following second-order equation (3.1) for the two dependent Y variables:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \beta_{ij} X_i X_j \qquad \text{(Equation 3.1)}$$

where β_0, β_i,, β_{ii},, β_{ij} are constants and regression coefficients of the model, and X_i and X_j are the dependent variables. Goodness-of-fit tests were performed on the model using the backward elimination procedure.

Table 3.1 Coded and actual values of the independent variables for the response surface experimental design to optimize protein extraction from quinoa bran [I].

Design point	Independent variables		
	Mean particle size of the bran (μm) (X_1)	Liquid-to-bran ratio (L/kg) (X_2)	Centrifugal force (× *g*) (X_3)
1	150 (-1)	10 (-1)	3500 (-1)
2	150 (-1)	10 (-1)	8500 (+1)
3	150 (-1)	20 (+1)	3500 (-1)
4	150 (-1)	20 (+1)	8500 (+1)
5	375 (+1)	10 (-1)	3500 (-1)
6	375 (+1)	10 (-1)	8500 (+1)
7	375 (+1)	20 (+1)	3500 (-1)
8	375 (+1)	20 (+1)	8500 (+1)
9	40 (-1.68)	15 (0)	6000 (0)
10	500 (+1.68)	15 (0)	6000 (0)
11	230 (0)	5 (-1.68)	6000 (0)
12	230 (0)	25 (+1.68)	6000 (0)
13	230 (0)	15 (0)	1000 (-1.68)
14	230 (0)	15 (0)	11000 (+1.68)
15	230 (0)	15 (0)	6000 (0)
16	230 (0)	15 (0)	6000 (0)
17	230 (0)	15 (0)	6000 (0)
18	230 (0)	15 (0)	6000 (0)
19	230 (0)	15 (0)	6000 (0)
20	230 (0)	15 (0)	6000 (0)

[I] Values in parentheses are the coded levels of the independent variables.

3.3.5 Protein concentration

3.3.5.1 Protein extraction

Protein was extracted from defatted bran material as well as from defatted and saponin-extracted bran under the optimized conditions determined according to section 3.3.4. To extract saponins, defatted bran was stirred with 60% (v/v) aqueous ethanol at room temperature for 5 hr and recovered by vacuum filtration using #2 filter paper (Whatman, Brentford, UK). The extracted bran was dried in a vacuum oven at 30ºC and reground in a UDY cyclone sample mill (UDY Lab Equipment and Supplies) equipped with a 0.5 mm screen.

3.3.5.2 Isoelectric precipitation (IEP)

Protein was precipitated from extracts at pH 4.5 using 2 *M* HCl according to Aluko and Monu (2003). The precipitated protein was recovered by centrifugation at 3,500 × *g* for 20 min. The precipitate was subsequently diluted with a quantity of water equivalent to the mass of the curd and its pH adjusted to pH 7 using 2 *M* NaOH. The neutralized product was then freeze-dried. The nitrogen solubility curve of WMF was created using AACC method 46-23 (AACC 2000). The nitrogen solubility was expressed as the Nitrogen Solubility Index (NSI), where NSI is defined as the protein that is soluble at a particular pH as a percentage of the total protein present in quinoa bran.

3.3.5.3 Ultrafiltration (UF)

An Amicon hollow fibre concentrator (Model DC2, Amicon Corporation, Lexington, MA) was used for the concentration of quinoa protein extracts. The unit was operated in concentration mode, and was equipped with a Romicon (Koch Membrane Systems Inc., Wilmington, MA) hollow fibre membrane cartridge (MWCO of 10,000 or 50,000). The membranes were evaluated with respect to permeate flux rate, maximum volume concentration ratio (VCR) obtainable and percentage protein (dry matter basis) in the retentate. To determine whether a significant amount of protein passed through the membrane, the permeates were heated for 2 min in a microwave, cooled in ice water and centrifuged at 2,000 × *g* for

10 min. Three different feed rates corresponding to pump speed control settings of 4, 5, and 6, and measured as flow rates to the membrane of 0.56, 1.04 and 1.67 L/min, were tested for their effect on permeate flux rate and the maximum VCR obtainable. The VCR was calculated as:

$$VCR = \frac{V_0}{V_r} = \frac{V_0}{V_0 - V_p} \qquad \text{(Equation 3.2)}$$

where V_0 (mL) is the initial volume of the extract, V_r (mL) is the volume of the retentate and V_p (mL) is the volume of the permeate.

The pressure in the membrane cartridge was controlled by a back-pressure valve at the outlet and was set to maximize the permeate flow, i.e. at the maximum operational pressure allowable.

The membrane was cleaned after each run. Cleaning consisted of circulating 0.1 *M* NaOH, and subsequently a 1% (w/v) solution of Terg-A-Zyme enzyme detergent cleaner (Alconox Inc., New York, NY), for at least 30 min. The membrane was rinsed with deionized water before, between and after each cleaning treatment. To determine whether the membrane was sufficiently cleaned, the flux rate of the membrane after cleaning was determined with deionized water. A recovery of 85-90% of flux rate was considered acceptable.

Recovery of permeate flux on cleaning was also confirmed by three repeated concentration runs with 500 mL of quinoa protein extract (1.5% total solids), the 50,000 MWCO membrane and a feed rate of 555 mL/min.

Protein extracts were concentrated in duplicate beginning with 500 mL of extract using the 50,000 MWCO membrane at a feed rate of 555 mL/min. The extract was concentrated to one-tenth of its original volume after which its pH was adjusted to pH 7.0 with 6 *M* HCl prior to freeze-drying.

3.3.6 Protein characterization

3.3.6.1 Protein functionality

Protein solubility, foaming capacity, foam stability and emulsifying properties of the protein products prepared from defatted quinoa bran or from defatted and saponin-extracted quinoa bran were compared. These products were concentrated by IEP or UF. The protein products were compared with values determined for a soy protein isolate (Pro Fam 781, ADM, Deactur, IL) and egg white. The egg white product was prepared by freeze drying egg white from fresh eggs. Protein functionality was tested on the basis of a fixed amount of protein (db). The colour of the protein concentrates was determined with a ColorFlex spectrophotometer (HunterLab, Reston, VA).

Solubility

Protein solubility was determined by preparing 1% (w/v) protein dispersions in 0.01 *M* phosphate buffer, pH 7.0, followed by vigorous mixing on a vortex for 2 min each. The samples were centrifuged at 20,000 × *g* for 20 min to obtain a clear supernatant, and the amount of soluble protein in the supernatant was determined by the method of Hartree (1972) and expressed as a percentage of the total amount of protein.

Foaming properties

Foaming capacity was determined according to the procedure described by Poole et al. (1984). Samples were dispersed in 0.01 *M* sodium phosphate solution, pH 7.0, to give final protein concentrations of 1% (w/v). The sample dispersions were homogenized for 30 s using a Polytron PT 10-35 homogenizer (Kinematica AG, Littan/Luzern, Switzerland) equipped with a 12 mm generator (foam generating model). The volume of foam obtained was expressed as a percentage of the initial volume of the protein solution. To determine foam stability, the volume of the foam that remained after standing at room temperature for 30 min was expressed as a percentage of the initial foam volume.

Emulsifying properties

Emulsifying properties were determined according to Aluko and Yada (1993) and Aluko et al. (2001). Aqueous dispersions of the protein products were prepared in 0.01 *M* sodium phosphate solutions, pH 7.0, such that the final protein concentration for each was 1% (w/v). Emulsions were prepared by adding 1 mL of commercial canola oil to 5 mL of the protein solution followed by homogenization for 1 min using the Polytron PT 10-35 homogenizer (Kinematica AG, Littan/ Luzern,Switzerland) equipped with a 20 mm generator. The mean droplet diameter [D 3,2] and the specific surface area (m^2/mL) of the emulsions were determined in a Mastersizer (Malvern Instruments Ltd.) with water as the dispersant. Emulsions were prepared with duplicate samples with two Mastersizer measurements per sample; therefore, results are means of four determinations. Emulsion stability was measured by replication of measurements on the emulsions 30 min after homogenization and was calculated as the specific surface area at 30 min as a percentage of the initial specific surface area of the emulsion.

3.3.6. Protein composition

The molecular weight distributions of the protein products were determined by sodium dodecylsulfate-polyacrylamide gel (10%, v/v) electrophoresis (SDS-PAGE) using a Pharmacia Biotech Phast System (Amersham Biosciences, Uppsala, Sweden) with Phast Gel gradient 8-25 gels (Amersham Biosciences) under reducing conditions (addition of 5% (v/v) mercaptoethanol). Freeze-dried samples were solubilized to a final concentration of 5 mg/mL in electrophoresis buffer and heated at 99ºC for 10 min. Fifteen micrograms of protein were loaded in each well and the electrophoresis was run at 10 mA for 20 min at 15ºC. The gels were stained for protein with Coomassie Blue R350 (Amersham Biosciences). A wide molecular-weight range marker M4038 (Sigma Aldrich, St. Louis, MO) was used. Amino acid composition was determined using a Pico-Tag analyzer (Waters Corporation). Amino acids analyses were performed by POS Pilot Plant Corp. (Saskatoon, SK).

3.3.7 Starch extraction from quinoa perisperm

Starch was extracted from the fine roller-milled (perisperm) fraction under alkaline conditions (pH 9) by blending the fines in sodium hydroxide solution at a liquid to fines ratio of 25:1 for 8 min at room temperature in an Osterizer blender (Sunbeam Products Inc.) at high speed. The starch was separated from the protein extract by centrifugation at 11,000 × *g* for 20 min at 20ºC, after which the supernatant was poured off. To increase the purity of the starch pellet, the slurry of fines in sodium hydroxide solution was screened through 200 (Tyler) mesh prior to centrifugation. The starch pellet was rewashed with the sodium hydroxide solution (pH 9) at a liquid to solids ratio of 2:1 (w/w), and the grey sediment which accumulated on top of the pellet was scraped off with a spatula. The protein extract and purified starch from the fines were termed protein "b", and starch "a", respectively.

A Rapid Visco-Analyzer (RVA) (Newport Scientific Pty Ltd., Narrabeen, Australia) was used to determine the pasting properties of starch "a", starch "b" and the fines (perisperm) that resulted from roller milling (Figure 3.1) according to AACC method 61-02 (AACC 2000).

3.3.8 A process for the fractionation of quinoa

A scheme for the fractionation of quinoa, based on the work described in previous sections, is outlined in Figure 3.2. Quinoa (WMF) was separated into a coarse (saponin-, protein- and oil-rich) bran fraction and a fine (starch-rich) perisperm fraction by roller milling of tempered seed. The bran was defatted and the saponins were extracted.

Protein was extracted from the coarse defatted/saponin-extracted bran material. The pellet from centrifugation was recovered quantitatively and was termed starch "b". The protein extract was concentrated by means of ultrafiltration and the concentrated product was brought to pH 7 using 2 N HCl prior to freeze drying. This protein product was named protein "a".

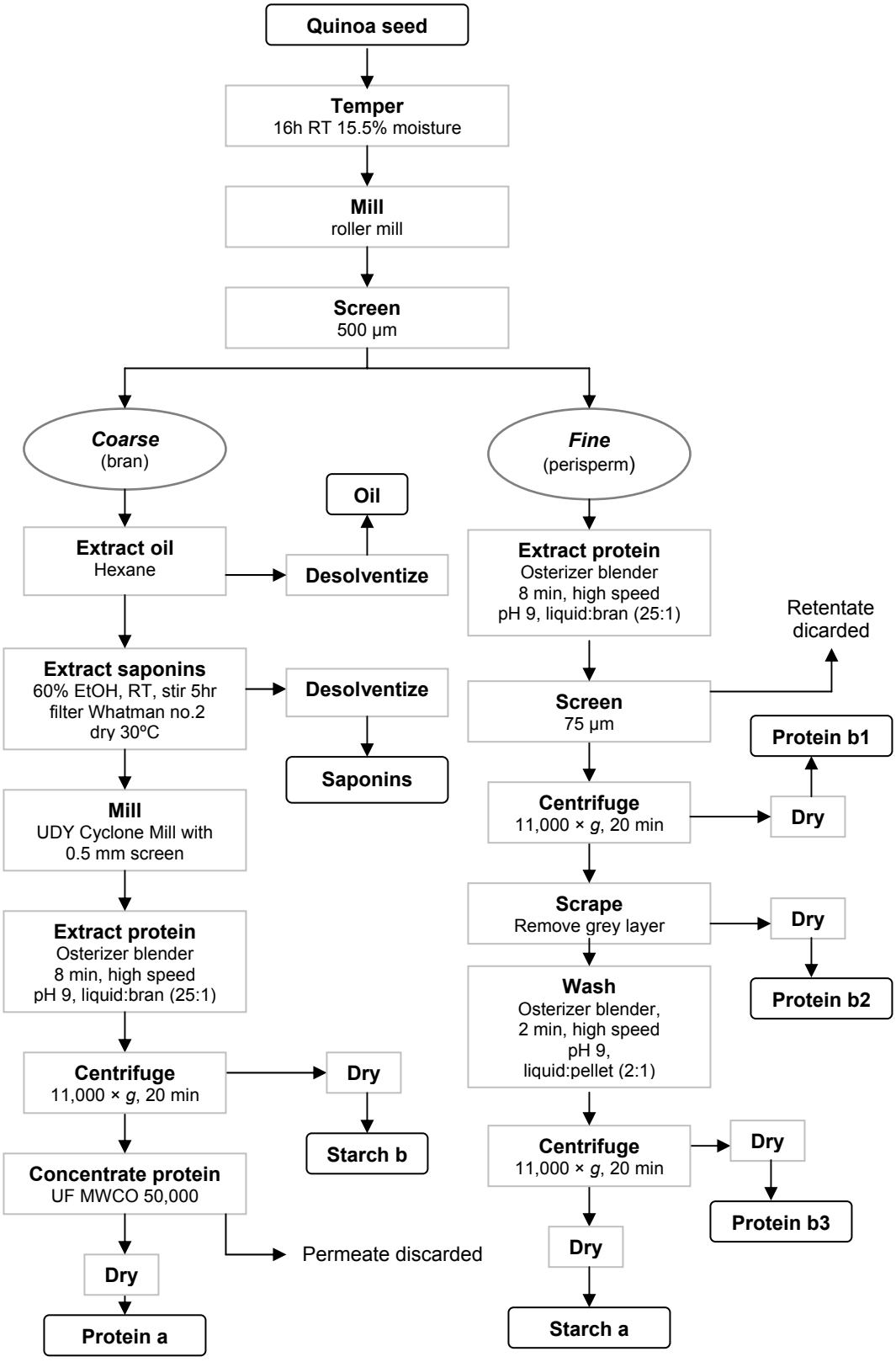


Figure 3.2 Processing schedule for quinoa fractionation.

The yield and dry matter content of all fractions were determined by AACC method 44-40 (AACC 2000). Protein “a”, protein “b”, starch “a” and starch “b” were analyzed for protein, starch, ash and crude fat content. Protein “a” was analyzed for saponin content. The fibre content was determined by difference.

3.3.8 Statistical analysis

Data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was used to analyze differences among the protein products (section 3.3.6.1) and to determine if starch pasting profiles differed among the starch-rich fractions (section 3.3.7). Least significant differences were calculated using the General Linear Model (GLM) procedure. Linear correlation coefficients between protein functionality and saponin content of the protein products were calculated.

4. RESULTS AND DISCUSSION

4.1 Characteristics of starch from eight quinoa lines

4.1.1 Amylose content

The amylose contents of the isolated quinoa starches, along with those of waxy and normal corn starches for comparative purposes, are presented in Table 4.1. The starches were analyzed without defatting, hence values obtained are apparent amylose contents. Except for waxy corn starch, the amylose contents determined colorimetrically were lower than those determined by HPSEC. A possible explanation for this could be incomplete solubilization of starch due to gel formation during the colorimetric assay, which occurred readily with the quinoa starch samples but not with the two corn starch samples. In general, a relatively large standard deviation was observed with the colorimetric method compared to the HPSEC method. Martinez (1996) used a similar colorimetric method to determine the amylose content of quinoa flour and also experienced a high standard deviation, which was attributed to the low amylose content (10.9%) of this sample. It was concluded that the colorimetric method lacked precision with samples containing less than 15% amylose. Therefore, all subsequent references to amylose content are to values determined by HPSEC.

4.1.2 Protein content

The protein contents of the isolated quinoa starches ranged from 0.1 to 1.2% (Table 4.1). With the exception of QC starch, all of the quinoa starches were lower in protein than the normal corn starch sample (0.69% protein). Corn starch typically has a protein content of approximately 0.35% (Watson 1984). The relatively high protein content of QC starch might reflect an actual compositional difference with this starch, as it was the only large, white-seeded quinoa sample examined. All other

Table 4.1 Amylose and protein contents of quinoa and corn starches[1].

Sample	Amylose (%) *HPSEC*	Amylose (%) *colorimetric*	Protein (%, db)
Ames 21926	3.5a	1.5ab	0.56ab
AAFC-1	4.6ab	0.3a	0.14c
NQC	6.4b	2.1ab	0.41abc
Ames 22155	11.5c	4.6bc	0.27bc
Ames 13745	12.7cd	6.3cd	0.36abc
WMF	14.4cd	9.3de	0.57ab
AAFC-2	15.1d	10.4e	0.60a
QC	19.6e	12.1e	1.23d
Normal corn	25.4	22.4	0.69
Waxy corn	1.0	2.8	0.82

[1] Values in the same column followed by the same letter are not significantly different ($p < 0.05$, $n = 3$).

quinoa lines were small-seeded and coloured (yellow, black, brown or purple). The higher protein content of QC starch may also reflect lower efficiency in refining of this sample.

4.1.3 Granule size

Six of the eight quinoa starches exhibited monomodal granule size distributions with granule sizes (volume mean diameters) of approximately 1.5 μm. An example is shown in Figure 4.1 (WMF). The waxy and normal corn starches also exhibited monomodal granule size distributions, with granule diameters of approximately 14 μm, which is within the range of 5-20 μm as reported by Jane et al. (1994). The NQC starch sample exhibited a major peak at 1.5 μm and minor peaks at 10 and 60 μm (Figure 4.1). QC starch exhibited a major peak at 1.5 μm and a shoulder extending to 30 μm (Figure 4.1). These apparent anomalies in granule size may have been due to aggregation, as reported by Varriano-Marston and DeFransisco (1984) who observed aggregates of 18-20 μm in diameter in quinoa starch. Aggregates are typical of most starches that consist of small granules, such as quinoa, amaranth and cow cockle (Lorenz 1990).

4.1.4 Thermal properties and retrogradation

Table 4.2 presents the thermal properties of quinoa and corn starches. The gelatinization onset and peak temperatures of the quinoa starches ranged from 44.6 to 53.7ºC and 50.5 to 61.7ºC, respectively, and the gelatinization enthalpies from 12.8 to 15.0 J/g of dry starch. The quinoa starches exhibited lower gelatinization temperatures than did the corn starches, as was reported by Inouchi et al. (1999). Quinoa and corn starches had similar gelatinization enthalpies. The quinoa starches differed significantly with respect to their onset and peak temperatures ($p < 0.05$), but differences in gelatinization enthalpy were not significant ($p > 0.05$). The onset and peak temperatures were positively correlated with amylose content ($p < 0.05$) (Table 4.3). This observation is in contrast to the negative correlation found by Fredriksson et al. (1998) for wheat, rye, barley and pea starches, but similar to results

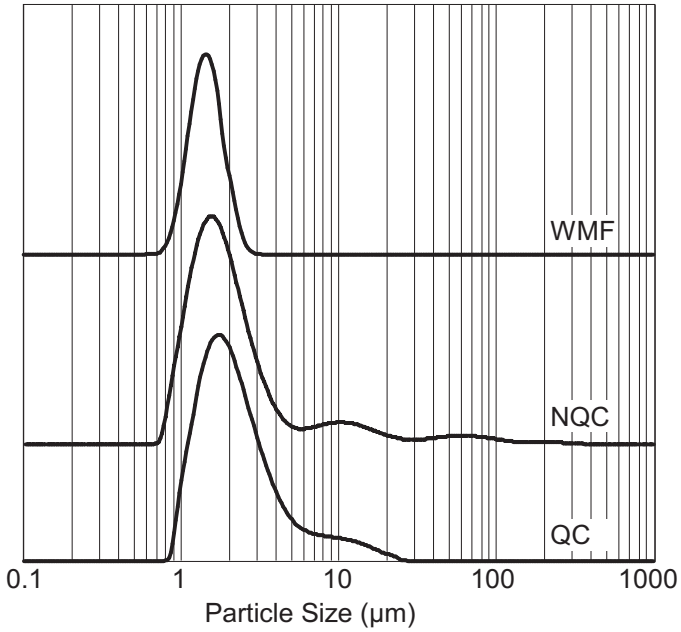


Figure 4.1 Granule size distributions of WMF, NQC and QC quinoa starches.

Table 4.2 Thermal properties and retrogradation of quinoa and corn starches as determined by differential scanning calorimetry[1].

Sample	T_o (°C)	T_p (°C)	T_c-T_o (°C)	ΔH (J/g)	Retrogradation (%)
Ames 21926	44.7a	50.7a	33.5ab	13.6ab	19.6a
AAFC-1	44.6a	50.5a	35.8a	14.3a	25.8ab
NQC	48.5b	56.1c	34.2a	15.0a	33.1bc
Ames 22155	46.6c	52.7c	34.1ab	13.6ab	25.9ab
Ames 13745	47.3d	52.7c	34.1ab	14.9a	28.7bc
WMF	53.7e	61.7d	32.7ab	14.5a	40.8d
AAFC-2	46.9cd	51.8e	30.8b	12.8b	32.4bc
QC	50.6f	57.4f	31.3b	14.2ab	36.1cd
Normal corn	61.7	69.1	22.1	13.1	75.0
Waxy corn	62.3	69.3	21.3	12.6	39.5

[1] Values in the same column followed by the same letter are not significantly different ($P < 0.05$, n=3). T_o, onset temperature; T_p, peak temperature; ΔH, gelatinization enthalpy; T_c, conclusion temperature; T_c-T_o, gelatinization range. All measurements were done at a 50% moisture content.

Table 4.3 Correlation coefficients between starch properties and amylose content[I].

Property	Correlation coefficient (r)	Property	Correlation coefficient (r)
Thermal		Solubility	
Onset temperature	0.60**	*65ºC*	-0.38^{ns}
Peak temperature	0.47*	*75ºC*	0.13^{ns}
Gelatinization range	-0.50*	*85ºC*	0.33^{ns}
Gelatinization enthalpy	-0.10^{ns}	*95ºC*	0.53*
Retrogradation	0.58**	Freeze-thaw	
		Cycle 1	0.47**
Pasting		*Cycle 2*	0.40*
Temperature	0.08^{ns}	*Cycle 3*	0.33^{ns}
Peak time	0.57***	*Cycle 4*	0.29^{ns}
Peak viscosity	-0.93***	*Cycle 5*	0.26^{ns}
Trough viscosity	-0.87***		
Final viscosity	-0.95***	Water-binding	-0.29^{ns}
Breakdown	-0.77***		
Setback	-0.90***	Shear stability	0.59*
Swelling power			
65ºC	-0.96***		
75ºC	-0.97***		
85ºC	-0.90***		
95ºC	-0.93***		

[I] *, **, ***, Significant at $p < 0.05$, 0.01, and 0.001; ns, not significant.

reported by Knutson (1990) for maize starch and by Varavinit et al. (2003) for rice starch. According to Gernat et al. (1993) and Fredriksson et al. (1998), starch crystallinity increases with amylopectin content. Hence, starches with higher amylopectin contents (i.e. lower amylose contents) would be expected to have higher onset, peak and conclusion temperatures. No explanation can be offered for the positive correlation between amylose content and gelatinization temperature in quinoa, nor was one proposed by Knutson (1990) or Varavinit et al. (2003) for maize or rice starches. There have been several reports that showed no correlation at all between peak temperature and amylose content (Biliaderis et al. 1986, Noda et al. 1998, Sasaki et al. 2000). The relationship between gelatinization characteristics and amylose content appears to be strongly species dependent. Furthermore, DSC gelatinization properties are influenced by factors other than amylose content. For example, it has been reported that the amount of extremely short chains of amylopectin is negatively correlated with the onset and peak temperatures (Noda et al. 1998, Noda et al. 2002, Vandeputte et al. 2003).

The term retrogradation describes changes that occur upon cooling and storage of gelatinized starch pastes, changes which often decrease the quality of starch-based foods. Retrogradation of the eight quinoa starches ranged from 19.6 to 40.8% of the initial gelatinization enthalpy and differed significantly among lines (Table 4.2). The retrogradation tendency was positively correlated ($p < 0.05$) with amylose content (Table 4.3). Amylose content is considered one of the most influential factors in starch retrogradation (Gudmundsson and Eliasson 1990, Chang and Lui 1991, Baik et al. 1997, Fan and Marks 1998, Kaur et al. 2002), with a higher level of amylose resulting in greater association of starch molecules and a higher degree of retrogradation. All of the quinoa starches, with the exception of WMF, exhibited less retrogradation than did waxy corn starch (Table 4.2). Apart from amylose content, there are other factors that influence the retrogradation of starch, including short term development of crystallinity (Miles et al. 1985, Sievert and Würsch 1993), the size and shape of the granules, and the presence/absence of lipid (Singh et al. 2003). Additionally, a high proportion of extremely short chains of amylopectin (dp 2-6) inhibits the retrogradation of starch (Würsch and Gumy 1994). Apart from that WMF

showed a higher tendency to retrogradate, it also had higher gelatinization onset and peak temperatures. The reason could be that this starch differed from the other starches in amylopectin fine structure. However, this was not investigated further.

4.1.5 Pasting properties

With the exception of pasting temperature, which ranged from 63.0 to 64.0ºC, significant differences in pasting characteristics were observed among the quinoa starches (Table 4.4 and Figure 4.2). Peak time ranged from 5.1 to 6.9 min and was positively correlated ($p < 0.05$) with amylose content (Table 4.3). A similar difference was found between waxy and normal corn starch, where the peak time was 3.8 min for waxy corn and 5.4 min for normal corn.

Viscosity parameters (peak, trough, final, breakdown and setback) were negatively correlated with amylose content ($p < 0.05$, Table 4.3). Figure 4.2 displays the pasting profiles of high (QC, 19.6% amylose), medium (Ames 22155, 11.5% amylose) and low (Ames 21926, 3.5% amylose) amylose quinoa starches. Reddy et al. (1994) also found a strong negative correlation between amylose content and viscosity parameters for rice starch if the paste concentration was less than 7% (w/v), as in the current study. At paste concentrations higher than 7% (w/v) they found a significant positive correlation between amylose content and paste viscosity. Wootton and Panozzo (1998) also found a highly negative correlation between RVA parameters, with the exception of setback, and amylose content in wheat. Setback reflects the degree of retrogradation of starch pastes and would be expected to be positively correlated with the amylose content of starch (Yasui et al. 1999, Grant et al. 2001, Abdel-Aal et al. 2002, Bhattacharya et al. 2002). As depicted in Figure 4.2 and described in Tables 4.3 and 4.4, setback was more pronounced for quinoa starches with lower amylose contents. The high setback as observed for Ames 21926 and AAFC-1 could be because these starches might have shorter amylose chains than the other starches. Shorter amylose chains are more mobile and are therefore able to interact more rapidly than longer chains.

Table 4.4 Pasting properties of quinoa and corn starches.[I]

Sample	P_{temp}	P_{time}	Viscosity (RVU)				
	(ºC)	(min)	PV	TV	FV	BD[II]	SB[III]
Ames 21926	63.3a	5.3a	25.8a	17.0a	28.0a	8.8a	12.3a
AAFC-1	63.9a	5.3a	24.4a	16.1a	28.9a	8.3b	12.8a
NQC	63.4a	6.7b	19.0b	17.9a	24.2b	1.1c	6.3b
Ames 22155	64.0a	5.1a	8.2c	7.0b	13.5c	1.1c	6.5b
Ames 13745	63.6a	6.7b	8.7c	8.1bc	14.5cd	0.5de	6.4b
WMF	63.0a	6.8b	11.4d	11.0d	16.4d	0.4e	5.4c
AAFC-2	64.0a	6.9b	10.3de	7.9ce	14.9cd	0.4e	5.0c
QC	64.0a	6.6b	3.6f	2.9f	4.2e	0.8d	1.3d
Normal corn	91.0	5.4	30.6	24.6	28.8	6.0	5.4
Waxy corn	71.3	3.8	57.8	36.4	48.2	21.4	6.3

[I] P_{temp}, pasting temperature; P_{time}, peak time; PV, peak viscosity; TV, trough viscosity; FV, final viscosity; BD, breakdown; SB, setback. Values in the same column followed by the same letter are not significantly different ($p < 0.05$, n=4).

[II] PV minus TV.

[III] FV minus TV.

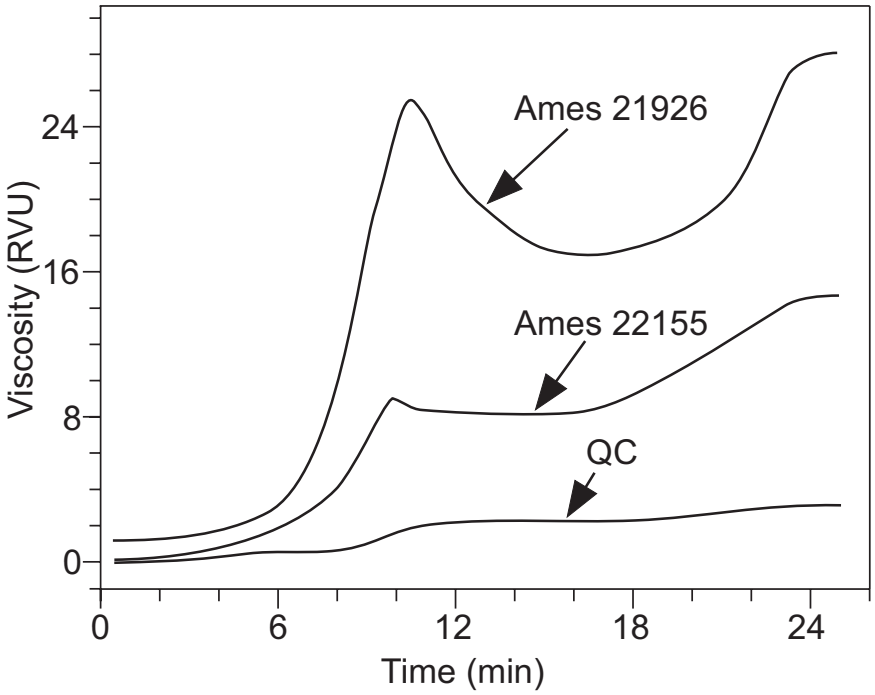


Figure 4.2 Pasting profiles of QC (19.6% amylose), Ames 22155 (11.5% amylose) and Ames 21926 (3.5% amylose) quinoa starches.

4.1.6 Swelling power and solubility, freeze-thaw stability, water-binding capacity and shear stability

Swelling power and solubility values for the quinoa starches are presented in Table 4.5. The data for solubility are incomplete due to the absence of a discernible supernatant at higher temperatures for some starches. Swelling power was strongly affected by amylose content, with a negative correlation between swelling power and amylose content ($p < 0.05$, Table 4.3). It is generally accepted that amylose acts as a restraint to swelling (Tester and Morrison 1990, Fredriksson et al. 1998, Noosuk et al 2003, Sasaki et al. 2003), and that waxy starches swell to a greater extent than their normal amylose counterparts (Tester and Morrison 1990). This was observed for the quinoa starches as well as for the normal and waxy corn starches in this study. Lorenz (1990) and Ahamed et al. (1996a) reported that quinoa starch had a very high swelling power compared to barley, wheat, rice, amaranth, potato and corn starches. However, in this study it was found that the swelling power of quinoa starch was strongly dependent on the line, in that the lower amylose quinoa lines (e.g. Ames 21926) exhibited higher swelling capacities, similar to waxy corn. Conversely, higher amylose lines (e.g. QC and AAFC-2) had swelling capacities similar to those of normal corn starch. The higher amylopectin content, and therefore the higher amount of crystalline regions, makes it possible for the granule to absorb large amounts of water without losing its structure.

Solubility reflects the leakage of amylose from starch granules (Ahamed et al. 1996a). At 95ºC a positive correlation ($p < 0.05$) between amylose content and solubility was found (Table 4.3). However, the solubility among the different quinoa lines did not differ significantly at the other temperatures at which solubility was determined. Values for both solubility and swelling increased slightly over the temperature range of 65-95ºC, due to progressive gelatinization of the starch granules.

A range of freeze-thaw behaviours was exhibited by the quinoa starches (Figure 4.3). As expected on account of its low amylose starch, waxy corn starch displayed high stability due to its resistance to retrogradation. Similarly, the high freeze-thaw stability of starch from AAFC-1 and Ames 21926 was expected in light of their low

Table 4.5 Swelling power, solubility, water-binding capacity and shear stability of quinoa and corn starches[I].

Sample	Swelling Power [II]				Solubility [III]				Water-binding (%)	Shear stability (%)
	65	75	85	95	65	75	85	95		
Ames 21926	20.7a	27.8a	53.8a	52.5a	2.5ab	2.3ab	-	0.1ab	59.6ab	59.6a
AAFC-1	18.0b	25.7b	34.0b	52.6a	3.2a	2.0bcd	3.1ab	-	75.9a	63.3a
NQC	17.9b	21.8c	34.6b	42.6b	2.2bc	2.4ab	4.4c	1.6abc	75.8a	72.8b
Ames 22155	13.4c	19.7d	27.6c	21.7c	1.9bc	1.8d	3.7d	2.5abc	70.5ab	58.6a
Ames 13745	13.6cd	18.9d	20.8d	21.9c	1.4c	2.2abcd	2.9b	2.8abc	64.2ab	60.8a
WMF	14.0c	18.5d	24.9e	23.5c	2.3abc	2.3abc	3.8d	4.7c	93.0a	74.8b
AAFC-2	11.8de	15.4e	17.4f	24.4c	1.8bc	1.9cd	2.3e	0.5ab	66.7ab	75.6b
QC	10.3e	12.6f	15.0g	16.4d	2.5ab	2.6a	3.3a	3.4bc	49.5b	80.5b
Normal corn	6.0	12.2	13.2	16.5	2.0	4.9	6.3	10.0	110.1	100.0
Waxy corn	6.2	54.2	54.8	54.9	2.2	-	-	-	116.7	86.1

[I] Values in the same column followed by the same letter are not significantly different ($p < 0.05$, $n = 3$).

[II] Swelling measured at temperatures of 65, 75, 85 and 95ºC.

[III] Solubility measured at temperatures of 65, 75, 85 and 95ºC.

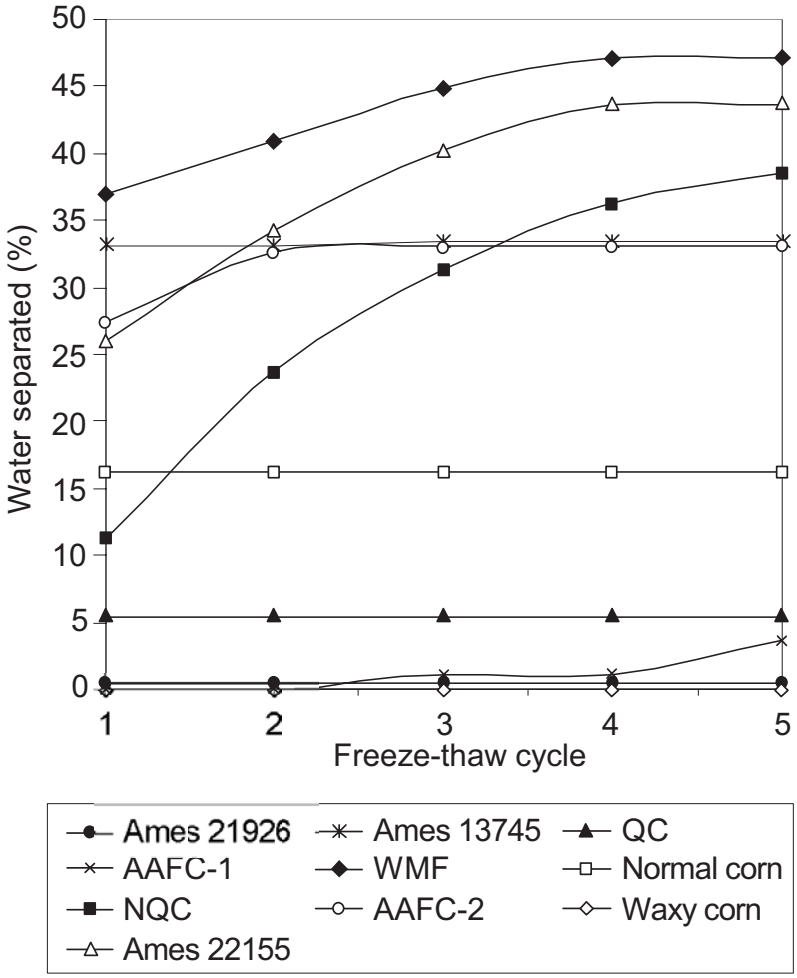


Figure 4.3 Freeze-thaw stabilities of quinoa and corn starches over five freeze-thaw cycles (n=4).

amylose contents (4.6% and 3.5%, respectively). However, QC had the highest amylose content of the quinoa starches studied, yet also exhibited freeze-thaw stability. A significant correlation between freeze-thaw stability and amylose content was not detected ($p > 0.05$, Table 4.3). Very high freeze-thaw stability for quinoa starch was reported by Ahamed et al. (1996a). As illustrated in Figure 4.3, however, it can be concluded that this was the case for some but not for all quinoa lines.

The water-binding capacities of quinoa starches ranged from 49.5 to 93.0%. These values were relatively low compared to those for normal and waxy corn starch (Table 4.5) and to the 118.5% reported by Lorenz (1990) for quinoa starch. A significant correlation between amylose content and water-binding capacity was not observed ($p > 0.05$, Table 4.3).

Quinoa starches differed significantly in their shear stability ($p < 0.05$, Table 4.3), which ranged from 58.6% to 80.5% (Table 4.5). Shear stability was positively correlated with amylose content ($p < 0.05$). A starch granule becomes increasingly susceptible to shear disruption as it swells, and those lines with lower amylose contents swelled more than their higher amylose counterparts. Therefore, lower amylose lines would be more susceptible to shear, as observed for both quinoa and corn starches in this study.

4.1.7 Scanning electron microscopy

Four quinoa starches ranging in amylose content from 4.3-19.6% were examined by scanning electron microscopy (Figure 4.4). No differences in starch granule morphology were observed. All exhibited the irregular, polygonal morphology typical of most small granule starches (Jane et al. 1994).

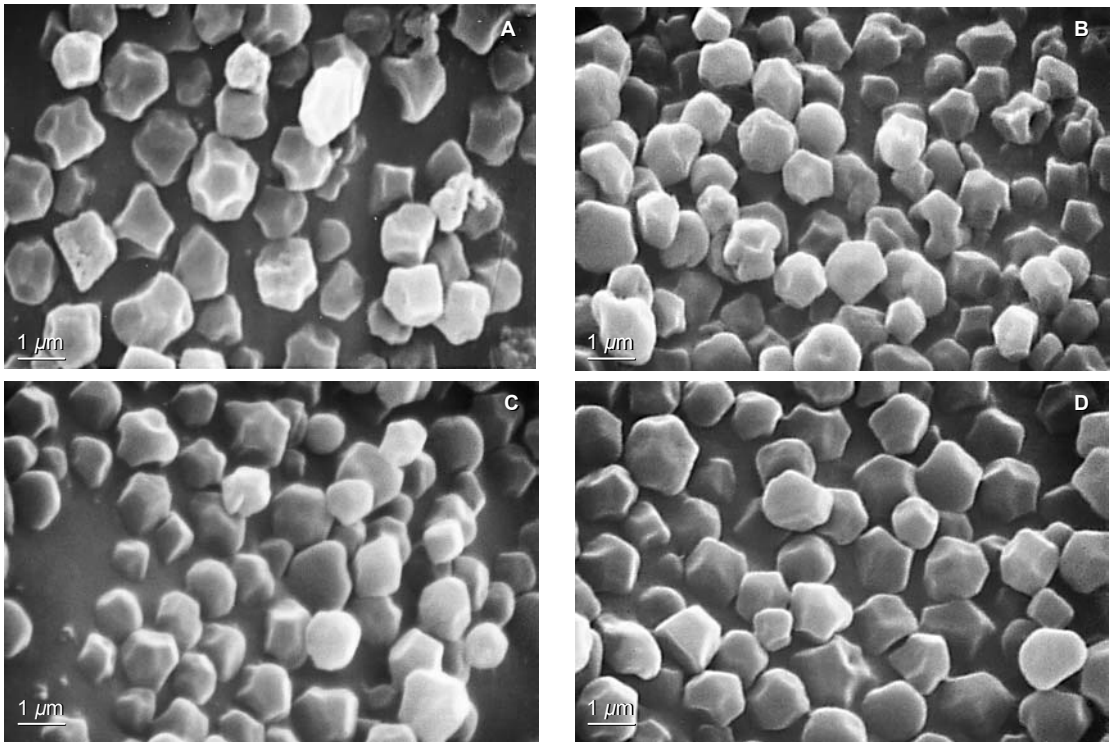


Figure 4.4 Scanning electron micrographs (10,000 × magnification) of quinoa starch samples differing in amylose content. a. Ames 21926 (3.5% amylose); b. NQC (7.5% amylose); c. WMF (14.4% amylose); d. QC (19.6% amylose).

4.2 Granule bound starch synthase I (GBSSI) in quinoa and its relationship to amylose content

4.2.1 Amylose and starch concentrations

All seeds used for the analyses, with the exception of those used for the analysis of starch synthase activity, were visually inspected to insure that only plump, well-filled seeds were selected. The amylose concentration in starch from sixteen quinoa lines ranged from 3.5% (Ames 21926) to 19.5% (Ames 22159) (Table 4.6). The lines analyzed in this research were grown under identical conditions and all amylose analyses were performed using HPSEC. Therefore, the variation in amylose is likely to reflect genotypic differences among lines. Some lines contained almost waxy starch (Ames 21926 and Baer). No line with high amylose starch was found (Table 4.6). The starch concentrations in quinoa seed ranged from 48.3 to 62.5% (Table 4.6).

4.2.2 GBSSI identification

SDS-PAGE analysis of quinoa starch granule bound proteins (SGP) revealed the presence of two predominant polypeptides having apparent molecular masses of 62 and 56 kDa, respectively (Figure 4.5, lanes 2 and 3). In addition, a few smaller polypeptides were detected. The 62 kDa polypeptide co-migrated with GBSSI polypeptides from wheat (Figure 4.5, lane 4). Antibodies specific to wheat GBSSI were used in an immunoblot analysis and were observed to react with both the 62 and the 56 kDa polypeptides from quinoa (Figure 4.6). This indicated that both polypeptides were isoforms of GBSSI. Antibodies against other wheat starch biosynthetic enzymes, such as starch synthase I and starch branching enzyme, did not cross react with the 62 and 56 kDa polypeptides from quinoa (results not shown). These antibodies did cross react with larger (> 70 kDa) polypeptides extracted from quinoa starch (results not shown).

Internal peptide sequence analysis of the 62 and 56 kDa polypeptides revealed similar sequences for both polypeptides, and the sequences were similar to those of GBSSI from other species (Table 4.7). These findings support the conclusion that the polypeptides are isoforms of GBSSI.

Table 4.6 Starch contents and amylose concentrations (± SD) of sixteen quinoa lines.

Line	Starch (%, db)	Amylose (%)
Ames 21926	52.4±1.0	3.5±1.0
Baer	56.7±0.3	4.4±0.5
Appelawa	53.5±2.0	7.5±0.3
95Y	54.2±1.6	11.7±1.5
Ames 13746	55.0±2.3	11.9±0.6
Ames 13745	53.2±0.9	12.7±0.2
Tango	50.0±0.6	12.8±0.3
407 Dave	57.3±0.2	13.7±0.3
Diverse	57.4±3.3	14.4±1.4
Ames 13747	53.6±0.4	15.8±0.5
Ames 21935	62.5±1.2	17.1±1.2
NSL 92331	53.0±1.2	17.6±0.4
Ames 22154	55.5±0.7	17.8±0.5
NSL 106396	49.4±1.7	17.9±0.6
Ames 13732	56.8±0.9	18.2±0.3
Ames 22159	48.3±1.9	19.5±0.2

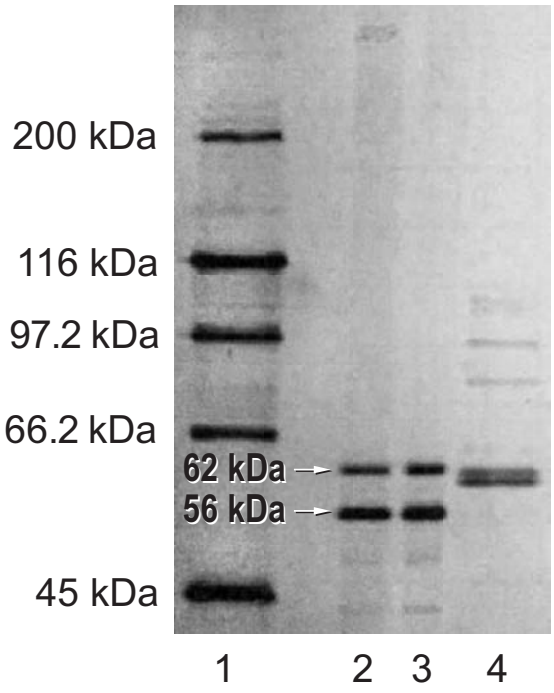


Figure 4.5 SDS-PAGE gel of starch granule bound protein extracted from quinoa (Ames 22159) (lanes 2, 3) and wheat starch (lane 4).

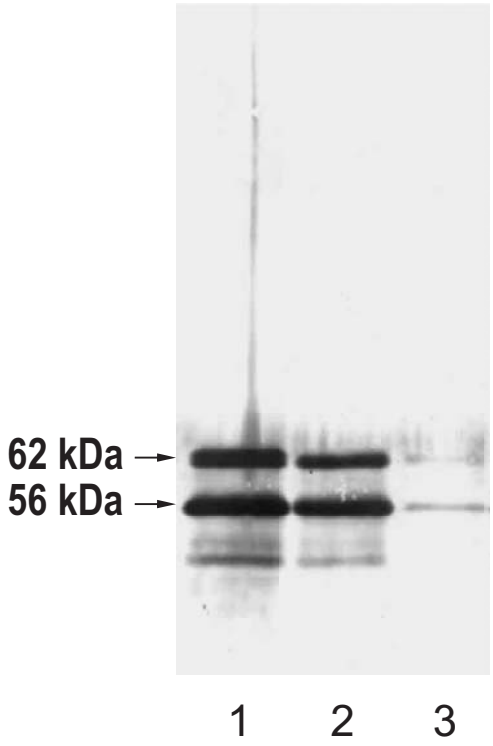


Figure 4.6 Immunoblot detection using rabbit antibodies against wheat GBSSI of starch granule bound protein extracted from quinoa starch: Ames 22159 (lane 1) containing 20.5% amylose, 95Y (lane 2) containing 13.5% amylose, and Ames 21926 (lane 3) containing 3.5% amylose.

The presence of two forms of GBSSI differing in molecular mass is unusual, but not unique. Vos-Scheperkeuter et al. (1986) and MacDonald and Preiss (1985) detected multiple forms of GBSSI in both amaranth and maize starches. The major proteins which reacted with rabbit antibodies against the 60 kDa GBSSI from potato were a 65 kDa protein from amaranth and a 61 kDa protein from maize. In addition, one minor cross-reacting polypeptide was detected in each species, which was suggested to be a minor isozyme of GBSSI. The 62 kDa and 56 kDa polypeptides in quinoa therefore might be isozymes. Another possibility is that the minor cross-reacting band is a precursor of GBSSI. A relationship between the two polypeptides is also indicated by the fact that both polypeptides are missing in waxy corn. It was suggested for amaranth that the minor 61 kDa polypeptide was an enzymatic digestion product of the major 65 kDa polypeptide (Vos-Scheperkeuter et al. 1986). This might also be the case in quinoa.

4.2.3 GBSSI content and starch synthase activity in quinoa and their relationship to amylose concentration

Immunoblot analysis of the SGP from mature seed of three quinoa lines (i.e., Ames 22159, 95Y and Ames 21926) was performed. The SGP fraction from quinoa starch with a relatively high amylose concentration (19.5%) exhibited much more intense and distinct peptide bands than did the SGP fraction from quinoa starch of intermediate amylose content (11.7%) or of low amylose content (3.5%) (Figure 4.6). Densiometry analysis of the polypeptide bands confirmed that the intensities of the 62 kDa and 56 kDa bands were proportional to the amylose contents of the starches from which they were extracted (Figure 4.7). This positive correlation between amylose content and GBSSI is not surprising, considering that GBSSI is intimately involved in amylose synthesis (Nelson and Rhines 1962, Baldwin 2001).

The concentration of starch in developing quinoa seeds was low in the early stages of seed development, but increased to near maximum levels after six weeks (Table 4.8). The concentration of amylose in starch also increased during seed development (Table 4.8), which has been observed in other species (Shannon and

Table 4.7 Internal peptide sequences of GBSSI proteins from species that matched the internal peptide sequences of the 56 kDa and the 62 kDa polypeptides from quinoa.

Polypeptide	Peptide sequence	Matching peptides	Score[a]
56 kDa	AGILESDR	GBSSI *Vauquelinia californica*	49
56 kDa	EALQAEVGLPIDR	GBSSI *Vauquelinia californica*	78
62 kDa	AGIIESDR	GBSSI *Perilla frutescens*	49
62 kDa	EALQAEVGLPVDR	GBSSI *Perilla frutescens*	72

[a] Score is -10*log(p), where P is the probability that the observed match is a random event. Individual ion scores >35 indicate identity or extensive homology ($p < 0.05$).

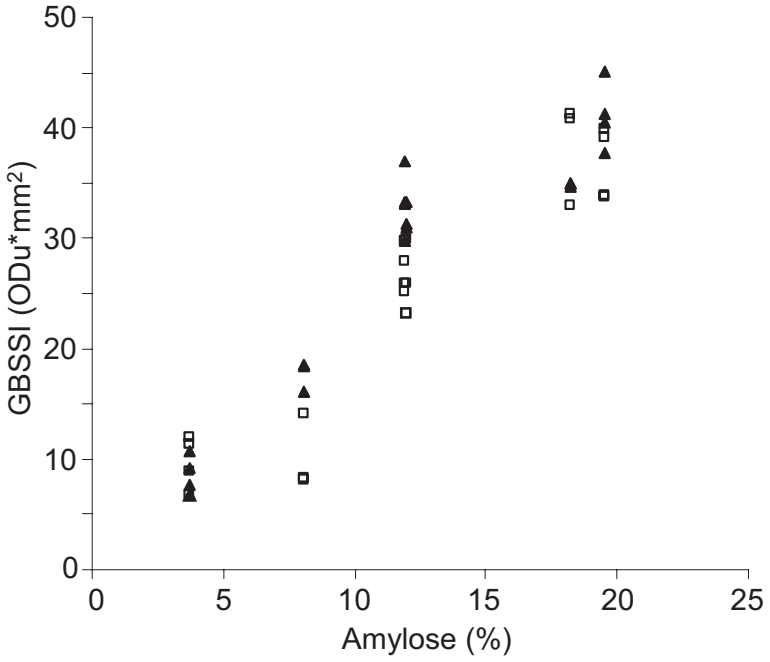


Figure 4.7 Relationship between amylose concentrations, as determined by HPSEC, and GBSSI content, as separated by SDS-PAGE, visualized using immunoblot analysis and quantified with densiometry (lines Ames 21926, Appelawa, 95Y, Ames 13746, Ames 13732, Ames 22159). The amounts of the 56 kDa (▲) and 62 kDa (□) polypeptides are expressed in density units of the peptide bands on the immunoblot membrane.

Table 4.8 Starch concentration in developing seed, amylose concentration in starch and starch synthase (SS) activity of three quinoa lines at three stages of maturity.

Line	Time after flowering (weeks)	Starch (%, db)	Amylose (%)	SS activity (cpm/µL)[a]
Ames 21926	4	7.3±0.2	1.8±0.2	0
	6	-[b]	-[b]	-[b]
	10	52.4±1.0	3.5±0.4	46.8±2.8
95Y	4	48.2±1.8	6.5±0.6	139.4±19.5
	6	52.7±2.5	11.0±0.7	234.5±5.6
	10	54.2±1.3	13.5±0.8	106.1±3.7
Ames 22159	4	40.7±1.5	15.8±1.0	262.0±4.7
	6	43.2±1.9	20.5±0.7	341.6±7.2
	10	48.3±1.9	20.7±1.6	404.5±16.6

[a] Amount of (U-^{14}C)glucose incorporated into amylopectin in 30 min and expressed as counts per minute (cpm) per µL cell lysate.

[b] Not determined due to shortage of material.

Garwood 1984). It appears that the synthesis of amylose is somewhat delayed compared with that of amylopectin. This is probably due to the timing of the biosynthesis of GBSSI, which appears later than some of the other starch synthases (Martin and Smith 1995).

Repeated attempts to determine GBSSI activity *in vitro* were not successful (data not shown). Others have reported that GBSSI has low enzymatic activity in standard assays (Nelson et al. 1978, MacDonald and Preiss 1985), so low that in some species it has been impossible to detect enzymatic activity *in vitro* (Smith 1990, Denyer et al. 1995). Analysis of cell lysates by SDS-PAGE revealed that the two peptides identified as GBSSI were the major peptides present in the lysates. Therefore, a strong relationship would be expected between total SS activity and GBSSI activity during seed development. Hence, total SS activity rather than GBSSI activity was measured in cell lysates of developing quinoa seed.

The SS assay conditions were optimized to detect SS activity. The various factors that could affect glucan synthesis for a given amount of cell lysate include the amount of substrate (ADP-glucose), the assay temperature, the pH of the reaction and the incubation time. Figure 4.8 shows the optimization of the SS activity assay, whereby activity was measured as the amount of U-^{14}C incorporated into glucan and expressed as counts per min (cpm) of (U-^{14}C)glucose. Vertical bars represent standard errors. As optimum conditions for determining the starch synthase activity, an ADP-glucose concentration of 5.2m*M*, an incubation time of 30 min, a reaction temperature of 32ºC and a pH of 7.5 were used. A positive and linear correlation was found between reaction time and the amount of U-^{14}C glucose incorporated into the amylopectin. The activities of the different samples, as displayed in Table 4.8, were measured under the optimized conditions. The line with the lowest amylose concentration in mature seed (Ames 21926) exhibited the lowest SS activity during seed development. The medium amylose line (95Y) had an intermediate activity. The high amylose line (Ames 22159) had the highest activity (Table 4.8). These results indicate that the concentration of amylose in starch in the mature quinoa seed and SS activity during seed development are related. In the high amylose line (Ames 22159),

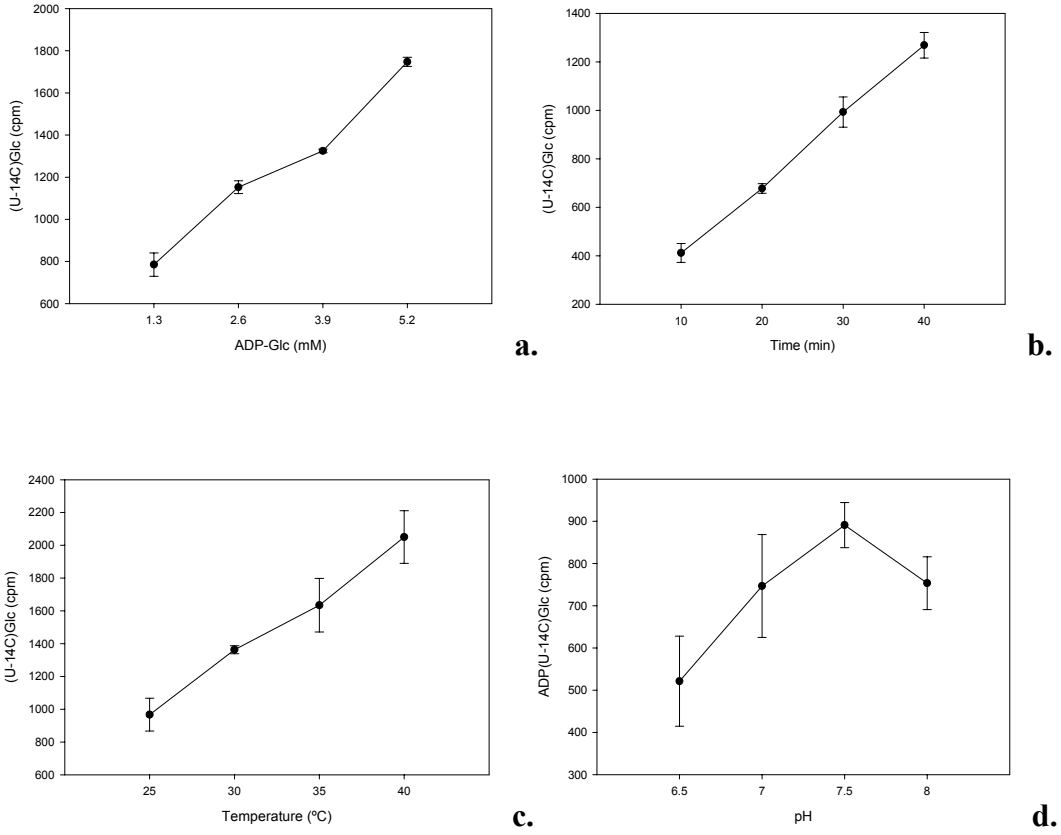


Figure 4.8 Effect of different levels of ADP-glucose (a), incubation time (b), incubation temperature (c), and pH (d) on starch synthase activity. Error bars represent the variation in starch synthase activity (n=3).

an increase in SS activity over the entire period of seed development was observed (Table 4.8). The medium amylose line (95Y) exhibited maximum enzyme activity six weeks after flowering. Not enough seed of the low amylose line at six weeks after flowering was available for analysis.

Denyer et al. (1997) found no correlation between the concentration of amylose in starch and the GBSSI concentration in different plant organs. Flipse et al. (1996) found a non-linear correlation between SGP activity and amylose concentration. At a certain level of SGP activity, a maximum amount of amylose was formed, but a further increase in SGP activity did not result in an increase in amylose concentration. Apart from GBSSI content and activity, other factors such as the presence of cofactors and the amylopectin matrix in which the GBSSI proteins are located might affect the amount of amylose synthesized (Koornhuyse et al. 1996, Denyer et al. 1997). The current study on quinoa did reveal a relationship between amylose content and both starch synthase activity and GBSSI concentration. However, other factors such as interactions between different biosynthetic enzymes, the physical characteristics of the enzymes (granule bound or located in the soluble phase) and the availability of substrates and cofactors should be studied to further clarify the relationship between starch synthase activity, GBSSI concentration and amylose content in quinoa starch.

4.3 Development of an integrated process for starch and protein purification from quinoa

4.3.1 Wet fractionation of flours from a variety of crops

To gain insight into the relative complexity of the wet fractionation of quinoa, the fractionation of quinoa flour was compared to that of barley, rice, buckwheat and corn flours. Because dehulling of quinoa seed is employed in practice to reduce its saponin content, flour from partially dehulled quinoa was also examined. The materials to which quinoa was compared were selected on the basis of their starch granule sizes. The actual sizes of the starch granules, as determined in starch isolated from the different crops, are presented in Table 4.9. The main objectives were to observe sedimentation, protein/starch separation and to recover the protein in an aqueous extract, leaving a starch-rich pellet after centrifugation of the various slurries. Figure 4.9 depicts the pellets formed on centrifugation. The various layers that comprised the pellets were separated, quantified and analyzed for starch and protein.

All flours yielded a starch-rich pellet and a supernatant enriched in protein. However, the appearance as well as the composition of the pellet varied from flour to flour (Table 4.10). The pellet obtained from quinoa consisted of more layers than did the pellets from any of the other flours tested, and these layers varied considerably in appearance and composition. For whole quinoa, the top layer of the pellet (layer 1) contained 32.8% protein and 14.5% starch, whereas layer 3 contained 77.6% starch and 3.9% protein. This was expected based on the higher density of the starch granules compared to protein (Gausman et al. 1952, Biss and Cogan 1988, Steinke and Johnson 1991). According to Stokes' law (see equation 2.2), starch would have a higher sedimentation velocity and, therefore, would sediment faster than protein. However, layer 4 had a lower starch content (47.0%) and a higher protein content (15.4%) than layer 3, which was unexpected. A possible explanation could be that some of the bran material, which is rich in protein (Table 2.1), was not ground finely enough and, therefore, sedimentated fastest. Partial dehulling of quinoa before fractionation strongly influenced the composition of this bottom layer of the pellet. The starch content of layer 4 increased by 16% and the protein content decreased by

Table 4.9 Granule sizes of corn, barley, buckwheat, rice and quinoa starches.

Sample	Granule size (μm)
Corn	19
Barley	25
Buckwheat	10
Rice	5.5
Quinoa	1.5

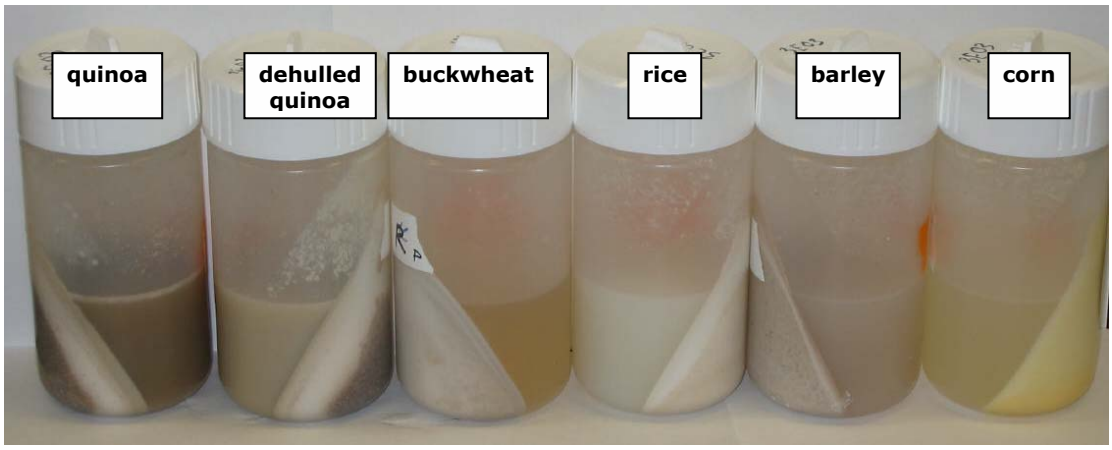


Figure 4.9 Comparison of pellets formed by centrifugation (3,500 × *g*, 20 min) of aqueous slurries (16%, w/v) of whole quinoa, dehulled quinoa, buckwheat, rice, barley and corn flours.

Table 4.10 Composition of the different layers of the pellet formed by centrifugation (3,500 × *g*, 20 min) of flour-in-water slurries (16%, w/v) of whole and dehulled quinoa, barley, rice, buckwheat and corn flours along with the viscosities of the slurries and the supernatants after centrifugation.

Sample	Viscosity (centiStokes)	Amount (g, dm[I])	Weight distribution (% of total)	Starch (%, db[I])	Protein (%, db[I])
Whole quinoa	10.7	18.0	100	52.2	14.9
Supernatant	1.7	3.6	20.6	20.9	29.5
Layer[II]: 1		1.7	9.8	14.5	32.8
2		2.9	16.8	56.6	9.7
3		4.7	27.2	77.6	3.9
4		4.4	25.7	47.0	15.4
Dehulled quinoa	5.7	18.3	100	58.5	14.1
Supernatant	1.6	4.1	22.2	23.4	30.6
Layer: 1		2.4	13.3	34.9	23.8
2		2.4	12.9	57.0	12.8
3		6.6	36.3	83.2	4.3
4		2.8	15.5	63.0	12.0
Barley	87.5	16.8	100	59.8	11.6
Supernatant	7.6	2.0	12.0	22.7	13.9
Layer: 1		1.9	11.3	43.4	29.3
2		7.9	47.2	61.4	11.2
3		4.9	29.6	76.1	7.1
Rice	2.9	17.0	100	99.4	5.61
Supernatant	1.2	0.7	4.0	31.6	16.4
Layer: 1		1.1	6.8	61.4	19.7
2		4.2	26.5	84.6	6.7
3		10.3	62.6	82.8	7.1
Buckwheat	7.6	17.0	100	70.4	8.84
Supernatant	2.1	1.3	5.5	8.8	31.5
Layer: 1		1.9	11.1	60.9	16.9
2		13.6	83.4	82.9	6.4
Corn	3.5	17.0	100	89.7	5.3
Supernatant	1.2	0.5	2.5	26.3	27.2
Layer: 1		16.4	97.5	90.8	5.0

[I] dm, dry matter; db, dry basis

[II] The layers are numbered from top to bottom, where layer 1 is the layer in contact with the supernatant.

3.4% (Table 4.10) when quinoa was dehulled prior to its fractionation. The pellets from the other flours all had the highest starch content and the lowest protein content in the bottom layer of the pellet. The pellets formed on wet fractionation could be refined further by washing and centrifugation or passing them through a series of hydrocyclones to yield high purity starch, as done in commercial corn starch production. It may be possible with quinoa to eliminate the problem of a bottom layer that is relatively rich in protein and low in starch by completely dehulling quinoa prior to wet fractionation. Grinding the quinoa finer is not likely an option because the quinoa in this experiment was already ground to pass a 200 μm screen. Bran is more resilient and rubbery than perisperm and, therefore, more difficult to grind, wet or dry. Sieving the extract slurry prior to centrifugation also might reduce the amount of coarse bran in layer 4, thereby increasing its starch and reducing its protein content, which would lead to an improvement in starch purity. However, this would also lead to the loss of protein and starch in the fraction retained on the screen.

The top layer of the quinoa pellet contained 14.5% starch and 32.8% protein for whole quinoa, and 34.9% starch and 23.8% protein for dehulled quinoa. When the upper layer was scraped off and discarded, to reduce the fibre and protein content of the final starch product, which is commonly done in some laboratory and industrial purification methods, a loss of starch occurred (2.7% of the starch from whole seed and 7.9% of the starch from dehulled seed). Starch granule size analysis was performed after removal of the first layer from the pellet during starch isolation (Table 4.9). Barley generally has a bimodal starch granule size distribution containing granules of 2-3 μm and of 12-32 μm (Lindeboom et al. 2004). However, only one group of granules with an average granule size of 25 μm was recovered. Apparently, the smaller granules in barley starch were lost during starch isolation. Loss of small starch granules has been shown to be a problem in the production of starches having a bimodal size distribution, as well as with small granule starches having a monomodal distribution (Lindeboom et al. 2004).

The supernatant from quinoa flour contained 3.6 g dry matter for whole seed and 4.1 g dry matter for dehulled seed, which was higher than that of the other crops (0.5-2.0 g dry matter). The starch content of the supernatant from quinoa (20.9% on a

dry basis for whole seed, and 23.4% on a dry basis for dehulled quinoa) was similar to that from barley (22.7%), lower than that from rice (31.6%) and corn (26.3%), and higher than that from buckwheat (8.8%). However, the absolute amount of starch in the quinoa supernatant (0.8 g for whole quinoa and 1.0 g for dehulled quinoa) was higher than that in the other supernatants (0.1-0.4 g). When the pellet and supernatant are separated during fractionation, more starch was therefore lost to the supernatant in quinoa than in any of the other crops examined.

Quinoa starch displayed the slowest sedimentation of the five starches studied (Figure 4.10), which is likely the cause of the high amount of starch in the quinoa supernatant. The slow sedimentation rate is attributable to its small starch granule size (Equation 2.2). Rice starch, which also has small starch granules (5.5 μm) compared to barley, buckwheat and corn, but larger ones than quinoa starch (1.5 μm), settled faster than quinoa starch, but much slower than starch from buckwheat, barley or corn (Figure 4.10). Sedimentation also effects the separation of the starch pellet and the supernatant. To obtain a firm starch pellet, which can easily be separated from the supernatant, a minimum centrifugal force of 400 × *g* is required for quinoa. Buckwheat and rice required a minimum centrifugal force of 200 × *g*, whereas barley and corn needed only 100 × *g* and 50 × *g*, respectively (Table 4.11). Additionally, the viscosities of the quinoa slurry and its supernatant were relatively high compared to those of the other crops, with the exception of barley (Table 4.10). This would further reduce the sedimentation rate of quinoa starch compared to that of the other crops studied (except barley), thereby making it more difficult to separate starch and protein from quinoa using centrifugation.

4.3.2 Comparison of the composition, α-amylase activity and pasting profiles of whole and dehulled seed from three quinoa lines

4.3.2.1 Composition

The composition and quality of the starting material will influence the yield and quality of products derived from the fractionation of quinoa. For example, in the event that a line is high in saponins, it may be necessary to extract these saponins prior to further fractionation of the seed. Also, to choose a particular line for the

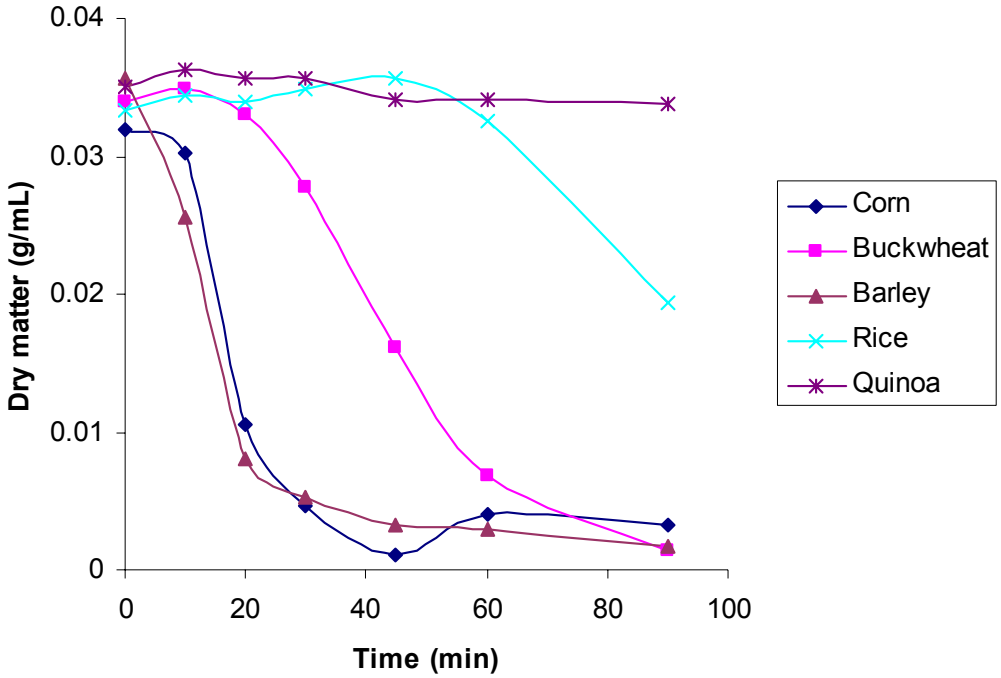


Figure 4.10 Sedimentation of corn, buckwheat, barley, rice and quinoa starches in water (0.35%, w/v).

Table 4.11 Sedimentation of quinoa, barley, rice, buckwheat and corn starches over a range of centrifugal forces.

Centrifugal force (× *g*)	Sedimentation description
400	All samples form a firm pellet
300	Quinoa forms a soft pellet, other samples form a firm pellet
200	Corn/barley/buckwheat/rice exhibit a clear supernatant and form a firm pellet Quinoa exhibits a cloudy supernatant and forms a soft pellet
100	Corn/barley exhibit a clear supernatant and form a firm pellet Buckwheat/rice exhibit a slight cloud above a firm pellet Quinoa exhibits a very cloudy supernatant and forms a small pellet
53	Corn exhibits a clear supernatant and forms a firm pellet Buckwheat/rice/barley exhibit a cloud above a firm pellet Quinoa exhibits a very cloudy supernatant and forms a small pellet
27	Corn/barley/buckwheat/rice exhibit cloudiness above a firm pellet Quinoa exhibits a very cloudy supernatant with no pellet formation

manufacturing of a specific end product, it would be necessary to be aware of any significant compositional differences among the lines available.

Seed of three commercial quinoa lines was obtained. Seed of all three lines was disk shaped, but differed in colour and size. Line NQC was yellow-seeded with a seed weight of 3.6 g/1000 seeds. Line QC was white-seeded with a seed weight of 4.1 g/1000 seeds. Line WMF was a mixture of black, yellow and pink seeds with a seed weight of 3.6 g/1000 seeds. The protein, total dietary fibre (TDF), ash, crude fat, starch and saponin contents of whole seed and dehulled seed of the three quinoa lines are presented in Table 4.12. The lines differed significantly in their composition. WMF had a protein content of 17.2 % (db), which was high compared to QC (13.4%, db) and NQC (13.0%, db); its starch content was 52.0% (db), which was low compared to QC (67.6%, db) and NQC (61.1%, db). In quinoa seed, starch is mainly located in the perisperm, whereas protein, along with oil, saponins, fibre and ash, are located mainly in the bran (Figure 2.1 and Table 2.1) (Varriano-Marston and DeFrancisco 1984, Becker and Hannes 1990). The higher protein content and lower starch content of WMF, along with its higher amounts of fibre, ash and oil, imply that WMF had relatively more bran and less perisperm than the other two quinoa lines. Variations in composition amongst the lines would be attributable to differences in the environments in which they were grown, as well as agronomic and genetic differences.

Saponin content differed significantly among lines. QC contained 2.2% (db) saponins, compared to 6.1% (db) in NQC and 8.1% (db) in WMF. Dehulling the seed reduced the saponin content significantly. In addition to a reduction in saponin content, a reduction in protein, total dietary fibre, ash, and crude fat occurred (Table 4.12). During abrasion milling, a large proportion of the protein-rich bran was removed, which is a concern because quinoa is often consumed because of its high level of high quality protein. Therefore, it might be better to remove the saponins by washing instead of abrasion or by a combination of washing and abrasion, as was suggested by Taylor and Parker (2002). These authors concluded that the combined action of washing and abrasion effectively reduced the level of saponins, while the loss of other nutrients was minimized.

Table 4.12 Composition, on a dry weight basis, of whole quinoa seed (ws) and dehulled seed (ds) of three commercial lines[I].

Constituent (%, db)	Line[II]					
	NQC		QC		WMF	
	ws	ds[III]	ws	ds	ws	ds
Protein[IV]	13.0a	11.7b	13.4c	10.7d	17.2e	15.6f
TDF[V]	9.7a	9.1ab	7.9c	6.8bc	11.8a	9.9d
Ash	2.9a	2.2b	2.1c	1.7d	3.5e	2.5f
Crude fat	6.4a	6.2a	6.8b	5.1c	6.2a	5.7d
Starch	61.1a	74.4b	67.6c	74.2b	52.0d	65.4e
Saponins	6.1a	2.9b	2.2c	1.4d	8.1e	3.0c

[I] Values in the same row followed by the same letter are not significantly different ($p < 0.05$, n=3).

[II] Material: commercially available quinoa seed from Northern Quinoa Corporation (NQC), Quinoa Corporation (QC) and West Mountain Farm (WMF).

[III] ds, dehulled seed was obtained after 9.7, 12.1 and 13.6% of the whole seed (ws) was removed as hull from NQC, QC and WMF, respectively.

[IV] N×6.25.

[V] Total dietary fibre.

The amino acid composition of the three quinoa lines is shown in Table 4.13, along with the amino acid profiles of wheat and soybean. The three quinoa lines had very similar amino acid profiles, except that the amount of glutamic acid was approximately 4% lower in WMF than in the other two lines. However, glutamic acid is not an essential amino acid. The levels of the essential amino acids valine, leucine, and lysine in quinoa were slightly higher than what was reported previously (Table 2.4 and 4.13). Also, the contents of the non-essential amino acids arginine and proline were higher than those previously reported (Table 2.4). Lysine is the limiting amino acid in most cereal proteins and is much lower in wheat (1.9%) than in quinoa (6.3-7.0%). This makes quinoa protein a good complement to a diet that is rich in cereals. The high methionine (2.2-2.7%) and cysteine (1.7-1.8%) contents also make quinoa protein a good complement to legumes. In general, quinoa protein showed more similarity to soybean protein than to wheat protein with respect to amino acid composition.

The fatty acid profiles of the three quinoa lines are shown in Table 4.14. The main fatty acids in quinoa were oleic acid (20.7-28.2%) and linoleic acid (44.7-50.8%) and, to a lesser extent, α-linolenic acid (8.5-13.1%). The last two fatty acids are essential fatty acids and comprise between 53.2% and 60.9% of the total fatty acids in QC and NQC, respectively. Oil from NQC was relatively low in oleic acid (20.7%) and high in α-linoleic acid (50.8%), whereas WMF oil was relatively high in linolenic acid (13.1%) (Table 4.14). The amount of α-linolenic acid in the three lines investigated was much higher than the 3.9% reported by Ruales and Nair (1993a).

4.3.2.2 Alpha-amylase activity and pasting profiles

The α-amylase activities and pasting properties of quinoa flour as affected by dehulling and an α-amylase inhibitor ($AgNO_3$) are shown in Table 4.15. As a comparison, wheat flour from CWRS (Canada Western Red Spring) of the variety AC Barry was analyzed. The viscosity values for flours from dehulled seed were much higher than those from whole seed. This is because the starch content of flour from dehulled seed is substantially greater (Table 4.12). Large differences in pasting

Table 4.13 Amino acid composition of whole seed of three quinoa lines (NQC, QC and WMF).

Amino acid[I]	**NQC**	**QC**	**WMF**	**Wheat** [II]	**Soybean** [II]
Essential					
Histidine	3.0	2.7	3.0	2.1	2.5
Isoleucine	4.2	4.3	4.5	3.5	4.7
Leucine	7.4	7.2	7.7	6.7	7.7
Lysine	6.3	6.3	7.0	1.9	5.1
Methionine	2.2	2.4	2.7	1.6	1.2
Cysteine	1.8	1.7	1.8	2.2	1.1
Phenylalanine	4.5	4.3	4.8	4.8	5.1
Tyrosine	3.3	3.6	3.3	3.2	3.4
Threonine	4.5	4.6	4.9	2.5	3.6
Tryptophan	1.2	1.2	1.4	-	-
Valine	5.4	5.3	5.3	4.1	5.2
Non-essential					
Alanine	4.9	5.7	5.4	2.8	4.1
Arginine	10.0	10.1	10.5	3.7	7.3
Apartic acid	9.5	9.7	9.6	4.1	11.7
Glutamic acid	15.5	15.1	11.2	33.1	18.6
Glycine	6.0	5.7	6.2	3.5	4.0
Proline	4.6	4.5	4.7	11.5	5.2
Serine	5.7	5.6	5.9	4.4	4.9

[I] In g/100 g of protein.
[II] From Friedman and Levin (1989).

Table 4.14 Fatty acid profiles[1] of oil from three quinoa lines (NQC, QC and WMF).

Fatty acid	NQC (%)	QC (%)	WMF (%)
C14:0 (Myristic)	0.24a	0.15b	0.22a
C16:0 (Palmitic)	8.66a	8.45a	8.45a
C16:1 *n*-7 (Palmitoleic)	0.21a	0.25b	0.17c
C18:0 (Stearic)	0.60a	0.79b	1.11c
C18:1 *n*-9 (Oleic)	20.70a	28.19b	23.81c
C18:2 *n*-6 (Linoleic)	50.81a	44.68b	44.96b
C18:3 *n*-3 (Linolenic)	10.12a	8.49b	13.12c
C20:0 (Arachidic)	0.41a	0.55b	0.44a
C20:1 *n*-9 (Eicosenoic)	1.47a	1.68b	1.45a
C20:2 *n*-6 (Eicosadienoic)	0.16a	0.10b	0.12b
C22:0 (Behenic)	0.61ab	0.68a	0.57b
C22:1 *n*-9 (Erucic)	1.38ab	1.48a	1.26b
C24:0 (Linocenic)	0.24a	0.26a	0.22a
C24:1 *n*-9 (Nervonic)	1.86a	1.59a	1.67b

[1] Values in the same row followed by the same letter are not significantly different ($p < 0.05$, $n = 3$).

Table 4.15 Alpha-amylase activity and pasting profiles[I] of quinoa flours and wheat flour as affected by dehulling and the α-amylase inhibitor $AgNO_3$ [II].

Accession	α-amylase activity (CU/g)[III]	Peak time (min)	Viscosity (RVU)				
			PV	HPV	BD	CPV	SB
		without $AgNO_3$					
NQC							
ws[IV]	0.24b	7.5d	107.3f	78.7f	28.6d	144.9e	66.2f
ds	0.14c	7.5d	199.5b	123.4d	76.2b	219.2c	95.8d
QC							
ws	0.24b	9.4a	118.2d	112.0e	6.2f	199.0d	87.0e
ds	0.07c	8.7b	295.8a	277.5b	18.2e	529.2a	251.6a
WMF							
ws	0.45a	7.7cd	45.1h	38.9h	7.1f	71.7g	33.6h
ds	0.23b	8.0c	99.5ef	81.8f	17.8e	148.3e	66.6f
wheat[V]	0.06d	8.9b	166.0c	110.6e	55.0c	219.5c	117.5c
		with $AgNO_3$					
NQC							
ws		7.5d	96.1f	79.8f	16.3e	142.9e	63.1f
ds		7.5d	160.3c	109.0e	51.3c	196.8d	87.7e
QC							
ws		9.4a	124.8d	109.4e	15.4e	195.8d	86.5e
ds		8.8b	299.2 a	284.7a	14.5e	528.9a	244.2b
WMF							
ws		8.0c	39.8h	34.0h	5.8f	67.1g	33.1h
ds		8.0c	78.0g	68.8g	9.2f	120.8f	52.0g
wheat		9.04ab	208.7b	140.6c	83.5a	240.5b	123.5c

[I] PV, Peak Viscosity; HPV, Hot Paste Viscosity; BD, Break Down; CPV, Cold Paste Viscosity.

[II] Values in the same column followed by the same letter are not significantly different ($p < 0.05$, n=3).

[III] One ceralpha unit (CU) is defined as the amount of enzyme, in the presence of excess thermostable α-glucosidase, required to release one micromole of *p*-nitrophenol from *p*-nitrophenyl maltoheptaoside in one min under defined assay conditions.

[IV] ws, whole seed; ds, dehulled seed.

[V] flour from AC Barry.

properties among quinoa lines were observed, due to factors such as amylose content and amylopectin fine structure (see section 4.1.5).

Wholeseed quinoa flour exhibited α-amylase activies of 0.24-0.45 CU/g, compared to 0.06 CU/g for wheat flour. This is in agreement with the findings of Lorenz and Nyanzi (1989). Alpha-amylase activity was significantly ($p < 0.05$) higher in WMF than in NQC or QC. Furthermore, whole seed exhibited significantly ($p < 0.05$) higher α-amylase activity than did dehulled seed (Table 4.15), which was opposite to what Lorenz and Nyanzi (1989) previously reported. The reason for this discrepancy is not known. The data presented indicate that α-amylase was present mainly in the peripheral tissues of quinoa seed.

For quinoa flours, significant differences ($p < 0.05$) in the pasting profiles in the presence or absence of $AgNO_3$ were found. These differences were very small, however, when compared to those observed for wheat in the presence or absence of α-amylase inhibitor (Table 4.15). For wheat flour, the peak viscosities were 209 and 166 RVU in the presence and absence of $AgNO_3$, respectively. Starch breakdown by α-amylase activity is likely to have caused this reduction in paste viscosity. Even though α-amylase activity in wheat was much lower than that in whole and dehulled quinoa flour, it affected the pasting properties of starch, while the high α-amylase activity had little effect on the pasting properties of quinoa starch. Varianno Marston and DeFransisco (1984) used scanning electron microscopy to study the germination of quinoa, and concluded that the starch located in the perisperm of the seed was relatively resistant to amylolysis. It can be concluded that for starch production from quinoa, α-amylase activity does not likely need to be considered. Besides, it is unlikely that the high α-amylase activity would affect the baking or cooking quality of quinoa.

4.3.3 Studies on abrasive and roller milling of quinoa

In quinoa seed, protein, oil and saponins are located mainly in the peripheral layers (pericarp, radicle and cotelydons) that comprise the bran, whereas starch is concentrated in the interior (perisperm) (section 2.1.1, Figure 2.1). Based on data in the literature (Reichert et al. 1986, Ridout et al. 1991, Chauhan et al. 1992), abrasive

milling was deemed worthy of study as a preliminary step in quinoa fractionation. Roller milling was also evaluated as means of separating quinoa seed into a protein-, oil-, and saponin-rich bran fraction and a starch-rich perisperm fraction.

4.3.3.1 Abrasive milling

Quinoa seed was abraded to different extents. Protein and crude fat contents of the abraded seed were determined (Figure 4.11). As expected, a decrease in protein and fat content due to abrasive milling was observed. A constant protein and fat content in the abraded seed was reached once 55% of the original seed weight had been removed. The protein content of this abraded seed was 4.6% (db), and the fat content 0.4% (db), compared to 17.2% (db) and 6.2% (db), respectively, in the whole seed (Table 4.16). Koziol (1993) reported that to remove as much protein and oil as possible without including significant starch from the perisperm in the fine fraction, only 25-30% of seed needed to be removed. By abrading 55% of the original seed weight, a marked proportion of the perisperm, principally from the edges of the disk-shaped seed, would be included in the bran fraction.

A major problem was encountered in attempting to scale up the abrasive milling process (i.e., from the TADD dehuller to a Satake mill) in that fines tended to accumulate in the Satake mill, likely due to their high fat content. Additionally, defatting of these fines prior to protein extraction was extremely difficult, as percolation issues were experienced with the Soxhlet apparatus on account of the fine particle size of the material.

4.3.3.2 Roller milling

Quinoa seed was roller milled, with and without tempering. The milled grain was separated into several fractions by sieving, and the different fractions were analyzed for their protein and starch contents. After roller milling, 23% of the flour from non-tempered quinoa and 48% of the flour from tempered quinoa was larger than 500 μm (Table 4.17). This coarse material contained 12.9% (db) protein and 48.8% (db) starch in flour from non-tempered quinoa, and 22.9% (db) protein and 32.1% (db) starch in flour from tempered quinoa. The fines derived from tempered quinoa

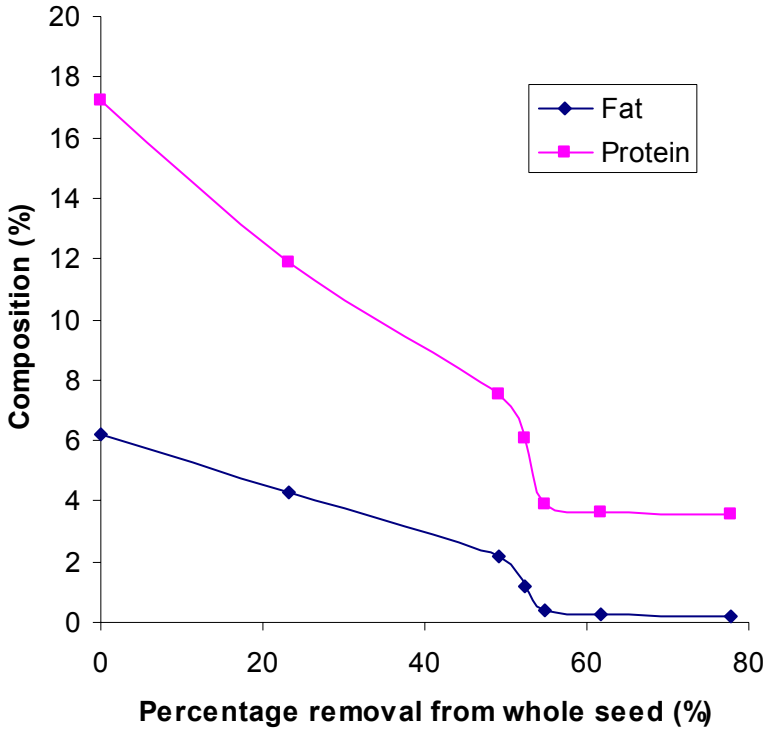


Figure 4.11 Effect of abrasive milling on the protein and fat concentrations in abraded quinoa seed.

Table 4.16 Yield and composition of fractions obtained from quinoa by abrasive and roller milling.

Mill fraction	Weight (%)	Starch (%, db)	Protein (%, db)	Crude fat (%, db)	Saponins (%, db)
Abrasive milling[I]					
Abraded seed (perisperm)	45	81.9	4.6	0.4	2.8
Fines (bran)	55	26.2	23.6	9.9	8.4
Roller milling[II]					
Fines (perisperm)	52	77.2	8.9	2.7	3.1
Coarse (bran)	48	32.1	22.9	8.8	7.4

[I] Abrasion milling was conducted using a Satake abrasive mill (Model TM 05, Satake Corporation, Hiroshima, Japan).
[II] Roller milling was conducted using a Quadrumat Junior mill (Brabender, Duisburg, Germany).

accounted for 52% of the original seed and contained 77.2% (db) starch, 8.9% (db) protein, 2.7% (db) oil and 3.1% (db) saponins. Tempering, roller milling and screening (500 μm) yielded a coarse fraction containing 70% of the total seed protein, whereas less than 20% of the total protein was present in the coarse fraction when quinoa was not tempered prior to roller milling. The fine and coarse fractions consisted primarily of perisperm and bran, respectively.

Unlike abrasive milling, material was not retained in the mill during roller milling, nor was it difficult to defat the coarse fraction so obtained. It is expected that by improving the tempering conditions (time, temperature and moisture) prior to roller milling, more selective hydration of the bran in quinoa seed could be achieved, thereby resulting in an even better separation of bran and perisperm.

4.3.4 Protein extraction from quinoa bran

The extraction of protein from quinoa bran was studied using a response surface design methodology whereby the values for the independent variables (X_1, X_2, and X_3) were compared to results for protein recovery and dry matter protein concentration in the protein extract (Table 4.18).

Mean bran particle size, liquid-to-bran ratio during the extraction and centrifugal force employed to separate the protein extract and the starch-rich pellet were chosen as the independent variables. The choice of independent variables was based on preliminary studies, where other factors such as pH, shear force and extraction time were shown to be of lesser importance than the independent variables optimized in this study. The particle size distributions of the bran are presented in Figure 4.12.

The highest protein recovery (82%) was observed at design point 8 (Table 4.18), whereas the highest protein content (58.5%) was at design point 4. Both design points utilized a centrifugal force of 8,500 × *g* and a liquid-to-bran ratio of 20.

The average particle sizes of the bran used for these extractions were different, i.e. 375 μm to obtain the highest protein recovery (design point 8) and 150 μm to obtain the highest protein content (design point 4).

Table 4.17 Distribution of particle sizes and composition of fractions obtained by roller milling and sieving of non-tempered and tempered quinoa.

Size (µm)	Weigh t (%)	Protein (%, db)	Protein (% of total protein)	Starch (%, db)	Starch (% of total starch)	Fat (%, db)	Fat (% total fat)
			Non-tempered				
>500 (coarse)	23.1	12.9	19.1	48.8	19.6		
<500 (combined)	76.9	16.4	80.9	60.1	80.4		
425	28.8	18.8	35.2	50.2	25.4		
300	20.2	18.6	24.4	57.6	20.5		
250	12.9	14.0	11.8	66.8	15.2		
187	7.8	10.9	5.6	73.2	10.1		
75	5.9	8.9	3.3	73.5	7.5		
<75	1.4	7.4	0.7	70.2	1.8		
			Tempered				
>500 (coarse)	48.2	22.9	70.4	32.1	28.5	8.8	75.0
<500 (combined)	51.8	8.9	29.6	77.2	71.5	2.7	25.0
425	18.7	15.6	18.7	60.8	21.0		
300	11.4	5.5	4.0	85.9	18.1		
250	8.2	5.0	2.6	87.1	13.2		
187	6.1	4.8	1.8	86.4	9.6		
75	5.8	5.0	1.8	87.1	9.3		
<75	1.5	6.0	0.6	85.4	2.4		

Table 4.18 Responses of dependent variables to conditions used to extract protein from quinoa bran[I].

Design point	Independent variables[II]			Dependent variables	
	Mean bran particle size (μm) (X_1)	Liquid-to-bran ratio (L/kg) (X_2)	Centrifugal force (× *g*) (X_3)	Protein content (%, db) (Y_1)	Protein[III] recovery (%) (Y_2)
1	150 (-1)	10 (-1)	3500 (-1)	51.0	69.1
2	150 (-1)	10 (-1)	8500 (+1)	49.4	77.9
3	150 (-1)	20 (+1)	3500 (-1)	50.3	77.8
4	150 (-1)	20 (+1)	8500 (+1)	58.5	78.4
5	375 (+1)	10 (-1)	3500 (-1)	50.2	67.7
6	375 (+1)	10 (-1)	8500 (+1)	47.4	69.6
7	375 (+1)	20 (+1)	3500 (-1)	52.1	76.3
8	375 (+1)	20 (+1)	8500 (+1)	51.1	82.0
9	40 (-1.68)	15 (0)	6000 (0)	51.0	79.9
10	500 (+1.68)	15 (0)	6000 (0)	50.4	74.2
11	230 (0)	5 (-1.68)	6000 (0)	44.5	71.2
12	230 (0)	25 (+1.68)	6000 (0)	52.2	78.9
13	230 (0)	15 (0)	1000 (-1.68)	45.6	76.4
14	230 (0)	15 (0)	11000 (+1.68)	52.1	78.5
15	230 (0)	15 (0)	6000 (0)	51.7	77.1
16	230 (0)	15 (0)	6000 (0)	51.7	76.6
17	230 (0)	15 (0)	6000 (0)	52.3	76.3
18	230 (0)	15 (0)	6000 (0)	52.6	76.2
19	230 (0)	15 (0)	6000 (0)	52.1	76.6
20	230 (0)	15 (0)	6000 (0)	52.4	77.1

[I] Means of three replications.

[II] Values in parenthesis are the coded levels of the independent variables.

[III] The amount of protein extracted as a percentage of the protein percent in the bran.

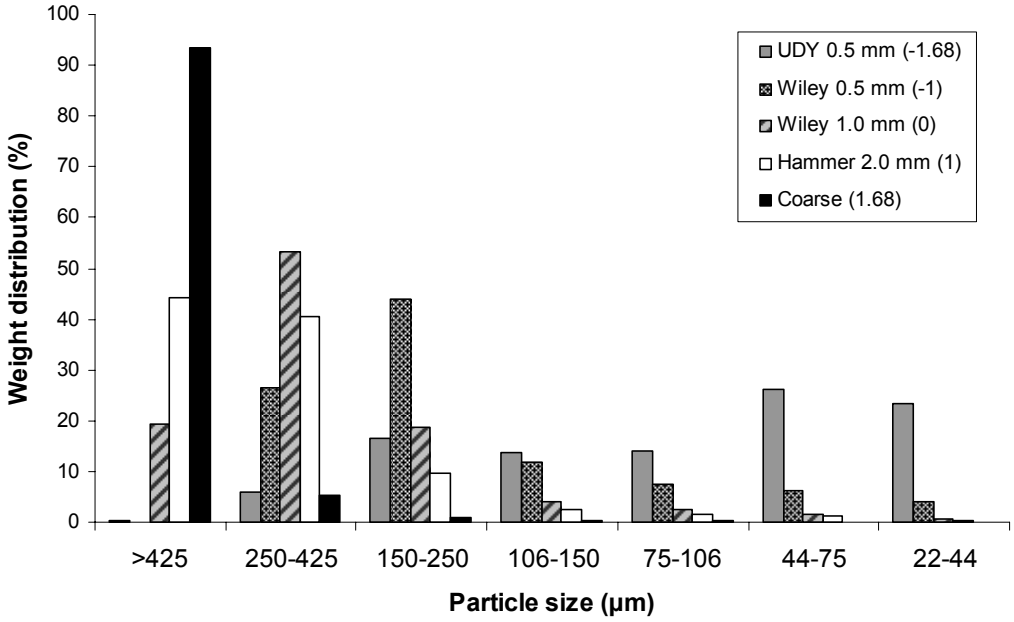


Figure 4.12 Particle size distribution of the bran materials (-1.68 = 40 µm, -1 = 150 µm, 0 = 230 µm, 1 = 375 µm and 1.68 = > 500 µm) used in the study of protein recovery from quinoa.

The models developed to describe protein recovery and protein content are given by the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \beta_{ij} X_i X_j \qquad \text{(Equation 4.1)}$$

When equation 4.1 was fitted to the experimental data from Table 4.18, the coefficients for the model were estimated (Table 4.19).

Analysis of the coefficient estimated for the two regression models indicated that the liquid-to-bran ratio (X_2) was the most important variable affecting protein content. The main effect of this independent variable was linear, i.e. first order for both protein content and protein recovery. An increase in the liquid-to-bran ratio resulted in an increase in protein content and recovery.

Figure 4.13 shows that the protein content increased markedy with an increase in the liquid-to-bran ratio at a constant centrifugal force, but was essentially independent of the bran particle size. In Figure 4.14, however, it can be seen that in order to obtain a higher protein content by increasing the liquid-to-bran ratio, an increase in the applied centrifugal force was also necessary. Only when both factors were increased, a maximum protein content was reached. Similar findings were observed for protein recovery (Figures 4.15 and 4.16). To obtain maximum protein recovery, a combination of a high liquid-to-bran ratio and high centrifugal force was necessary (Figure 4.16). However, in contrast to protein content, protein recovery was dependent on bran-particle size, in that the highest recovery was obtained with the smallest particle size (Figure 4.15). Both an increase in the centrifugal force and a decrease in the bran particle size led to an increased protein recovery.

The coefficients predicting the effect of centrifugal force and bran particle size on protein content were not significant ($p > 0.1$), nor were the interactions among the variables and their effect on protein recovery and content, to the extent that a separate coefficient for the interaction needed not be included in the two models. The maximum protein recovery, as predicted by the full model, was 81.2% and the highest protein content 62.2%. To attain these maxima, protein should be extracted

Table 4.19 Regression coefficients and analysis of variance of the second-order polynomial model[I] for the effect of mean bran particle size, liquid-to-bran ratio and centrifugal force on protein content and protein recovery in the extraction of protein from quinoa bran.

Coefficient		Protein content (Y1)	Standard error of Y1	Protein recovery (Y2)	Standard error of Y2
	β_0	52.073	1.030	76.722	0.843
Linear					
	β_1	0.679	0.684	-1.267**	0.599
	β_2	1.965***	0.684	3.166***	0.599
	β_3	1.018	0.684	1.510**	0.599
Quadratic					
	β_{11}	0.065	0.666	-0.206	0.545
	β_{22}	0.892	0.666	-0.924**	0.545
	β_{33}	0.713	0.666	-0.082*	0.545
Interactions					
	β_{12}	0.341	0.893	1.488	0.731
	β_{13}	1.292	0.893	-0.225	0.731
	β_{23}	1.443	0.893	-0.538	0.731
Variability explained (R^2)		0.388		0.635	
F		1.08		2.52	
Probability of F		0.394		0.056	

* Significant at 0.1 level ** Significant at 0.05 level *** Significant at 0.01 level

[1] $$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \beta_{ij} X_i X_j$$

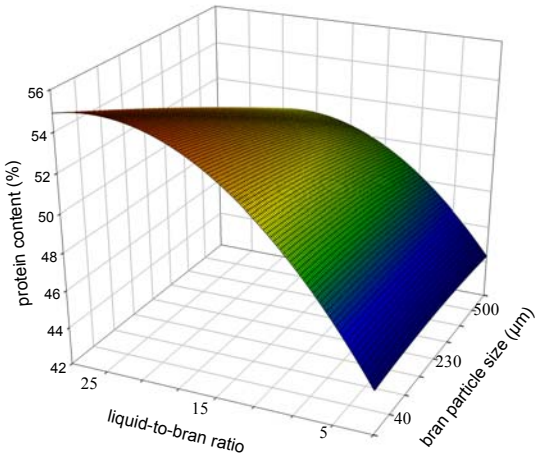


Figure 4.13 Response surface for the effect of liquid-to-bran ratio and bran particle size on protein content at a centrifugal force of 6,000 × *g*.

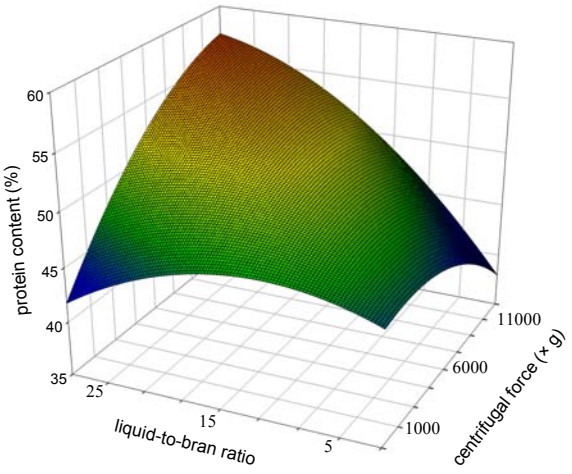


Figure 4.14 Response surface for the effect of liquid-to-bran ratio and centrifugal force on protein content at a bran particle size of 230 μm.

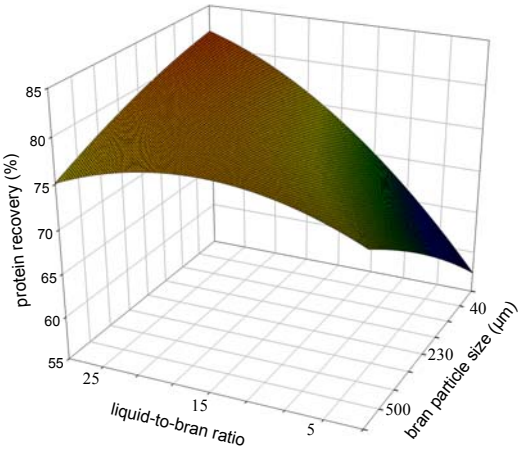


Figure 4.15 Response surface for the effect of liquid-to-bran ratio and bran particle size on protein recovery at a centrifugal force of 6,000 × *g*.

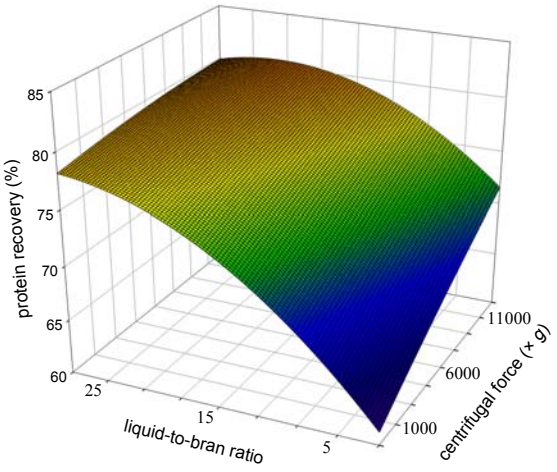


Figure 4.16 Response surface for the effect of liquid-to-bran ratio and centrifugal force on protein recovery at a bran particle size of 230 µm.

from bran of a very small mean bran particle size (40 μm) using a centrifugal force of 11,000 × *g*. For maximum protein recovery, a liquid-to-bran ratio of 20 kg/L should be employed, whereas for maximum protein content, the ratio should be 25 L/kg. When the model is reduced to include only the coefficient estimates that were significant ($p < 0.1$), the maximum predicted protein recovery is 86.7% and the highest protein content is 55.4%. Maximum recovery as well as protein content would, according to this model, be reached with a 40 μm bran particle size, a liquid-to-bran ratio of 25 L/kg and a centrifugal force of 11,000 × *g*. In practice, however, a protein recovery of 84% was obtained, along with a protein content of 53.8%, using the extraction conditions of a bran particle size of 40 μm, a liquid-to-bran ratio of 25 L/kg and a centrifugal force of 11,000 × *g*. This is very close to what was predicted by the reduced model. However, during the preliminary experiments carried out to develop the model, a maximum protein content of 58.5% was attained (Table 4.18).

The equations developed were tested for adequacy and goodness-of-fit by analysis of variance. The model developed for protein content ($R^2 = 0.383$, Table 4.19) was not adequate in explaining the variability because the F value was not significant ($p > 0.1$). The model fit the experimental data poorly and, therefore, would not adequately predict further experiments. The model developed for protein recovery ($R^2 = 0.635$) was shown to be adequate for explaining the variability because the F value was significant ($p < 0.1$). This indicates that the variability among results can be explained by the model. However, the model tends to overestimate protein recovery at low values, and underestimates it at high values.

The poor fit of the model could be due to the very high variability of the data, especially for protein content. When coarse bran was used for extraction in combination with a very low centrifugal force, the reproducibility of the data was poor. After centrifugation, the grey sediment accumulating on top of the pellet tended to pour off easily and was included in the supernatant, resulting in enhancement of its protein content and recovery. Unfortunately, the extent to which this happened was variable, and therefore reduced the reproducibility.

4.3.5 Protein concentration

Isoelectric precipitation (IEP) and ultrafiltration (UF) were evaluated as methods of concentrating quinoa protein extracts. The protein extracts prepared from defatted bran and defatted/saponin-extracted bran that contained 53.8% (db) and 54.7% (db) protein, respectively (Table 4.20).

4.3.5.1 Isoelectric precipitation (IEP)

To determine the pH most suitable for precipitation of quinoa protein, a nitrogen solubility curve was prepared by extraction of WMF quinoa flour at a variety of pHs (Figure 4.17). The solubility of quinoa protein exhibited a minimum at pH 3.5; this is the best pH at which to precipitate protein from the extract. However, it was difficult to maintain this low pH. Therefore, pH 4.5 rather than pH 3.5 was chosen for precipitation. Aluko and Monu (2003) also used pH 4.5 for the precipitation for quinoa protein. By using pH 4.5 for protein precipitation, less alkali was needed to neutralize the protein concentrate after precipitation and prior to drying. Maximum protein solubility was observed at pH 9, which supported the use of pH 9 in the initial protein extraction experiments.

Protein recoveries, expressed as a percentage of the amount of protein present in the original extract, of 78.5 and 75.4% were obtained for extracts from defatted bran and defatted/saponin-extracted bran, respectively (Table 4.20). The recoveries did not differ significantly ($p > 0.05$) and were slightly lower than what would be predicted based on the nitrogen solubility index (NSI) of approximately 15% at pH 4.5 (Figure 4.17). During defatting of the bran, some protein might have been denatured, which would therefore reduce its extractability. Moreover, whole quinoa flour was used in generating the NSI curve, whereas the protein extracts were prepared from defatted bran and defatted/saponin-extracted bran. Protein extraction from whole flour appeared to be more efficient than protein extraction from bran. The reason for this might be the differences in protein composition and type between the protein in the bran and that in whole flour. The IEP products prepared from defatted and defatted/saponin-extracted bran contained 71.5% (db) and 88.5% (db) protein, respectively (Table 4.20). Apparently, protein precipitation resulted in concentration

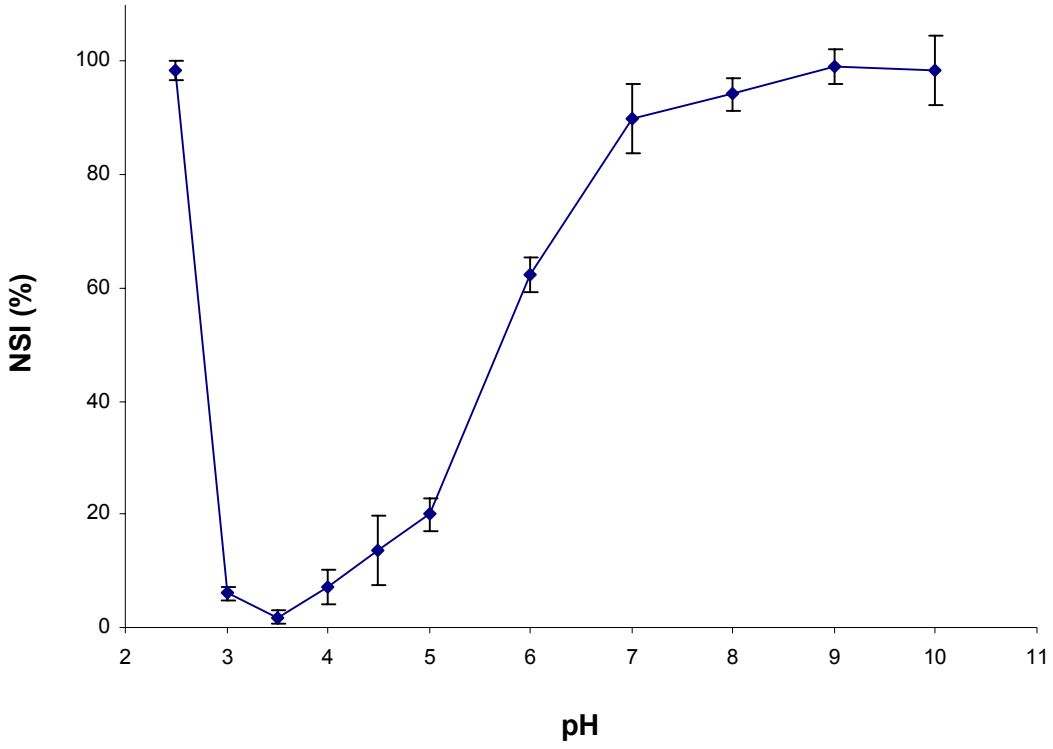


Figure 4.17 Nitrogen solubility index (NSI) curve prepared by extraction of quinoa flour (WMF) at a variety of pHs.

Table 4.20 Recovery and concentration of protein in products prepared from quinoa bran by alkaline extraction and isoelectric precipitation (IEP) or ultrafiltration (UF)[I].

Protein extract	Protein content (%, db)	Recovery		
		extract (%)[II]	bran (%)[III]	seed (%)[IV]
Defatted bran				
Unconcentrated extract	53.8a			
IEP	71.5b	78.5a	65.9a	46.2a
UF	67.9c	83.9b	70.5b	49.4b
Defatted/saponin-extracted bran				
Unconcentrated extract	54.7a			
IEP	88.5d	75.4ac	63.3ac	44.3ac
UF	77.2e	77.0c	64.7c	45.3c

[I] Values in the same column followed by the same letter are not significantly different ($p < 0.05$, $n = 3$).

[II] Recovery calculated as the ratio of the total protein recovered in the precipitate (IEP) or the retentate (UF) and the total protein in the protein extract.

[III] Recovery calculated as the ratio of the total protein recovered in the precipitate (IEP) or the retentate (UF) and the total protein in the bran.

[IV] Recovery calculated as the ratio of the total protein recovered in the precipitate (IEP) or the retentate (UF) and the total protein in whole quinoa seed.

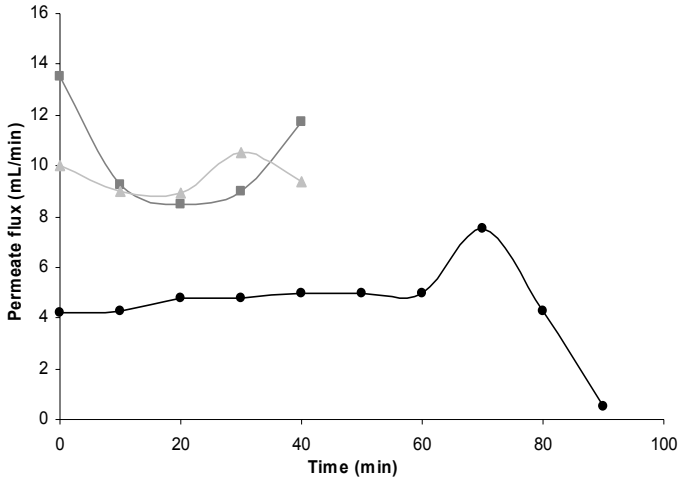
as well as purification of quinoa protein extracts. The extraction of saponins from the bran markedly increased the protein content of the precipitated protein. Co-precipitation of saponins or other endogenous substances in protein extracts from non-saponin extracted bran would account for the lower protein contents of the corresponding IEP protein products.

4.3.5.2 Ultrafiltration (UF)

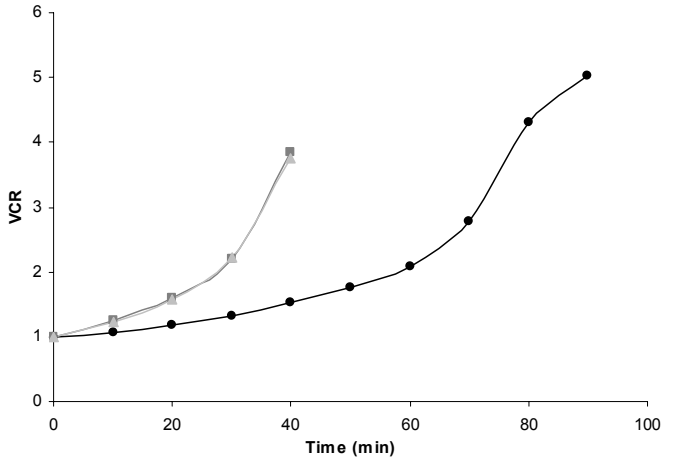
A bench-scale, hollow-fibre concentrator was used to concentrate protein extracts from defatted and defatted/saponin-extracted quinoa bran. This system was used because compared to other UF systems, it could produce a high shear rate, thereby reducing concentration polarization during operation and hence improving throughput. Furthermore, a hollow fibre system has a high surface to volume ratio which yields a high volumetric permeate flow rate compared to other membrane designs (Diosady et al. 1984).

For concentration of the protein extracts, two membranes with molecular weight cut-offs (MWCO) of 10,000 or 50,000 were evaluated. When the protein extract was concentrated with the 50,000 MWCO membrane, a volume concentration ratio (VCR) of 5.0 was achieved at a feed rate of 555 mL/min. The maximum attainable VCR using the 10,000 MWCO membrane was 3.8, after which the system automatically shut down due to overpressurization of the membrane (data not shown). The permeates from both membranes were analyzed for residual protein by first heating and then subsequent determination of the amount of material precipitated. No protein was detected in permeates from either membrane. This indicates that both membranes were able to effectively retain quinoa protein.

For the concentration of protein by UF, different feed rates can be used. To reduce the time needed for concentration, a high feed throughput is preferred. Unfortunately, a high feed rate tended to result in over pressurization of the system, i.e. excessive trans-membrane pressure. To prevent membrane damage, a maximum transmembrane pressure was set which, if exceeded, resulted in automatic shutdown of the UF system. The permeate flux rates as well as the maximum VCRs obtained at three different feed rates are presented in Figure 4.18. Feed rates of 1040 and 1665



a



b

Figure 4.18 Effect of feed rate [555 mL/min (●), 1040 mL/min (■) or 1665 mL/min (▲)] on permeate flux (a) and volume concentration ratio (VCR) (b), using a membrane with a molecular weight cut-off (MWCO) of 50,000.

mL/min gave permeate fluxes of 10.0 and 13.5 mL/min, respectively. By employing a feed rate of 555 mL/min, the permeate flux was only 4.0 mL/min, and concentration would take much longer. However, the maximum VCR attainable at the low feed rate was greater than 5, whereas at the two higher feed rates, VCRs higher than 4 were not possible. The reason for this was the excessive pressure that developed at higher feed rates and higher VCRs, which resulted in shutdown of the system. Therefore, a feed rate of 555 mL/min was deemed most suitable for concentration of quinoa protein extracts on account of the higher VCR achieved.

According to reports by Brinegar and Goundan (1993) and Brinegar et al. (1996), quinoa protein extracts are expected to contain proteins with molecular masses smaller than 50 kDa. Normally, then, the 50,000 MWCO membrane would not have been expected to retain these relatively small proteins. Apparently, the polarized layer that formed on the membrane and which was observed as membrane fouling, i.e. a decrease in flux rate over time (Figure 4.18), changed the selectivity characteristics of the membrane system and generated a lower effective MWCO.

When UF is utilized by industry, it is important that the membranes can be cleaned relatively easy and the original permeate flux rate can be recovered. The procedure used to clean the membrane in this study (section 3.3.5.3) recovered the total transmembrane flux, as evident in Figure 4.19, where three consecutive concentration cycles of approximately 125, 150 and 175 min are shown, with cleaning of the membrane in between the first and second, and second and third, cycles.

Approximately 84% of the protein present in the extract from defatted bran was recovered using UF (Table 4.20). Saponin extraction from the bran prior to protein extraction reduced the recovery of protein to 77.0%. The protein products prepared by UF contained 67.9% (db) and 77.2% (db) protein for extracts from defatted bran and defatted/saponin-extracted bran, respectively (Table 4.20). Clearly, UF resulted in purification as well as concentration of protein in extracts from quinoa bran.

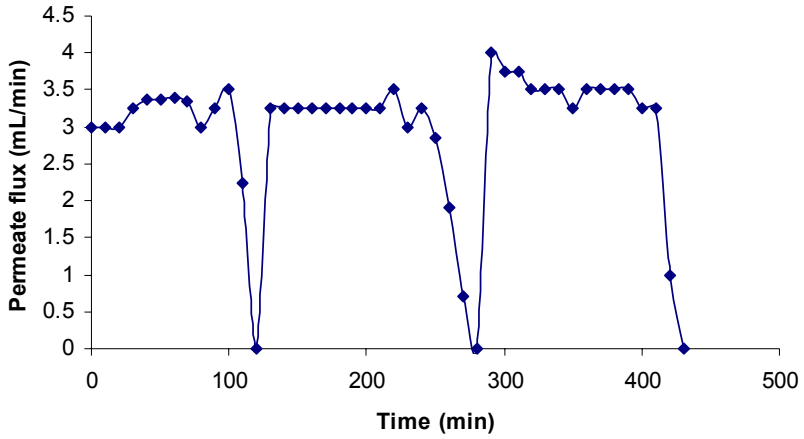


Figure 4.19 Recovery of transmembrane flux after membrane cleaning (at approximately 125 min and 275 min) as achieved during concentration of protein extracts from quinoa bran.

4.3.5.3 Isoelectric precipitation (IEP) in comparison to ultrafiltration (UF)

Significantly more protein was recovered from protein extracts by UF than by IEP (Table 4.20, $p > 0.05$). Similar results were found for the recovery of protein from soy, coconut, rapeseed and *Rosa rubiginosa* (Lawhon et al. 1981, Chakraborty 1985, Tzeng et al. 1988, Moure et al. 2001) by UF and IEP. The highest overall recovery, i.e. from seed, in this study was approximately 50%.

The protein content of the concentrated protein product on a dry matter basis was significantly lower when the protein product was acquired by UF than IEP ($p < 0.05$). This was attributed to more extensive co-concentration of non-protein constituents by UF. It might have been possible to further increase the protein content of both the UF and the IEP concentrated protein products by washing them, as was done for *Rosa rubiginosa* where the protein content was increased by three to thirteen percentage units (Moure et al. 2001). Based on protein terminology commonly applied to soybean products, the concentrated quinoa protein products would qualify as concentrates, i.e. all contained 65-70% or more of protein on a dry weight basis (Fuhrmeister and Meuser 2003). The IEP protein product from defatted/saponin-extracted bran was essentially a protein isolate, i.e. it contained 90% or more of protein on a dry weight basis. Other constituents in the protein products include fibre and starch. Due to the small granule size of quinoa starch, it is not completely removed from the protein extract by centrifugation. The same applies to fine fibre particles suspended in the protein extract. Extracting saponins from the defatted bran material increased the protein content of the final protein products by six to eight percentage units depending on the method used to concentrate the protein extract (Table 4.20). Other impurities in the concentrated protein products would include soluble constituents in the protein extracts, which were occluded or entrapped in the UF retentate or IEP precipitate, respectively.

4.3.6 Protein characteristics

The functional properties of the four protein products prepared from defatted or defatted/saponin-extracted quinoa bran were compared to those of soybean protein and egg white (Table 4.21).

The protein contents of the protein products were discussed in the previous section. The products also differed in their saponin contents (Table 4.21). Ultrafiltration resulted in protein products that contained significantly less saponins than their corresponding IEP products. The purpose of washing the bran with 60% (v/v) aqueous ethanol was to extract the saponins prior to protein extraction. The saponin contents of the defatted bran and the defatted/saponin-extracted bran was 2.9% (db) and 0.8% (db), respectively. Saponin extraction reduced the saponin content of the UF and IEP concentrated protein products from 2.7% (db) to 2.2% (db) and from 6.2% (db) to 4.1% (db), respectively (Table 4.21).

Because saponins have substantially lower molecular weights than the proteins, UF provided an effective means of removing these undesirable endogenous constituents. In contrast, it appears that saponins were entrapped within the protein matrix during IEP. Although the phytate contents of the protein products were not determined, it is expected that phytate is also removed during UF, since at alkaline pH, at which the protein was extracted and concentrated by UF, the phytate-protein complex stability is low. This would result in the passing of the phytate through the UF membrane whereas protein would be retained, as was found in the extraction and concentration of protein from yellow mustard meal by UF (Xu et al. 2003).

Protein recovered by UF (saponin-extracted or not) was not bitter in flavour (Table 4.21). The bitterness of IEP-prepared products was attributed to their higher saponin contents, which would make them less applicable in food products. The saponin extraction step had a large influence on the colour of the protein products (Table 4.21) in that the saponin-extracted products had significantly higher L values ($p > 0.05$), and lower a and b values. Clearly, 60% (v/v) ethanol was an effective solvent to remove coloured impurities in quinoa protein products. The method of concentration also affected the colour of the protein products. UF resulted in a less yellow product (Table 4.21). Apparently, the coloured constituents can pass through the UF membrane, whereas they co-precipitated, at least in part, with the protein during IEP. Which compound was responsible for the colour of the protein products, was not determined. Saponins have been related to bitterness and hemolysis,

Table 4.21 Functional properties of quinoa protein products as compared to soybean protein and egg white[I].

Property	***Product***					
	UF	**UF-saponin[II]**	**IEP**	**IEP-saponin[II]**	**Soybean**	**Egg white**
Protein content (%, db)	67.9a	77.2b	71.5c	88.5d	87.7d	90.7e
Saponin content (%, db)	2.7a	2.2b	6.2c	4.1d	-	-
Bitterness	-	-	+	+	-	-
Colour						
L	52.3a	62.8b	50.9a	59.3b	74.3c	88.6d
a	3.5ab	2.9c	3.7a	3.2bc	1.5d	0.6e
b	13.7a	11.4b	14.6c	13.2d	10.6e	16.9f
Solubility (%)	91.2ab	86.5a	93.0b	47.0c	64.7d	95.6b
Foaming properties						
Foaming capacity (%)	246.1a	246.1a	204.2b	219.9b	303.7c	91.6d
Foam stability (%)	38.2ab	40.5a	35.9b	38.1ab	27.6c	60.0d
Emulsifying properties						
Specific surface area (m^2/g)	1.7a	0.7ab	5.3c	1.1ab	1.4ab	0.4b
Droplet diameter (μm)	3.8a	9.0b	1.1a	5.4ab	4.4ab	15.3c
Emulsion stability (%)[III]	230abc	327ab	125c	121c	331.6a	126.8bc

[I] Values in the same row followed by the same letter are not significantly different ($p < 0.05$, $n = 3$).

[II] Protein product made from bran that was washed with 60% ethanol to remove saponins.

[III] '-' the product did not taste bitter, '+' the product tasted bitter.

[IV] $$\text{Emulsion Stability} = \frac{\text{Specific Surface Area (t=30min)}}{\text{Specific Surface Area (t=0min)}}$$

but not directly to the formation of yellow or dark-coloured substances (Gee et al. 1989). However, most white-coloured quinoa varieties are low in saponins, whereas the more yellow lines contain higher levels (Fleming and Galwey 1995), as was also found with the three commercial quinoa lines in this study (section 4.3.2). Phenolic compounds, or their decomposition products formed during processing, may contribute to the colour of quinoa protein products. Phenolic compounds afforded a dark colour to protein isolates from rapeseed meal. Quinones formed by oxidation of the phenolic compounds. These quinones reacted with protein, forming dark substances (Tzeng et al. 1988). Some phenolic compounds also have a bitter flavour. In this study, the starting material for the preparation of quinoa protein was a bran fraction from WMF quinoa, which was dark-yellow in colour. The use of a white line, such as QC, which is also lower in saponins (Table 4.9), may have made it unnecessary to include a saponin/colour extraction step.

4.3.6.1 Protein functionality

The quinoa protein products exhibited solubilities of 47.0 to 93.0%, depending on the material extracted (saponin-extracted or not) and the method of protein concentration. With the exception of the precipitated protein from saponin-extracted bran, the solubilities of the quinoa protein products were significantly higher than that of soybean protein ($p < 0.05$) and similar to that of egg white ($p < 0.05$). The solubility (47.0%) of the precipitated protein from defatted/saponin-extracted bran was lower than that of the other protein products, including the IEP protein from defatted bran (Table 4.21). On precipitation, protein aggregates were formed that were hard to solubilize. Aluko and Monu (2003) also found that the solubility of protein decreased on saponin extraction. Hence, the solubility of precipitated protein from bran that did not undergo the saponin-extraction step was high (93.0%). Saponins and additional substances that co-extracted with the saponins in 60% (v/v) ethanol, such as polyphenols, might influence the structure, denaturation, precipitation and resolubilization of protein. However, no correlation between solubility and saponin content was found (Table 4.22, $p > 0.05$).

Quinoa protein foamed much better than egg white, but less than soybean protein (Table 4.21). The foaming capacity was not affected by saponin extraction. These findings were in contrast to those reported by Aluko and Monu (2003) and Chauhan et al. (1999b). According to Aluko and Monu (2003), quinoa protein had a very low foaming capacity, which was due to the globular nature of the protein. This globular nature reduced its ability to form interfacial membranes around air bubbles. This foaming capacity was reduced further by saponin extraction. Protein products concentrated by UF foamed significantly better than those concentrated by IEP ($p < 0.05$). This is also opposite to the theory put forward by Aluko and Monu (2003). It appears that the unfolding of protein during isoelectric precipitation, whereby the globular nature of the proteins was lost, did not increase their ability to form interfacial layers around air-bubbles. A strong negative correlation ($r = -0.87$, $p < 0.01$) was found between saponin content and foaming capacity. Because the concentration of saponins is strongly reduced by UF, it is not possible to determine if the foaming capacity is affected mainly by the amount of saponins present or by differences in protein structure caused by the methods used for concentration.

The foam stabilities of the four quinoa protein products were similar and significantly higher than that of soybean protein, and lower than that of egg white protein (Table 4.22) ($p < 0.05$). Fat content is known to have a detrimental effect on foam stability. In other systems, it has been found that protein obtained by IEP had a higher fat content than that obtained by UF (Fuhrmeister and Meuser 2003). An explanation for this would be that with the unfolding of the protein at low pH, the hydrophobic regions become exposed and more binding to fat can occur. However, the bran material from which the protein was extracted had a crude fat content of less than 0.1%, and consequently the crude fat contents of all of the protein products were less than 0.1%. Saponin extraction prior to protein extraction did not affect the foam stabilities of the protein products (Table 4.22, $p > 0.05$). This was opposite to the findings of Chauhan et al. (1999b) who found increased foam stability following saponin extraction. Even though little difference in foaming capacity was observed among products, a negative correlation was found between foam stability and saponin content ($p < 0.01$).

Table 4.22 Correlation coefficients between the functional properties of protein products and their saponin contents.[1]

Property	Correlation coefficient (r)
Colour	
L	-0.56 ^{ns}
a	0.64 ^{ns}
b	0.78 *
Solubility (%)	-0.01 ^{ns}
Foaming properties	
Foaming capacity (%)	-0.87 **
Foam stability (%)	-0.61 ^{ns}
Emulsifying properties	
Specific surface area (m^2/g)	0.86 **
Droplet diameter (μm)	-0.79 *
Emulsifying stability (%)	-0.73 *

[1] *, **, ***, Significant at $p < 0.05$, 0.01, and 0.001; ns, not significant.

The emulsification parameters measured, namely specific surface area of the emulsion, droplet diameter and emulsifying stability, varied among the protein products. The IEP product extracted from non-saponin-extracted material exhibited a significantly higher specific surface area ($p < 0.05$) and a significantly smaller droplet diameter ($p < 0.05$) than did the other protein products, including soybean protein and egg white. The denaturation of protein during IEP might have exposed hydrophobic moieties that previously were folded into the protein, and which now could stabilize the hydrophobic canola oil droplets in water. This product also had the highest saponin content, hence the impact of saponins on emulsification properties cannot be ignored. In general, for protein products prepared from non-saponin-extracted material, the specific surface areas were larger and the droplets smaller (Table 4.21). A strong positive correlation ($p < 0.01$) was found between specific surface area and the saponin content of the protein products, and a negative correlation was observed between droplet diameter and saponin content ($p < 0.05$). This suggests that saponins, or the interaction between protein and saponins, or both, improved the capacity of quinoa protein to form emulsions of oil and water. Chauhan et al. (1999b) also found a higher emulsifying capacity for quinoa protein that contained more saponins. A decrease in the specific surface area of the emulsion after 30 min was expected, as the oil droplets would fuse together over time resulting in emulsifying stability values of less than 100%. As evident in Table 4.21, however, the emulsifying stability for all products was greater than 100%. A reason for this may be that the sample on which emulsion stability was determined was taken near the top of the emulsion where oil droplets had concentrated over time. When emulsion stability was compared among products, the means of protein concentration was shown to have a major effect. UF-concentrated protein generated much more stable emulsions than did IEP-concentrated protein. A negative correlation was found between saponin content and emulsion stability, which is in accordance with the results of Chauhan et al. (1999b) who reported that stability was higher for saponin-extracted material.

The quinoa protein products were freeze-dried. Commercially, protein products are typically spray dried, which might influence their colour. Tian et al. (1999)

obtained a much whiter field pea protein powder by spray drying than by freeze drying. They attributed this darkening of the freeze-dried protein concentrate to a greater oxidation of components such as polyphenols during freeze drying as compared to spray drying. Oxidation of polyphenols might have caused, in part, the relatively dark colour of some of the quinoa protein products. The use of UF and aqueous ethanol extraction would have reduced the levels of polyphenols in three of the four protein products, which most likely accounts for their lighter colours (Table 4.21).

4.3.6.2 Protein composition

It was not apparent whether the differences in functionality observed (Table 4.21), especially those related to the concentration process employed, were attributable to protein conformation only or also to protein composition. To determine whether UF or IEP concentration impacted protein composition differentially, the molecular weight distributions of the protein products were determined under reducing conditions. The protein products contained polypeptides ranging in size from 14 to 120 kDa (Figure 4.20). The major polypeptides were estimated to have molecular masses of 19, 21, 27, 37, 49, and 120 kDa. No obvious differences were found in the molecular weight distributions of the various products (Figure 4.20). The group of 8-9 kDa polypeptides described by Brinegar and Goundan (1993), and which were identified as 2S polypeptides (Brinegar et al. 1996), was not present in any of the products. These 2S storage proteins are soluble at pH 4.5-5 and, therefore, would not be expected to be present in the IEP products. Apparently, the UF membrane did not retain these polypeptides due to its high MWCO (50,000). The polypeptides that were present in the products were mainly comprised of the 11S polypeptide fraction of quinoa, which was reported to contain polypeptides having molecular masses of 22-23 kDa and 32-39 kDa (Brinegar and Goundan 1993).

Few differences in the amino acid profiles were found between the IEP- and UF-concentrated protein products (Table 4.23), yet differences in the amino acid composition of the protein products and whole seed flour from WMF were evident.

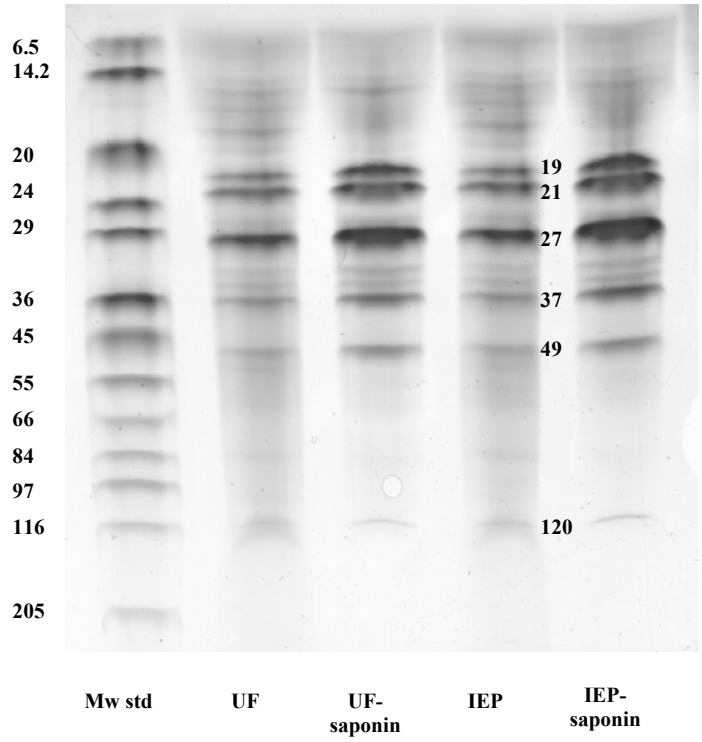


Figure 4.20 SDS-PAGE gel showing the size distribution of proteins present in four protein products from quinoa (UF = UF-concentrated protein extract from defatted bran; UF-saponins = UF-concentrated protein extract from defatted/saponin-extracted bran; IEP = IEP-concentrated protein extract from defatted bran; IEP-saponins = IEP-concentrated protein extract from defatted/saponin-extracted bran).

Table 4.23 Amino acid profiles of protein products prepared from saponin-extracted quinoa bran by ultrafiltration (UF) or isoelectric precipitation (IEP) in comparison to the profile of whole flour.

Amino acid[I]	**Whole flour**[II]	**UF**[III]	**IEP**[IV]
Essential			
Histidine	3.0	3.0	2.9
Isoleucine	4.5	4.7	4.5
Leucine	7.7	7.0	7.0
Lysine	7.0	4.4	4.1
Methionine	2.7	2.4	2.2
Cysteine	1.8	1.1	1.2
Phenylalanine	4.8	4.3	4.3
Tyrosine	3.3	3.5	3.3
Threonine	4.9	3.2	3.0
Tryptophan	1.4	1.2	1.0
Valine	5.3	5.2	4.9
Non-essential			
Alanine	5.4	4.1	3.8
Arginine	10.5	11.5	11.4
Apartic acid	9.6	11.2	10.8
Glutamic acid	11.2	17.3	18.0
Glycine	6.2	5.5	5.2
Proline	4.7	4.4	4.2
Serine	5.9	5.9	5.6

[I] g/100 g of protein.
[II] from whole flour from WMF quinoa seed.
[III] protein recovered by UF from the bran fraction of WMF quinoa seed
[IV] protein recovered by IEP from the bran fraction of WMF quinoa seed

The levels of lysine was reduced to 4.1-4.4% of protein, and threonine to 3.0-3.2%, as compared to levels of 7.0 and 4.9%, respectively, in the whole seed flour. This may be a concern, because a reduction in the levels of these essential amino acids could cause quinoa protein products to become nutritionally incomplete for children (see Table 2.3).

4.3.7 Starch extraction from quinoa perisperm

The fine, i.e. < 500 μm, roller-milled fraction, was used for the production of a refined starch product. Starch was extracted by mixing the fines with aqueous NaOH solution at pH 9 (25:1 liquid:fines ratio) to solubilize the protein. The slurry of fines in NaOH solution was passed through a 75 μm screen prior to centrifugation. This was to remove the relatively coarse and dark-coloured bran material that did pass through the 500 μm screen. However, the amount remaining on the screen was so minute that it was not analyzed further.

Removal of the grey layer, which accumulated on top of the starch pellet after centrifugation, increased the starch content and decreased the protein content of the final starch product by 2.5 and 1.6 percentage units, respectively (Table 4.24). Rewashing the pellet with alkali increased the starch content and decreased the protein content further, yielding a final starch product containing 96.9% starch and 1.2% protein.

The pasting properties of the fine perisperm flour, starch “a” and starch “b” (see Figure 4.21), and those of starch prepared by a wet milling process under non-alkaline conditions (section 3.1.2) are presented in Table 4.25. Starch “b” was recovered after extraction of protein from defatted and saponin-extracted bran. The pasting temperature of starch “b” was significantly higher ($p < 0.05$) than that of the other starches, which might have been due to the extraction process used to remove the fat from the bran. This sample also had a much lower starch content than the other starch samples. No significant differences in peak times were observed among the starches ($p > 0.05$).

The starch products varied significantly in starch content ($p < 0.05$), hence it is difficult to compare their paste viscosities. It is likely that the high starch content in

Table 4.24 Effect of scraping and washing (with alkali) of the starch pellet after centrifugation on the composition of the final starch product[1].

Process	Starch (%, db)	Protein (%, db)
Without scraping and washing	93.5a	3.5a
With scraping only	96.0b	1.9b
With scraping and washing	96.9b	1.2b

[1] Values in the same column followed by the same letter are not significantly different ($p < 0.05$, $n = 3$).

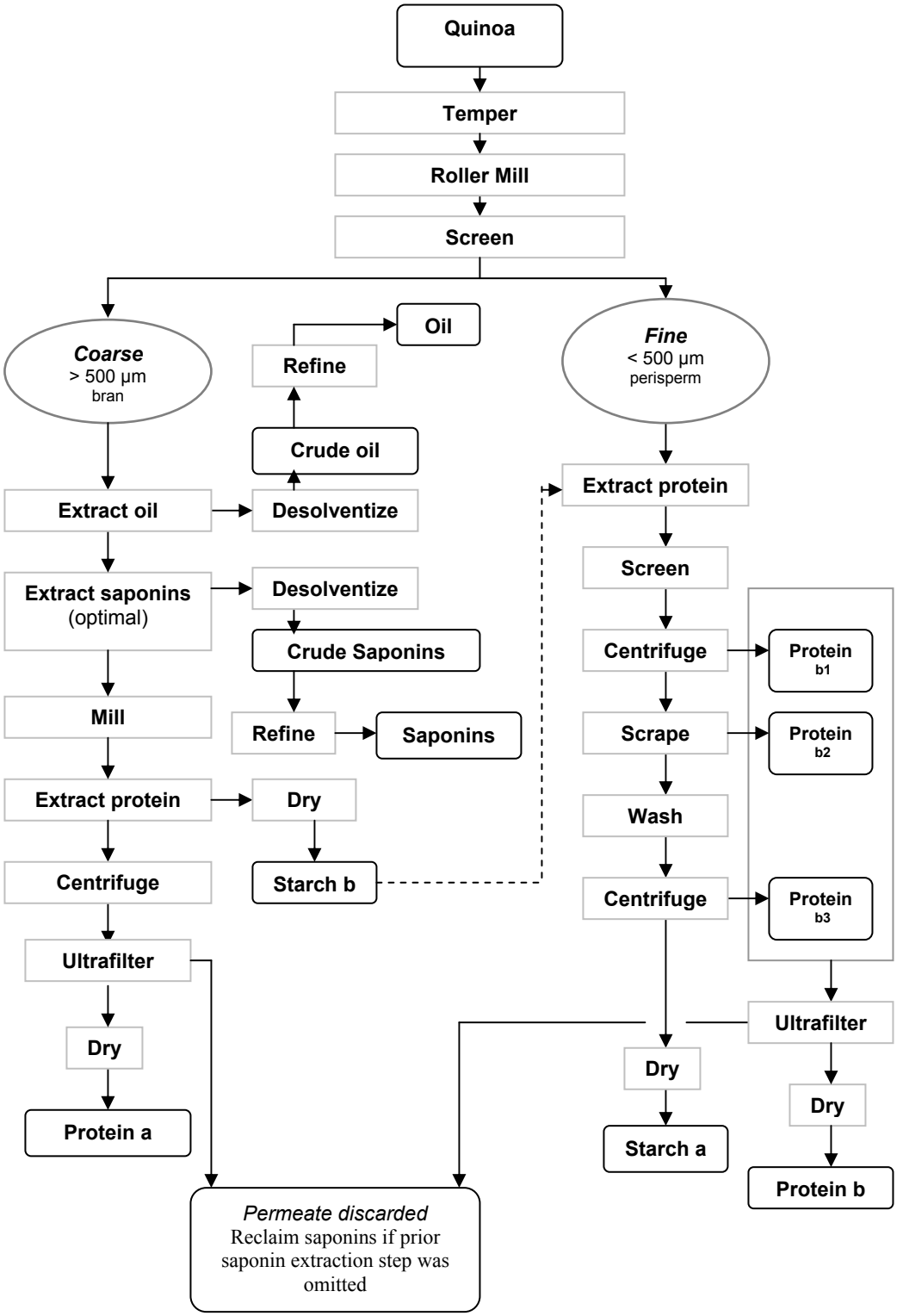


Figure 4.21 Process for the fractionation of quinoa.

Table 4.25 Pasting properties[I] of starch-rich fractions from quinoa[II].

Sample	Starch (%, db)	P_{temp} (°C)	P_{time} (min)	Viscosity (RVU)				
				PV	TV	FV	BD[III]	SB[IV]
Fine perisperm	77.2a	63.0a	6.7a	8.6a	7.4ab	8.5a	1.2ab	1.1a
Starch a	96.9b	63.0a	6.7a	19.9b	18.7c	23.0b	1.2ab	4.3b
Starch b	51.2c	65.0b	5.9a	6.3a	4.7a	5.9a	1.6a	1.2a
WMF starch[V]	98.5b	63.0a	6.8a	11.4a	11.0b	16.4c	0.4b	5.4c

[I] P_{temp}, pasting temperature; P_{time}, peak time; PV, peak viscosity; TV, trough viscosity; FV, final viscosity; BD, breakdown, SB, setback.

[II] Values in the same column followed by the same letter are not significantly different ($p < 0.05$, $n = 3$).

[III] PV minus TV.

[IV] FV minus TV.

[V] Starch extracted using wet milling as described in section 3.1.2.

starch “a” and wet-milled (WMF) starch was responsible for the high viscosity values observed for these samples. No significant differences in peak time, pasting temperature or pasting viscosity were observed between starch “a” and wet-milled WMF starch ($p > 0.05$). This suggests that the mild alkaline conditions used for the extraction of starch ”a” were not damaging, nor was dry-milling.

4.3.8 A process for the fractionation of quinoa

The compositions of the different fractions obtained in the developed quinoa fractionation process are given in Table 4.26. From whole quinoa, 42% was recovered as starch “a”, 36% as starch “b”, 7% as protein “a”, 10% as protein “b”, 4% as crude oil and 1% as crude saponins (Table 4.26).

Of the protein present in the quinoa seed, 41% was recovered as a product containing 77% (db) protein, whereas 24% was recovered as a powder with a protein content of 45% (db) (Table 4.26, Figures 4.21 and 4.22). Normally, the protein fractions “b1”, “b2” and “b3” would be combined, concentrated by means of UF and dried. However, in this study these fractions were combined and dried without a concentration step. It is expected that the protein content would increase, and protein recovery would decrease, on concentration by UF prior to drying.

Sixty-eight percent of the starch present in whole quinoa seed was recovered as a refined product with a starch content of 97% (db) and a protein content of 1.2% (db), while another 29% of the initial starch was recovered as a product that contained 53% (db) starch and 18% (db) protein (Table 4.26, Figures 4.21 and 4.22). A higher yield of starch “a” could have been obtained by purifying starch “b”. Because starch “b” had a different pasting profile than did starch “a”, it may not be appropriate to combine these streams.

The process developed to fractionate quinoa began with a roller milling process to separate the seed into a coarse (bran) fraction enriched in protein, oil and saponins and a fine, starch-rich (perisperm) fraction (Figure 4.21). If quinoa flour from whole seed instead of the fine and coarse fraction from roller milling was used for protein extraction, a protein-rich product containing 37% (db) of protein and a starch product containing 71% (db) starch and 5% (db) protein would have been obtained. Clearly,

Table 4.26 Mass balance and end-product composition of the quinoa fractionation process.

Whole quinoa (WMF) *100%* [I]	
Starch	52.0% (db)
Protein	17.2% (db)
Fibre	11.8% (db)
Oil	6.2% (db)
Saponins	8.1% (db)
Ash	3.5% (db)

Coarse >500µm (bran) *48%* [I]		**Fines <500µm (perisperm)** *52%* [I]	
Starch	32.1% (db)	Starch	77.2% (db)
Protein	23.6% (db)	Protein	8.9% (db)
Fibre	22.3% (db)	Fibre	6.6% (db)
Oil	8.8% (db)	Oil	2.7% (db)
Saponins	7.4% (db)	Saponins	3.1% (db)
Ash	5.8% (db)	Ash	1.5% (db)

Starch b *36%* [I]		**Protein a** *7%* [I]		**Oil** *4%* [I]	**Saponins** *1%* [I]	**Starch a** *42%* [I]		**Protein b** *10%* [I]	
Composition (%, db)									
Starch	52.9	Starch	5.8			Starch	96.9	Starch	10.6
Protein	17.8	Protein	77.2			Protein	1.2	Protein	44.7
Fibre	23.9	Fibre	8.8			Fibre	1.4	Fibre	29.1
Oil	0.1	Oil	0.1			Oil	0.1	Oil	10.5
Saponins	n.d.	Saponins	2.2			Saponins	n.d.	Saponins	n.d.
Ash	5.3	Ash	5.0			Ash	0.3	Ash	n.d.

[I] The mass of the fraction as percentage of whole seed.

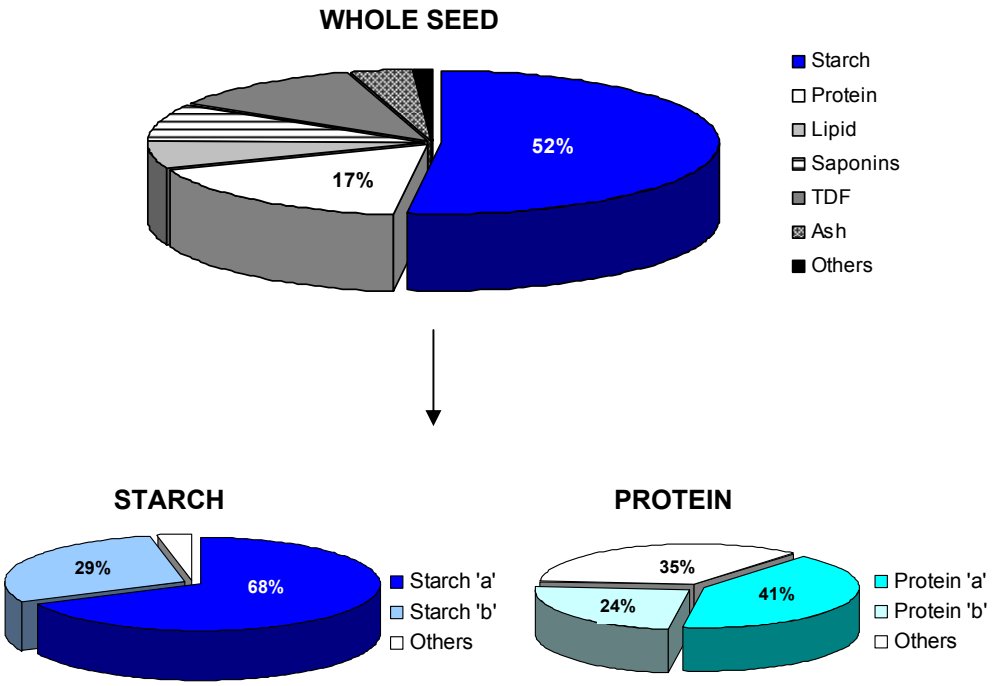


Figure 4.22 Distribution of starch and protein in the products obtained using the developed quinoa fractionation process. Yields are expressed as a percentage of the starch/protein that was present in whole seed.

fractionation by means of roller milling prior to protein and starch extraction was a useful step.

Oil was extracted from the bran prior to protein extraction. This was necessary as the concentration of oil in the final protein product would negatively impact its functional properties. Extraction of saponins from bran might be unnecessary, as they were in large measure removed during UF.

5. SUMMARY AND CONCLUSIONS

The amylose contents (3 to 20%) and physicochemical characteristics (thermal, retrogradation and pasting properties, swelling and solubility behaviour, freeze-thaw stability, water-binding capacity, shear stability, granule size and morphology) of starches isolated from eight quinoa lines were compared. The gelatinization onset and peak temperatures and retrogradation tendencies differed among starches and were positively correlated with amylose content. The starches had similar gelatinization enthalpies. With the exception of pasting temperature, large variations in pasting characteristics as well as differences in swelling, solubility, freeze-thaw stability, shear stability and water-binding capacity were observed among starches and were correlated to amylose content. Although amylose was an important factor in determining the characteristics of quinoa starches, not all observed differences in starch characteristics could be attributed to variations in their amylose contents.

In quinoa, two peptides were identified by immunoblot analysis and peptide sequencing as isoforms of GBSSI, the enzyme responsible for amylose synthesis. These peptides had apparent molecular masses of 56 and 62 kDa, and the amounts in which they were present in starch granules from different quinoa lines were positively correlated to the amylose contents of the respective starches. Total starch synthase activity, of which GBSSI activity is a part, was measured during seed development and was positively correlated to the amylose content of starch and the starch concentration in the seed during seed development. The identification of GBSSI in quinoa and its relationship to amylose content could be used in the development of quinoa lines that contain starches with high or low amylose contents and particular physicochemical characteristics.

Three commercially-available quinoa lines differed with respect to their seed morphology and composition, especially their saponin contents and their α-amylase

activities. Quinoa displayed relatively high α-amylase activity compared to wheat flour. However, this did not appear to affect the properties of quinoa starch. The line used in the fractionation studies was relatively high in saponins, lipid, fibre and protein, and low in starch, compared to the other lines investigated.

Wet fractionation of whole quinoa was complicated by its small starch granule size, the presence of endogenous substances that increased the viscosity of quinoa flour-in-water slurries and the composition of the pellet formed on centrifugation of the slurries. Dry-separation of the seed by roller milling and screening into a protein-oil- and saponin-rich bran fraction and a starch-rich perisperm fraction was found to be a useful first step in the fractionation of quinoa.

Protein extraction from roller-milled bran was optimized by means of response surface methodology using a modified central composite design for liquid-to-bran ratio, flour particle size and centrifugal force, with five levels of each factor. From the bran, 84% of the protein was recovered as a protein product which contained 52% (db) protein. A high liquid-to-bran ratio was used for protein extraction. Therefore, a concentration (and purification) step employing either ultrafiltration (UF) or isoelectric precipitation (IEP) was required prior to freeze-drying of the protein extract. Ultrafiltration recovered more protein than did IEP, and the UF protein products were higher in protein and lower in saponins and would be expected to contain less phytate. Additionally, the UF-concentrated protein products exhibited better colour, taste and functionality, with the exception of emulsifying capacity, than did the IEP-concentrated protein products. Extraction of saponins from the bran prior to protein extraction reduced the saponin content and improved the colour and emulsifying properties of quinoa protein products.

Starch was extracted from the fine, roller-milled perisperm fraction under conditions similar to those used for protein extraction from the bran. The dry milling and alkaline extraction conditions employed did not appear to cause damage to the starch granules. The fractionation process recovered 41% of the protein present in the seed as a product that contained 77% (db) protein and 6% (db) starch, and 24% as a protein product that contained 45% (db) protein and 11% (db) starch. Sixty-eight percent of the total starch was recovered as a starch product that contained 97% (db)

starch and 1.2% (db) protein, and 29% as a product containing 53% (db) starch and 18% (db) protein.

The production and consumption of quinoa are poised to increase because of its pest resistance, hardiness, non-GMO status and image as a health food and ancient grain. At the moment, however, the cost of quinoa seed is high, which works against economic component separation. It is therefore essential that any fractionation process applied to quinoa will generate, to the greatest extent possible, high value products with little unusable by-product. This study has shown that it is possible to generate highly functional, starch-rich and protein-rich fractions from quinoa, and it would seem that the saponin and oil co-products might also contribute economically to the process. On the negative side, the oil content of quinoa and quinoa bran may be too low for economic oil extraction, and the inability to prepare a protein product containing at least 90% (db) protein may be a significant problem. Future work should address these shortcomings and others related to starch and protein product yields, and identify potential uses for products derived from quinoa.

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