Evaluation of technical feasibility of dry fractionation as a method to obtain novel protein foods from quinoa

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Preface

With great pleasure, I can show the result of my master thesis of Food Technology, in which the technical feasibility of dry fractionation as a method to obtain novel protein foods from quinoa was evaluated. Since quinoa has been an important part of basic nutrition of the Andean region for many years, as Ecuadorian, I was very happy to work with the selected topic, and to collaborate with the recent revival and interest that quinoa has shown lately.

During this period I gained a lot of knowledge from literature and from the experimental performed work. Working in the department of FPE- WUR, has been a great opportunity for me, for hands-on learning, utilizing some of the most sophisticated laboratories and equipment available anywhere.

Along the project I had many responsibilities and freedom in the choices to take for the development of my research, which was a challenge in the beginning but in the end, it made me aware of my skills and competencies.

I would like to give special thanks to my supervisor Francisco Rossier that despite of the distance, he was able to support me with valuable feedback and supervision on this thesis. Furthermore, thanks to the lab-technicians: Jos Sewalt, Maurice Strubel, Jarno Gieteling and Martin de Wit for the help they gave me with new equipment and lab analyses. Finally, I would like to thank my boyfriend, my parents and all my family and friends for supporting and cheering me up from “far, far away”.
Summary

Quinoa has remarkable nutritional properties because of its protein content and amino acid balance, which give this grain a great potential for food applications. This work aimed to explore and compare different fractionation technologies to separate and exploit the benefits from individual components or enriched fractions of quinoa. Understanding the grain structure and its behavior under different processing conditions sheds new light on the optimal conditions to rationally develop a more efficient fractionation process.

The major anatomical parts of quinoa grain are: (1) the pericarp, a thin layer that covers the grain, (2) the perisperm, the main storage tissue composed mostly of starch granules, and (3) the embryo (radicle and cotyledons), a yellowish ring-like structure, surrounding the perisperm and that, unlike other grains, represents 30% of the seed’s volume. The protein content in the full quinoa meal, pericarp, perisperm and embryo was found to be 16.84%, 10.56%, 42.91% and 3.16% respectively.

The main quinoa components (fiber, starch, oil and protein) were easily separated in an alkaline medium, obtaining a protein concentrate of 77.36% purity with a yield of 43.41% with respect to the initial flour. However, the extreme pH used caused protein denaturation and starch damage, as verified by DSC. Furthermore, this process involved the use of large amounts of water and considerable energy to dry the final products.

For dry fractionation, quinoa was pin-milled at two different speeds and temperatures. The milling temperature showed a big influence on the particle size of the resulting meals. Freezing of the grains in liquid nitrogen just before milling resulted in considerably smaller particles than milling at room temperature. However, independent of the particle size distribution of the flour, milling resulted into a product were the particle sizes of the protein and starch fractions largely overlapped. Nevertheless, the fractions could be further purified successfully in a dry process. Applying electrostatic separation on meals with a typical protein content of 14%, protein enriched fractions of up to 27.57% and protein depleted fractions down to 5.85% could be obtained. Coarseness in the flour showed a positive effect on the electrostatic separation.

The fracture properties of quinoa seed were evaluated by a compression method, finding that a force of 30N applied over a displacement of only 200 µm were sufficient to separate the cotyledon from the rest of the seed without damaging of the structure. Based on that observation quinoa seed was roller-milled and sieved into three different fractions. The coarse fraction contained mainly pericarp (hulls) and fragments of perisperm, the medium fraction was composed mostly of cotyledons, and the finer fraction contained smaller starch particles. These preliminary results showed that by breaking the grain under controlled conditions making use of its structure, a mild and efficient fractionation process could be develop that keeps the native functionalities of quinoa intact.
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1. INTRODUCTION

Quinoa (Chenopodium quinoa Willd) is a well-known grain that was domesticated in the Andes; in the region of Ecuador, Peru, Bolivia, Argentina, and Chile [2]. In Pre-Columbian times, quinoa was the major crop of these cultures in Latin America. Unfortunately, after the Spanish colonization its use, consumption, and cultivation was almost eliminated and only remained in the farmers' traditions. Therefore, outside that region, quinoa was virtually unknown. However, recent interest in the study of this cereal has been shown, due to its excellent nutritional food quality [3]. Demand for quinoa is increasing in the United States, Europe, and Asia, but the supply in the quinoa producing countries of South America is insufficient. It is believed that an increased consumption of quinoa in the developed countries will enhance the internal, urban markets in countries with a traditional production of quinoa [4].

Quinoa is a product of high nutritional qualities with worldwide importance because of its protein content, which exceed most of the major cereals. Nevertheless, the real value consists in its protein quality, namely the combination of a higher proportion of essential amino acids for human consumption, which gives this seed a high biological value [5]. It has protein quality comparable to that of whole dry milk in terms of balanced amino acid composition [6], being rich in lysine, methionine, and cysteine [7]. Compared with other cereals, some types of wheat come close to matching quinoa protein, but cereals such as corn and rice generally have less than half its protein content [8]. An important advantage is that quinoa is a gluten-free raw material, making it appropriate for people suffering of celiac disease [9].

Because of the recent revival of quinoa, most of the research has been focused on agricultural aspects rather than technological aspects [9]; giving rise for plenty of opportunities for investigation in this area. As the world population increases, there will be a greater pressure for the direct consumption of plant products in foods possessing aesthetic and organoleptic appeal, e.g., meat analogues. This development will place high emphasis on the need for proteins with multiple functional properties [10].

The use of protein isolates and concentrates has increased in the food industry because of several factors such as high protein level, good functionality, and low content of anti-nutritional factors [11]. Besides the high nutritional value of quinoa proteins due to the high quality of amino acids, their use as food ingredients in the form of protein rich fractions depends also on their functional properties. These properties are correlated to their structure, that is, to the degree of unfolding of the protein isolates, greatly affected by the extraction conditions [12], namely wet and dry extraction. Wet fractionation is a conventional technique that has been used to produce protein isolates and concentrates, obtaining relatively pure products. However it uses large amounts of water and energy, producing equally large waste streams. Moreover, it has a negative influence on the
functionality of the obtained ingredients. Harsh conditions during the fractionation (pH and temperature), and the final drying of ingredients into powders are detrimental to the functionality of the ingredients. In contrast, dry fractionation is a collection of mature technologies that uses no or less water requires no stabilization process after the separation and specially focuses on preserving the native functionality rather than on molecular purity. The replacement of wet fractionation routes for production of protein concentrates is expected to have considerable sustainability benefit compared to the other applications [13]. This report evaluates the technical feasibility of dry fractionation as a method to obtain novel protein foods from quinoa.

1.1. Aim
Considering the great potential that quinoa has as an extraordinary nutritive grain and the importance of developing new products to meet market demand for ingredients with applications in food formulation, the aim of this study comprises:

- A clear understanding of the structure of the quinoa grain, which involves the differentiation of the main tissues and the distribution and proportion of the major components (starch, protein and fiber) within them.
- To explore the wet fractionation process to extract the individual components from quinoa, to set a basis for an objective evaluation between this method and other possible fractionation techniques that could be applied for quinoa.
- The evaluation of the technical feasibility of (a) dry fractionation route to obtain protein enriched fractions from quinoa that could be used in the food industry.
- The study of the fracture behavior of quinoa seed under controlled conditions, to investigate the possibility of making use of the structure of the grain to rationally develop efficient fractionation processes.

1.2. Approach
In this work, quinoa seed structure was studied giving special attention to the morphology of starch and protein particles and the way in which they are arranged within the grain, to gain a better understanding for a further fractionation processes. Additionally, the distribution of protein in the three main parts of the grain: pericarp, embryo and perisperm, was determined. Via wet fractionation procedure the main components from quinoa (oil, protein, starch and fiber) were extracted and its protein fraction evaluate regarding purity and yield. Moreover, the effect of the extraction conditions on proteins and starch were analyzed using DSC. A dry fractionation route was studied, starting with a dry milling process, which took into account two variables: speed and temperature. The effect of those variables on particle size distribution was investigated. Electrostatic separation was applied to further enrich quinoa proteins fractions.
Considering the structure of the grain and the protein composition in each of its parts, preliminary fracture testing was done to advice on optimal breakage for minimal processing and a mild fractionation. Finally, some potential applications of these protein-enriched fractions were explored in terms of protein functionality. Foaming capacity was the selected as the technical functionality to be tested, leaving an open window for further research in this area.
2. QUINOA: THEORETICAL BACKGROUND

2.1. Seed structure
Quinoa grain has a disc-shape, of about 2 mm in diameter and 0.5 mm in thickness [14]. It ranges in color from white and yellow to red, brown and black [15]. The weight of the seeds vary from 1.9 to 4.3 g/1000 seeds [16]. The major anatomical parts of the grain are the pericarp, the perisperm and the embryo [17] (Figure 2-1).

The pericarp is a thin layer that covers the entire seed [1]. It contains saponins, which transmit the characteristic bitter taste of quinoa. However, since this layer is easily removed when rubbed or washed [18], saponin content can be easily and significantly reduced [19].

The embryo is a yellowish structure that consists of a radicle and two cotyledons, which surround the perisperm like a ring [18]. Unlike other seeds, it comprises approximately 30% of the seed volume, measuring 3.54 mm long and 0.36 mm wide, in some cases it reaches a length of 8.2 mm and occupies 34% of all seed [17]. Around 57% of total protein of the grain is located in the embryo [14] and reaches protein levels of 35-40% as an individual structure [17].

The perisperm is a white structure that represents roughly 60% of the seed volume [17], it is the main storage tissue and is mostly composed of starch grains inside uniform, non-living, thin-walled cells [1]. Low levels of protein content, approximately 7% has been found to be present in this individual structure [14].

Figure 2-1 Quinoa seed structure
Prego et al., (1998) [1].
2.2. Chemical and nutritional aspects

The chemical composition of the macronutrients of whole quinoa seed is summarized in Table 2-1.

<table>
<thead>
<tr>
<th>Components</th>
<th>%</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>11.2</td>
<td>All values are on a dry basis (%)</td>
</tr>
<tr>
<td>Protein</td>
<td>12-23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Abugoch James [20]</td>
</tr>
<tr>
<td>Fat</td>
<td>4.4-8.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Becker and Hanners [7]</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>12.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Schoenlechner, Siebenhandl [21]</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>73.6-74&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

2.2.1. Proteins

Quinoa is a grain with high nutritional value due mainly to its high content of good quality protein [14]. The seed protein content is high, about 15% in average [22]. In comparison with other cereals, corn and rice have in general less protein than quinoa but some types of wheat come close to matching its protein content [20]. Nevertheless, the real value of quinoa consist in its protein quality, because of a wider amino acid spectrum than cereals and legumes, with higher lysine and methionine contents [23].

Two main storage proteins are present in quinoa: the first one is the 11S salt soluble globulin (chenopodin), which accounts for 37% of the total protein and is made of subunits each of which consists of a basic and an acidic polypeptide, with molecular mass of 20–25 and 30–40 kDa, respectively. The second major protein that represents 35% of total is a 2S-type protein also known as albumin and has a molecular mass of 8–9 kDa. Both proteins are stabilized with disulphide-bridges in their native state, but their major difference consists in their solubility at pH 5, where most of the 11S is precipitated, while the 2S remains soluble [24] [25]. The distinctive structural and solubility characteristics of these two proteins suggest that their functional properties differ markedly [21]. Prolamins are found in a very low amount in quinoa, which indicates that is a gluten-free seed, suitable for people suffering celiac disease [26].

2.2.2. Carbohydrates

Carbohydrates are the major components in quinoa, represented mainly by starch, which comprises approximately 55% of the total mass [27]. The starch is located in the perisperm of the seeds. It is unique that quinoa starch can be present as simple units from 0.5 to 1µm or as spherical aggregates with a size from 20-25µm [1].
In most cereals, starch consists of 20% amylose and 80% amylopectin, being this ratio an essential factor determining important industrial properties and applications. The amylose content of quinoa starch is relatively low; it varies between 3% and 20%, similar to some rice varieties, but higher than some barley varieties. Amylose content affects the functional and physico-chemical properties of starch, including its pasting, gelatinization, retrogradation, and swelling properties [27]. This wide variation within the amylose content is therefore responsible for the differences in physical properties of starch [28]. It has been reported that quinoa starch has an average molar mass of $11.3 \times 10^6$ g/mol, comparable to amaranth, higher than wheat but lower than waxy maize starch [15].

In quinoa starch the amylopectin content is approximately 77.5%. It has a unique chain length distribution as a waxy amylopectin, with 6700 glucan units for the amylopectin fraction of quinoa starch. Quinoa amylopectin, like that of amaranth and buckwheat, contains a large number of short chains from 8 to 12 units and a small number of longer chains of 13–20, compared to the endosperm starches of other cereals [15].

The gelatinization properties of starch are related to a variety of factors including the size, proportion and kind of crystalline organization, and the ultrastructure of the starch granule [20]. Quinoa starch exhibits low gelatinization temperatures ($T_o = 46.1$-$57.4^\circ C$, $T_p = 54.2$-$61.9^\circ C$, $T_c = 66.2$-$68.5^\circ C$) which is generally the case for small granules of starch [27] and gelatinization enthalpies from 12.8 to 15 J/g of dry starch. The gelatinization temperatures are positively dependent of amylose content [15].

Dietary fiber is another important carbohydrate; it is known to have beneficial effects on human health. The fiber content of quinoa lies within the range of other cereals and is mostly present in the pericarp and embryo [1].

### 2.2.3. Lipids

Quinoa has been considered an alternative oilseed crop because its oil contains high amounts of polyunsaturated fatty acids and is particularly stable due to the presence of high amounts of natural antioxidants [29]. The lipid content of quinoa is between 2 and 3 times higher than in other cereals such as maize and wheat [28]. Quinoa fat content ranges from 4.4 to 8.8 %, it is higher than maize (4.7 %) and lower than soy (18.9 %). Cytochemical and ultrastructural analyses show that lipid bodies are the storage components of the cells of the endosperm and embryo tissues [1].

### 2.3. Protein functionality

In general, functional properties are those non-nutritional properties which are capable of imparting specific technological and desirable features in a given product [30]. Functional properties of proteins connote physicochemical properties which affect the
behavior of proteins in food systems during preparation, processing, storage and consumption. They are not only necessary in determining the quality of the final product, but also in facilitating processing [10].

Plant proteins, to be effectively and successfully used in food applications, should ideally possess several desirable functional properties. Many factors have been reported to affect the functional properties of proteins. External factors that may modify the protein, during processing, and hence its effect on the product characteristics, include heat, shear, presence of salts and other hydrocolloids. Intrinsic factors such as size, shape and conformation [31] may also affect protein functionalities. The method and conditions of isolation of fat was also reported to affect the functional properties of protein [32].

Major functionalities of food proteins include solubility, emulsification, gelation, foaming, water binding capacity and heat stability. This chapter includes a theoretical approach on the functionalities of quinoa proteins in relation to foaming.

### 2.3.1. Foaming

A foam can be defined as a two-phase system in which a distinct gas bubble phase is surrounded by a continuous liquid lamellar phase [33]. It can be formed by mixing or injecting gas in a solution under a certain pressure. Different methods have been used to induce foam formation and it has been proven that they have a large effect on foam behavior. Therefore, observed results are not merely a property of the protein [34].

When air is injected into a protein solution, entrapment of the formed bubbles occurs due to the absorption of the protein molecules at the bubble surface [35]. In most food proteins, only the soluble protein fraction will be involved in foam formation [36]. The basic requirements for a protein to be good foaming agent are: (a) the ability to adsorb rapidly at the air–water interface during bubbling; (b) undergo rapid conformational change and (c) rearrangement at the interface and form a cohesive viscoelastic film via intermolecular interactions [37]. The first two criteria are essential for better foam capacity, whereas the third is important for the stability of the foam. The sparging method defines foam capacity as the foam volume immediately after injection of the gas over the volume of the injected gas. The Foam half life time \( t_{1/2} \) is a parameter used to measure the foam stability and it is expressed as the time needed to reduce the foam volume to half of its original value.

Table 2-2 lists some factors that affect foaming properties of proteins, which might be the causes for the variances in the results reported by different authors. Some studies have been done in quinoa as a foaming protein. Aluko and Monu (2003) measured foam capacity and stability (expressed as %). Protein concentrate showed the smaller foam expansion (<20%), but a protein hydrolysate presented values over 160%. Foam stability was better with a protein concentrate. In contrast, Lideboom (2005) reported that quinoa protein had a much better foam capacity than egg white, but less than
soybean protein. On the other hand, foam stabilities of quinoa protein were higher than that of soybean protein, and lower than that of egg white protein [16].

Table 2-2 Factors affecting foaming properties of proteins

<table>
<thead>
<tr>
<th>Stabilizing Factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Surface Viscosity:</strong></td>
<td>Denaturation and association of proteins.</td>
</tr>
<tr>
<td><strong>2. Concentration:</strong></td>
<td>Solubility, diffusion rate, concentration in disperse phase.</td>
</tr>
<tr>
<td><strong>3. Electrical double layer:</strong></td>
<td>Repulsion affected by counter ions in solution.</td>
</tr>
<tr>
<td><strong>4. pH:</strong></td>
<td>Theoretically maximum near isoelectric pH.</td>
</tr>
<tr>
<td><strong>5. Complementary Surfactants:</strong></td>
<td>-- Other proteins, polysaccharides (not lipids).</td>
</tr>
<tr>
<td><strong>6. Denaturants:</strong></td>
<td>Limited denaturation may aid film formation.</td>
</tr>
<tr>
<td><strong>7. Marangoni Effect:</strong></td>
<td>Ability of surfactant solute to rapidly concentrate at a stress point in the film.</td>
</tr>
</tbody>
</table>

Kinsella (1979) [38]

2.4. Fractionation Processes

Different studies have been done on fractionation of quinoa on a laboratory scale. Mostly, protein and starch have been extracted for individual characterization. However, none of them have been applied in industrial processes. 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 be economic [20].

2.4.1. Wet fractionation

Wet fractionation is a process applied to different products to extract individual components from them (e.g., proteins, starch, fiber and lipids). It is widely used in the production of protein isolates (85-90% protein) and concentrates (48-70% protein). Relatively pure isolates can be obtained with this technique, however, it is associated with the use of great amounts of water and energy and goes along with production of large amounts of waste. Furthermore, it has negative influence on the functionality of the final ingredient [39].
The extraction of protein from quinoa by a wet fractionation technique, consists of the following steps: a) Milling of quinoa seeds, b) flour defatting, c) solubilization of the protein under alkaline conditions, d) isoelectric precipitation, and e) drying.

\textbf{a) Preconditioning and milling}

In an industrial scale, before proceeding to milling, grain must undergo cleaning to remove impurities such as dust, plant waste, foreign particles, and others. Then the grain must be conditioned, if necessary, to keep moisture suitable for grinding, that is 14% maximum [40]. Washing is another step used to remove the saponin content and reduce bitterness of the grain. Sweet varieties of quinoa have the advantage of containing very low amounts of saponin compared to bitter varieties [3]. Once completed the pretreatment of the seed, milling is required to create cell disruption and lose the proteins that are usually contained in protein bodies inside the cell walls, so they can be totally solubilized and extracted [41].

\textbf{b) Defatting}

Prior to protein extraction, quinoa flour that contains relatively high levels of oil must be defatted. This is to prevent emulsion formation during protein extraction, to produce oil-free protein products and to prevent further fat oxidation, thus increasing the shelf life of the final product. Mechanical pressing as well as solvents such as hexane and petroleum are used for fat removal [20]. The preferred ratio of quinoa to solvent is about 1:1 (w/v) and residence time in the extractor is 60 min, however, this ratio and residence time can be adjusted depending on the solvent and a given sample of quinoa [42].

\textbf{c) Protein solubilization}

Protein solubilization is generally employed to separate proteins in the sample selectively from different substances that may interfere in the extraction process [41]. 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) [15]. The optimal medium to solubilize quinoa protein is an aqueous salt solution and the ratio of the defatted quinoa four to alkaline solution is 1:10 (w/v), however, this ratio can be adjusted, if necessary, and the molarity of the alkaline solution and defatted quinoa four suspension can be adjusted to obtain a pH in the range of 8-12. The temperature is not critical for this step and can be readily modified. The length of the extraction should be adjusted to maximize protein recovery[42].
The solubilization process strongly affects the quality of the final results and thus determines the success of the entire experiment. For example, it has been shown that for amaranth protein isolates obtained by two distinct methods, alkaline extraction-isoelectric precipitation and micellization, the alkaline extraction had a greater protein yield and protein content than micellization [12]. On the other hand, Abugoch (2008) evaluated a quinoa protein isolate, extracted at pH 11 and found out that the protein was denatured due to its extreme pH extraction, concluding that an alkaline pH has a negative effect on the structure of quinoa protein [12]. In addition of protein denaturation, the protein quality can also be altered by alkaline processing due to undesirable reactions involving racemization of amino acids, formation of toxic compounds, reduction of digestibility, loss of essential amino acids, and decrease in nutritive value. Furthermore, the remaining alkali needs to be washed thoroughly from the final product, leading to generation of a large amount of waste-water [43].

**d) Isoelectric precipitation**

Protein extraction is followed by centrifugation to separate the soluble proteins (supernatant) from the insoluble material [22]. The result, is an intermediate that is much diluted with the extraction medium. Therefore, the next step in protein production is concentration. The main objective of the isoelectric precipitation is to convert the soluble proteins to insoluble ones by altering their structure (surface characteristics) or changing the environment [44]. This leads to supersaturation, which in turn results in nucleation followed by aggregation. Several strong acids, such as HCl, H₂SO₄ and H₃PO₄, have been used for isoelectric precipitation [45]. At isoelectric point, a protein has no net charge and as consequence solubility is reduced because the protein is unable to interact (dipole-dipole and electrostatic interactions) with other proteins and will then fall out of solution. After precipitation, the proteins can be recovered by means of centrifugation, settling or filtration.

**e) Drying**

The final step of protein extraction by wet fractionation is drying. Protein is typically dried to a powder by spray or freeze-drying, with the consequent associated energy consumption.

**2.4.2. Dry Fractionation**

Dry-fractionation processes have been developed to separate and make better use of all the different parts of materials [46]. Until now, dry fractionation has been most successfully applied to pulses and cereals, which is correlated to their specific tissue architecture and milling behavior [13]. One limitation of the dry fractionation processes is that, in contrast to wet processing, fractionation is not possible at the molecular scale.
However, it has the advantage of retaining a matrix effect that may be beneficial for the biological activity of nutritionally important compounds, it is more energy efficient and is able to produce enriched fractions that retain native functionality. Also, in terms of process viability, the absence of water consumption and effluent production presents a clear advantage [47].

Most fractionation processes include two steps. The first one is dry milling, where bran tissues are broken down and dissociated. Depending on the fragmentation process, this can be done by applying different types of stresses (impact, compression, crushing, shearing, etc.) to the bran. The second one is the separation step, in which the diverse particles are sorted out according to certain characteristics, such as their size, shape, mass, density, or dielectric properties. Different separation methods that can be used include sieving and air-classification, or recently developed techniques like electrostatic separation [46] and will be briefly described below.

a) Dry Milling

Conventional milling of grains aims to dissociate and separate the starchy endosperm from the outer layers with the highest possible yield and purity [46]. Due to the small size of the quinoa seed and its botanical peculiarities, specific adaptations of the milling procedure are required. While the production of whole meal flour from quinoa is not complicated, specific demands arise during grinding and separation when producing flour fractions with different chemical compositions and physical properties. In addition to differences in compositions, there are also differences regarding the quality. Therefore, the mill and the milling technology used plays a key role in determining the quality of the resulting flour fractions [21].

The production of quinoa flour fractions with different chemical composition has been only poorly investigated. Several different mills (disk mills, hammer mills and a pin mill using several pin configurations) have been tested by Becker and Hanners (1990), however, all only shattered the seeds and produced whole meal flour. They also tested the use of a stone mill and found that 33-40% of the seed can be removed as a bran fraction, indicating a high abrasion [48].

Some other novel milling techniques like cryogenic grinding might also be worth to study to evaluate the influence of particle size on dry fractionation of quinoa. Although just few studies of the use of cryogenic grinding on cereal products have been carried out, it has been demonstrated the advantages of it in the case of grinding spices. The use of cryogenic cooling, using liquid nitrogen, increases the degree of brittleness of seeds and cryogenic grinding prevents the particles from sticking together and agglomerating, allowing the production of considerably smaller particle than grinding at ambient temperature, particularly for fibrous products [46]. A drastic decrease in temperature also ensures the protection of heat-labile components, that could be damaged if ground
at higher temperature. Decreasing the temperature of the grain could be another way to influence the physical properties of its constitutive tissues and thus to ease its fragmentation [49].

**b) Air classification**

Air classification and sieving are generally combined to generate a separation process that takes advantage of properties such as particle size and density, to yield fractions with distinct compositions. A drawback of this process is that in some cases, it doesn’t allow production of high purity fractions, due to the low differentiation in size and density of the particles generated from the different tissues after grinding [50]. The protein content of one fraction depends on the initial protein content of the flour, the dispersibility of the flour and the cut point. The cut point is the size at which a particle has a 50% chance to move either to the fine fraction or to the coarse fraction. It can be adapted by selection of the appropriate air classification conditions, such as the classifier wheel speed and the air flow [39].

**c) Electrostatic separation**

Electrostatic separation consists in a first step of charging of the particles, followed by a second step in which the charged particles are separated in an electric field, depending on their acquired charge. An electrostatic separator usually consists of a feeding charging and sorting zones. The charging process is a key factor in any separator. Solid materials are usually charged by induction, corona or tribo-charging. The separating capability depends on the difference in polarity and in amount of charge acquired by pieces or particles to be separated [51]. The tribo-charging separation is a good alternative for materials that exhibit similar conductivities, thus it has been applied in cereals. During this process, the particles acquire charges of opposite signs by contacting each-other and by impacting on the walls of the tribo-charging device. The composition of the particles, has a big influence on their contact electrification [52].

This promising process has successfully been used to produce aleurone-rich fractions from medium-ground wheat bran and it has revealed that the increase dissociation of the bran’s structures generated by ultrafine grinding may lead to a better separation of the particles during electrostatic separation. Furthermore, grinding may modify the charging behavior of the different particles [53]. However, no reports have been publicized yet for quinoa, which might represent an opportunity for further research in this area.
2.5. Analysis of individual grains for an optimal fractionation process
In order to rationally develop optimal fractionation processes, the various properties of the different parts of the grain must be taken into account. For example, it would be advantageous to be able to monitor fractionation processes and understand the fates of grain tissues, to determine the histological composition of the generated fractions, and to understand the mechanical properties of tissues and the local compositions of tissue interfaces [46].

2.5.1. Chemical composition of different tissues
Differences in chemical composition can be used to quantify the histological composition of technological fractions. As a first step, new tools were developed for monitoring separation processes, based on fresh insights into grain tissue structure, properties and composition. New mechanical devices and innovative technologies coupled with microscopy were developed for determining the local compositions and properties of tissues and their interfaces as a prerequisite for underpinning the development of improved fractionation processes. However one major limiting factor comes when the tissues of the specific grain are so small that it becomes hard to obtain enough tissues to determine their composition. Moreover, different authors showed that the natural variability among the grains, might represent a difference in the amount of components in the same tissues [47].

2.5.2. Fracture properties of individual grains
Seed hardness has always been a major concern of millers. The hardness of the seeds, determines the grinding time, energy costs, as well as the properties and appearance of the final product [54]. Seed hardness also affects the dry fractionation process, where milling is used as pretreatment. Both, the yield and composition of starch and protein concentrates were found to be related to the hardness of the seeds. Therefore, it is important to evaluate the hardness of the seeds in order to optimize a fractionation process. Cereal grains are mostly ground on roller mills, where the major breaking mechanism is compression and shear. Thus, for prediction of energy consumption and breaking behavior, a single kernel tester gives good results [55].

Granule strength is not well defined in the literature, and measured strength is heavily dependent on the particular experimental technique used [56]. The strength of a material can be interpreted as the resistance of the material to permanent deformation and fracture during a stressing event. It is normal to attribute material strength to be a maximum allowable stress value before fracture occurs. Hence, the stress distribution arising when a material is loaded plays a significant role in determining the fracture behavior of the material [57].
The different failure modes of the grains can be classified into three categories: brittle, semi-brittle and ductile failures depending on the extent of plastic deformation undergone by the material during fracture. Brittle failure occurs without significant plastic deformation whereas substantial plastic deformation can be found if the material fails in a ductile manner. An intermediate case where brittle fracture occurs at the boundaries of a small plastically deformed region is termed semi-brittle failure. Granular material is a cluster of small particles held together by inter-particle bonds. The inter-particle bonds within a granular solid may be ruptured causing the particles at the point of load application to be sheared apart before the load can be transmitted throughout the solid as in a homogenous elastic system [58]. With this, it becomes clear that the strength of a granular medium is governed by inter-particle bonding mechanisms rather than the strength of individual constituent particles. Additionally, the load transmission in a granular medium is affected by its internal particle packing [59].
3. MATERIAL AND METHODS

This chapter mentions the materials used for the experiments and describes the methods followed in this research. A general list of materials and pictures of the different setups, can be found in Appendix 7.1 and 7.2.

3.1. Materials
Quinoa seeds (*Chenopodium quinoa* Willd) sweet varieties, “Atlas” and “Pasto” were supplied by the Agricultural Research Institute (INIA), Santiago, Chile. Quinoa seeds var. “Atlas” were used for wet fractionation experiments, whereas Quinoa seeds var. “Pasto” were used for dry fractionation experiments.

3.2. Structure Analysis by Scanning electron microscope (SEM)
For the observation of dry quinoa seed structure three samples (cuts) were prepared at room temperature: longitudinal cut, transversal cut and slightly crushed in a mortar. After preparing the samples, they were mounted onto aluminum stubs, applied conductive carbon cement to make sure all particles were properly stuck, and placed in the scanning electron microscope (Phenom G2 Pure, Eindhoven, The Netherlands) for imaging. Samples from milling experiments were studied using the same sample preparation scheme if needed. The applied voltage for all pictures was 5kV, the focal distance and the magnification varied and they are specified in the bottom of each picture.

3.3. Hand-dissected tissues
Quinoa grains were hand dissected as described by Hemery et al., (2009) [60], three different strips comprising different tissues were separated using a scalpel. The outer strip corresponds to the pericarp, the inner strip to the starch-rich perisperm and the intermediate layer are the cotyledons.

3.4. Wet Fractionation Process
A combination of two methods was used in the wet fractionation process to extract the individual components from quinoa (protein, oil, starch and fiber). For protein and oil extraction, a modified version of the method proposed on the US 2010/0184963 A1 patent, published by Scalin (2010) was used [42], and for starch and fiber extraction, it was used the method described by Perez (1993) [61]. A general scheme of the combined method is shown in Figure 3-1 and the detail of each step is given below.
Evaluation of technical feasibility of dry fractionation as a method to obtain novel protein foods from quinoa

Figure 3-1: Flow diagram of wet fractionation process to obtain individual components from quinoa
3.4.1. Fat extraction
Quinoa seeds were grinded with a lab scale mill (Fritsch Mill Pulverisette 14, Idar-Oberstein, Germany), at 7000 rpm and sieved through a 200µm sieve, obtaining fine flour. Quinoa oil was extracted from quinoa flour with hexane as solvent, using lab model Buchi extractor (Soxhlet Buchi extraction system B-811, Flawil, Switzerland). The solvent was recovered from the quinoa oil and the quinoa marque was desolventized and dried overnight. See compositional analysis (fat content) for details on the method of soxhlet standard program.

3.4.2. Protein Extraction
30 grams of defatted quinoa flour was suspended in 300ml of 0.03 M NaOH and stirred for 4 h at room temperature (20±2 °C) to maximize the solubility of the protein. The pH needs to be monitored on this stage to ensure that remains in the range from 8-12.

To increase transparency of the supernatant, the resultant slurry was stored (4°C for 18h) and centrifuged afterwards (6000×g for 30 min at 10°C); obtaining in this way a supernatant with the soluble protein (Supernatant 1) and a pellet containing fiber, starch and insoluble protein (Pellet 1).

The pH of the resultant supernatant was adjusted to 4.5 using a 1M HCl solution to precipitate the proteins. The precipitated proteins were isolated by centrifugation (13000 × g for 30 min at 10 °C), giving as a result a supernatant containing soluble sugars (Supernatant 2) and a protein pellet (Pellet 2). The obtained protein pellet was re-suspended in water, neutralized at pH 7 and freeze-dried (Chris Epsilon 2-6D Freeze Dryer, Osterodeam Harz, Germany) as protein concentrate.

3.4.3. Starch and Fiber Extraction
The starch-fiber separation consisted on a series of consecutive centrifugation/separation/re-dispersion steps. The pellet 1 from the previous process was suspended in MilliQ water (1:3 w/w), stirred for 4 hours to ensure re-dissolution of the pellet, and sieved through four consecutive sieves with mesh size 250, 125, 80 and 45 µm respectively, to separate the starch from the fiber. The residues on the sieves containing most of the insoluble fiber were removed. The remaining suspension was centrifuged (500 × g for 1 min at 20°C) to sediment the hulls. The supernatant was collected and centrifuged (3000 × g for 7.5 min at 20°C). The crude starch was suspended and stirred in 0.05M NaOH for 48 hours at room temperature (20°C). The last centrifugation (3000 × g 7.5min) resulted in a white starch layer covered with a grey protein layer, which was mechanically removed. The starch was re-suspended in water and centrifuged again. This step was repeated four times. After that, the starch slurry was dried at 40°C, 40mbar in a vacuum oven (Binder Vacuum Oven VD53, Tuttlingen, Germany).
3.5. Compositional Analysis

3.5.1. Fat Content
The fat content was determined by soxhlet standard program. Settings of the program are showed in Table 3-1.

<table>
<thead>
<tr>
<th>Step</th>
<th>Heating</th>
<th>Cycle</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction</td>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Rinsing</td>
<td>2</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Drying</td>
<td>3</td>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>

(Soxhlet Buchi extraction system B-811)

Four samples of approximately 25g each were weight into cellulose extraction thimbles. The fat was extracted and collected in pre-weighted oil beakers. After extraction, thimbles and oil beakers were weight again. The extracted oil was determined by the difference in weight of the oil beakers, before and after the extraction (1). The moisture loss of quinoa flour was also calculated (2). Finally, the fat content was obtained dividing the extracted oil by the difference between the sample before extraction and the moisture loss (3).

- **Equation (1)**

\[
\text{Extracted Oil (g)} = \text{oil beaker aft. extraction (g)} - \text{oil beaker bef. extraction (g)}
\]

- **Equation (2)**

\[
\text{Moisture loss (g)} = (\text{thimble + cotton + sample bef. extraction + support})(g) - (\text{thimble + cotton + sample aft. extraction + support})(g)
\]

- **Equation (3)**

\[
\text{Fat Content (%) = } \left( \frac{\text{extracted oil (g)}}{\text{Sample bef. extraction(g) - moisture loss(g)}} \right) \times 100\%
\]
3.5.2. Protein Content

The protein content was analyzed using the Dumas combustion method. Its working principle is to break down the bonds in the peptide chains, releasing the nitrogen through complete combustion of the sample. Samples of 10-15 mg were weighted into tin cups, closed and put in the devise (Dumas Flash EA 1112 Series NC analyzer, Wigan, UK). Nitrogen content was measured, and the percentage of protein was calculated applying a conversion factor of N × 5.7, following Chauhan [62]. All measurements were done in duplicate.

3.6. Differential scanning calorimetry (DSC)

The thermic characterization of the proteins and starch was performed following a modified version of the method reported by Abugoch (2008) [12], and of the method reported by Tang (2002) [63] for starch. In short, for each DSC run, 20% w/w suspensions of protein fractions and 40% w/w of starch fractions were prepared in distilled water and stirred until a homogenous mixture was obtained. DSC samples consisted of hermetically sealed stainless steel aluminum pans filled with 12-15 mg suspensions. They were run twice in a calorimeter (Perkin Elmer Diamond DSC, Massachusetts, USA) at a heating rate of 10°C/min from 20°C to 140°C, and a double, empty pan was used as a reference. After each run, the dry matter content was determined by puncturing the pans and heating them overnight at 107°C. The denaturation parameters were calculated with the proprietary software of the setup, considering the denaturation temperature ($T_p$) as the value corresponding to the maximum transition peak, whereas the transition enthalpy ($\Delta H$) as the total integrated zone below the thermogram peak, which indicates total heat energy uptake by the sample after suitable baseline correction affecting the transition [64]. Determinations were performed in duplicate.

3.7. Functional Properties

Protein foam capacity, foam half-life and foam density tests were performed on the Quinoa Protein Concentrate (QPC) obtained by wet fractionation. The pH was set on 6.2, which correspond to the pH of a benchmark commercial protein drink.

3.7.1. Foaming

Foaming capacity of the samples was determined by the sparging method described by O’Neill and Kinsella (1985) [33], using a Foamscan (Teclis Foamscan IT Concept, Longessaigne, France). This device creates foam by injecting a gas into the liquid. During sample analysis the rising foam was captured by a video camera, which photographed every 60 s. and the evolution of the samples’ conductivity was followed. QPC were
suspended in MilliQ water at a concentration of 1% w/v. The pH of the suspensions were adjusted to 6.2, stirred for 2.5h at room temperature and centrifuged (13000 × g for 30 min at 20°C). The solutions were filtered with a 0.2 µm syringe before putting them in the Foamscan. Nitrogen gas was injected (60s, 200ml). Foaming capacity (FC), foam half lifetime and foam density (MD) were measured. Experiments were performed in triplicate. The detailed settings of the experiment can be found in Appendix 7.3.

Foam capacity was calculated using the following expression:

- **Equation (4)**

\[ FC = \frac{V_{foam_0}}{V_{gas_0}} \]

Where \( V_{foam_0} \) is the volume of the formed foam after 60s, and \( V_{gas_0} \) is the volume of the gas introduced for the formation of this foam.

The foam half-life time \( (t_{1/2}) \) is the time taken to obtain the half of the foam volume after the end of the bubbling.

The liquid content in the foam was characterized by the maximum foam density:

- **Equation (5)**

\[ MD = \frac{V_{liq_0}}{V_{foam_0}} \]

Where \( V_{liq_0} \) is the volume of the liquid entrapped in the formed foam.

### 3.8. Dry Fractionation Process

#### 3.8.1. Dry Milling

Quinoa seeds were pin milled with a Multi-mill (Hosokawa 100-AFG Multimill, Augsburg, Germany), using different parameters (speed and temperature), obtaining in this way, four samples. Parameters are shown in Table 3-2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Speed</th>
<th>Temperature</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT/17500</td>
<td>17500 rpm</td>
<td>25°C</td>
<td>All samples were pre-milled before the final milling, using a speed of 7500 rpm.</td>
</tr>
<tr>
<td>RT/17500</td>
<td>17500 rpm</td>
<td>25°C</td>
<td></td>
</tr>
<tr>
<td>CRYO/20000</td>
<td>20000 rpm</td>
<td>Cryogenic</td>
<td></td>
</tr>
<tr>
<td>CRYO/20000</td>
<td>20000 rpm</td>
<td>Cryogenic</td>
<td></td>
</tr>
</tbody>
</table>
For the cryogenic milling, quinoa seeds were dipped in liquid nitrogen and cooled down. Temperature control was not possible during the process.

### 3.8.2. Particle Size Distribution (PSD)

The particle size distribution was determined by laser diffraction using a Mastersizer (Malvern Mastersizer 2000, Worcestershire, UK) equipped with the Scirocco 2000 dry dispersion unit. Samples of 2 grams were dosed into the handling unit and measured in a single pass. Volume percentages plots given by the Mastersizer were analyzed. The particle median diameter \( d(0.5) \) and the granulometric distribution (expressed in % of the total particle volume) were determined to characterize the fractions. Detail of the parameters used for this method can be found in Appendix 7.4.

### 3.8.3. Electrostatic Separation

Approximately 2 grams of each sample was manually fed into a pilot electrostatic separator (FUG HCP-140-35000, Schechen, Germany). The flour particles were then conveyed by compressed nitrogen (gas flow rate 7lt/min) into a charging line where they were charged by tribo-electricity, by impacting against each other and impacting against the walls of the charging line. The charged flour particles were then introduced in a separation chamber containing a grounded and a positive electrode (20000 V), where the positively charged particles were attracted by the negative/grounded electrode and the negatively charged particles were attracted by the positive electrode. The material captured on each electrode was collected separately by careful brushing.

### 3.9. Texture Analyzer

Fracture properties of quinoa seeds were determined with a texture analyzer (Instron Texture Analyzer, Massachusetts, USA), brittle particles were fractured by compressive loading and the fundamental properties of the fracture process were studied more effectively by a well-controlled experiment on single particles. The speed of the compressor cylinder was set at 100 \( \mu \)m/min. During the test the required force (stress) to break the quinoa and the distance from the top of the grain to the fracture point (penetration depth) were recorded. The test stopped automatically once the grain was broken or it was compressed until half of its diameter (500\( \mu \)m).
In this chapter the results are presented and discussed according to the scientific literature previously introduced. First, some remarkable aspects of the wet fractionation process are mentioned. Then, the evaluation of dry fractionation as a feasible technology for quinoa is outlined, after which the optimal breakage for minimal processing fractionation is analyzed. Finally as a preliminary test, foaming as functional property of quinoa protein concentrate obtained by wet fractionation is mentioned.

### 4.1. Quinoa Structure for optimal fractionation design

The structure of the quinoa seed was studied with SEM, to identify its different structures, with special attention on morphological features of the ones containing proteins and starch. The identification of these structures helps in the evaluation of opportunities to optimize the seed breakage for minimal processing fractionation.

The three major anatomical parts: pericarp, embryo (cotyledons and radicle) and perisperm were observed in seeds cut longitudinally and transversally and were easy to identify as previously reported in literature [1]. Details are shown in Figure 4.1.

Figure 4-1 Scanning electron microscopy of quinoa seed

C: Cotyledon; P: perisperm; SC: pericarp (seed coat); R: radicle
a) Longitudinal cut. Bar=500μm, 140x
b) Transversal cut. Bar=300μm, 210x
c) Embryo-cotyledons. Bar=300μm, 215x
d) Embryo-radicle. Bar=300μm, 260x
Localization of stored reserves inside the seeds of quinoa showed a clear separation of structures. In accordance with Prego et al., (1998) [1], carbohydrate reserves were found principally in the perisperm, while proteins were located mostly in the embryo, which represents a natural opportunity to separate the seeds in starch-rich and a protein-rich fraction.

**a) Embryo**

The embryo consisted of a hypocotyl-radicle axis and two cotyledons (Fig 4.1 c, d). It represented approximately the 33% of the total volume of the seed, which is in line with the average 30% reported in literature by Mujica (2013) [17].

Protein bodies varied in diameter from 0.5 to 3.5µm and they were present as small spheres as it might be seen in Figure 4-2. All embryo cells stored abundant protein.

![Figure 4-2 Scanning electron microscopy of quinoa embryo cells and their contained protein](image)

*a) Embryo cells storing abundant protein bodies. Bar=30µm, 2500x*

*b) Embryo single cell showing protein bodies in the inside. Bar=30µm, 2750x*

*c) Detached protein bodies shaped as small spheres. Bar=20µm, 2950x*

**a) Perisperm**

Starch accumulation was observed in the perisperm (Figure 4-3). The angular shaped cells are rich of starch granules, which were present as single starch granules (0.5-1µm) but also as oblong aggregates (20-25 µm). It represented approximately the 63% of the total volume of the seed, in line with the observations of Mujica (2013)[17].
The protein content of the milled grain and each of the three individual parts of quinoa grain was analyzed and is shown in Table 4-1. The protein content in the milled grain, pericarp, embryo and perisperm was 16.84, 10.56, 42.91 and 3.16% respectively.

Table 4-1 Protein content of quinoa grain fractions

<table>
<thead>
<tr>
<th>Protein(a)</th>
<th>Milled Grain</th>
<th>Pericarp</th>
<th>Embryo</th>
<th>Perisperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.84</td>
<td>10.56</td>
<td>42.91</td>
<td>3.16</td>
<td></td>
</tr>
</tbody>
</table>

All values are on a dry basis (%) and are the mean of two separate measurements.

\(a\) \(N \times 5.7\)

Figure 4-4 graphically shows the protein distribution inside quinoa grain, from this, it can be concluded that the protein is localized mostly in the outer part of the grain and confirms that the embryo is a protein rich structure.
Similar studies were done by Ando et al., (2002) [14], where the food components in fractions of quinoa seed were analyzed. They reported values of protein content in the milled grain, pericarp, embryo and perisperm of 13.3, 6.1, 23.5 and 7.2% respectively. These values differ significantly from the stated results in this report, especially in the protein content of the embryo. The reason could be the fractionation method applied. In our case, the complete embryo was carefully separated by hand from quinoa seeds, keeping its natural structure and integrity, while these authors used a sieving technique to separate the fractions.

In general, the embryo or germ functions as a storage organ and in most of the cereals is relatively high in protein [65]. However, as mentioned before, a noteworthy characteristic of quinoa is that the embryo corresponds to the 33% of the total volume of the seed, unlike most of the cereals where it makes up 2.5-3.5% of the kernel. This remarkable property makes quinoa protein fractionation very appealing; since one could get up to 142 g of protein in its native state per kilogram of quinoa seeds if a well-designed relatively simple mechanical separation is applied.

Furthermore, during the preparation of the samples for SEM tests, it was observed that in some cases the cotyledons fell apart easily while cutting the seed (Figure 4-5).

![Figure 4-4 Protein distribution in the a) pericarp, b) embryo and c) perisperm of quinoa seed](image_url)
This can be explained because granular material is a cluster of small particles held together by inter-particle bonds. The inter-particle bonds within a granular solid may be ruptured causing the particles to be sheared apart at the point of load application before the load can be transmitted throughout the solid as in a homogenous elastic system [58]. From this it can be concluded that the strength of a granular medium is governed by inter-particle bonding mechanisms rather than the strength of individual constituent particles. Moreover, the load transmission in a granular medium is affected by its internal particle packing [59]. It is clear that the perceived strength of a granule will be a function of the nature and concentration of its internal bonds, which in case of quinoa it was showed that it has a rather weak inter-particle bonding allowing the structures to fell apart easily.

The characteristic ease of separation of the cotyledon, high protein content of the embryo, high mass of the embryo and its strategic localization in the outer part of the seed, gives insight on the possibility for making use of the quinoa’s structure for a smart and mild fractionation.

### 4.2. Wet Fractionation Process for Quinoa high purity fractions

As a benchmark for future fractionation processes a wet fractionation scheme was developed to extract the different macro-components from quinoa, with emphasis on the protein fraction (see Chapter II “Materials and Methods” for details). It should be mentioned that the purpose was not to optimize the wet fractionation process, but to
obtain a protein concentrate following a general extraction condition. Therefore the pH for protein solubilization was set on 10 and the pH for protein precipitation was set on 4.5.

Quinoa flour was dispersed overnight in an alkaline medium giving as result a solution with layers of different particle sizes and densities, clearly differentiated to the naked eye (See Figure 4-6-I). The layers consisted of soluble protein with a foam layer on the top, followed by a white starch layer, and sedimented fiber at the bottom. Centrifugation yielded a starch/fiber rich pellet and a supernatant enriched in protein (Figure 4-6-II). Precipitation of the proteins at pH 4.5 resulted in a final protein concentrate.

![Figure 4-6 Graphic wet fractionation process of Quinoa](image)

- **I)** Solubility of protein in alkaline medium,
- **II)** Centrifugation of the solution showing a starch/fiber rich pellet and a supernatant enriched in protein,
- **III)** Individual components from quinoa, fiber, starch and protein

Wet fractionation of quinoa seed to obtain protein is a simple process. However, the protein quality can be diminished by the alkaline processing due to undesirable reactions involving racemization of amino acids, formation of toxic compounds such as lysinoalanine, reduction of digestibility, loss of essential amino acids, and decrease in
nutritive value. Furthermore, the remaining alkali needs to be washed thoroughly from the final product, leading to generation of a large amount of waste-water [41].

For starch isolation, the obtained pellet was re-solubilized and sieved in order to remove the fibers, followed by sequential washing of the starch, generating a large amount of waste-water as well. It was necessary to repeat the washing step several times because the obtained starch rich pellet showed a grey layer of protein on top of it. When carbohydrates are separated, also a part of the proteins remains because not all the protein fraction precipitates at pH 4.5 [66]. Those proteins settle as a layer on top of the starch due to the higher density of the starch granules compared to protein [15].

As indicated in the mass balance of water consumption in wet fractionation of quinoa, (Appendix 7.5) large quantities of water were used in order to isolate protein and starch. When evaluating the individual processes, 13 l of water per kg of quinoa flour processed for protein fractionation were needed; in the case of starch isolation, the value was lower; 6 l of water per kg of pellet 1 was required. The use of large amounts of water for protein extraction is the result of the alkaline medium to solubilize it, followed by the addition of NaOH in a low concentration (0.03M) to neutralize the stream. Some authors recommend lower concentrations of NaOH for protein solubilization [62], which leads to even higher water- waste streams. In the case of starch, the amount of water used for washing would also depend on the amount of insoluble proteins presented on top of the pellet after centrifugation. The more protein present, the more washing repetitions needed.

Furthermore, as protein and starch need to be dried after the extraction, this step normally implies high energy consumption. The most common drying method is convection drying using hot air systems. However, the high temperatures reached in this process would damage the starch and protein concentrates, thus different drying methods need to be used. Specially for proteins, freeze-drying is considered one of the best ways to preserve them, with the potential to yield products that are stable as well as convenient to ship and handle [67]. In general terms, if one compares the energy consumption within these two methods, it would be needed 2056kJ, and 2208kJ to dry 1kilogram of raw material with 80% of initial water content with convection and freeze drying respectively. From this, it can be concluded that taking care of the final protein concentrate would represent an additional 7% energy cost.

4.2.1. Compositional Analysis
Quinoa flour showed a fat content of 8.2%. Defatting of the flour prior to a wet fractionation process is a common procedure for protein concentrates/isolates production. This, results in a highly pure and stable product protected from lipid oxidation. However, this step might also have an influence on the final properties of the product and sustainability of the process.
The extraction of quinoa protein in a (defatted) flour-water relation 1:10 (w/v), in alkaline medium pH 10.85, and precipitation at isoelectric point 4.5, allowed a QPC of 77.36% of protein content and a yield of 43.38% equivalent to 9.42g of protein concentrate/100g quinoa defatted flour (QDF) (Table 4-2).

<table>
<thead>
<tr>
<th>QF (g)</th>
<th>Protein (QDF) (%)</th>
<th>PC (g)</th>
<th>Purity (QPC) (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>16.8</td>
<td>9.42</td>
<td>77.36</td>
<td>43.38</td>
</tr>
</tbody>
</table>

Similar values were reported by Martinez (1996) [68] in protein extraction of amaranth, where the yield, expressed as grams of isolate per 100 g of defatted flour was in a range from 4.9 to 12.4 g/100g. From literature, it is expected that purities up to 83.6% can be obtained if the conditions of the wet fractionation process are optimized for quinoa protein extraction [69]. Optimization on the use of different buffers for protein solubilization could reduce or avoid protein modification, aggregation, or precipitation that may result in the reduction of protein extraction yield [68].

Starch isolation delivered 21.8% yield, which is in line with results of previous studies of quinoa starch isolation [70]. Because starch granules are also susceptible to damage by alkaline solutions or other hydrogen bond breaking agents, starch loss may occur during protein extraction with these agents and an alteration in the functional properties might also be the case. Wilhelm et al. found that starch granules of amaranth and quinoa were damaged irreversibly in an alkaline medium, resulting in a lower paste viscosity [71]. Minimizing starch loss is desirable not only to increase the starch yield but also to reduce the organic waste occurring during the starch isolation [72].

4.2.2. Thermal characterization of protein and starch

Figure 4-7 shows the thermogram for quinoa protein concentrate obtained by DSC. The protein concentrate showed two endotherms, the first one (T1) at 57°C and the second one (T2) at 79.8°C. By comparing these thermograms with those of the protein fractions reported by Martinez and Añon (1996) [68], it can be estimated that all fractions, but mainly albumins-2 and globulins, contribute to the second (of higher Td) endotherm, while only albumins-1, glutelins, and the minor components of globulins contribute to the first. The denaturation enthalpy in both peaks were very low, 0.1 J/g in the first and 0.32 J/g in the second. That indicates almost full denaturation of the quinoa protein, due to its extraction at pH near to 11. This result is in line with what is reported
in literature, where thermograms of quinoa protein isolates extracted under comparable conditions showed an absence of endotherm indicating the state of denaturation of the protein isolate due to its extreme pH extraction [12]. Based on literature, it is known that alkali causes cleavage and unfolding of disulfide bonds of the subunits [38]. l-l-S globulins, (the major fraction present in QPC), might have been more affected by alkali due to the high content of disulfide groups, explaining high degree of denaturation.

Figure 4-8 shows the thermogram of quinoa starch obtained by DSC. Quinoa starch presented a gelatinization temperature (Tp) at 69.15°C, with a gelatinization enthalpy of 2.7 J/g. Quinoa starch gelatinizes at a relatively low temperature (Tp = 54.2-61.9°C) which according to Goering and DeHaas (1972) [73], is generally the case for small granules of starch. With respect to gelatinization temperature the result is in the range of 64.5-85.7°C of a previous study on amaranth starch [74]. However, the gelatinization enthalpies measured are very different (2.7 versus 9.07 J/g). Nevertheless, for this thermal transition, the reported enthalpies (1.66 - 12.2 J/g) are in line with the results reported for quinoa starch by Qian (1999) [71]. Differences in gelatinization enthalpies might imply a more compact inner molecular configuration and a higher degree of crystallinity among them. Moreover, the reduction of the melting enthalpy might also be the consequence of mechanically damaged granules due to prior milling and the extraction conditions [75].

![Figure 4-7 Differential scanning calorimetry thermal curve of quinoa protein concentrate](image-url)
It should be mentioned that one of the measurements of the thermal transition of starch during this experiment, showed an unusual thermogram with two gelatinization peaks (Appendix 7.6). Based on literature, quinoa starch normally shows one transition peak as mentioned before, so no conclusions could be drawn from that measurement, but is included for completeness of the generated information during the development of this work.

### 4.3. Dry Fractionation Process for Quinoa protein enriched fractions

The resulting flours (RT/17500, RT/20000, Cryo/17500, Cryo/20000) from pin milled quinoa were studied with respect to their particle size distribution. In addition, to clarify what was analyzed with the Mastersizer, some microscopic views of the fractions were also evaluated.

#### 4.3.1. Particle Size Distribution

Figure 4.9 shows plots of the four quinoa flours of volume percent of particles over a range of particle diameter as determined by light scattering analysis. No big difference in the PSD between the different speeds in the milled flours was found, while temperature showed a big difference. As expected, cryogenic grinding resulted in smaller particle sizes than room temperature grinding. Indeed, the median diameter of the particle size distribution $d_0.5$ of all samples in cryogenic grinding was three times smaller than room temperature grinding (Table 4-3). This difference is attributed to the increase of...
the degree of brittleness of seeds by the use of liquid nitrogen in cryogenic grinding, which prevents the particles from sticking together and agglomerating, allowing the production of considerably smaller particle than grinding at ambient temperature [76]. On the other hand, RT milling presented bigger PSD but the smaller span value indicates a narrower particle size distribution than cryogenic milling.

| Table 4-3 Particle size distribution (D10, D50, D90) of four quinoa pin milled flours |
|-----------------|----------------|----------------|-----------------|----------------|
| PSD             | RT/17500       | RT/20000       | Cryo/17500      | Cryo/20000      |
| d(0.1)          | 18.3µm         | 16.2µm         | 8.5µm           | 7.2µm           |
| d(0.5)          | 157.0µm        | 148.3µm        | 50.7µm          | 41.8µm          |
| d(0.9)          | 375.7µm        | 371.2µm        | 226.9µm         | 209.8µm         |
| Span            | 2.28           | 2.39           | 4.31            | 4.85            |

The lower size distribution was spread within the 1–50 µm particle size range for each sample, with a peak somewhere between 17 and 20 µm. The larger size distribution occurred in the 10–600 µm range, with the actual range and peak varying somewhat with the sample. Most likely, the smaller size particles are individual starch granules and protein bodies, while the larger particles correspond to undissociated or partially dissociated starch aggregates and protein fractions.
Conventional milling of grains prior to dry fractionation aims to dissociate and separate the starchy endosperm from the outer layers with the highest possible yield and purity [46]. Results from the previous SEM analysis showed that protein bodies are between 3 and 3.5 µm, single starch granules are between 0.5 and 1 µm and the starch agglomerates are around 25 µm. To facilitate protein enrichment, quinoa seeds should be ground to particles with a diameter of less than 25µm to detach the protein from other cellular compounds. However, less than 10% of the RT and Cryogenic flours, consisted of smaller particles (below 25 µm), indicating that in the samples there were more particles present as bigger fractions of starch and protein combined, than single starch and protein granules.

4.3.2. Analysis of the fractions after milling

Figure 4-10 shows SEM images containing starch and protein fractions found in quinoa flour. They visually confirm that starch and protein were not present as individual components, but as bigger fractions. Protein bodies were mainly present as fractions composed by a surrounding fibrous shell which varied in shape and size, whereas starch was present as individual aggregates, but also bigger fractions composed by their agglomeration (starch clumps) were found.

Figure 4-10 Scanning electron microscopy of quinoa protein and starch fractions

a) Starch aggregate (left) protein fraction (right). Bar=20µm, 3100x
b) Protein fraction surrounded by fibrous shelf (left) starch aggregate covered by single starch granules (right). Bar=30µm, 2350x
To complete the results of particle size distribution, some protein and starch particles were individually examined with SEM and their shape and size were measured (Figure 4-11; Table 4-4). However, this procedure was done just to get a general idea of the particles found in the samples, it should be mention that this method of measuring particles sizes is not for routine use and much more measurements should be done in order to have meaningful results.

![Figure 4-11 Scanning electron microscopy of quinoa protein and starch fractions' sizes](image)

*Bar=80μm, 1000x*

**Table 4-4 Starch and protein fractions sizes found in quinoa pin milled flour**

<table>
<thead>
<tr>
<th>No.</th>
<th>Fraction</th>
<th>Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Starch clump 1</td>
<td>67.7</td>
</tr>
<tr>
<td>II</td>
<td>Starch clump 2</td>
<td>56.4</td>
</tr>
<tr>
<td>III</td>
<td>Protein clump 1</td>
<td>43.3</td>
</tr>
<tr>
<td>IV</td>
<td>Protein clump 2</td>
<td>25.9</td>
</tr>
</tbody>
</table>
Figure 4-12 shows a comparison within the four pin milled quinoa flours done with SEM. The resulting pictures show that for all samples, independent of their particle size distribution (coarse or fine flour), milling resulted into a flour having largely overlapping particle sizes of the protein and starch fractions which might difficult an air classification for a subsequent protein fractionation.

**Figure 4-12 Scanning electron microscopy of four quinoa pin milled flours**

*Comparison of the different milling conditions. Bar 80μm, 1000x*
For protein-starch separation by air classification, the optimum cut size is around 10 µm, which is just below the size of most starch granules [13]. However, this could not be applied for quinoa, since the starch granules are smaller than other common cereals. Furthermore, when applied air classification to several common cereals, sieve analysis has demonstrated that the finest particles of the flours contain higher protein levels than the coarser fractions of the flours [77]. Contrary to this prediction, it has been shown that quinoa behaves the other way around. The coarser fraction contains higher protein levels than the finer fraction of the flour [15]. Therefore, it is not possible to follow general fractionation procedures developed for other products and special attention and unique techniques need to be investigated.

On the other hand, despite the particle size overlapping, it was observed that starch and protein were separated in the coarse and fine milling, making electrostatic separation a potential method for dry fractionation.

### 4.3.3. Electrostatic Separation

As a result of the small difference of the PSD between the speeds from the previous test, it was decided just to consider the temperature of the milling as a variable and to narrow this experiment to two samples: cryogenic and room temperature milled quinoa.

Table 4.5 shows the of protein content after electrostatic separation of the protein enriched fraction (PEF) and the protein depleted fraction (PDF) obtained by room temperature milling and by cryogenic milling. The original protein content (OPC) of the respective flours, are also specified.

<table>
<thead>
<tr>
<th></th>
<th>RT Flour (%)</th>
<th>Cryogenic Flour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEF</td>
<td>27.57</td>
<td>24.93</td>
</tr>
<tr>
<td>OPC</td>
<td>13.61</td>
<td>14.21</td>
</tr>
<tr>
<td>PDF</td>
<td>5.85</td>
<td>9.96</td>
</tr>
</tbody>
</table>

During electrostatic separation, protein fractions were negatively charged and were attracted by the positive electrode, whereas starch particles were positively charged and got attracted by the negative electrode. Because the proportion of starch is higher than protein in the initial flour, more positively charged particles were collected and a faster saturation of the negative electrode was observed. This observation should be taken into consideration when designing an industrial process.

Due to the small-scale equipment used for this experiment, yields could not be calculated. However, it is known that the charge acquired by the particles has also an
impact on the separation yields [50]. Average yields mentioned for other cereals are 20, 14, 17.5 and 27% for soft wheat, hard wheat, barley and oat respectively [13].

A clear difference between the colors of the positive and negative fractions could be noted between both milling samples (Figure 4-13). Unlike room temperature milling, cryogenic milling showed that the positively charged fractions (PDF) were more whitish than the negatively charged fractions (PEF), which were more brownish. Same observation is mentioned by Hemery (2011) in his study of electrostatic separation of wheat. This difference in color is attributed to the difference in composition (starch vs. protein) and the difference of particle sizes (the finer, the lighter) [50].

![Figure 4-13 Electrostatic separation of cryogenic quinoa flour](image)

**Figure 4-13 Electrostatic separation of cryogenic quinoa flour**

*Protein enriched fraction (PEF) and protein depleted fraction (PDF)*

This white characteristic color owned by starch, confirms the principle of cryogenic grinding of increasing brittleness of particles and thus an easier particle break up into smaller fragments, which according to Hemery (2009), could be potentially beneficial for dry fractionation[78]. Indeed, different authors [79] affirm that, the smaller the particles, the higher the specific charge, due to the greater number of particles/charging device collisions. Contrary to this statement, the obtained results showed a higher increase of protein in the PEF of the coarser flour (RT) than of the cryogenic flour. This increase was proportional to the decrease of protein in the PDF for both flours. Figure 4-14 captures this relation with respect to the OPC for RT and Cryogenic flour.
This can be explained by the fact that the tribo-charging of the starch and protein fractions was influenced not only by the size of the particles, but also by their surface composition [78]. It is known that the composition of materials greatly influences their contact electrification [52]. Therefore, the differences in structure and composition of the fragments inside the grain, might explain the different tribo-charging behaviors of these materials, leading to the difference in purities. Another reason might be attributed to the dispersability of the powder mixture, which is an important parameter that determines the performance of fractionation and is negatively influenced by particle agglomeration [80]. In the case of cryogenic milling, the smaller starch particles might have been adhered to the larger protein fractions, resulting in detrimental effects on the yield of the protein fraction and its purity [13].

One limitation of the dry fractionation processes is that, in contrast to wet processing, fractionation is not possible at the molecular scale. However, it has the advantage of retaining a matrix effect that may be beneficial for the biological activity of nutritionally important compounds. Also, in terms of process viability, the absence of water consumption and effluent production presents a clear advantage [47]. Furthermore, it seems to be an energy efficient process, since the main energy consumption depends on the efficiency of the power supply (in most cases 90%) and the gas flow used to carry the powders.

**4.4. Optimal Breakage for minimal processing fractionation**

Individual seeds were used to study the fracture properties of quinoa. Fundamental understanding of the basic particulate fracture processes is required to ensure that the technology will develop in a way that will lead to greater operating efficiencies. Understanding the properties of single granules that determine their strength can be
linked with meso- and macro- scale studies allowing better understanding of the granulation process and control of granule properties. [59]

Figure 4-15 gives the result of the stress vs. the penetration depth (distance) as recorded with the texture analyzer. The peak force at the end of the measurement is the force recorded at the moment the quinoa seed breaks, with the area under the curve giving the total energy needed for the fracture. The slope of the curve gives an indication of the hardness of the seed. Hardness of the seed is an important parameter to consider in dry fractionation process, where milling is used as pre-treatment. In the case of starch and protein concentrates prepared by pin milling/air classification of grain legumes, both, the yield and the composition of these fractions were found to be related to the hardness of their seeds [55].

![Figure 4-15 Results of texture analyzer measurements (compression method) for individual quinoa grains](image)

A particle will be broken only if it is stressed beyond its strength which is determined by the intrinsic properties of the material, the presence of micro flaws which act as stress raisers when the particle is under load and the state of stress that is experienced by the particle [81]. In the case of quinoa, the stress needed to break the seed was approximately 20-30N and a penetration depth (distance) of 0.2mm. Similar values were reported by Dijkink (2002) for peas with low moisture content. Although moisture was not a variable considered in this test, the mentioned author, showed that at higher moisture content the penetration depth increases and the hardness of the seed decreases. However below moisture content of 11% the stress; penetration depth and hardness are almost independent of the moisture content [55]. This could represent an advantage for quinoa, since the average moisture content of the seed is 11-12%, thereof no extra preconditioning would be needed.
After breaking the seed with these controlled conditions, it was visually evaluated and it could be observed that the perisperm was disintegrated into smaller particles; consequently, the cotyledons were expelled out of the seed with a minimum of particle damage. Figure 4.16 depicts this observation. This breakage behavior might be attributed to the breakage pattern that the seed experienced, the different composition of the structures and the inter-particle bonding within the grain. Subero and Ghadiri (2001) classified the failure of agglomerates into two main patterns: localized disintegration and fragmentation. Here, localized disintegration occurs at relatively low impact velocities, where the damage due to impact is limited to the impact site with some production of fines. According to this, quinoa perisperm experienced a localized disintegration because the cylinder of the texture analyzer was placed in the center of the grain, where the perisperm is located and the compression velocity was very slow (100µm/min).

![Quinoa seed breakage behavior after applying controlled conditions](image)

**Figure 4-16 Quinoa seed breakage behavior after applying controlled conditions**

*Texture analyser (compression method).*

It was showed in previous results that the composition of the embryo and perisperm of quinoa seed differ significantly, the great amount of starch located in the perisperm and the way the single starch particles are packed together make the perisperm a brittle structure. In contrast, embryo showed to be a semi-brittle structure, which might be confer to the loosely arrangement that the protein bodies presented inside it. Brittle failure occurs without significant plastic deformation, whereas substantial plastic deformation can be found if the material fails in a ductile manner. An intermediate case
where brittle fracture occurs at the boundaries of a small plastically deformed region is termed semi-brittle failure [82].

Finally, inter-particle bonds among the structures also played an important role in this breakage behavior that quinoa seed experienced. The previous reported observation by SEM, showed that quinoa might have a very weak bonding between the cotyledons and the perisperm, this is why at the moment of the perisperm disintegration, and the cotyledons were easily expelled out of the grain with a minimum of damage.

### 4.4.1. Alternative milling process

As a conclusion from the breakage behavior that quinoa grain showed, it could be said that it is important to consider the properties and the different parts of the grain, in order rationally develop efficient processes. This characteristic behavior gives the insight for an alternative milling process, which could be adapted to quinoa to carry out the same outcomes at industrial levels. Alternative technologies have been designed or are under development to improve processing to exploit the potential of the grain more fully [46]. So far in this research, quinoa flour obtained by a pin mill under different temperatures was evaluated. In addition, a small-scale test using a conventional roller mill combined with sieving was also done as a preliminary experiment. Pictures of the obtained fractions are shown in figure 4-17.

![Figure 4-17 Different fractions obtained from roller-milled quinoa seeds](image-url)
The samples were evaluated qualitatively by visual inspection on aspect, color and sizes, obtaining a general insight of the outcome. Chemical composition of these fractions is to be determined in future research. The process yielded a coarse, medium and fine fraction, which went through sieves of 1mm, 0.6mm and 0.3mm respectively. Although the speed and distance between the rolls could not be controlled during milling, remarkable characteristics were observed within the fractions. The coarser fraction presented mostly pericarp with some perisperm fragments attached to it, which most likely were not able to disintegrate to finer starch particles during the milling. The medium fraction was composed mainly of small broken pieces of cotyledons easily recognized for their yellowish color, but also some starch fragments were present. Finally, it could be noted mostly smaller starch particles in the finest fraction, which had also a characteristic white color owned by the starch.

Based on these observations, it seems that a slightly modified roller mill could be more favorable for a preliminary step in quinoa fractionation. Unlike conventional roll mills, the obvious option would be to use two smooth rolls at the same speed, where one can set very carefully the distance between the rolls. Although nowadays, roller milling is a process very refined and advanced with high degree of automation, the tuning of the roller mill machines is still done manually, which does not allow a careful control of the process [83]. Quinoa grains are about 1 mm in diameter; so according to the observed penetration depth from the experiment done with the texture analyzer, an inter-roll distance of 800 µm would suffice to gently break the grain.

Some authors have already tried a roller mill for quinoa. However none of them have done this taking care of the distance between the rolls and the stress applied to the grain. A laboratory roller mill has been used by Chauhan (1992) to separate the bran fraction from the flour fraction. About 40% was removed as a protein-and fat rich bran fraction, leaving out about 50% of starch-rich flour fraction[84]. Schoenlechner (2008) affirmed that in quinoa the combination of technical roller mill and plansifter allows a better separation between starch-rich-flour, middlings fractions, and protein-rich semolina fractions[21]. In addition, quinoa seed was also roller milled, with and without tempering by Lideboom (2005). The coarse flour obtained by this process 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) [15].

4.5. Proposed process for the fractionation of quinoa

Figure 4-18 shows the proposed process to fractionate the individual components from quinoa.
Figure 4-18 Proposed process for the fractionation of quinoa

Alternative process
Process
Alternative wet fractionation

Quinoa whole grain
- Roller Mill
- Sieve

Coarse 1000µm
- Roll Mill
- Sieve 300µm

Medium 500µm
- Defatting
- Electrostatic separation

Fine 300µm
- Mill d(0.5): 150 µm

Starch enriched fraction
- Dispersion
- Scrape
- Wash
- Concentrate

Protein concentrate

Fiber (Hulls)
- Dispersion
- Centrifugation
- Freeze-dry

*Insoluble protein
*fiber
*starch

Starch Concentrate
- Dispersion
- Scrape
- Wash
- Dry

Protein enriched fraction

Oil

Protein (Native) Protein enriched fraction

Protein (Defatted) Protein enriched fraction
The process begins with roller milling to obtain a coarse, medium and fine fraction with different compositions. From the coarse fraction that contains the hulls (pericarp) and some attached perisperm fragments, it is suggested to perform another milling to detach the perisperm fragments from the hulls and disintegrate the starch into smaller particles, followed by sieving (>300μm), obtaining in this way a starch enriched fraction. If one would like to purify the starch, a wet fractionation would be recommended.

If the proper control of the stress, speed and distance between the rolls is used in the process, it is expected that the medium fraction would be already a protein enriched fraction composed mainly of cotyledons, which could reach up to 40% of protein content in its native state. This fraction would also contain a high content of oil, thus a defatting process could be an alternative because the concentration of oil in the final protein product would negatively impact its functional properties [15]. Another alternative to increase the purity of the protein in this fraction could be to perform a finer milling and undergo a subsequent electrostatic separation. However, a finer milling might damage the protein and starch particles in a certain extent.

Finally, a finer milling with a subsequent electrostatic separation is proposed for the fine fraction. As this fraction is expected to be composed mainly of starch, the electrostatic separation might be very beneficial to obtain highly pure starch enriched fractions and take advantage of their functional properties for novel applications. A following wet fractionation might be also an alternative to purify that starch in a higher degree.

4.6. Functional Properties towards novel food product applications

4.6.1. Foaming

As shown in Figure 4-19, the foam volume and the amount of liquid incorporated into the foam of QPC increased during the bubbling time (60s). After bubbling, the liquid drainage and the foam destabilization began. The foam generated reached a volume of 176 cm3 at the end of bubbling. The foam capacity (FC) showed that for the case of QPC, 88% of the injected gas was trapped by the foam, explaining a relatively good FC. However, just 33% of the volume of the liquid was retained inside the foam bubbles at the end of the sparging, giving a foam density (MD) of 0.07. This confirms that a protein that has a good foam capacity, not necessarily has a good foam stability [33].

Some factors might have influenced the foam capacity of QPC. The first one can be attributed to the saponin content of quinoa. Because a sweet variety of quinoa (low level of saponin) was used and because the concentration of saponins is strongly reduced by hexane in the defatting step, 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 method used for protein extraction. However, Chauhan (1999) affirms that saponins have a marked effect on the surface properties of quinoa proteins, based on the results that showed a higher FC of the desaponized protein with respect to the
whole seed flour, and the lower foaming stability in the presence of saponines [67]. Furthermore, the presence of lipid materials in preparations is very detrimental to foaming because they destabilize the protein films. Thus, hexane treatment used in defatting, might have removed neutral and bound polar lipids, respectively, enhancing in this way the foaming properties [38].

In contrast with these findings, Aluko and Monu (2003), reported that 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[22]. A different finding was reported by Lideboom (2005), where quinoa protein had a better FC capacity than egg white, but less than soybean protein [15]. This contradiction of FC estimations might be due to the different methods used for the foaming measurements. According to Kinsella et al., (1985) there is a dramatic difference between sparging and agitation as the means to incorporate air into bubbles. In the whipping method, agitation is continued for a fixed amount of time and those proteins most able to rapidly stabilize films tend to produce a greater number of bubbles; hence a lower density, a higher overrun. Conversely, sparging introduces a fixed amount of air and those proteins with better surface properties are able to stabilize these bubbles more rapidly. Therefore, bubble collapse and drainage from the surrounding liquid phase are less as the foam column accumulates [33]. In this case, where foam volume is constant, the quantity of solution retained by the foam corresponds to enhanced foaming ability and density increases.
Bubbles generated from QPC are shown in Figure 4-20. The first picture was taken after 1 minute when the gas injection stopped; the second picture was taken after 25 min and the third picture after 50 min when the foam collapse began.

![Figure 4-20 Evolution of the bubbles on time of QPC generated by sparging method with foamscan](image)

It is notorious that the diameter of bubbles generated from the system gradually becomes larger with time. The shape of bubble changes from a sphere to polyhedron, agreeing with the observation by Barik and Roy (2009) [85]. Bubbles coarsen by the gas diffusion from smaller bubbles to larger bubbles following the Laplace law. The liquid between the bubbles drains out from the liquid channels and plateau borders under gravity. When the liquid film is thin enough, the adjacent bubbles coalesce [33]. So the bubble diameter increases and liquid volume in foams decreases. Generally speaking, the drainage process reduces the liquid in foam films and brings the bubbles closer to each other. The gas diffuses from the smaller bubbles to the larger bubbles, which have lower pressure. So the larger bubbles increase and smaller ones are continually eliminated. This drainage process continues until equilibrium is reached between gravity and capillarity pressure [40].

The half-life time \( (t_{1/2}) \) of the QPC was about 12236 s (3.4h), this value is used as an estimation of foam stability. However, the pictures show the collapse of the bubbles after 50 min already, explaining that the presence of bubbles detected in this method and revealed as the half-life time, does not necessarily mean a good foam stability due to the large sizes of the bubbles.

Even though the FC and foam stability of quinoa protein was evaluated just under one scenario (pH 6.8 and concentration 1%), and no conclusions of the optimal condition for its performance could be drawn, quinoa protein showed potential for applications in food products that requires a good foam capacity and a short-time stability. The fact that quinoa does not contain gluten could be pointed as one of its advantages, benefiting
people suffering from celiac disease. In this way, there is an increasing interest in their application in the production of nutrient-rich gluten-free products [28]. Gluten free beer could be a possible application for quinoa protein. At the moment, only few publications exist on the utilisation of this grain for brewing purposes. Quinoa beer was produced by Zweytick, Sauerzopf, and Berghofer (2005) and the authors reported a slightly opaque yellow product with acceptable foam and taste [86].

4.7. Present and future food applications of quinoa

Because of the increased knowledge of protein technology and chemistry and the increased demand for biodegradable polymers, research on technical applications of proteins has resulted in the last decades in new protein-based products [87]. In the case of quinoa, the high amount of protein possessing an attractive amino acid balance for human nutrition represents a compromise between nutritional improvement and achievement of satisfactory sensory and functional properties for food applications. Therefore, the specific advantageous properties of quinoa for industrial uses must be identified and exploited, and process technologies enabling exploitation of such properties must be developed. To be successful these products must compete with other raw materials that are often cheap, readily available, and of acceptable quality [4].

Currently, quinoa is mostly available as whole grain or ground as flour. Several studies on methods to process quinoa grains into single “highly “pure components have been done, and the evaluation of their individual functionalities have shown promising applications in the food industry. Some of these applications include: fortified foods for infants, toddlers and elderly, meat analogs, ice creams, whipped toppings, baked products, and salad dressings and the like, to reduce water activity, reduce fat, bind ingredients, emulsify, and/or stabilize foams. [42].

However, none of the studies have been focused on evaluating functional enriched fractions of quinoa obtained by dry fractionation preserving the native functionality of the grain. Studies done on pea and faba beans, in which solubility of protein enriched fractions and isolates obtained via dry and wet processing respectively, were compared showed that dry fractionation retains (native) solubility of the pea and faba beans protein enriched fractions to a large extent [13]. These examples could be used for quinoa as a starting point for a new focus on the development of different food processing methods that take advantage of the functionality of the enriched fractions rather than the purity of the protein concentrates.
CONCLUSIONS

The main components from quinoa are easily separated using an alkaline medium in the wet fractionation route. However, due to the harsh extraction conditions, proteins were denatured and starch damage was also observed. Furthermore, a great amount of water was needed to solubilize the proteins and to wash the starch.

Dry fractionation to obtain protein enriched fractions seemed to be a feasible technique to be applied in quinoa. Nevertheless, it should be consider that quinoa is a pseudo-cereal with different structure, properties and nutritional composition than common pulses and cereals and dry fractionation methods commonly applied on these kind of products will not be necessarily the most efficient for quinoa. As shown, conventional milling will produce mixed particles of starch and protein, making hard to separate them afterwards in a dry route. Although the electrostatic separation of quinoa fractions has not been optimized yet, these preliminary results showed a promising potential of the electrostatic separation coupled with tribo-electric charging of quinoa flour. Structure and properties of the different parts of quinoa grain must be taken into account in order to rationally develop efficient separation processes.

Different experiments for an optimal breakage, making use of the structure of the grain, showed that by pressing the grain between two flat surfaces and applying 30 Newton in a displacement of only 200 µm, the starch, which is a brittle structure located in the middle, was disorganized into small granules and all the protein that surrounds the grain was released and remained intact due to its higher flexibility. A potential option for breaking the grain would be the use of two rolls working at the same speed, where one can set very carefully the distance between them; an inter-roll distance of only 800 µm would suffice to gently break the grain. Additional processes of sieving and air classification need to be performed in order to obtain the different fractions. Separation of protein from starch in this way would allow a mild fractionation obtaining protein enriched fractions up to 47% protein content and would keep their native functionalities intact.

Independent of the fractionation processes applied, particular importance must be attached to the energetic costs of the new products or ingredients. Even if the process allows the production of nutritionally and functional interesting fractions, it may not be economically viable because of the very high-energy consumption. Nevertheless, it must be assumed, that improved knowledge on the technology and the proper evaluation of the advantages and disadvantages that each method brings, will allow a considerable reduction in the production costs.
6. RECOMMENDATIONS

This study involved a general exploration of some of the fractionation methods that could be applied for quinoa. Now that the bases are settled and it is known how quinoa grain behaves under different processing conditions, some recommendations can be done:

- Evaluate and compare the functional properties of the quinoa protein concentrates obtained by wet fractionation vs. the protein enriched fractions obtained by dry fractionation techniques.

- The evaluation of dry fractionation processes should not be based on a single parameter, in this case protein concentration. Therefore, optimization of the electrostatic separation process coupled with tribo-electric charging of quinoa flour should be done, focusing also, on the evaluation of the yield, protein separation efficiency and protein shift.

- Perform a careful milling process of quinoa seeds with the use of a roller mill with controlled conditions (smooth rolls, same speeds, 800µm inter-roll distance), to produce fractions displaying different compositions and to take care of their native functionalities. In addition, the chemical composition of these fractions should be determined.

- Besides from quinoa protein functionality and its industrial applications, quinoa starch with its uniformly small granules might be worth a deeper research, since it has several potential industrial applications. Possible industrial products suggested from quinoa starch are flow improvers to incorporate into starch flour products, fillers in the plastic industry, anti-offset and dusting powders, etc. Moreover, it has been proven that intact quinoa starch granules efficiently stabilize oil droplets creating Pickering-type emulsions.
7. APPENDIX

7.1 Overview of materials and Devises

Tables 7-1 and 7-2 show an overview of the suppliers of the materials and devises used in the experimental section.

Table 7-1 Suppliers of materials and chemicals

<table>
<thead>
<tr>
<th>Product</th>
<th>Supplier</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinoa (Chenopodium quinoa Willd) seeds var. “Pasto” and “Atlas”</td>
<td>INIA</td>
<td>Santiago, Chile</td>
</tr>
<tr>
<td>n-Hexane PEC grade</td>
<td>Actu-All Chemicals b.v</td>
<td>Oss, The Netherlands</td>
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<tr>
<td>NaOH (diluted 0.03M)</td>
<td>Sigma-Aldrich Co. LLC</td>
<td>St. Louis, USA</td>
</tr>
<tr>
<td>HCl 37% (diluted to 1M)</td>
<td>Merk KGaA</td>
<td>Darmstadt, Germany</td>
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<tr>
<td>Carbon cement</td>
<td>Plano GmbH</td>
<td>Wetzlar, Germany</td>
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Table 7-2 Suppliers of devises

<table>
<thead>
<tr>
<th>Devise</th>
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</thead>
<tbody>
<tr>
<td>Mill PULVERISETTE 14</td>
<td>FRITSCH GmbH</td>
<td>Idar-Oberstein · Germany</td>
</tr>
<tr>
<td>Soxhlet Buchi extraction system B-811</td>
<td>Buchi Labortechnik AG</td>
<td>Flawil, Switzerland</td>
</tr>
<tr>
<td>Multitherm-HP6ST Magnetic Stirrer</td>
<td>Variomag</td>
<td>Daytona Beach, USA</td>
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<tr>
<td>SevenMulti pH meter</td>
<td>Mettler Toledo</td>
<td>Schwerzenbach, Switzerland</td>
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<td>Sorvall Legend XFR Centrifuge</td>
<td>Thermo Fisher Scientific Inc.</td>
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<td>CE Instruments Ltd.</td>
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<td>Chris Epsilon 2-6D Freeze Dryer</td>
<td>Martin Christ Gefriertroknungsanlager GmbH</td>
<td>Osterodeam Harz, Germany</td>
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<td>Binder Vacuum Oven VD53</td>
<td>BINDER GmbH</td>
<td>Tuttlingen, Germany</td>
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<tr>
<td>Teclis Foamscan IT Concept</td>
<td>Teclis Instruments</td>
<td>Longessaigne, France</td>
</tr>
<tr>
<td>Equipment</td>
<td>Supplier</td>
<td>Location</td>
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<tr>
<td>---------------------------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------</td>
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<tr>
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</table>

### 7.2 Experimental Setup

![Figure 7-1 Teclis Foamscan IT Concept](image1.png)

![Figure 7-2 Malvern Mastersizer 2000](image2.png)

![Figure 7-3 Soxhlet Buchi extraction system](image3.png)

![Figure 7-4 Phenom G2 Pure SEM](image4.png)
7.3 Foaming Experiment Details: Teclis Foamscan IT Concept Settings

**Experiment details:**

**Settings:**

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7.4 PSD Experiment Details: Mastersizer 2000 Parameters

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<tr>
<td><strong>Result units:</strong></td>
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</tbody>
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Evaluation of technical feasibility of dry fractionation as a method to obtain novel protein foods from quinoa.

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7.5 Mass balance: Water consumption in quinoa’ wet fractionation process
7.6 Differential scanning calorimetry of quinoa starch

Figure 7-1 shows an unusual thermal curve of quinoa starch with two peaks. Reported gelatinization enthalpies for native starches are generally in the range 5-20J/g. Genetic variability in physical properties of starch, as quickly detected by DSC may be useful in breeding programs aimed at screening germ plasm for desire properties of starch. Starches from corn mutants with the ae gene frequently show broad endothermic peaks or even a double melting transition for starch crystallites in excess water, indicative of the heterogeneity of ordered structures inside their granules [75]. However, it was not possible to find any support in literature for a possible double transition peak for quinoa starch. Further research might be needed to investigate this behavior.

Figure 7-1 Differential scanning calorimetry thermal curve of quinoa starch
8. REFERENCES


15. Lindeboom, N., *Studies on the characterization, biosynthesis and isolation of starch and protein from quinoa (Chenopodium quinoa Willd.).* 2005, University of Saskatchewan.
Evaluation of technical feasibility of dry fractionation as a method to obtain novel protein foods from quinoa


42. Laurie A. Scanlin, A., CO (US); Martha B. Stone, Fort Collins, CO (US); Claire Burnett, Erie, CO (US), *Quinoa protein concentrate production and functionality*. 2010, KEEN INGREDIENTS, INC.,: United States.


