Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Natural Resources and Agricultural Sciences**
**Department of Food Science**

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*Christine Thuresson*

**Supervisor:** Olof Böök, Aventure AB

**Assistant Supervisor:** Roger Andersson, Swedish University of Agricultural Sciences, Department of Food Science

**Examiner:** Lena Dimberg, Swedish University of Agricultural Sciences, Department of Food Science

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## Abstract

The aim of this project was to develop and make studies on a liquid suspension based on quinoa (*Chenopodium quinoa*). Quinoa is an Andean pseudo-cereal from South America that is considered to be gluten free. A screening was set up to standardize the method for making a beverage. The standardization included trying different quinoa grains, mixing techniques, heat treatment, additives, enzyme treatment and types of filtration. As a reference, a plant based beverage from oats was used in order to achieve the same viscosity, pH, dry matter and palatability in the quinoa beverage. Another pseudo-cereal, cañahua *(Chenopodium pallidicaule)* was also used for preparation of beverage, however the main focus was on the quinoa beverage. Quinoa beverage was analyzed chemically and with other measurements such pH, viscosity and dry matter. A scale up was performed and the palatability of the beverage was evaluated with a sensory test of acceptance with 61 untrained participants. The results showed that making a quinoa beverage has potential and it is possible to achieve, same parameters as the reference beverage, the oat beverage. The high protein content was confirmed. Result from the scale up showed that the enzymes increased the sugar formation and the sweetness of the beverage. The test of acceptance showed that the quinoa beverage had a score of 5.5 on the hedonic scale, which is between, *Neither like nor dislike* and *Like slightly*. This is lower than the score for the oat beverage which received a score of 7.3. Due to this the taste can be improved in order to produce a product that is accepted by consumers.

*Keywords:* Quinoa (*Chenopodium quinoa*), cañahua *(Chenopodium pallidicaule)* plant-based beverage, α-amylase, β-amylase

## Sammanfattning

Syftet med detta projekt var att utveckla och göra studier på en flytande blanding baserat på quinoa *(Chenopodium quinoa).* Quinoa är ett pseudo-spannmål från Anderna i Sydamerika. Genom att sålla ut olika steg i metoden för att framställa drycken kunde metoden standardiseras för framställningen av drycken. Standardiseringen inkluderade försök med olika quinoagryn, blandningstekniker, värmebehandling, tillsatser, enzymbehandlingar och filtreringmetoder. Som referens användes en växtbaserad dryck från havre för att uppnå samma viskositet, pH, torrsubstans och smaklighet i quinoadrycken. Ett annat pseudo-spannmål, cañahua *(Chenopodium pallidicaule)* användes också för att framställa en dryck. Dock var fokus på quinoa drycken. Quinoadrycken analyserades kemiskt och andra mätningar såsom pH, viskositet och torrsubstans togs. En uppskalning av drycken utfördes och prover genomfördes för att utvärdera smakligheten av drycken. Detta gjordes med ett sensorisk acceptanstest, där 61 otränade deltagare deltog. Resultaten visar att producera en quinoadryck har potential och det är möjligt att uppnå samma parametrar i quinoadrycken som i referensdrycken, havredrycken. Det höga proteininnehållet bekräftades. Resultat från proverna som togs under uppskalningen av drycken visade att enzymerna medförde en ökning av sockerbildningen och därmed sötman av drycken. Acceptanstestet visade att quinoadrycken erhöll en poäng av 5,5 på den hedoniska skalan, vilket innebär mellan; *Varken gillar eller ogillar* och *Gillar något*. Det är dock något lägre än för havredrycken som fick en rankning på 7.3. Med hänsyn till detta bör smaken bli förbättrad för att producera en produkt som är accepterad av konsumenter.

*Nyckelord:* Quinoa (*Chenopodium quinoa*), cañahua *(Chenopodium pallidicaule),* växtbaserade drycker, α-amylas, β-amylas

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# 1 Introduction

There is an increasing trend of consuming plant based beverages due to health aspects such as lactose intolerance, milk allergies and stomach diseases. Today there are many varieties of plant based beverages on the market and the most common plant milk is soymilk. Other plant beverages are for example based on oat, rice, coconut and almond. The quinoa grain is a potential fundament in plant beverage and the interest of the quinoa grain is wide due to its unique nutritional value. Quinoa is an Andean crop that do not belong to the *Graminae* family, which is the family for common cereals like wheat, rye or barley. Pseudo-cereals, like quinoa, and cañahua are important staple food of the Inca people and former cultures. These native crops are considered to have high nutritional value and potential health benefits (Gallego Villa et al., 2014; Repo-Carrasco-Valencia et al., 2010). Pseudo-cereals are not real cereals but they can be used as common cereal crops and for example be milled into flour. These grains are rich in phenolic compounds and especially cañahua has a high content of dietary fibre. Comparing with common cereals, they have a higher content of calcium, iron and zinc. These are all essential minerals that is required for diverse physiological and biochemical functions (Repo-Carrasco-Valencia et al., 2010).

This paper deals with the development of a new product based on quinoa; a quinoa beverage. The first experimental part (screening) is performed in the laboratory at Aventure AB, Lund in Sweden. The second experimental part is completed in the laboratory at University de Mayor San Andres (UMSA), La Paz in Bolivia. The work has been made together with Swebol Biotech, which is a Swedish-Bolivian venture project, within Aventure AB. Swebol aims at adding value to natural products such as quinoa in order to boost Bolivian export. In La Paz continued attempts were performed with quinoa flour and cañahua flour in order to develop a beverage. The quinoa beverage was produced in a larger scale. Evaluation of the beverage was completed with a consumers test, designed as a hedonic acceptance test.

# 2 Objectives

The main objective of this study was to write a protocol for making a liquid suspension based on quinoa *(Chenopodium quinoa).* A quinoa beverage.

Specific objective 1: Collect, treat grains and choose method

Specific objective 2: Standardize a method for making a quinoa beverage and write a protocol of the process for the beverage

Specific objective 3: Scale up the process

Specific objective 4: Test of acceptance

Specific objective 5: Comparison with a beverage made on raw cañahua and roasted cañahua

# 3 Literature review

## 3.1 Plant beverages

Beverages based on plants may be water dispersions of cereals, pseudo-cereals, oil seeds or legumes that appears similar as cow's milk. Today there are several plant based beverages on the market such as soy milk, almond milk, oat beverage, rice beverage and other milk substitutes. An increasing trend of consuming plant based beverages is due to reasons like health aspects, sustainable food systems and interest of the foods origin. Plant beverages are an alternatives to dairy products and as lactose intolerance is getting more common the interest of plant based beverages is growing (Mäkinen et al., 2015). Development of functional foods that promote health as well as the awareness of avoiding diseases are also aspects of an alternative to cow's milk. High quality food products are increasing and the product´s geographical origin is considered as more valuable (Luykx and van Ruth, 2008; Prado et al., 2008).

Further reasons why consumption of plant beverages are of interest are for example that plant beverages are considered as functional foods with positive impact on health. The plant beverage becomes a natural lifestyle choice for those who have concern about their health. People who suffers from inflammatory bowel diseases have shown to have higher sensitivity to dairy compared to the average population and are recommended to avoid dairy products. Lactose intolerance and allergy are examples of medical causes why consumers decide to exclude dairy products from their everyday diet (Mäkinen et al., 2015; Mishkin, 1997).

Another health issue is intolerance of gluten or gluten sensitivity. This can cause inflammatory response in the body. Gluten is a protein that exists in wheat and other cereals like rye and barley and triggers an immune mediated enteropathy called coeliac disease. Unfortunately wheat is one of the most consumed foods in the world (Arendt and Dal Bello, 2008). Trends show that the prevalence of people

in Europe who suffer from this disease is increasing. Substitute to bread and the market for gluten free products are small and the products considered having poor nutritional value (Elgeti et al., 2014). Studies have been made on pseudo cereal-containing gluten-free breads and are evaluated to be rich in protein, fat, fibre, ash and minerals. Pseudo-cereals such as quinoa, cañahua and amaranth have good health benefits but are not common on the market (Alvarez-Jubete et al., 2009). Unlike most common cereals, pseudo-cereals do not contain allergic proteins like gluten and create new opportunities to develop gluten free products (Elgeti et al., 2014). According to many studies in the western world the most widely consumed plant based beverage today is soymilk (Diarra et al., 2005).

Awareness of climate change and global warming are promoting the change towards more sustainable food systems. The fact that the dairy industry is responsible for a large share of greenhouse gas emission, the substitution to plant based beverages increases from an ecologically perspective (Mikkola and Risku-Norja, 2014).

## 3.2 Morphology and culture aspects of quinoa and cañahua

The Andean area of South America is considered as an important area for food crops. The region holds common crops like potatoes, corn, peanuts and tomatoes. The Andeans is also an area of lesser known crops, as mentioned earlier, quinoa *(Chenopodium quinoa)* and cañahua *(Chenopodium pallidicaule)*. These pseudo-cereals have had an important impact on people for centuries due to their nutritional value and health aspects. The crops are not only good sources of several nutrients but they are also well adapted to high mountain environment (Bhargava et al., 2006; Repo-Carrasco-Valencia et al., 2010). The crops are tolerant to frost and can grow at high altitude under ecological conditions in the Andeans (Bhargava et al., 2006). Quinoa is mainly cultivated in Argentina, Bolivia, Chile, Colombia, Ecuador and Peru. It is imported to Europe and because of its high protein content crop it is also imported in North America, Asia and Africa (Bhargava et al., 2006).

The quinoa plant develops from a height of 90 to 180 cm and is extended from the stalk leaves. The leaves exhibit polymorphism, which means that the upper leaves are lanceolate and the lower leaves are rhomboidal. At the end of the stalk the seeds grow. They are applied in large thick clusters and differ in color depending on the species (Bhargava et al., 2006; Brady et al., 2007). The plant has a well-developed, much branched tap-root system and is present as deep as 1.5 m below surface and this defends it from lack conditions. A feature of quinoa is the hermaphrodite and unisexual female flowers. The hermaphrodite is located at the distal end and has five perianth lobes, five anthers and a superior ovary with two

or three branches (Bhargava et al., 2006). The quinoa seeds can achieve colors like pink, orange, black, tan, purple or red. The seeds can be consumed as they are or milled into flour. Quinoa is a pseudo-cereal and not categorized in the grass family, even though the milling and processing of the seeds resemble that of a cereal crop. This makes it an alternative to other cereals (Brady et al., 2007). The grain is divided in the parts, the perisperm, embryo and endosperm. The perisperm is the storage of starch and the endosperm and embryo contain the protein and the lipids (Bhargava et al., 2006).

Cañahua *(Chenopodium pallidicaule)* is an annual diploid ($2n = 2x = 18$) species of the poorly studied family *Chenopodioideae,* which is also the family of quinoa. The cañahua plant reach a height of 25-60 cm, which is smaller than the quinoa plant. It takes 95-173 days for the crop to mature and the wide range depends on what type of ecotype the crop grows in. In south America, there are two major centres for cultivation of cañahua; One on the northern Altiplano next to Lake Titicaca, which is in the departments of La Paz, Bolivia and Puno in Peru. Smaller cultivations of cañahua extends of the Bolivian departments of Oruro, Cochabamba and Potosi. The yields of cañahua range from 375 to 2968 kg/ha in the northern Altiplano (A. Vargas, 2010). The area of using cañahua includes milling the seeds into a flour or *pito* made from roasted cañahua grains which are milled into flour (A. Vargas, 2010).

A unique parameter of quinoa and cañahua is the adaptability to different agro-ecological regions. The plants can survive harsh climate conditions like growing at humidity of 40% to 88%, temperatures between -4°C to 38°C. The crops are also tolerant to lack of soil and can produce yields even if the rainfall is around 100 to 600 mm (A. Vargas, 2010; Gallego Villa et al., 2014; Jacobsen, 2003).

## 3.3 Chemical composition of quinoa and cañahua

As Table 1 shows the quinoa and cañahua seeds have an excellent nutritional value and the average protein in quinoa is higher in average than in common cereals like wheat, rice and oat. The protein content in the quinoa seeds can vary from 8% to 22% (Valencia-Chamorro, 2003). Quinoa contains all the essential amino acids that the body needs and the grain has a high content of the amino acid lysine, which is not excessively abundant in the vegetable world. Quinoa is also high in the amino acid methionine, which makes it a good complement to legumes, which are low in lysine and methionine (Valencia-Chamorro, 2003). The cañahua seed contains a protein content from 12 to 19 % with a great balance of essential amino acids which is comparable soybean (A. Vargas, 2010; Repo-Carrasco-Valencia et al., 2010). Both quinoa and cañahua are rich in phenolic compounds and especially

cañahua has a high content of dietary fibre (Gallego Villa et al., 2014). The phenolic components in cañahua has a high antioxidant activity (A. Vargas, 2010).

The average fat content in both quinoa and cañahua is higher than the average fat content in the crops oat, wheat and rice, but lower than in soy. Quinoa and cañahua have a very similar content of fatty acids and the main fatty acids in both pseudo cereals are linoleic acid, oleic and palmitic acid. The saturated fatty acids is 28.6 % of methyl ester mixture in cañahua and 22.7% in quinoa. Due to this the amount of unsaturated fatty acids is 71.4% in cañahua and 72.5% in quinoa (Gallego Villa et al., 2014). The quinoa and the cañahua grains have an exceptional balance between protein and fat and the crop is referred to as a pseudo-oilseed (Gallego Villa et al., 2014). All Andean cereals, like quinoa do not belong to the grass family and are therefore not real cereals, since the crop produce seeds that can be milled into flour and used as cereal crops. The quinoa seeds are larger and are common to boil and as ingredients in products (Brady et al., 2007).

Table 1. *Chemical composition of quinoa and some cereals and legumes (g/100 g dry weight)*

| | Quinoa[1] | Cañihua[2] | Oat[3] | Wheat[1] | Soy[1] | Rice[1] |
|---|---|---|---|---|---|---|
| Protein (%) | 16.5 | 12.8 | 11.1 | 14.3 | 36.1 | 7.6 |
| Fat (%) | 6.3 | 7.0 | 4.6 | 2.3 | 18.9 | 2.2 |
| Carbohydrates (%) | 69.0 | 59.9 | 54.6 | 80.4 | 34.1 | 80.4 |
| Dietary fibre (%) | 3.8 | 6.3 | 0.3 | 6.4 | 5.6 | 6.4 |
| Ash (%) | 3.8 | 3.1 | 2.9 | 3.4 | 5.3 | 3.4 |
| Energy (kcal/100g)[4] | 399 | 354 | 304 | 392 | 451 | 372 |

[1]Valencia-Chamorro 2003 [2]Gallego Villa et al., 2014 [3] Johnsson and Croissant 1985 [4]4× (% protein + carbohydrates) + 9× (% fat)

Comparing with common cereals, these Andean cereals have a higher content of calcium, iron and zinc. These are all essential minerals that is required for diverse physiological and biochemical functions (Repo-Carrasco-Valencia et al., 2010). One issue for developing products of quinoa is the high content of saponins in the outer layer of the seed. The saponins gives the quinoa a bitter taste (Ruales and Nair, 1993).

Quinoa and cañahua, especially quinoa has received great attention due to an increasing awareness of nutritious and pure food products (Jacobsen, 2003). The major protein fraction in quinoa is albumins and globulins (44-77% of total protein). The quinoa considered to be gluten free since the grain contains very small amount of or no prolamin. For people with gluten intolerance or who suffer from celiac disease, quinoa is a good food source (Valencia-Chamorro, 2003).

## 3.4 Technology

### 3.4.1 Raw material of quinoa and pretreatment

Cultivation of quinoa is spread over large parts of the Andean region. Conferring to Vargas[1], who is telling about the cultivation in Bolivia, the mature seeds are first harvested and delivered to factories that treat the quinoa grain. The first part of the treatment is sorting, separation from rocks, sticks and dust followed by washing to reduce the layer of saponins contained within the shell around the seeds. The cleaned, pure quinoa can be used to further process steps for developing products.

According to Vargas[2], the saponins are a toxic alkaloids and create a bitter taste of the grain. Because of this it is important to treat and clean the grains before other processes. After the elimination of saponins through washing the following processes are drying, vented, selected after size, separation from stones and impurities. The bags with harvested quinoa grains are delivered to Andean Valley from the farmers. A sample is taken from each bag and then the quinoa is stored in large silos for 1 to 1.5 day. The grains are washed for 5 minutes, not more because there is a risk to lose important minerals and vitamins. The wet quinoa is then dried under control for 35 minutes to a moisture content of 11%. The saponin free quinoa grains are then going through another cleaning step, where further separation from tiny stones is made. This process contains three steps in one and by shaking, weighting and aeration the small stones can be eliminated. The greatest grains are packed and delivered to customers, the smaller grains are used for making flours or animals feed. Vargas[1] suggest to use precooked quinoa flour to make a beverage, since this flour is free from saponins and that reduces bitterness. Precooked flour is made with extrusion and after the grains have been rinsed followed by drying they are grinded into a flour which is followed by the last step of extrusion.

### 3.4.2 Heat treatment

By accomplish different heat treatments the starch of quinoa can be modified. The starch in quinoa starts to gelatinize around 65 °C. Processes like autoclaving, cooking or drum drying of the flour can be used to measure the modification level by modifying properties such as water-absorption, water solubility, swelling ability, viscosity development and degree of gelatinization. The autoclaving showed the lowest degree of gelatinization (32.5% measured by DSC method) while drum drying and precooking gave a higher degree of gelatinization (97.4% by DSC method) (Ruales Nájera, 1992). By gel chromatographic separation it was shown

1. Ariel Vargas Production manager Andean Valley, interview March 12 2015
2. Ariel Vargas Production manager Andean Valley, interview March 12 2015

that the cooked seeds had higher starch polymer degradation than in the autoclaved seeds. Starch in heat treated quinoa is more unstable to amylase hydrolysis than starch in raw quinoa. The starch in precooked and drum dried quinoa had the highest degree of hydrolysis, followed by extruding seeds, cooked for 60 minutes, extruded seeds cooked for 30 minutes and autoclaved seeds and the raw quinoa (Ruales Nájera, 1992).

Process like roasting the raw material before milling and further processes enhances the flavor and the aroma of the product (Hinds et al., 1997).

### 3.4.3 Starch degradation, extraction and separation

Extraction is an important part of developing beverages and has an impact of the final product. The yield of the extraction can be increased by modification of pH or by using enzymes (Rustom et al., 1991). Enzymes hydrolyze proteins or polysaccharides which increases the yield (Rustom et al., 1993). Starch can be degraded by α-amylase and β-amylase into smaller molecules like monosaccharides, disaccharides, oligosaccharides and dextrins (Muller, 1991). The breakdown of dextrins can be made by β-amylase, which otherwise has a lower impact on starch comparing with α-amylase. The thermal stability is another parameter that differs between these enzymes. α-Amylase is the more stable enzyme when it comes to high temperature (Muller, 1991). According to the patent of Mitchell et al., (1988) the liquefaction can be made using α-amylases and to saccharificate the suspension, β-amylases can be used. Separation of the slurry after the extraction step can be done my filtration, decanting or centrifugation (Diarra et al., 2005). The liquefaction can take place before or after the filtration (Lindahl et al., 1995). This process will change the viscosity and increase the sweetness of the final product.

### 3.4.4 Homogenization and formulation of product

Plant beverage substitutes contain insoluble coarse material, like protein, starch, fiber and other cellular material. When this is removed other ingredients can be added to the beverage in order to form a product (Rustom et al., 1995). These ingredients include sweeteners, flavorings, stabilizers that contains mono- or diglycerides, guar gum or carrageenan (Hinds et al., 1997). Other possible stabilizers that are improving the stability of e.g. oat based beverages are sodium stearoyl-2-lactylate (SSL), which bind specifically to oat proteins (Chronakis et al., 2004). Since the stability of plant based beverages is a problem, emulsifiers and hydrocolloids are often used to increase the viscosity and the stability of the suspension (Rustom et al., 1995). Addition of food nutrients can be necessary to enhance the nutritional value of the plant product. Additives, like micronutrients, for example salt, affect the nutritional value and enhance the flavor of the product (Flynn et al.,

2003). Since these are denser then water, they make the beverage product unstable. The smaller particles the more stable is the product (Durand et al., 2003). Coagulation is common during heating of plant based beverages due to the unfolding of proteins. The non-polar amino acid residues are then exposed to water which increases the surface hydrophobicity. Result of this is increasing protein-protein interactions that can promote aggregation, sedimentation or gelling. The stability of proteins depends on ionic strength, pH and if there are other compounds like minerals or carbohydrates present (Damodaran, Parkin & Fennema, 2008). Homogenization improves the stability of the plant based beverage due to the decreasing of particle size and minimizing the size of lipid droplets. This increases the stability in plant based beverages as peanut milk and soy milk (Malaki Nik et al., 2008; Rustom et al., 1995).

# 4 Experimental

The main objective of this study was to write a protocol to make a liquid suspension based on quinoa, a “quinoa beverage”. The experimental design was set up as a screening in order to collect the grains and choose treatment of grains. The second objective was to standardize a process to make a beverage. As a fundament for developing the process, a plant based beverage from oats was used as a reference. Parameters like pH, viscosity and dry matter of the reference (oat beverage) were fixed to obtain a quinoa beverage with same parameters. *See Table 3.*

Experiments were performed in two laboratories, first part was performed in the laboratory at Aventure AB, Lund Sweden. This included collecting grains and standardization of the process for making quinoa beverage. The second part was performed in the laboratory at University of Mayor San Andres (UMSA), La Paz Bolivia. At UMSA other products like precooked flour from quinoa, flour from cañahua and *pito* (precooked, roasted flour) of cañahua were tested as a base for the beverage. The preparation of beverage still had the outputs from the reference’s fixed parameters. The different grains, products and reference that were used are presented in Appendix 1.

A scale up of the quinoa beverage was performed at IIDEPROQ (Research Institute and Development of Chemical Processes, UMSA) La Paz.

A consumers test was designed as a hedonic acceptance test. The test was performed at UMSA on a panel of 61 untrained participants.

## 4.1 Ingredients and enzymes

Red quinoa, tricolor quinoa, white quinoa, precooked white quinoa flour, precooked, roasted cañahua flour, cañahua flour, oat beverage were used. (See Appendix 1) as well as oil, salt, water, maltodextrins and the enzymes α-amylase, β-amylase, β-glucanase and xylanase.

## 4.2 Formulation of quinoa beverage

The screening set up included trials with different grain of quinoa and other parameters such as degree of dilution, additives, heating temperature and time for mixing. In the screening experiments different enzymes were added and some samples were treated with $N_2$. The screening process is presented in Appendix 2 and 3. The viscosity, pH and dry matter were not measured in all beverages. Red, tricolored and white quinoa were used to prepare beverage. First two references were used, the oat beverage and a quinoa beverage from Ecomil. The oat beverage was used because it was available on Aventure AB and as it is a Swedish product with good organoleptic qualities. The quinoa beverage was chosen because it is one of few existing quinoa beverage on the market today. This quinoa beverage was used only in the screening set up, in order to compare the parameters like viscosity, pH and moisture content. In the continuing studies only the oat beverage was used as reference in order to obtain the parameters and for palatability. During the screening, different amount of quinoa were mixed with different amount of water. The seeds were processed with different methods; grinding before diluted in water, cooked before mixing or roasted before grinding. Quinoa grains were optionally rinsed and non-rinsed before grinding. The grinding techniques varied from grinded in blender, with a stick blender or in a coffee grinder. Ingredients like maltodextrins, enzymes, salt and oil were added to enhance the quality of the product and to reach the fixed parameters, viscosity, pH and moisture content. At UMSA other Bolivian products were tested as a base for the quinoa beverage, such as pre-cooked white quinoa flour. Two types of cañahua flour were also tested to make a beverage. After screening, the base for standardized method was the pre-cooked white quinoa flour.

The standardization of the method was divided into three steps: Pre-preparations, heat-treatment and addition of enzymes. The pre-preparation included mixing, filtration, addition of salt and oil followed by homogenization. These pre-preparation steps were comparable in lab scale and in scale up, but with different equipment. The heat treatment and additions of enzymes were modified in the scale up. *See Figure 1.*

### 4.2.1 Pre-preparation

The pre-preparation steps involved mixing of flour with water, where mixing techniques with stick blender and Osterizer blender were tested followed by filtration which included filtration through coffee filter, sieve (VWR #120 U.S. Standard testing sieve), cloth bags and filtration pump. The amount of oil and salt was calculated to match the amount of oil and salt in the reference product. Preparation in the lab scale a coffee filter was used during the screening part in Lund, while a

120 µm sieve was used in La Paz. During the scale up the filtration pump was tested but the continued filtration was made through cloth bags.

#### 4.2.2 Heat-treatment

The beverage was heated to 65 °C in order to gelatinize the starch, followed by addition of enzymes. The fixed parameters of the reference (viscosity, pH and moisture content) were controlled.

4.2.2.1 The reheating of beverage was made after enzyme treatment to 95-100 °C, in order to pasteurize the product and inactivate enzymes. The incubation in the fermenter was not performed in lab scale, only in scale up.

#### 4.2.3 Addition of enzymes

The enzymes that were tried in order to achieve desired parameters were α-amylase, β-amylase, xylanase and β-glucanase. To obtain the desired viscosity, dry matter and pH, the quinoa beverage was treated with various enzymes. Further preparation of the drink included only α-amylase and β-amylase, due to their positive impact on the quality of the beverage. The sugar formation after enzymes were added was measured in °Bx. The enzymes were added directly to the suspension, when the beverage had a temperature of 60-65 °C

### 4.3 Scale up of quinoa beverage

Scale up of quinoa beverage was performed at IIDEPROQ (Research Institute and Development of Chemical Processes, UMSA) La Paz. The scale up of the beverage was made to an amount of 10 liter. The procedure followed the same as lab level, but with modification of the temperature, filtration and addition of enzymes. See Figure 1. The scale up was made twice. First scale up was made without reheating after addition of enzymes, the second scale up was reheated after addition of enzymes. The beverage was stored in a fermenter during 24h with fixed temperature of 50 °C, pH 7, agitation with 320 rpm. Samples were collected every 10 minutes for measuring the glucose content in order to evaluate the activity of the enzymes. In scale up the enzymes were first dissolved in a small amount of the beverage with a temperature of 60-65 °C and then the solution was added to the large scale beverage. The filtration was made with 4 layers of cloth bags. The glucose content was measured during 2 hours in both trials and the pH was only measured in the first trial. pH, dry matter and viscosity were measured in both trials after 24 hours in the fermenter.

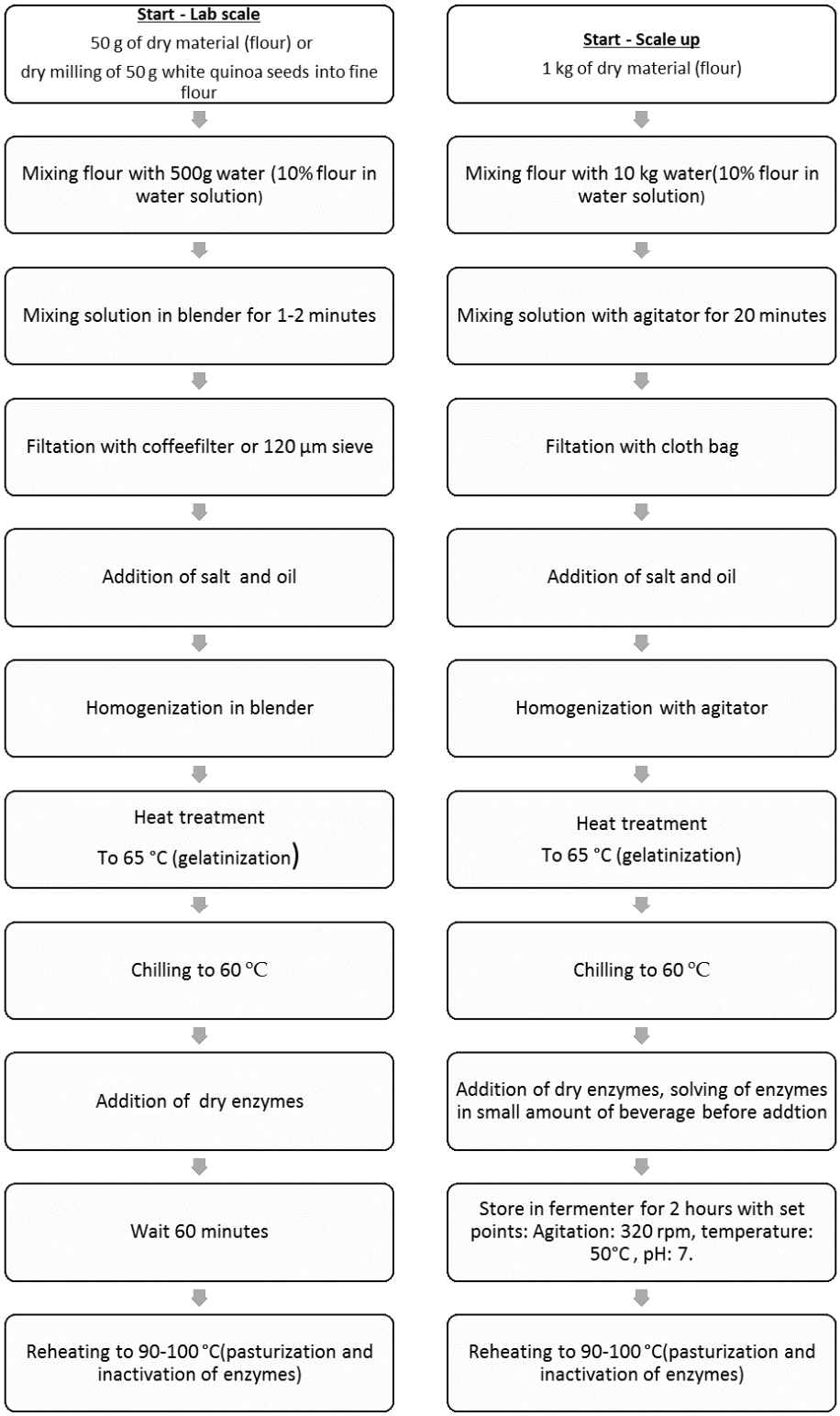


***Figure 1.*** Flow chart to prepare quinoa/cañahua beverage in lab scale (left) and scale up of quinoa beverage (right).

## 4.4 Test of acceptance

Acceptance test measure the degree of liking or disliking according to rating scales. The objective of this test was to apply a Hedonic scale of 9 steps (Lawless and Heymann, 2010). In order to evaluate the acceptance of the product. The evaluation of the spontaneous reaction of the acceptance of like/dislike, accept and not accept the products without any training of panel before was used (Gustafsson et al., 2014). The test was performed on 61 untrained Bolivian participants from UMSA, which included professors and students. The participants were served the quinoa beverage and the reference oat beverage. The two products were coded A and B and served together with a glass of water, that was recommended to drink between the coded products. The products had a temperature of 15 °C and were served in a semi-dark room, in order not to be able to discern the difference between the beverages due to the color of the beverages. Each participant rated the drinks on a paper form.

To ensure credibility if there was a significant difference in acceptance of the two beverages, a variance analysis of ANOVA was made, were the beverage type and participants were the factors. Paper forms that were used in the test can be seen in Appendix 4.

## 4.5 Chemical analysis

### 4.5.1 Chemical composition of quinoa beverage and cañahua beverages

Determination of water, ash, fat and crude fiber was made using gravimetric methods. Water content was determined with drying. Ash was determined as Bolivian Standard 664. The fat determination was done by extraction with petroleum ether by Bolivian standard 665. Crude fiber was determined by Bolivian standard 663. Protein was determined by using the Kjeldahl method (Barbano and Clark, 1990), Bolivian Standard 666. Carbohydrates were calculated as the difference of 100 percent from the contents of water, ash, protein and fat. All Bolivian standards are compiled in IBNORCA (http://www.ibnorca.org, 2014).

### 4.5.2 Total starch content and sugar content

The determination of sugar equivalents from glucose was made with a polarimetric method using a POLAX Polarimeter (ATAGO). This method is based on measuring the change in optical rotation which mainly depends on the optically active material content in the sample. The method involves reading the optical rotation of hydrolyzed samples (Huanca Lopez, 2014).

The total starch of the quinoa beverage and the two cañahua flour was measured by AOAC method 996.11 and AACC method 76.13 (Megazyme kit). The total

starch of quinoa beverage A and cañahua beverage A, this results are presented in Table 3.

### 4.5.3 Total phenolic compounds

The total phenolic compounds were determined using the Folin-Ciocalteu reagent which oxidizes the phenolic compounds to phenolates at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-tungsten complex as it is described by Peñarrieta et al., 2008. The Folin-Ciocalteu reagent, diluted 10 times (2.5 mL), and 2 mL of saturated sodium carbonate (75 g/L) and 50 µL of the sample (diluted ten times with water) were mixed for 10 s and heated for 30 min at 45 °C. The absorbance at 765 nm was read after cooling to room temperature. The absorbance of each sample was compared with those obtained from the standard curve made from gallic acid. The data were expressed as mg gallic acid equivalents per liter. Standard deviation (SD) and coefficient of variation (CV) were calculated (n=6). The samples were Quinoa beverage A, made of precooked quinoa flour, and cañahua beverage A made of cañahua flour.

### 4.5.4 Cadmium and copper content

The presence of metals copper and cadmium in samples quinoa flour and cañahua flour was determined by atomic absorption according to 990 Analytical Atomic Absorption Spectrophotometer, Flame and Graphite Analysis (www.ispch.sl/lab_amb, 2009).

### 4.5.5 Viscosity and dry matter

The viscosity was measured with two types of viscometers depending on in what laboratory the experiment was performed. Vibro Viscometer (SV-10) was used in La Paz and in Lund a Brookfield viscometer was used. The dry matter of the beverages was measured with the same equipment in the both laboratories. The purpose was to achieve the same viscosity and dry matter as the reference product, the oat beverage. All measuring of viscosity and dry matter were repeated three times in order to ensure the collected data.

# 5 Results

## 5.1 Formulation of quinoa beverage

The screening in Lund showed that raw white quinoa grain was the best material to formulate a beverage. The grains were grinded in a coffee grinder directly into fine flour. The grains were not treated with heat, not rinsed nor cooked before grinding. Quinoa flour was mixed in a blender with water into a 10% quinoa solution. Treatments with $N_2$, or addition of maltodextrins were not included in continued experiment after the screening. Addition of enzymes like α-amylase and β-amylase was standardized in the process. The result from the screening experiment, the required parameters was achieved by pre-preparations steps, heat treatment and addition of enzymes showed in Table 2. The preparation in La Paz, excluded the grinding of white quinoa seeds and instead a precooked white quinoa flour was shown more suitable as base for the beverage. The results of the screening process is presented in Appendix 2 and 3. The protocol for the beverage formulation is shown in Figure 1.

Pre-preparation differed between quinoa beverage A and quinoa beverage B. Quinoa beverage A was made from precooked white quinoa flour and quinoa beverage B was made on raw grinded white quinoa seeds (Table 2). Cañahua flour was used as base for cañahua beverage A and cañahua beverage B was made of a precooked and roasted cañahua flour (Table 2). As Table 2 shows the viscosity was much higher in quinoa beverage B compared to the other beverages. The quinoa beverage A was most similar in viscosity and dry matter to the reference. The pH of quinoa beverage A was lower than the reference. The quinoa beverage B and cañahua beverage A had pH more comparable to the reference. The viscosity of quinoa beverage B was higher than the reference and the beverage contained a lot of small particles which made the beverage not to smooth. The dry matter of quinoa beverage B and cañahua beverage A were lower than in the reference.

Table 2. *Viscosity, dry matter and pH of beverages. Quinoa beverage A: Precooked quinoa flour. Quinoa beverage B: Grinded white quinoa seeds into flour. Cañahua beverage A: Cañahua flour. Cañahua beverage B: Roasted and cooked cañahua flour. Reference: Oat beverage.*

| Beverage/Parameters | Viscosity (cP) | Dry matter (%) | pH | Temperature (°C) | Comment |
|---|---|---|---|---|---|
| Quinoa A | 5.5 | 9.1 | 5.4 | 14-16 | White, smooth, small particles and tasty, good gelatinisation |
| Quinoa B | 13 | 6.8 | 6.4 | 11 | Lot of particles, less gelatinisation |
| Cañahua A | 3.9 | 6.8 | 4.9 | 14-16 | Dark colour, lot of coarse particles, and not to good gelatinisation |
| Cañahua B | ---[1] | 5.2 | ---[1] | 14-16 | No gelatinisation of starch during heating |
| Reference (oat) | 7.3 | 10.8 | 6.7 | 14-16 | White, smooth, no particles, taste of oat and sweet |

[1]Not analysed

Table 3 shows the chemical content of the quinoa beverage A, cañahua beverage A and the values from literature of the reference (oat beverage) were included in the table for comparison. Dry extract of quinoa beverage was 9.54 g/100 mL and the cañahua beverage had a dry extract content of 5.5 g/100 mL. The reference have a dry extract value of 10 g/100mL. Protein content in quinoa beverage was 1.43 g/100 mL 0.83 g/100 mL in cañahua beverage and the reference has a protein value of 1.0 g/100 mL. As Table 3 shows the carbohydrate content was 7.33 g/100 mL in quinoa beverage, 3.89 g/100mL in cañahua beverage and the reference had a value of 6.5 g/100mL. Fat content in the quinoa beverage and cañahua beverage

were 0.49 g/100mL and 0.44 g/100 mL and were lower than in the reference beverage (1.5 g/100mL). The fiber content of quinoa beverage is 0.16 g/100 mL, 0.28 g/100 mL in the cañahua beverage and the oat beverage has a fiber content of 0.8 g/100 mL. Ash content is 0.29 g/100 mL in the quinoa beverage and 0.34 g/100 mL in the cañahua beverage. Sugar equivalents was highest in quinoa beverage (9.7%) and the cañahua beverage had a value of 3.7%. Total starch of quinoa beverage was 1.67 mg/100 mL and 0.97 mg/100 mL in the cañahua beverage (Table 3). The total starch in cañahua beverage B was 1.23 mg/100 mL. As Table 3 shows the total phenolic compounds in quinoa beverage were 152 mg gallic acid equivalents/L and 157 mg gallic acid equivalents/L in the cañahua beverage. The total phenolic compounds were measured on cañahua beverage B and received a value of 178 mg gallic acid equivalents/L. Viscosity and pH are included in Table 3 for comparison with reference beverage.

Table 4 compares content of protein, carbohydrates, fat, dietary fiber and ash with literature. The percent values (Table 4) are achieved from the parameters by calculating the values from Table 3 and divided with the dry extract from Table 3. Table 4 shows that the quinoa beverage A had a protein content of 14.9%, fat content of 5.1%, carbohydrates of 76.8%, dietary fiber of 1.6% and ash of 3%. The literature shows that quinoa beverage has a protein content of 16.5 %, fat of 6.3% carbohydrates of 69.0%, dietary fiber of 3.8% and ash of 3.8%. The cañahua beverage A has a protein content of 15%, fat content of 8.0%, carbohydrates of 70.0%, dietary fiber of 6.1% and ash of 5.1% The literature claims that cañahua beverage has a protein content of 12.8%, fat content of 7.0%, carbohydrates of 59.9%, dietary fiber of 6.3% and ash of 3.1% (Table 4).

Table 3. *Result of chemical analysis of quinoa beverage, cañahua beverage and a reference beverage*

| Parameter/Beverage | Quinoa beverage A | Cañahua beverage A | Reference (Oat beverage)[1] |
|---|---|---|---|
| Water content (g/100 mL) | 90.46 | 95.50 | 90 |
| Dry extract (g/100 mL) | 9.54 | 5.5 | 10 |
| Protein (g/100 mL) | 1.43 | 0.83 | 1.0 |
| Carbohydrates (g/100 mL) | 7.33 | 3.89 | 6.5 |
| Fat (g/100 mL) | 0.49 | 0.44 | 1.5 |
| Fibre (g/100 mL) | 0.16 | 0.28 | 0.8 |
| Ash (g/100 mL) | 0.29 | 0.34 | |
| Sugar equivalents (%) | 9.7 | 3.7 | |
| Total starch (mg/100ml) | 1.64 | 0.97 | |
| Total phenolic compounds (mg gallic | 152 | 157 | |

| | | | |
|---|---|---|---|
| acid equivalents/L) | | | |
| Viscosity (cP) | 5.5 | 3.9 | 7.3 |
| pH | 5.4 | 4.9 | 6.9 |

[1](http://www.oatly.com/products/sweden/havredryck/)

Table 4. Comparing result of chemical analysis with analysis from literature.

| Parameter (%) /Beverage | Quinoa beverage A[3] | Quinoa beverage[1] | Cañahua beverage A[3] | Cañahua beverage[2] |
|---|---|---|---|---|
| Protein (%) | 14.9 | 16.5 | 15.0 | 12.8 |
| Fat (%) | 5.1 | 6.3 | 8.0 | 7.0 |
| Carbohydrates (%) | 76.8 | 69.0 | 70.0 | 59.9 |
| Dietary fiber (%) | 1.6 | 3.8 | 6.1 | 6.3 |
| Ash (%) | 3 | 3.8 | 5.1 | 3.1 |

[1](Valencia-Chamorro, 2003) [2](Gallego Villa et al., 2014) [3]Parameters/dry extract from Table 3

## 5.2 Scale up

The scale up was performed twice; the first time the beverage was made without reheating after addition of enzymes. This resulted in a decreasing of pH (Figure 2). The second scale up was made with reheating of beverage and resulted in more stable viscosity, moisture content and sugar formation (Figure 3) as the first scale up, but with an increasing pH. The final pH after 24h in the reheated beverage was 7.58. The pH in the non-reheated beverage was 4.14 and the parameters viscosity, moisture content and pH is presented in Table 3. There was a large difference in pH and the non-reheated beverage had a much lower pH of 4.14 then the reheated scale which received a pH of 7.75 after 24 hours in the fermenter. Data of pH from reheated beverage was not collected during 2 hours, only after 24 hours as Table 5 shows.

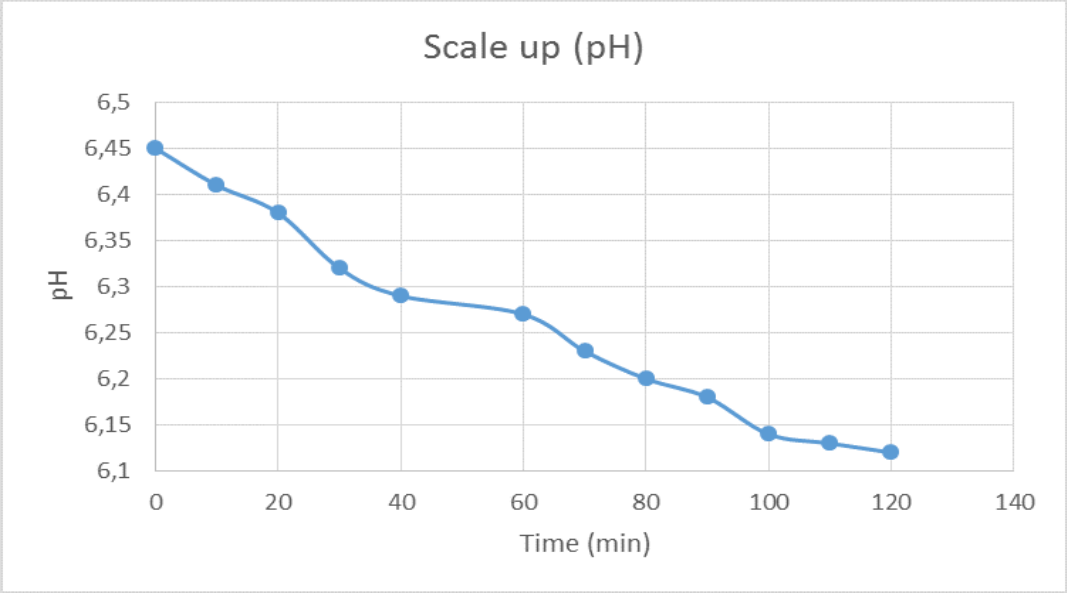


*Figure 2.* pH data from 0-120 minutes while quinoa beverage is stored in fermenter. Beverage was not reheated after adding enzymes. Set points of fermenter: Temperature 50 °C, pH: 7.0, agitation: 320 rpm.

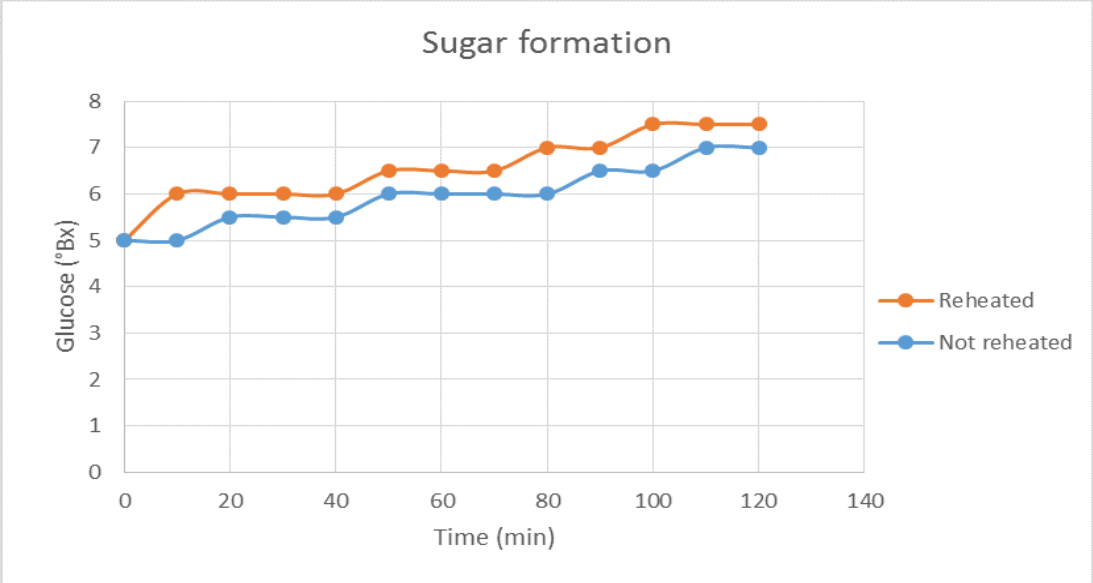


*Figure 3.* Sugar formation in quinoa beverage after addition of enzymes. Samples were collected during 120 minutes under storage in fermenter. Set points of fermenter: Temperature 50°C, pH: 7.0, agitation: 320 rpm.

Table 5. *Scale up of quinoa beverage. Data was collected during scale up that was untreated with heat and treated with heat after addition of enzymes.*

| Parameter/Quinoa beverage | Non re-heated | Re-heated |
|---|---|---|
| pH | 4.1 | 7.5 |
| Moisture content (%) | 93.7 | 92.9 |
| Viscosity (cP) | 7.6 | 7.0 |

## 5.3 Test of acceptance

Evaluation of quinoa beverage by 61 participants gave a mean value of 5.5, which is “Neither like nor dislike and “Like slightly” according to the hedonic scale (Figure 5). The reference product, the oat beverage, received a mean score of 7.3, which is “Like moderately” to “Like very much” according to the hedonic scale. Comments about the quinoa beverage were for example; feeling of small particles, similar to soy milk, to strong flavour of quinoa, to less taste and an intense smell of quinoa.

The analysis of variance of scores according to the hedonic scale showed a significant difference between the beverages ($P<0.001$).

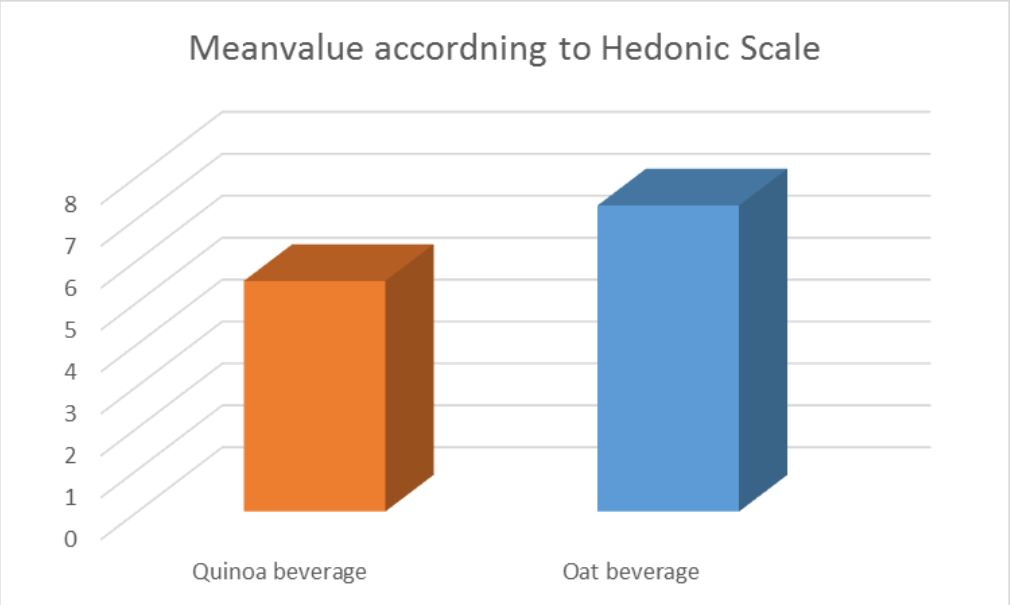


*Figure 5.* Result from test of acceptance. Score 1: Dislike extremely. Score 2: Dislike very much. Score 3: Dislike moderately. Score 4: Dislike slightly. Score 5: Neither like nor dislike. Score 6: Like slightly. Score 7: Like moderately. Score 8: Like very much. Score 9: Like extremely.

### 5.3.1 Analysis of pesticides and heavy metals in quinoa and cañahua flour

Analysis of pesticides was made on the quinoa flour and the cañahua flour. This was made by Marcelo Bascope O. at the Centro de Investigations Quimicas S.R.L. The flour showed no positive result and the control indicated that the method was working properly.

The flours were also analysed for cadmium and copper content and the cañahua flour contained 3.95E-05 mg/g cadmium and 0.0042 mg/g copper. The quinoa flour showed 1.35E-05 mg/g cadmium and 0.0010 mg/g copper.

# 6 Discussion

## 6.1 Preparation of quinoa beverage

In the experiments to formulate a quinoa beverage only the oat beverage was used as a reference in order to reach the parameters (viscosity, pH and dry matter). The other reference product (quinoa beverage from Ecomil) was excluded quite promptly since the taste was undesirable. The pre-preparation steps in the screening set up resulted in the standardization of the process and a quinoa beverage with the desired parameters. The standardized method was applied in the laboratory at UMSA, La Paz. The formulation of beverage suggested that the white precooked quinoa flour should be chosen as a base for the quinoa beverage. The precooked flour was grinded as fine as possible to reduce the sticky mouth feeling of coarse particles. The raw quinoa grains, which were milled into flour during the screening in Lund, gave a more sticky mouth feeling. As Table 2 shows the quinoa beverage A had a much lower viscosity, 5.5 cP, than the oat beverage, which had 7.3 cP. This might depend of a new type to viscometer that was used in only this batch. Since the cañahua beverage A was measured with the same equipment and showed a really low viscosity of 3.9 cP it is believable that it has to do with the type of viscometer. As Vargas[3] suggests, the precooked quinoa flour is the optimal choice for making a beverage. As Table 2 shows, the viscosity was much higher in quinoa beverage B, which was made of raw quinoa seeds. This might depend on the lower temperature and that this beverage was made of raw white seeds. It is also difficult to get the same dry matter as the reference without using more than 10% quinoa in water. This could depend on all the coarse particles that cannot be disperse well in the water. Optional is to use another grinding technique and not the coffee grinder. Even though this resulted in a very fine flour it is not as fine as the precooked quinoa flour. This is better to use in order to reach the desirable dry matter of 10%.

3. Ariel Vargas Production manager Andean Valley, interview March 12 2015

The beverages, made with precooked and raw quinoa seeds, were treated and prepared as Figure 1 demonstrates. Another reason for using precooked flour was for the taste. Comparing the flour that was made from raw quinoa seeds (quinoa beverage B) with quinoa beverage A, made of precooked flour, the beverage B had an unpleasant beany taste while quinoa beverage A had a better taste and the unpleasant beany taste was reduced in beverage A. According to Vargas[1], the amount of saponins is less in the precooked flour which reduce the bitter taste. The reason why choosing white quinoa as a base for the beverage is mainly because a more desirable color might affect the impression of the product. White quinoa is also used because of less bitter taste, since colored quinoa contains more bitter compounds like saponins. As Appendix 2 shows the evaluation of colored quinoa led to exclude this in the preparation of beverage. Another alternative to get rid of the beany raw taste was to cook the quinoa and then mix it with water. However, the cooked quinoa absorbs water during cooking, the water content is higher and it is more difficult to make a beverage with a dry matter of 10%, as the reference has. This might decrease the yield of beverage.

The pre-preparation steps included mixing flour with water, filtration, addition of oil and salt and homogenization. In lab scale the flour was mixed with water in a blender on high speed. This made the particles separated and to be more homogenized in the water. It might be a necessary step to dilute the flour in the water and then optimize the flavor in the beverage. The second step of the pre-preparation was the filtration, which was made both by coffee filter and a sieve. Both alternatives were suitable for the beverage but the beverage still contained some coarse particles from the flour. During the scale up, there was an attempt to filter the beverage trough a filtration pump. This filtration technique did not succeed since the filter was too fine. There are more alternatives for filtration of plant beverages, such as centrifugation or decantation. These methods were not tested and might be better options for filtration of quinoa beverage.

The result from the experiments in this study were similar to those from the literature (Table 4). This means that the good quality of protein and other macronutrients is confirmed. As can be seen in Table 3, the parameters are also quite similar to the parameters of the oat beverage, which was the goal. For example the dry extract was 9.54 %, and the target was to achieve a beverage with a dry matter of 10%.

Table 3 summarizes the result from the chemical analysis. As the result shows, there was not a very big difference in the chemical composition between the oat beverage and the quinoa beverage. The expected and biggest difference was in the protein content that was larger in the quinoa. The protein content in cañahua agreed with the literature and cañahua had higher amount of the macronutrients that were analyzed (Table 4).

The difference between protein in the pseudo cereals and the oat is moderate and an analysis of amino acid composition would be good in order to confirm that the quinoa contains the important amino acids like lysine and methionine as Valencia-Chamorro (2003) states. Table 4 it is seen that the chemical composition of the prepared quinoa beverage had a similar chemical composition according to Valencia-Chamorro, 2005.

### 6.1.1 Heat treatment

The heat treatment to 65 °C generated gelatinization of starch which is suitable according to Ruales Nájera (1992). If the beverage has a high amount of coarse particles, it seems more difficult for the starch to gelatinize. The beverage prepared from cañahua did not gelatinize, which might depend on the more fibers which encapsulate the starch. Result in Table 2 shows the level of gelatinization. As Ruales Nájera (1992) attempts, in a heat treated quinoa, the starch is more unstable to amylase hydrolysis than the starch in raw quinoa. The precooked and drum dried, extruded quinoa had the highest degree of hydrolysis. Hydrolysis of starch is important due to viscosity and palatability of the beverage and according to Lindahl et al., (1995) the liquefaction will change the viscosity and increase the sweetness, if the starch have been hydrolyzed by amylases. This confirmed Vargas[1] suggestion for using precooked quinoa flour as a base for making a quinoa beverage.

### 6.1.2 Effect of enzymes

As Muller (1991) explains, the temperature and mash thickness has an affect both on mash performance and enzyme activity. Amylases like α- and β-amylase are inactivated during heat treatment but α-amylase is shown to be more resistant to heat treatment then β-amylase. Heating to 65-80 °C have effect on both enzymes, especially β-amylase. Temperatures of 85 °C and over have a significant effect on α-amylase when it comes to inactivation of the enzyme (Muller, 1991). Due to this, the quinoa beverage was heated to boiling point to inactivate the enzymes. The addition of amylases changed the viscosity and enhanced the sweet taste as can be seen by the sugar formation in Figure 3. According to Lindahl et al., (1995) the α-amylases liquefies the beverage while the β-amylase is more responsible for sweet taste, since it produces smaller glucose molecules. The usage of enzymes is an optional to achieve a sweeter taste in the beverage without adding sugar. The β-amylase activity promotes maltose components, in the presence of α-amylase.

## 6.2 Scale up

The scale up of the quinoa beverage was performed as the flow chart to the right in Figure 1 shows. One step in the flow chart that was modified in scale up was the time of agitation due to the less force to a larger amount of liquid. It needed to blend for about 20 minutes in the agitator, comparing with the time of 2 min needed for mixing only 1 liter of beverage. This might depend on the big amount of flour that has to be dispersed in a large amount of water in a big pot. In lab scale the beverage was mixed in a blender, with smaller volume of beverage and with higher force. Also the homogenization is important to decrease the particle size and later, after addition of fat, it is important to agitate the beverage in order to improve the stability (Malaki Nik et al., 2008; Rustom et al., 1995). The filtration was made through 4 layers of filtering cloth bags instead of through a sieve or coffee filter as for the lab scale experiment. The modification of filtration in the scale up was mainly changed in order to save time, since the coffee filter resulted in a very slow filtration.

The scale up experiment was performed twice, one without reheat treatment and one with reheat treatment. Samples for pH and sugar analyses were collected every 10 minutes for 2 hours and a last sample was collected after 24 hours. The non-reheated beverage received a pH of 4.14 after 24 hours, while the reheated had a pH of 7.75 after 24hours in the fermenter. This large difference in pH might depend on still active enzymes in the non-reheated beverage. The reheated beverage was heated to boiling point, which is a temperature of inactivation of enzymes (Muller, 1991). Furthermore, the decrease in pH could also depend on contamination of microorganisms since the non-reheated beverage might be a less sterile product. These microbes can affect the pH.

## 6.3 Test of acceptance

The panel comprised 61 untrained participants with variation of preferences such as liking or disliking the quinoa beverage. See Figure 5. An untrained panel was mainly used because no trained panel was accessible and the time did not allow training of respondents for a trained panel.

According to Stone and Sidel (1993) it is important for the assessors to be able to use the preference scale in order to evaluate the product. In the experiment, this was not the case, because the panel was untrained. This could make the result of the hedonic acceptance test not creditable. The statistical analysis of two way variance (ANOVA) strengthens the result of the acceptance test which is shown in Figure 5. The analysis of variance together with the comments from the participants showed that the product with best palatability was the reference product. This is seen Figure 5 in the result.

## 6.4 Pesticides and heavy metals

The quinoa and cañahua flour were tested for contamination of pesticides and heavy metals. Due to the result, which was negative, the flours can be considered to be safe when it comes to pesticides and heavy metals.

Heavy metals like copper and cadmium were tested since equipment to run those tests was available in the laboratory. The cañahua flour contained 3.95E-05 mg/g cadmium and 0.0042 mg/g copper. The quinoa flour showed 1.35E-05 cadmium and 0.0010 mg/g copper. Since a high intake of cadmium can lead to negatively impact on health, the World Health Organization recommends a highest weekly intake of cadmium at 7 µg/kg of body weight. The weekly value agrees to a daily intake of 70 µg of cadmium for the average man of 70-kg and 60 µg of cadmium per day for the average woman (60-kg) (WHO, 2010). Due to this recommendation, the cañahua and quinoa flour can be considered safe to consume. The daily limit of intake of copper is around 2 or 3 mg/day (WHO, 2004) which means that the coppercontent in the quinoa and cañahua flour is low and you have to consume a lot of these flour to exceed the limit recommendation.

## 6.5 Suggestions for continued studies

Results from Table 3 indicate that quinoa beverage had a high protein which agrees with literature according to Valencia-Chamarro (2003). This only includes the total protein content, therefore further analysis of specific amino acid composition is suggested in order to ensure the assertion of high content of the important amino acids methionine and lysine (Valencia-Chamorro, 2003).

Other suggested studies to perform:

- Pasteurization by Ultra-High-Temperature (UHT), to see if the product nutritional status remains and to guarantee the stability of the product.
- Filtration by methods such as decanting or centrifugation to see if all coarse particles can be removed
- Making a beverage mixed with other vegetal beverages to determine the performance of beverage
- Finding an emulsifier to prevent separation
- Calculating if it is economically sustainable to use precooked quinoa flour from Bolivia, or if it is possible to process the grains similar in Sweden.

# 7 Conclusion

- Making a beverage on quinoa has potential, due to viscosity and dry matter
- The chemical composition is similar to oat beverage
- The addition of enzymes promotes sugar formation and gives a similar viscosity as oat beverage
- Decreasing pH is a stability problem
- The quinoa beverage must be shaken before use to counteract sedimentation
- The hedonic acceptance test showed values below oat beverage
- The taste has to be developed

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# Appendix 1. Products and reference material

Table 6. *Quinoa product, Cañahua products and reference material.*

| Product name | Brand | Ingredients | Source | Result |
|---|---|---|---|---|
| Red quinoa | Risenta | Ecologic red quinoa | No source | Parameters do not match with reference |
| Tricolore quinoa | Garant | White quinoa 50%, black quinoa 25%, red quinoa 25% | Peru | Parameters do not match with reference |
| White quinoa | Garant | Ecologic white quinoa | Peru | Grinded into a fine flour, agreed with fixed parameters, but beany flavour |
| White quinoa flour (precooked) | Andean Valley | Organic white quinoa | Bolivia | Agreed with fixed parameters |
| Cañahua flour (precooked and roasted) | Irupana | Cañahua | Bolivia | Parameters do not match with reference |
| Cañahua flour | UMSA | Cañahua | Bolivia | Parameters do not match with reference |
| Reference (Oat drink) | Oatly | Oat base (water, oats 10%), rapeseed oil, calcium, salt, vitamins (D2, riboflavin and B12). | Sweden | Viscosity |

# Appendix 2. Screening in Lund

Table 7. *Different varieties of quinoa and processes during screening in Lund*

| Product | Process | | Result | | Appearance |
|---|---|---|---|---|---|
| | Mixer | Time (min) | Viscosity (cP/%) Spider 61, speed 100rpm | Dry matter (%) | |
| Red in 250g | Blender | 2 | | 20.86 | Thick, coarse particles, bitter taste, dark |
| Red in 250g( raw) | Stick | 2 | | | Thick, coarse particles, bitter taste, dark |
| Red in 500 g | Blender | 4 | | 17.47 | Thick, coarse particles, bitter taste, dark |
| Tricolor in 150g | Blender | 4 | | 35.41 | Thick, creamy, grey, beany taste |
| White in 250g* | Blender | 2 | 39.3/13.2 | 16.69 | Thick, creamy, yellow |
| White in 333g | Blender | 2 | 16.5/27.8 | 23.38 | Thick, creamy, nutty, yellow |
| White 333g | Stick | 2 | | | Thick, creamy, high fiber, heterogeneous |
| White in 500g | Blender | 2 | 14.0/8.52 | 27.04 | Fairly creamy, nice taste, |
| White in 1000g | Blender | 2 | 4.44/7.4 | 22.47 | Watery, no taste, nice yellow |
| Ref.1 | | | 7.32/12.3 | 10.84 | Smooth, no fiber |

# Appendix 3. Screening process, heat treatment, additives to white quinoa grains

Table 8. *Samples of quinoa beverage was prepared in several ways. Raw; unrinsed, rinsed, heat-treated and roasted. Different additives where tested to see the effect of viscosity, pH and moisture content. Experiments with no effect no change in viscosity, or bad influence on appearance or taste, further analysis of dry matter, viscosity and pH were not measured.*

| Heat treatment and processes | Additives: Maltodextrins, Enzymes, N2, salt and oil | Viscosity (cP) | Dry matter (%) | pH | Taste and appearance |
|---|---|---|---|---|---|
| Raw, unrinsed | | 6.0 | 6.6 | 4.11 | Salty taste, and little bit watery. |
| Raw, rinsed | | 4.2 | 6.4 | 5.0 | Salty taste |
| Raw, unrinsed | Dextrin 01910 (5g) | 6.0 | 6.6 | | Sweet, artificial sweetness |
| Raw, rinsed | Dextrin 01314 (5g) | 4.8 | 6.4 | | Sweet, artificial sweetness |
| Heated to 85-90 °C | β-glucanase | 6.4 | 5.0 | | No reaction, very thick |
| Heated to 85-90 °C | α- amylase | 14 | 6.4 | 7.6 | Viscosity much lower |
| Heated to 85-90 °C | β -amylase | | | | No reaction in viscosity |
| Heated to 85-90 °C | Xylanase | | | | No reaction |
| Roasted in oven for 20 min 125°C | | 8.5 | 5.1 | | Low dry matter, taste of roast |
| $N_2$ treated | Salt(0,1%) and oil(1.5%) | | | | Not a difference in taste, more watery and not as nice as sample below |
| Heated to 85-90 °C | Salt(0.1%) and oil (1.5%) | | | | Not too salt, nice, but a little bit grey colour. |
| Heated to 85-90 °C | Salt(0.05%) | | | | Watery, less flavour and moth feeling |
| Reference | | 7.3 | 6.7 | 10.8 | White, sweet, non coarse particles, good viscosity and not sour |

# Appendix 4. Paper forms for test of acceptance

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EVALUACIÓN DE ACEPTABILIDAD

ESCALA HEDÓNICA

Nombre..... Fecha.....

Test: Escala Hedónica Producto Leche de quinua

Sírvase a degustar las muestras que se presentan y señale su reacción de agrado o desagrado según la escala adjunta

1: Me disgusta extremadamente
2: Me disgusta mucho
3: Me disgusta moderadamente
4: Me disgusta levemente
5: No me gusta ni me disgusta
6: Me gusta levemente
7: Me gusta moderadamente
8: Me gusta mucho
9: Me gusta extremadamente

| MUESTRAS | PUNTAJE | OBSERVACIONES |
|---|---|---|
| ..... | ..... | ..... |
| ..... | ..... | ..... |
| ..... | ..... | ..... |
| ..... | ..... | ..... |

# Appendix 5. Popular scientific summary

There is a general need and requirements for alternatives to ordinary cow's milk. Even if there are well-established cereal-based milk alternatives, based on oats etc., today they cannot in full meet the gluten-free requirements that are increasing within this category. Thus, there is a need to investigate and study how to develop milk-like product based on raw materials that in absolute terms can be defined as "gluten-free" and, in addition, has a macro-nutrients profile that can meet the requirements consumer puts on milk-like products.
In this project the focus will be on quinoa *(Chenopodium quinoa)* as a raw material for milk-like, vegetable suspension that is gluten free and has good nutritious profile. Different types of quinoa will be tested (white, red, tricolored) and mixtures thereof. Different enzymatic methods will be used in this process. To verify the final products, chemical measurements together with sensory analysis will be used. Analysis of the macro-nutrients, such as carbohydrates, starch, proteins and fat will be analyzed.

A screening was set up to standardize the method for making a beverage. The standardization included trying different quinoa grains, mixing techniques, heat treatment, additives, enzyme treatment and forms of filtration. As a reference a plant based beverage from oats was used in order to achieve the same viscosity, pH, dry matter and palatability in the quinoa beverage. Another pseudo-cereal, cañahua *(Chenopodium pallidicaule,* was also used for preparation of beverage. A scale up of the beverage was performed and the palatability of the beverage was evaluated with a sensory test of acceptance on 61 untrained participants.

The results showed that making a beverage on quinoa has potential, due to the chemical analysis and parameters like viscosity, pH and dry matter were comparable to the reference. There is more work to do in order to improve taste and stability. Since the pH is rapidly decreasing, this is a parameter to analyze further and search for reasons of the declining. The protein content was 1.43 g/100ml, as expected, higher than the reference product, the oat beverage. The addition of enzymes promotes sugar formation and the beverage get a natural sweeter taste. The enzymes gives the beverage a desirable viscosity, similar as the oat beverage. The quinoa beverage must be shaken before use to counteract sedimentation. The hedonic acceptance test showed values below oat beverage and the quinoa beverage had a score of 5.5 on the hedonic scale, which is between, *Neither like nor dislike* and *Like slightly*. Which is lower than the score for the oat beverage, due to this the taste can be improved in order to be a product for consumers.