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Influence of salinity, germination, malting and fermentation on quinoa nutritional and bioactive profile

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ABSTRACT

The depletion of freshwater resources, as well as climate change and population growth, are threatening the livelihoods of thousands of people around the world. The introduction of underutilized crops such as quinoa may be important in countries with limited productivity and/or limited access to water due to its resistance to different abiotic stresses and its high nutritional value. The aim of this review is to assess whether techniques such as germination, malting and fermentation would improve the nutritional and bioactive profile of quinoa. The use of nitrogen oxide-donating, oxygen-reactive and calcium-source substances increases germination. The ecotype used, temperature, humidity and germination time are determining factors in germination. The presence of lactic acid bacteria of the rust-type phenotype can improve the volume and texture during baking of the doughs, increase the fiber content and act as a prebiotic. These techniques produce a significant increase in the content of proteins, amino acids and bioactive compounds, as well as a decrease in anti-nutritional compounds. Further studies are needed to determine which conditions are the most suitable to achieve the best nutritional, functional, technological, and organoleptic quinoa properties.

KEYWORDS

Quinoa; germination; malting; fermentation; salinity; nutritional; bioactive

Introduction

Sustainable development and the livelihoods of thousands of people around the world are at risk due to factors such as desertification, depletion of freshwater resources, increased food loss and waste, loss of biodiversity, greenhouse gas emissions, climate change, as well as increased population and urbanization (FAO 2018). This situation has been exacerbated by the COVID-19 pandemic and the ongoing war in Ukraine. The introduction of underutilized crops, such as ancient cereals and pseudocereals with high nutritional value and resistance to different abiotic stresses, can be relevant in countries with limited productivity and/or restricted access to protein sources (United Nations 2022).

Quinoa (*Chenopodium quinoa* Willd.), a species native to the Andes of Bolivia and Peru, was the main food of ancient Andean cultures. Great civilizations such as the Tiahuanacota and Inca civilizations were involved in its domestication and conservation some 7000 years ago. The year 2013 was declared by the United Nations General Assembly as the “International Year of Quinoa (IYQ)” for its important role in the fight against food insecurity and poverty, as well as to promote environmentally friendly agriculture (FAO and CIRAD 2015). Its seeds are high in protein, with a good balance of essential amino acids, vitamins,

minerals, antioxidants and dietary fiber. Its protein, with a higher content of lysine, cystine and methionine than cereals, is similar to milk casein (Martinez-Villaluenga, Peñas, and Herná et al. 2020; Bilal Pirzadah and Malik 2020; Rana et al. 2020). It is an outstanding source of vitamins, mainly pyridoxine (B₆) and folic acid (B₉), as well as vitamin E, riboflavin (B₂), niacin (B₃), thiamin (B₁) and vitamin C (Martínez-Villaluenga, Peñas, and Hernández-Ledesma 2020). In terms of minerals, it is notable for its calcium, iron, magnesium, potassium, phosphorus, zinc and manganese content (Martínez-Villaluenga, Peñas, and Hernández-Ledesma 2020). It contains a high amount of unsaturated fatty acids, linoleic acid (ω -6) the main unsaturated fatty acid and palmitic acid the most abundant saturated fatty acid (Rana et al. 2020). It has a low glycaemic index (GI) of 35-53 and is gluten-free (Boukid et al. 2018; Martinez-Villaluenga et al. 2020). This low GI helps maintain blood glucose levels and body weight, as well as lowering the risk of diabetes, cardiovascular disease and certain types of cancer (Gordillo-Bastidas et al. 2016). Multiple secondary metabolites with important physiological functions have been identified. Among these secondary metabolites are: phenolic acids, flavonoids (kaempferol and quercetin), terpenoids, steroids and nitrogen-containing metabolites

(betalains) (Martínez-Villaluenga, Peñas, and Hernández-Ledesma 2020; Lin et al. 2019).

There is currently a greater interest in the consumption of vegetable proteins in substitution of animal proteins, due to a greater demand for healthier and/or more environmentally sustainable foods. In view of healthy properties, quinoa has great potential as a functional ingredient that can be incorporated into different foods or as a raw material in gluten-free foods.

Quinoa is a facultative halophilic plant with a large genetic variability, which allows its cultivation in extreme environment such as frost, drought, saline soils, marginal lands, etc. Since it is known that excessive soil salinity alters the germination of plants, decreasing their growth, development and resistance by modifying their metabolic processes, decreasing their cell division and expansion and/or altering their membrane, among others (Panuccio et al. 2014). There is therefore a need to review how soil salinity affects the nutritional properties of quinoa.

There are processes such as germination and malting that could modify the nutritional composition of pseudocereals, cereals and legumes. Germination comprises the stages from imbibition or water absorption to the prominence of the radicle by breaking the seed coat as the endosperm and testa weaken (Carrera-Castaño et al. 2020; Nonogaki 2019). In the case of malting, it consists of a first germination stage followed by a baking or drying stage, the purpose of which is to inactivate the enzymatic processes and increase the stability and storability of the dried product. Sometimes there may be a third roasting stage to give the product optimal sensory characteristics (color, flavor, aroma, etc.). The advantage of these processes lies in the fact that they could produce certain changes at a nutritional, functional, technological or sensory level, which would result in an increase in the concentration of certain nutrients (vitamins, minerals, bioactive substances, etc.), a decrease in anti-nutritional factors, improved digestibility, reduced bitterness, etc. However, these effects vary according to the conditions of germination and crop variety. It is therefore necessary to review existing studies on quinoa in order to be able to draw conclusions on the nutritional benefits that could be derived from this pseudocereal.

Healthy dietary styles such as veganism, as well as the emergence of new food pathologies, are encouraging a growing interest in the production of fermented products from vegetable sources. Fermentation has already been used for the preparation and preservation of foodstuffs, especially those of animal origin, for some 6.000 years ago. There is increasing scientific evidence of the beneficial functions of human microflora on health. The acidification of the medium that is achieved through fermentation increases the activity of endogenous phytases, resulting in a decrease in phytic acid, an important anti-nutritional factor that produces a decrease in the bioavailability of minerals, as well as of the basic amino acids (Carrizo et al. 2016). The conditions of fermentation and the lactic acid bacteria used can cause variations in the results obtained in quinoa and its derivatives.

No reviews on the effects of soil salinity, germination, malting and fermentation on the nutritional composition of quinoa have been reported in the literature. These processes constitute a potential means of improving and developing quinoa as a functional food. Therefore, the main objective of this paper is to summarize the published articles the evaluation of the nutritional changes that occur in seeds of different quinoa varieties during the processes of germination, fermentation and malting.

Effect of salinity on the nutritional composition of quinoa

Quinoa can adapt and grow in harsh conditions. The use of saline hydro sources together with the cultivation of quinoa is a promising solution to the shortage of water resources. Quinoa can grow sustainably and productively in saline soils, making this crop suitable for cultivation. In addition to affecting crop yields, saline irrigation can change soil composition and increase aridity and infertility (Hajihashemi et al. 2020). High soil salinity alters the germination of plants, decreasing their growth, development and resistance by modifying their metabolic processes, decreasing their cell division and expansion and/or altering their membrane, among others (Panuccio et al. 2014).

Soil salinity causes osmotic and ionic stress in plants. Osmotic stress results in a decrease or inhibition of the root's ability to absorb water, resulting in reduced growth. Ionic stress, resulting from the accumulation of toxic ions such as sodium (Na^+) and chlorine (Cl^-) in the cell and essential ions such as potassium (K^+) and calcium (Ca^{2+}) (Causin, Bordón, and Burrieza 2020) and interference with enzymes, causes alterations in processes such as photosynthesis, protein synthesis, as well as advancement of senescence, chlorosis or necrosis of older leaves (Panuccio et al. 2014). In addition, reactive oxygen and nitrogen species (ROS and RNS) accumulate in response to this stress and can damage cell structures (Hajihashemi et al. 2020).

Quinoa varieties that grow in saline areas are more adapted to these conditions than varieties from non-saline areas. Similar to other halophilic plants, quinoa has salt glands or salt bladders, which retain and secrete excess salt from the tissues. The mechanisms used by quinoa to mitigate high salt levels in the soil may be: ionic homeostasis by membrane transporters (SOS1, NHX1, H^+ -ATPase, HAK and HKT) (Adolf, Jacobsen, and Shabala 2013; Cai and Gao 2020), non-enzymatic antioxidants (glutathione reductase, glutathione, mannitol, proline, etc.), accumulation of proteins, soluble sugar and proline in leaves, K^+/Na^+ ratio of leaves and roots (more K^+ in leaves and more Na^+ in roots), better tolerance to ROS accumulation and optimal control of stomatal development and opening (Adolf, Jacobsen, and Shabala 2013; Cai and Gao 2020).

Table 1 lists the objectives, conditions of germination, parameters analyzed and quinoa ecotypes used in the different studies used in this review, which address the issue of salinity and the repercussions on the nutritional value of quinoa seeds.

Table 1. Objectives, germination conditions, parameters analyzed and varieties used in the salinity studies.

Objectives of the study	Conditions for germination	Parameters analyzed	Varieties used	References
To study how seawater (SW) and different solutions of NaCl, KCl, CaCl ₂ and MgCl ₂ affect the germination and biochemical composition of quinoa seeds.	<ul style="list-style-type: none"> • Sterilization with 20% NaClO for 20 minutes. Washing and soaking in distilled water for 1 h. • Nine replicates of 50 seeds with seawater (SW) at 25%, 50%, 75% and 100% salinity and solutions of NaCl (0, 100, 200, 300 and 400 mM), KCl (0, 2.54, 5.08, 7.62 and 10.2 mM), CaCl₂ (0, 2.54, 5.08, 7.62 and 10.2 mM) and MgCl₂ (0, 13.4, 26.7, 40.1 and 53.5 mM) • Petri dishes sealed with parafilm: 3 ml of each solution and seeds on filter paper. Germination: darkness, 25 °C and 70% relative humidity. 	<ul style="list-style-type: none"> • Total antioxidant activity (AA) and total phenolic compounds (TPC). 	<ul style="list-style-type: none"> • <i>Danish-bred quinoa</i> (<i>Chenopodium quinoa</i> cv.Titicaca). 	Panuccio et al. (2014)
To study the effects of pretreatment with CaCl ₂ , H ₂ O ₂ and sodium nitroprusside (SNP) on the germination of quinoa seeds exposed to salinity.	<ul style="list-style-type: none"> • Imbibition of 100 mg of seeds in 0, 0.1 and 0.2 mM SNP, 0, 2.5 and 5 mM H₂O₂, 0, 2.5 and 5 mM CaCl₂ or a combination of the 3 (0.1,2.5 and 2.5; 0.2,5 and 5) at room temperature for 60 minutes. • 10 Petri dishes: 100 seeds and addition of 10 ml NaCl at 0, 50, 100 and 200 mM. Germination: 25 °C, 16 h light and 8 h darkness cycle for one week 	<ul style="list-style-type: none"> • α- and β-amylase activity, soluble proteins, total amino acids, water-soluble sugars, glucose and starch. 	<ul style="list-style-type: none"> • <i>Chenopodium quinoa</i> Willd. 	Hajjhashemi et al. (2020)
To examine the alterations caused by NaCl at different concentrations on antioxidant composition and lipid peroxidation during germination in three quinoa varieties, as well as the influence of betalains on salt stress tolerance.	<ul style="list-style-type: none"> • Seeds: soaked in 2% NaOCl for 12 minutes and washed. • Germination: Petri dishes moistened with 5 ml NaCl at different concentrations: 0 (control), 150, 300 and 400 mM and 21 °C. 	<ul style="list-style-type: none"> • Soluble proteins, total antioxidant capacity and betalain concentration. 	<ul style="list-style-type: none"> • <i>Chenopodium quinoa</i> var. CICA (Puno region, Peru) • <i>Chenopodium quinoa</i> var. Villarrica (Araucania region, Chile) • <i>Chenopodium quinoa</i> var. Chadmo (Lagos region, South of Chile). 	Causin, Bordón, and Burrieza (2020)

In their study Panuccio et al. (2014) analyzed the impact of different concentrations of seawater (SW) and different solutions of NaCl, KCl, CaCl₂ and MgCl₂ on the germination and biochemical composition of Danish-bred quinoa (*Chenopodium quinoa* cv.Titicaca) seeds. They germinated the seeds with the different solutions in darkness, at 25 °C and a relative humidity of 70%, observing an increase in antioxidant activity in all samples with respect to the control sample, reaching the highest value of 4.13 ± 0.15 μmol α-tocopherol/g FW (fresh weight) in the 50% seawater sample. As well as a significant increase of total phenolic compounds in the presence of NaCl and especially seawater, the maximum being 625 ± 20 mg TAET (tannic acid equivalents)/g DW (dry weight) in 75% seawater.

Despite the decrease in germination observed at higher seawater concentration, increases in both antioxidant activity and total phenolic compounds were observed, so that this variety of quinoa could be suitable for cultivation in high salinity areas. This may be due to the activation of antioxidant enzyme systems (superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, glutathione S-transferase, guaiacol peroxidase and catalase) and non-enzymatic systems such as the accumulation of glycine-betaine, betalains and/or polyamines and structural and physiological variations to maintain adequate osmosis of water and ions (Causin, Bordón, and Burrieza 2020).

Hajjhashemi et al. (2020) soaked *Chenopodium quinoa* Willd. seeds in 0, 0.1 and 0.2 mM sodium nitroprusside (SNP), 0, 2.5 and 5 mM H₂O₂, 0, 2.5 and 5 mM CaCl₂ or a combination of the 3 (0.1,2.5 and 2.5; 0.2,5 and 5). They then germinated them with 10 ml of NaCl at 0, 50, 100 and 200 mM at 25 °C and a cycle of 16 h of light and 8 h of darkness for one week, with the aim of evaluating the effects of pretreatment with CaCl₂, H₂O₂ and sodium nitroprusside (SNP) on the germination of these seeds exposed to salinity. They observed that pretreatments with SNP, H₂O₂, CaCl₂ and combinations of these significantly increased: α- and β-amylase activity, protein content, total amino acids, water-soluble sugars and glucose. There was also a decrease in starch content. Thus, the most effective pretreatment is the combination of 0.2 mM SNP, 5 mM H₂O₂ and 5 mM CaCl₂. In relation to these results, Hajjhashemi et al. (2020) concluded in their study that, although quinoa is a halophilic plant, salt stress causes a decrease in its germination. Pretreatments with NO-donor (SNP), O₂-reactive (H₂O₂) and Ca²⁺-source (CaCl₂) substances decrease the adverse effects of salt stress on germination and induce α- and β-amylase enzymes. These enzymes hydrolyze starch into small glucose molecules, which activate seed germination and growth. The combination of the 3 substances increases germination above control levels.

In another study, Causin, Bordón, and Burrieza (2020) investigated the alterations caused by NaCl at different concentrations on antioxidant composition and lipid peroxidation during germination in three quinoa varieties (*Chenopodium quinoa* var. CICA (Puno region, Peru), *Chenopodium quinoa* var. Villarrica (Araucanía region, Chile) and *Chenopodium quinoa* var. Chadmo (Lagos region, southern Chile). As well as the influence of betalains on tolerance to salt stress. The data they collected at the end of their research were: the germination initiation time in the control sample and in the saline samples, in order of highest to lowest, was Chadmo > Villarrica > CICA and CICA > Villarrica > Chadmo, respectively. This means that the Chadmo variety is the most sensitive, while the CICA variety is the most resistant to salt stress. However, there is a decrease in antioxidant activity in the following order Villarrica > CICA > Chadmo, as well as a four- to five-fold increase in Na⁺ levels in the seed coat in the 300 mM samples, with the highest value for the Villarrica variety. While in the case of Cl⁻ it is not so clear and there is no noticeable variation in Ca²⁺. Regarding K⁺ levels, a slight decrease was observed at the same concentration of 300 mM NaCl and a clear increase in the Na⁺/K⁺ ratio in the canopy of the 3 quinoa varieties studied. Based on these results, Causin, Bordón, and Burrieza (2020) concluded that, of the 3 varieties studied, var. CICA is the most resistant to salt stress and oxidative damage, while var. Chadmo is the most sensitive to NaCl variations. In addition, the seed coat of all 3 varieties is a major obstacle to Na⁺ penetration. In the case of the var. CICA it seems that the penetration of Na⁺ reduces the osmotic effect, making it a more tolerant variety.

Germination

The consumption of germinated sprouts emerged in Asian countries, with interest in this technique now also spreading to Europe, the United States and Australia (D'Ambrosio et al. 2017).

Table 2 shows the objectives, germination conditions, parameters determined in the flour production process, as well as the ecotypes used and the references of the different studies.

Hager, Mäkinen, and Arendt (2014) concluded that the most suitable germination temperature was 15 °C, as this was where the lowest number of non-germinated and/or abnormally germinated seeds were detected. Significant changes in α -amylase action as well as in sugars at 24 hours are a sign of the beginning of starch hydrolysis. Germination causes an increase in enzyme activity. Starch grains are found mainly in the perisperm of the seed in the form of single units or spherical aggregates. Hydrolysis of starch (amylose and amylopectin chains) into glucose molecules at the radicle growth stage starts by the action of α - and β -amylase, debranching and α -glucosidase enzymes (Hager, Mäkinen, and Arendt 2014). In such a way, there is a decrease in starch due to the mobilization of its reserves and an increase in reducing sugars (sucrose and other sugars). The embryo appears to participate in the synthesis and

accumulation of α -amylases (Hager, Mäkinen, and Arendt 2014).

The study of modifications at the structural and chemical level of germination on proteins and starch and how these may affect the practical uses of germinated quinoa flour was carried out by Suárez-Estrella et al. (2020a). For this purpose, they used whole and pearled seeds of *Chenopodium quinoa* Willd. Var. Titicaca germinated at 22 °C and 90% relative humidity for 12, 24, 48 and 72 h. The results of their study were that the starch and sucrose content decreased in the first 24 h. At the end of germination, they observed an increase in sucrose as the activity of the enzymes that synthesize sucrose increased. The β -amylase activity did not change, however, the α -amylase activity underwent a 4-fold increase in the first 12 h. Despite the structural changes of the proteins due to endogenous protease activity, the content of accessible thiols remained unchanged. These alterations in the protein part generated a decrease in foaming capacity at 48 or 72 h. The conclusions drawn by Suárez-Estrella et al. (2020a) were that germination can improve both nutritional and functional properties of quinoa by producing changes in protein structures due to increased action of endogenous proteases. And also by producing a decrease in starch due to increased α -amylase activity. In addition, the longer the germination time, the greater the stability of the foam formed and the lower the foaming capacity.

Variations in the phenolic and proximate composition of Peruvian quinoa seeds subjected to germination and baking was the purpose of the study by Pilco-Quesada et al. (2020). They used seeds of *Chenopodium quinoa* var. Chullpi that germinated at 22 °C, 90-95% relative humidity for 72 h, which they subsequently dried at 40 °C for 1 h. At the end of the study, the results recorded were that the total amount of protein increased 72 h after germination, from 9.6 to 26.0 g/100 g dry weight. This increase is due to the mobilization of sucrose together with proteins and amino acids from the embryo to the radicle. Lipid content decreases because they are involved in respiratory activity and are a source of energy in germination. There is also a decrease in ash and carbohydrate content, while phenolic compounds (coumaric acid being the most abundant) increase in germination. This increase in phenolic compounds is due to the activity of endogenous esterases that release the content of phenols bound to the cell wall and/or their neosynthesis

In the work of Carciochi et al. (2016a), they used seeds of *Chenopodium quinoa* Willd. var. Real and germinated them in darkness, at 20 °C and 90% relative humidity for 72 h with the aim of analyzing changes in antioxidant capacity and phenolic substances produced by germination. Vitamin C and α -tocopherol levels were increased to a maximum of almost 16 times the initial level and 134% at 72 h, respectively. There was also a 101% increase in total phenolic compounds (mainly p-coumaric acid and vanillic acid) and a doubling of antioxidant activity at 72 h as a consequence of the increase in vitamins and phenolic compounds with antioxidant capacity.

Table 2. Objectives, conditions of germination, parameters analyzed and varieties used in flour production in germination studies.

Objectives of the study	Conditions of germination	Parameters analyzed	Ecotypes used	References
To explore the progression of amylolytic activities occurring in the perisperm and embryo of quinoa seed during germination.	<ul style="list-style-type: none"> Desaponification of seeds by abrasion with spring water. Imbibition of seeds in 3% H₂O₂ for 1 minute and germination with 1 ml of water in darkness at 15°C. 	<ul style="list-style-type: none"> α-amylase and total starch hydrolysis activity. Starch and sugar content. 	<ul style="list-style-type: none"> <i>Quinoa roja réal</i> (Priméal, France). 	Hager, Mäkinen, and Arendt (2014)
To study the structural and chemical changes of germination on proteins and starch and how these may affect the practical uses of germinated quinoa flour.	<ul style="list-style-type: none"> Whole and pearled seeds were soaked in water for 14 h at 22°C. Germination: 22°C and 90% relative humidity for 12, 24, 48 and 72 h. Drying: to 7-10% humidity at 55°C for 6 h. Enzyme activity samples: treatment with liquid N and freeze-drying. Rest of samples: grinding to size < 250 µm. 30 g of quinoa seeds: disinfection with 70% (v/v) ethanol solution, rinsing and subsequent wetting with distilled water in a 1:20 ratio (seeds: distilled water) for 24 hours. Germination: 22°C and 90-95% relative humidity for 72 h, wetting every 12h. Samples germinated at 24, 48 and 72 h: drying in oven at 40°C for 1h. Half of the samples germinated at 72 h: drying at 90°C for 5 minutes. Subsequent grinding to particle size < 0.5 mm. 200 g seeds: disinfection with 2.5% NaClO and rinsing with distilled water. Germination: in darkness, at 20°C and 90% relative humidity for 72 h. Samples taken every 24 h, dried at 50°C and ground to 0.5 mm particle size. 	<ul style="list-style-type: none"> Content of proteins, soluble sugars, starch, saponin, metals, α- and β-amylase and proteolytic activity. Protein aggregation state, solubilized proteins, thiol accessibility and protein profile. Foaming capacity and stability and viscosity properties. Values of: moisture, protein content, lipids, ash, fiber and phenolic compounds. 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. Var. Titicaca. 	Suárez-Estrella et al. (2020)
To study variations in the phenolic and proximal composition of Peruvian quinoa seeds subjected to germination and baking.	<ul style="list-style-type: none"> Germination: 22°C and 90-95% relative humidity for 72 h, wetting every 12h. Samples germinated at 24, 48 and 72 h: drying in oven at 40°C for 1h. Half of the samples germinated at 72 h: drying at 90°C for 5 minutes. Subsequent grinding to particle size < 0.5 mm. 200 g seeds: disinfection with 2.5% NaClO and rinsing with distilled water. Germination: in darkness, at 20°C and 90% relative humidity for 72 h. Samples taken every 24 h, dried at 50°C and ground to 0.5 mm particle size. 	<ul style="list-style-type: none"> Ascorbic acid (vitamin C), tocopherols, vitamin E activity, total phenolic compounds (TPCs), antioxidant activity. 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. var. Real. 	Pilco-Quesada et al. (2020)
To study the effects of germination on the antioxidant capacity and phenolic substances of quinoa seed	<ul style="list-style-type: none"> Seeds: sterilization and soaking in distilled water at 25°C for 6h. Germination: darkness for 96h. Drying: up to 12% humidity at 30, 45 and 60°C. 	<ul style="list-style-type: none"> Total phenolic content, total flavonoid content, phenolic acid profile, antioxidant activity, ABTS, FRAP and hydroxyl radical scavenging capacity. 	<ul style="list-style-type: none"> Bolivian red quinoa (<i>Chenopodium quinoa</i>). Chilean white quinoa (<i>Chenopodium quinoa</i> var. Regalona). 	Zlotek et al. (2019)
To study how germination and subsequent drying affect phenolic and antioxidant composition.	<ul style="list-style-type: none"> Sample germinated seeds: 100 g of seeds moistened in 500 ml of NaClO for 30 minutes at 15°C, Germination: darkness, 28°C for 24 and 72 h. Quinoa protein isolate (QPI): extraction at pH 8.0 followed by isoelectric precipitation at pH 4.5. Simulated in vitro hydrolysis of germinated and non-germinated QPI samples: gastric and duodenal phase using pepsin and pancreatin enzymes, respectively. 	<ul style="list-style-type: none"> Germination percentage. Percentage protein content of QPI of germinated and non-germinated seeds by BCA method. DPPH, ABTS and ORAC method. 	<ul style="list-style-type: none"> White, red and black quinoa (<i>Chenopodium quinoa</i> Willd. var. Real). 	Piñuel et al. (2019)
To evaluate the antioxidant activity of protein isolates from germinated seeds of white, red and black quinoa (<i>Chenopodium quinoa</i> Willd.) in zebra larvae (<i>Danio rerio</i>).	<ul style="list-style-type: none"> 30 g quinoa seeds: wetted with 0.1% NaClO solution for half an hour at room temperature, rinsed with distilled water to neutral pH and wetted with distilled water for 6 h with shaking every half hour. Germination: 90% relative humidity and with a temperature and time between 12-28°C and 12-72 h, respectively. 	<ul style="list-style-type: none"> GABA content by high performance liquid chromatography (RPHPLC). Total phenol content (TPC). Phenolic compounds. Antioxidant activity (AA) by ORAC method. 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd var. INIA-415 Pasankalla. 	Paucar-Menacho et al. (2018)
Using response surface methodology (RSM) to find out the best germination conditions to maximize phenolic, GABA content and antioxidant activity of quinoa seeds.	<ul style="list-style-type: none"> Germination: 90% relative humidity and with a temperature and time between 12-28°C and 12-72 h, respectively. 	<ul style="list-style-type: none"> Antioxidant activity (AA) by ORAC method. 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd var. INIA-415 Pasankalla. 	Paucar-Menacho et al. (2018)

(Continued)

Table 2. (Continued)

Objectives of the study	Conditions of germination	Parameters analyzed	Ecotypes used	References
To study changes in the nutritional composition of quinoa seed flour subjected to germination.	<ul style="list-style-type: none"> Wash seeds with tap water to remove saponins, soak in tap water for 6 h at room temperature. Germination: at 22-24°C, 80-90% relative humidity and darkness until 1.00-1.50 cm radicle size (24 h). Drying at 50°C and grinding. 	<ul style="list-style-type: none"> Germination capacity Moisture, fat, ash, total nitrogen, total protein and protein digestibility, total carbohydrates, dietary fiber (total, soluble and insoluble), starch (resistant, total and digestible), reducing and total sugars, amylose and thermal behavior. Daily seed counts and final germination after 5 days. Total phenol content (TPC) and flavonoid content (TFC). 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> var. C1ca. <i>Chenopodium quinoa</i> var. Kamiri. <i>Chenopodium quinoa</i> var. Inga Pirca. 	Jiménez et al. (2019)
To assess the antioxidant capacity of quinoa seeds during germination.	<ul style="list-style-type: none"> Conventional and hydroponic method. Germination: darkness at 25°C, until radicle size > 4 mm 	<ul style="list-style-type: none"> Determination of L (brightness), a (red-green), b (yellow-blue) and texture (elasticity, cohesiveness, chewiness and gumminess). Appearance, color and odor. Ethanol and acetaldehyde content. Total phenolic content (TPC) and antioxidant activity (AA). 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. 	Vrancheva et al. (2020)
To evaluate the chemical and sensory properties of quinoa seeds after 4 days of germination and quality in passive modified atmosphere (MAP) packaging and storage.	<ul style="list-style-type: none"> Seeds soaked in tap water for 3 hours. Germination: 20°C, 70% humidity and photoperiod of 24 h and 4 days. 25 g fresh sprouts packed in microperforated polypropylene (PP) bags at 5°C, 7 days in passive MAP. 	<ul style="list-style-type: none"> Determination of L (brightness), a (red-green), b (yellow-blue) and texture (elasticity, cohesiveness, chewiness and gumminess). Ethanol and acetaldehyde content. Total phenolic content (TPC) and antioxidant activity (AA). Catabolic profiling at community level (Shannon diversity index, richness and evenness of substrates), enumeration, isolation and identification of LAB and yeasts. Chemical characterization of raw and germinated flours (protein, starch, fat, ash, total free carbohydrates, total dietary fiber and total phenolic content). 	<ul style="list-style-type: none"> Chilean variety (<i>Chenopodium quinoa</i> Willd. var. Regalona Baer). Bolivian var. (<i>Chenopodium quinoa</i> Willd. var. Real). 	D'ambrosio et al. (2017)
To investigate the effects of germination on lactic acid bacteria and yeasts of quinoa, barley, wheat, chickpea and lentil seeds.	<ul style="list-style-type: none"> Seeds were germinated based on the protocol of Donkor et al. (Perri et al. 2020). 	<ul style="list-style-type: none"> Catabolic profiling at community level (Shannon diversity index, richness and evenness of substrates), enumeration, isolation and identification of LAB and yeasts. Chemical characterization of raw and germinated flours (protein, starch, fat, ash, total free carbohydrates, total dietary fiber and total phenolic content). Metabolites, total saponin content (TSC), total soluble protein (TSP). 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. 	Perri et al. (2020)
To find the differences between the three ecotypes of quinoa through their metabolomic profile, to identify each ecotype after a washing, cooking and germination process and to identify the metabolites that make it possible to assess the consequences of each treatment on nutritional values	<ul style="list-style-type: none"> Seeds grown and harvested under the same conditions. Five treatments (control (C), washing (W), cooking (CK), washing with cooking (WCK) and germination (G)). Five groups: group C, group W, group Ck, group WCK and group G. Group G: washed seeds, stratified at 4°C for 24 h and germination at 10°C, >10,000 lx in a 16 and 8 h day-night cycle for 3 days. Nuclear magnetic resonance (NMR) analysis. 	<ul style="list-style-type: none"> Metabolites, total saponin content (TSC), total soluble protein (TSP). 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> var. Chauca. <i>Chenopodium quinoa</i> var. Tunkahuan. <i>Chenopodium quinoa</i> var. "Pata de Venado". 	Lalaleo et al. (2020)

Likewise, Zlotek et al. (2019) studied how germination and subsequent drying affect phenolic and antioxidant composition in Bolivian red quinoa (*Chenopodium quinoa*) and Chilean white quinoa (*Chenopodium quinoa* var. Regalona) seeds. The seeds were germinated in darkness for 96 h and dried at 30, 45 and 60 °C until a relative humidity of 12% was achieved. Red quinoa has a higher concentration of phenolic compounds (including ferulic acid) than white quinoa. In both cases, germination increases the content of flavonoids and total phenolic compounds, mainly vanillic acid and ferulic acid. The subsequent drying process barely modifies the antioxidant values, so germination could be used for the manufacture of functional nutrients.

Similar results were obtained by Vrancheva et al. (2020) where they assessed the antioxidant capacity of quinoa seeds *Chenopodium quinoa* Willd during germination and compared them with different grains (chia, common oats, proso millet, amaranth, buckwheat, flaxseed and einkorn). Quinoa was the one with the highest content of total flavonoids and total phenolic compounds, with ferulic acid being the major compound.

Piñuel et al. (2019) used protein isolates by isoelectric precipitation from germinated seeds of white, red and black quinoa (*Chenopodium quinoa* Willd. Var. Real) to evaluate antioxidant activity and the ability to inhibit reactive oxygen species (ROS) in zebra (*Danio rerio*) larvae. The antioxidant activity is affected both by the germination time and by the seed variety used. The use of germinated quinoa and germinated quinoa protein isolates could be used for the manufacture of foods with high antioxidant and protein concentration in view of the growing demand by consumers for this type of products.

Paucar-Menacho et al. (2018) used response surface methodology (RSM) to determinate the best germination conditions that maximally increase phenolic, GABA content and antioxidant activity of quinoa seeds. They germinated seeds of *Chenopodium quinoa* Willd var. INIA-415 Pasankalla at 90% relative humidity and temperature and time between 12–28 °C and 12–72 h, respectively. After analysis, a significant increase in GABA, total phenolic compounds and antioxidant activity was obtained. Within polyphenols, both flavonoid, non-flavonoid and total compounds increased, with quercetin glucuronide and kaempferol dirhamnosyl-galactopyranose being the majority phenols. With these data, Paucar-Menacho et al. (Paucar-Menacho et al. 2018), concluded that the most favorable conditions for increasing GABA content, total phenolic compounds and antioxidant activity are 20 °C and 42 h of germination time. The increase in antioxidant activity is due to the increase in vitamin C that originates during germination. As well as the increase in phenolic compounds due to the accumulation of phenolic compounds, the release of phenolic compounds that were bound to the cell wall and the neosynthesis by the pathway of phenylpropanoids.

In their work Jimenez, Lobo, and Sammán (2019) evaluated the modifications in the nutritional composition in the seed flour of three quinoa varieties (*Chenopodium quinoa* var. Cica, *Chenopodium quinoa* var. Kamiri and *Chenopodium quinoa* var. Inga Pirca) subjected to

germination. The seeds were germinated at 22–24 °C, 80–90% relative humidity and darkness until radicle size of 1.00–1.50 cm (24h), dried at 50 °C and ground to obtain flour. After the study, the following results were obtained:

- Increase in protein content, except in Inga Pirca.
- Increase in total sugar content, protein digestibility percentage and significant decrease in starch content (total, resistant and digestible).
- Increase in total fiber and insoluble fiber in all varieties.
- Significant increase in the content of reducing sugars, with the highest in the Kamiri variety.
- There were no significant changes in gelatinization temperature, but there was a significant decrease in gelatinization enthalpy, with the highest in the Cica variety. Significant increase in the percentage of retrogradation, with the highest in the Kamiri variety.

From the study of Jimenez, Lobo, and Sammán (2019), it is concluded that germination increases the protein concentration, protein digestibility and starch retrogradation, ash, total and insoluble fiber, total and reducing sugars, as well as a decrease in gelatinization energy. Consequently, there is an increase in the nutritional value of the seeds and structural and functional changes due to the proteolytic activity, which can produce modifications in the technological, functional, rheological and/or sensory properties when these germinated seed flours are used in the manufacturing of foodstuffs.

These flours have been used for the production of pasta products (Table 3) and their properties have been evaluated in the developed foods.

Demir and Bilgiçli (2020) studied how to improve the quality of noodles with the addition of germinated black quinoa powder by improving the functional, nutritional and organoleptic properties by adding different proportions of raw quinoa flour (RQF) and germinated quinoa flour (GQF). In the study, they observed that the germinated quinoa flour increased the ash content (due to a decrease in carbohydrates), crude protein (due to amino acid neosynthesis), total phenolic compounds, antioxidant activity and all minerals, with the highest increase of calcium (88.3%), potassium and phosphorus, as well as a reduction in crude fat content (possible source of energy for seed growth) and phytic acid. During germination, there is an increase in the activity of enzymes such as endogenous esterases and the enzyme phytase. Due to the action of the former, new phenolic compounds are synthesized. Phytase, by hydrolyzing phytates, decreases phytic acid and increases the bioavailability of minerals. However, the concentration of quinoa played a role in the quality of the pasta. The translucent bright yellow color, a sign of pasta quality, decreases in pasta with a higher proportion of quinoa flour. The parameters of water absorption, volume and cooking loss also increase as the proportion of germinated quinoa flour (GQF) increases, reaching maximum in the sample with 30% of this flour. Therefore, increasing the proportion of germinated

Table 3. Objectives, conditions of germination, parameters analyzed and varieties used in quinoa flour-based products in the studies on germination.

Objectives of the study	Conditions for germination	Parameters analyzed	Ecotypes used	References
Improve the quality of noodles with addition of germinated black quinoa powder by improving the functional, nutritional and organoleptic properties by adding different proportions of raw quinoa flour (RQF) and germinated quinoa flour (GQF).	<ul style="list-style-type: none"> Seeds washed and disinfected with 2.5% NaClO solution for 10 minutes. Raw quinoa flour (RQF): dried and ground seeds. Germinated quinoa flour (GQF): seeds soaked in water for 3 hours. Germination 2 days at 20 °C and 80-90% humidity. Oven-dried at 45 °C and milled. Control dough: 100% wheat semolina in a 100:30 semolina:water ratio. RQF and GQF samples: substitution of 0, 10, 20 and 30% (w/w) wheat semolina. 	<ul style="list-style-type: none"> Values of ash, crude protein and fat, phytic acid, total phenolic content (TPC) and antioxidant activity (AA) in wheat semolina, RQF, GQF and pasta samples. Physical parameters: color of raw materials and samples (L (brightness), a (red-green) and b (yellow-blue)), firmness, cooking properties (water absorption (WU), volume increase (VI) and cooking loss (CL)) of the samples. Organoleptic parameters (odor, flavor, appearance and approval). 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. 	Demir and Bilgiçli (2020)
To find the level of enrichment with germinated quinoa to achieve maximum productivity in bread making and then compare it with pearled quinoa (PQ) to estimate its usefulness in the bread making process.	<ul style="list-style-type: none"> Germination: at 22 °C for 48h. Drying: at 55 °C for 6h. Pulverization of germinated and pearled seeds to size < 250 µm. Commercial wheat flour (WF) with 123 mg/g db protein. 5 mixtures: 10:90, 20:80 and 30:70 of SQ:WF (sprouted quinoa:wheat flour), 100% WF and 20:80 of PQ:WF. Once the different breads had been made, they were examined at 2, 24 and 72 h after baking. 	<ul style="list-style-type: none"> Sticking, gluten aggregation, mixing and fermentation properties. Color and texture and firmness of crumb, electronic tongue. 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. var. Titicaca. 	Suárez-Estrella et al. (2020)
To evaluate the nutritional and functional composition of gluten-free (GF) pasta with the addition of raw and sprouted quinoa flour (QF).	<ul style="list-style-type: none"> Germination: 20 °C for 48 h, wetting every 12 h. GF paste: drying at 45 °C to 10% moisture and milling to size < 500 µm, obtaining raw and germinated QF. Control GF sample: with rice (RS) and maize semolina (CS) in proportion (50:50), 400 ml water and 3% guar gum. Other samples: with RS:CS (50:50) plus raw/sprouted QF at 10, 20 and 30%, 400-436 ml water and 30 g guar gum. 	<ul style="list-style-type: none"> Parameter L (brightness), a (red-green) and b (yellow-blue) Ash, phytic acid, fat and crude protein content of both raw materials used and samples. Total phenolic compounds (TPC), minerals (Ca, Fe, K, Mg, P and Zn). Water absorption (WU), volume increase (VI), loss of solids (CL). Organoleptic characteristics (taste, odor, appearance and general acceptability), firmness and texture of the different samples. 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. 	Demir and Bilgiçli (2021)

quinoa flour results in pasta of lower technological quality and lower firmness.

Suárez-Estrella et al. (2020b) used germinated and pearled seeds of *Chenopodium quinoa* Willd. var. Titicaca in their study with the aim of finding the level of enrichment with germinated quinoa to achieve maximum productivity in bread making and then comparing it with pearled quinoa (PQ), thus estimating its usefulness in this process. They germinated the seeds at 22 °C for 48h, dried them at 55 °C for 6 h and then pulverized them milled to obtain the flour. The addition of sprouted flour resulted in a significant decrease in the breakage and viscosity values after cooling. As the concentration of sprouted flour increased, there was a reduction in the gluten aggregation time due to a decrease in gluten. A higher proportion of sprouted flour led to an increase in the degree of softening and gas production, decreasing the dough's retention capacity. A comparison of

bread with sprouted flour versus pearl flour showed an increase in water absorption and softening, as well as a decrease in development time and stability in the sample. The color of the crumb is redder, yellower and softer in the sample with sprouted flour. Sprouted quinoa flour in bread produces a softer crumb by decreasing starch retrogradation. In addition, the inclusion of this flour reduces rancidity, which can be useful for bread making by increasing the shelf life of the product. Therefore, in this study it was concluded that the use of germinated quinoa can be an alternative with greater potential than pearl in the production of bread and derived products with a higher fiber and protein with high biological value.

In another research by Demir and Bilgiçli (2021), they evaluated the nutritional and functional composition of gluten-free (GF) pasta when raw and germinated quinoa flour (QF) was added. They used *Chenopodium quinoa*

Willd. seeds that were germinated at 20 °C for 48 h, wetting them every 12 h. The germinated versus raw quinoa flour paste had higher mineral content (calcium, iron, potassium, magnesium, phosphorus and zinc), ash, total protein, total phenolic compounds and antioxidant activity, as well as lower fat and phytic acid content. Additionally, from an organoleptic point of view, the sample with 10% sprouted quinoa flour had better taste, aroma, appearance and acceptability. In this study, it was found that germinated quinoa flour increases the nutritional properties of gluten-free pasta. Increases in fat, protein, total phenolic compounds, antioxidant activity and mineral content were directly proportional to the percentage of sprouted quinoa flour. Although this leads to a decrease in yellow color and brightness and an increase in reddish color, the latter due to the increase in phenolic compounds during germination. There is also an increase in firmness and loss of solids which may affect in terms of consumer acceptability and technological properties of the pulp, respectively.

Different seed varieties, light, temperature, humidity and germination time are determining factors in the nutritional quality of sprouts (D'ambrosio et al. 2017). Ready-to-eat sprouts have a short shelf life and can easily deteriorate if improperly stored. They may suffer from oxidation reactions, loss of their sensory characteristics or nutritional value. As a result, strategies such as post-packaging and cold storage can increase their shelf life.

Among the different types of packaging, modified atmosphere packaging (active or passive) may be suitable for increasing the shelf-life of quinoa germinated sprouts. The advantages of modified atmosphere packaging (MAP) include reduced respiration, enzymatic activities and ethylene production (D'ambrosio et al. 2017). In MAP packaging, it is important to take into account factors such as product respiration, temperature, fill volume and weight, film surface area and permeability.

In their study D'ambrosio et al. (2017) evaluated the chemical and sensory properties of two quinoa seed varieties (Var. chilena (*Chenopodium quinoa* Willd. var. Regalona Baer) and Var. boliviana (*Chenopodium quinoa* Willd. var. Real)) after 4 days of germination and quality in passive modified atmosphere packaging and storage. Seeds were germinated at 20 °C, 70% humidity and photoperiod of 24 h and 4 days and 25 g of fresh sprouts were packed in micro-perforated polypropylene (PP) bags for 7 days at 5 °C in passive MAP. The Regalona Baer variety has a higher germination power, total phenol content, antioxidant activity, acetaldehyde and ethanol content. In both cases, passive MAP packaging decreased oxygen levels and increased carbon dioxide levels. The Real variety had more unpleasant odors than the Regalona Baer variety.

In addition to producing changes such as an increase in proteins, bioactive compounds or a reduction in anti-nutritional factors, among others, germination can also lead to changes in the microbiota (lactic acid bacteria (LAB) and yeasts). Perri et al. (2020) investigated the effects of germination on lactic acid bacteria and yeasts in quinoa (*Chenopodium quinoa* Willd.), barley, wheat, chickpea and lentil seeds. There was a lower number of LAB but a greater

variety of strains in the germinated flour than in the raw quinoa flour. Yeast (*Clavispora lusitaniae* and *Debaryomyces*) did increase in the germinated flour. It was also observed that germination increases the use of carbohydrates and nitrogen as energy sources, as well as the content of total phenols and insoluble dietary fiber. The metabolic profile of the flour varies depending on the changes in the microbiota. Furthermore, there is a correspondence between germination time and increases in both nutritional composition and microbiota composition. This may have both technological and sensory implications for germinated quinoa flour and its application in baking.

Metabolomics makes it possible to determine and quantify the metabolites that are present in a plant or plant organ and to know their energetic, oxidative, reproductive and anabolic status at a given time. The nuclear magnetic resonance (NMR) analytical platform provides different perspectives for targeted and untargeted metabolic fingerprinting studies. These techniques are used to recognize differential biomarkers of different species grown in different regions. In particular, the 1H-NMR technique allows the detection of secondary metabolites (flavonoids, saponins, etc.) and primary metabolites (amino acids, organic acids, etc.) simultaneously. In their study Lalaleo et al. (2020) used three ecotypes produced and consumed in Ecuador: *Chenopodium quinoa* var. Chaucha, *Chenopodium quinoa* var. Tunkahuan and *Chenopodium quinoa* var. "Pata de Venado". The results obtained show significant differences in metabolites between the germinated seed group and the rest of the non-germinated groups (control, washed, cooked, washed with cooking), but not between the different ecotypes. It was found that 30 metabolites were detected, including 12 amino acids, 8 organic acids, saccharides, nucleosides and choline. The sample with the highest amount of metabolites was the germinated quinoa. Regarding these results, Lalaleo et al. (2020) conclude that the increases in nutritional values observed during germination correlate with increased bioavailability of macro- and micronutrients, mainly due to increased enzyme activity during germination, decreased saponins and phytic acid, and a decrease of saponins and phytic acid.

Malting

The malting process involves a first germination stage followed by a kilning or drying stage, in order to inactivate the enzymatic processes and increase the stability and storability of the dried product. Sometimes there might be a third roasting stage to give the product the optimal sensory characteristics (color, flavor, aroma, etc.). Some authors use germination and malting interchangeably, although germination itself comprises only imbibition until radicle elongation.

Table 4 lists the objectives, conditions of germination and drying, parameters analyzed, quinoa ecotypes used in each study and the reference of the different studies used to evaluate the effects of malting on the nutritional characteristics of quinoa seeds.

Table 4. Objectives of the study, conditions of germination and malting, parameters analyzed, ecotypes used and references.

Objectives of the study	Conditions for germination and malting	Parameters analyzed	Ecotypes used	References
To assess the effect of malting quinoa seeds on phenolic content and antioxidant activity, as well as the conditions suitable for increasing antioxidant activity.	<ul style="list-style-type: none"> Methodology of response surface methodology (RSM): germination time (10, 20 and 30°C), soaking degree (36, 40 and 44%) and germination time (24, 48 and 72 h). Germination: darkness, constant temperature, 80-90% relative humidity and radicle > 2 mm. Malting: 100 g seeds, sterilization with 2.5% NaClO for 5 min, washing with distilled water to neutral pH, soaking to 36% moisture and germination in darkness, 23°C for 72 h. Baking of sprouts: 50°C for 24 h obtaining quinoa green malt (G). Roasting: 20 g of G at 100, 145 and 190°C for half an hour. 	<ul style="list-style-type: none"> Daily seed count, final percentage of germinated seeds and vigor index (VI or germination speed). Raw and malted quinoa: soluble and total phenolic compounds (TPC), total flavonoid content (TFC), antioxidant activity (AA), reducing power, phenolic acid and flavonoid identification, Maillard reaction products (MRP), fluorescent intermediate compounds (FIC) and melanoidins (MRPs). 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. 	Carciochi et al. (2016a)
To determine the effect of malting on folate in quinoa, amaranth and buckwheat seeds.	<ul style="list-style-type: none"> 50 g seeds soaked in distilled water (1:10) 1:10 at 30°C and two days in darkness. Germination: darkness, 23°C and 48 h. Drying: 42°C to humidity < 5% for 10 h and grinding. 	<ul style="list-style-type: none"> Total folate (TF) content by α-amylase treatment, protease and rat serum and by ultra high pressure liquid chromatograph mass spectrometry (UPLC-MS/MS) and folate retention (%TR). 	<ul style="list-style-type: none"> <i>White quinoa (Chenopodium quinoa)</i> 	Motta et al. (2017)
To assess the effects on the nutritional composition of malting on the seeds of 3 Peruvian quinoa varieties.	<ul style="list-style-type: none"> Malting: same procedure as Carciochi et al. study (Carciochi et al. 2016b) with some variations. Saponin-free seeds: washed, sterilized with 2.5% sodium hypochlorite for 5 min and soaked in distilled water in 2:3 ratio (seed:water) at 25°C for 4 h with 45-55% humidity. Germination: 25°C for 48 h, wetted with water every 8 h for 100% humidity. Drying: 55°C for 24 h to 5.8% moisture when radicle between 7-10 mm. Removal of radicles by hand and grinding to 0.25 mm. 	<ul style="list-style-type: none"> Total phenolic compounds (TPC), total flavonoids (TFC), antioxidant activity (AA), ash, protein, total fat, reducing sugars and vitamin C. 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. var. Inia Salcedo <i>Chenopodium quinoa</i> Willd. var. Red Pasankalla <i>Chenopodium quinoa</i> Willd. var. Black Collana 	Aguilar et al. (2019)
To investigate the effects on the technological, nutritional and sensory properties of gluten-free muffins to which different amounts of whole quinoa flour (WQF) and malted quinoa flour (MQF) have been added.	<ul style="list-style-type: none"> Whole quinoa flour (WQF): washing, drying at 11% moisture content and milling to a particle size of 0.25 mm. Malted quinoa flour (MQF): wetting at 20°C for 2 h, germination at 25°C for 24 and 72 h and milling to 0.25 mm particle size. Muffin or cupcake doughs: control (F0 = 100% rice flour (RF)), RF:WQF (F1 = 70:30), RF:MQF24 (F2 = 70:30) and RF:MQF72 (F3 = 70:30). 	<ul style="list-style-type: none"> Ash content, total fat, total protein, carbohydrate and amino acid profile. Size, texture (firmness, texture, crumb elasticity, crumb and crust color (parameters L (brightness), a (red-green) and b (yellow-blue)). Sensory properties of the muffins 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. 	Miranda-Villa et al. (2019)
To analyze how germination and malting affect the mineral and polyphenol content, anti-nutritional factors and Maillard reaction products (MRP) of malted quinoa flour	<ul style="list-style-type: none"> Seeds: disinfection with 2.5% NaClO for 5 minutes. Soaked in 2:3 deionized water, 4 h at 25°C. Germination: darkness, 95-100% humidity, 24°C for 24, 48, 72 and 96 h. Drying: in oven, 50°C and 24 h. Milling of germinated and malted grains: size < 250 μm 	<ul style="list-style-type: none"> Ash, protein, moisture and reducing sugars. Minerals, saponins, phytic acid and tannins. Antioxidant activity (AA), total phenolic compounds (TPC) and total flavonoid content (TFC). High performance liquid chromatography (HPLC). Maillard reaction products (MRP), available lysine, fluorescent intermediate compounds (FIC), FAST index and browning index (BI) 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. var. white. <i>Chenopodium quinoa</i> Willd. var. black. 	Bhinder et al. (2021)
To compare how boiling, steaming and malting affect amino acid content	<ul style="list-style-type: none"> 50 g seeds soaked in distilled water (1:10) at 30°C and 48 h in darkness. Germination: darkness, 23°C and 24 h. Drying: 42°C to humidity < 5% for 10 h and grinding. 	<ul style="list-style-type: none"> Moisture, protein and amino acids. Amino acid score (AAS), essential amino acid index (EAAI) and actual retention (%TR). 	<ul style="list-style-type: none"> <i>Chenopodium quinoa</i> Willd. 	Motta et al. (2019)

The assessment of the effect of malting quinoa (*Chenopodium quinoa* Willd.) seeds on phenolic content and antioxidant activity, as well as the conditions suitable for increasing antioxidant activity were the subject of the study by Carciochi et al. (2016c). For this purpose, the response surface methodology (RSM) was used for suitable germination conditions: germination time (10, 20 and 30°C), soaking degree (36, 40 and 44%) and germination time (24, 48 and 72 h). Seeds were germinated in darkness, at constant temperature, 80-90% relative humidity and radicle > 2 mm. To malt the seeds, they germinated them first in darkness at 23°C for 72 h and baked the sprouts at 50°C for 24 h to obtain green quinoa malt (G). Finally, they roasted the quinoa green malt at 100, 145 and 190°C for half an hour. These conditions are the same as in a previous study with raw quinoa seeds (Carciochi et al. 2016b). These authors showed that the factors that most affect the germination percentage are germination temperature and the degree of soaking. More precisely, the temperature of 23°C is where both the germination percentage and speed of germination reach their maximum. On the other hand, at 145°C the highest level of total phenolic compounds, antioxidant activity, reducing power, fluorescent intermediate compounds and melanoidins was reached. This reaction is also favored by the increase in temperature during malting. Roasting green quinoa malt results in a significant increase in quinoa antioxidants and can therefore be used to enrich gluten-free products and beverages.

Motta et al. (2017) studied the effect of malting on folate in quinoa, amaranth and buckwheat seeds. White quinoa (*Chenopodium quinoa*) seeds were germinated in darkness at 23°C for 48 h, dried at 42°C to below 5% humidity for 10 h and milled. There was a decrease in folic acid (FA), 5-methyltetrahydrofolate (5-MTHF) and total folate (TF) values and a slight increase in the 10-formyltetrahydrofolate (10-CHOTHF) value of malted quinoa compared to raw quinoa. In the case of quinoa, the malting technique produces a decrease in values except for the 10-CHOTHF value, in contrast to the other pseudocereals.

In their study Aguilar et al. (2019) analyzed the effects on the nutritional composition of malting on the seeds of 3 Peruvian quinoa varieties (*Chenopodium quinoa* Willd. var. Inia Salcedo, *Chenopodium quinoa* Willd. var. Red Pasankalla and *Chenopodium quinoa* Willd. var. Black Collana). Seeds were germinated at 25°C for 48 h, moistened with water every 8 h to have 100% moisture, dried at 55°C for 24 h to 5.8% moisture when radicle between 7-10 mm, removed the radicles by hand and ground. Malting was carried out according to the study of Carciochi et al. (2016c) with some variations. According to the results obtained in the malted sample compared to the unmalted sample, the contents of total phenols, total flavonoids, vitamin C and antioxidant activity increased. On the contrary, ash content, total fat content and reducing sugars decreased. The conclusions of the study were that malting affects each of the varieties used differently. The variety with the most advantages in terms of nutritional composition, phenolic compounds and antioxidant activity is Red Pasankalla followed by Black Collana. Black Collana also showed a

significant increase in protein values and could be used alone or in combination with Red Pasankalla (increased reducing sugars, phenolic compounds and vitamin C) in the manufacture of more nutritious foods.

Miranda-Villa et al. (2019) used quinoa (*Chenopodium quinoa* Willd.) seeds to study the effects on the technological, nutritional and sensory properties of gluten-free muffins to which different amounts of whole quinoa flour (WQF) and malted quinoa flour (MQF) have been added. The addition of quinoa flour and malted quinoa increases the protein, mineral and amino acid content of the muffins in the final product. In addition, it increases firmness and chewiness without changing the elasticity, cohesiveness and strength of the crumb. The use of 24 h malted quinoa flour is the closest to the final product without quinoa flour. The sample with this flour has the best color and brightness characteristics of the crust and crumb, being the second sample in the preference ranking test. As the malting time increases, the color of the crumb changes, becoming darker as the amount of phenolic compounds increases during malting as a result of the Maillard reaction. Therefore, the use of this type of flour increases nutritional quality but significantly affects sensory parameters such as odor, flavor, color and texture.

In their work Bhinder et al. (2021) analyzed the changes in mineral content, polyphenol content, anti-nutritional factors and Maillard reaction products (MRP) produced by germination and malting in malted quinoa flour of two varieties (*Chenopodium quinoa* Willd. var. white and *Chenopodium quinoa* Willd. var. black). They germinated the seeds in darkness at 95-100% humidity at 24°C for 24, 48, 72 and 96 h, dried them in an oven at 50°C for 24 h and milled them. In this work they found differences in nutritional content and bioactive profile between the different quinoa flours used. The contents of protein, ash, moisture, reducing sugars, calcium, iron, manganese and available lysine are higher in white quinoa flour (WQ) than in black quinoa flour (BQ). On the contrary, total free flavonoid content (FTFC), total flavonoid and phenol content (TFC and TPC), free, bound and total antioxidant activity (FAA, BAA and TAA) are higher in BQ than in WQ. The malting process increases the content of reducing sugars, flavonoids and phenols (total, bound and free), antioxidant activity (total, free and bound) and fluorescent intermediate compounds (FIC). There is also a decrease in ash content, moisture content, mineral content (calcium, potassium, magnesium and zinc) and anti-nutritional factors (saponins, phytic acid and tannins) as a result of malting.

The aim of the research by Motta et al. (2019) was to compare the amino acid content of pseudocereal seeds by applying different techniques (boiling, steaming and malting). In the case of quinoa, they used *Chenopodium quinoa* Willd. seeds, germinated them in darkness at 23°C for 24 h, dried them at 42°C to less than 5% humidity for 10 h and milled them. Malting produced an increase in protein and in all essential amino acids except cysteine. Also of all non-essential amino acids (alanine, aspartic acid, glutamic acid, serine), with glutamic acid being the most important amino acid during malting.

Fermentation

Lactic acid bacteria (LAB) are interesting from a rheological and technological point of view. It has been shown that the presence of LAB can improve the volume and texture during baking of doughs, increase the fiber content and act as a prebiotic. LAB can act as preservatives by inhibiting different pathogens such as *Listeria*, *Bacillus*, *Aspergillus* and *Penicillium* (Ruiz Rodríguez et al. 2016).

The pH, temperature, humidity and type of feed are factors that affect the activity of the phytase enzyme. The use of techniques such as imbibition, fermentation, germination or malting can also increase it, as well as the use of exogenous phytase of plant or microbial origin (BAL) (Castro-Alba et al. 2019).

Table 5 summarizes the objectives, fermentation and culture conditions, parameters analyzed and LAB used from the studies used in the review.

Carrizo et al. (2016) studied the use of lactic acid bacteria (LAB) from quinoa seeds (QG) and spontaneous sourdough (QSS) as starter cultures in the manufacture of gluten-free fermented products. For sourdough fermentation they used commercial quinoa flours Q1 and Q2 and quinoa seeds (QG) of three varieties (CHQ, RCQ and RHQ). In this work they identified 44 different patterns belonging to *Lactobacillus* strains in both QSS and QG. *L. plantarum* and *P. pentosaceus* stood out in Q1SS and Q2SS, respectively. *L. rhamnosus* CRL 1963 was the strain that produced the highest concentration of riboflavin. While all strains showed phytase activity, with the highest values in *E. mundtii* CRL 2007, *E. casseliflavus* CRL 1988, *Leuc. mesenteroides* CRL 2012 and *L. rhamnosus* CRL 1983. Based on these results, LAB may have potential in the production of gluten-free fermented products enriched in folate and riboflavin, as they are optimal for the preparation of quinoa sourdough starter cultures. *L. rhamnosus* CRL 1963 stands out, as it produces folate and riboflavin and shows both phytase and amyolytic activity.

Ruiz Rodríguez et al. (2016) conducted an investigation on the technological, nutritional and food safety characteristics of the different LAB present in the spontaneous fermentation of quinoa sourdough, which took 10 days to acidify. There was a significant increase of LAB and total mesophiles in the samples until the sixth day, after which they stabilized, reaching their maximum microorganism counts value on the tenth day. *L. plantarum* CRL1905 and *Leuc. mesenteroides* CRL1907 are the strains with the best profile for use as starter cultures. These strains are safe for food use as they have no antibiotic resistance, antimicrobial activity (against *Bacillus* and *Aspergillus*) and acidification power.

Castro-Alba et al. (2019) studied the effect of fermentation and dry roasting on the biochemical composition and organoleptic characteristics of quinoa. They used quinoa flour, quinoa seeds and *L. plantarum* 299v[®]. In all cases there was a decrease in pH and phytate without significant variation with fermentation time. The sample with raw flour fermented for 4h and toasted for 3 minutes at 120°C had the best sensory characteristics. Fermentation and roasting

significantly affect the sensory attributes of the seeds, especially the aftertaste and flavor, which are clearly involved in consumer approval. To improve color and flavor, it is best to roast the seeds first and then ferment. Both roasting and fermentation produce significant decreases in the molar ratios of minerals to phytate (phytate to calcium, zinc and iron), which results in an increase in the bioavailability of these minerals, especially when fermentation is followed by roasting.

In their study Lorusso et al. (2018) evaluated whether the addition of fermented quinoa flour to pasta improves the nutritional and technological characteristics of the pasta. The LAB they inoculated were *L. rossiae* T0A16 and *L. plantarum* T6B10 grown 12h to late exponential phase of growth. An increase in protein, total dietary fiber, ash, total phenol content, lipid content and antioxidant activity was observed in the quinoa pasta and fermented quinoa pasta compared to the control pasta. Besides, there was a significant decrease in the starch content. Therefore, the addition of fermented quinoa flour can further improve the nutritional properties of the pasta, without significantly affecting its rheological characteristics, as it does not modify either the strength or the cohesiveness.

The objectives of the study by Castro-Alba et al. (2019) were to analyze the effects of spontaneous and *L. plantarum* 299v[®] (Lp299v[®]) fermentation on phytate, as well as mineral bioavailability before and after fermentation. There was a decrease in phytate content in the fermented and inoculated seeds and flours. Fermentation with Lp299v[®] results in a more controlled fermentation, increased lactic acid, lower pH and increased phytate degradation. As well as, accessibility and bioavailability of iron, zinc and calcium improved. Quinoa flour fermented with Lp299v[®] has the best values compared to the other two pseudocereals used (canihua and amaranth).

Future challenges and trends

Figure 1 shows a summary infographic with the different mechanisms influencing the variables studied (salinity, germination, malting and fermentation) affecting quinoa quality. Germination, malting, and fermentation are inexpensive and simple processes that produce physical, chemical, functional, technological and organoleptic changes in quinoa seeds and the products made from them.

The future trend for quinoa due to its high nutritional value, genetic variability and adaptability to different climatic conditions should be an increase in the expansion of its crop, improvements in its genome, a decrease in the amount of saponins in its seed coat, as well as improvements in germination, malting and/or fermentation conditions. All of this may serve to obtain a more resistant quinoa with better nutritional properties, as well as contribute to the production of functional foods with suitable organoleptic, sensory and nutritional properties. Therefore, quinoa will be able to diversify the diet and counteract facts such as the growing increase in world population and the demand for animal protein with an increase in vegetable protein. Moreover, its

Table 5. Objectives of the study, conditions of fermentation and cultivation, parameters analyzed, LAB and references.

Objectives of the study	Conditions for fermentation and cultivation	Parameters analyzed	LAB	References
To assess the possible use of LAB as starter cultures for the production of gluten-free fermented products.	<ul style="list-style-type: none"> Sourdough fermentation: commercial quinoa flours Q1 and Q2. 1st fermentation: 100g quinoa flour + 100 ml sterile water + 2g glucose. Incubation 24h at 30°C. Retardation every 24h for 10 days to obtain spontaneous sourdough (QSS): 10% dough first fermented with flour and water. Quinoa seeds (QG): CHQ, RCQ and RHQ varieties. 2.5g seeds + 22.5ml MRS-5 broth incubation 30 minutes at 30°C. Filtering and removal of seeds. Incubation 10 days at 30°C, in anaerobiosis 	<ul style="list-style-type: none"> pH, microbial count and LAB isolation from Q1SS and Q2SS samples at 0, 1, 3, 6, 8 and 10 days. pH, microbial count and LAB isolation from CHQ, RCQ and RHQ seed samples at 0, 2, 7 and 10 days. DNA isolation and identification of LAB. Acidification capacity, amyolytic activity of LAB from QSS and QG. Folate, riboflavin and inorganic phosphate concentration (phytase activity) for identification of folate, riboflavin and phytase producing LAB 	<ul style="list-style-type: none"> <i>L. plantarum</i> in Q1SS. <i>P. pentosaceus</i> in Q2SS. <i>E. gallinarum</i> in CHQ, RCQ and RHQ 	Carrizo et al. (2016)
To investigate the technological, nutritional and safety characteristics of the different LAB present in the spontaneous fermentation of quinoa sourdough.	<ul style="list-style-type: none"> Samples: commercial quinoa flour Yin Yang (QY) and natural Real Hornillos (QR). Mixture of 100 ml sterile water + 100 g flour, with a yield of 200. Mature sourdough: incubation 24 h at 30°C. 10g of mature sourdough in 90ml of sterile water and 100g of flour and incubation 24h at 30°C, repeating 10 days and analysis on days 0 (dough), 2, 3, 6, 8 and 10 (sourdough). Microbiological cultures at 20°C in PCA and MRS-5 medium. Culture of total mesophilic bacteria in aerobiosis and PCA and putative LAB in anaerobiosis. Incubation 48h at 30°C. Acidification and proteolytic activity: sterile flour extract (SFE), LAB isolates in and incubation 72h at 30°C. MRS culture medium 16h at 30°C and cell suspensions inoculated with 2% SFE and incubation 72h at 30°C. Amyolytic activity: spot inoculation of active BAL isolates in MRS starch medium and incubation for 48h at 30°C. GABA production: spot inoculation in MRS medium and incubation 72h at 30°C. 	<ul style="list-style-type: none"> Total mesophilic bacteria and putative LAB count. Genotypic identification by RAPD-PCR and 16S rRNA gene sequence of LAB isolates. Molecular typing by culture-independent PCR-DGGE. Acidification activity: pH measurement at 0, 4, 8, 24, 48 and 72h. Proteolytic activity: spectrophotometrically by OPA (o-phthalaldehyde). Amyolytic activity, rust phenotype. GABA (L-aminobutyric acid) production: pH at 24, 48 and 72h. Antimicrobial activity against <i>Listeria monocytogenes</i> and <i>Bacillus subtilis</i>, antifungal activity against <i>Aspergillus oryzae</i> and <i>Penicillium roqueforti</i> and production of biogenic amines. Antibiotic susceptibility: ampicillin (Amp), vancomycin (Van), chloramphenicol (Chl), gentamicin (Gen), streptomycin (Str), kanamycin (kan), tetracycline (Tet), erythromycin (Ery) and clindamycin (Cln). Moisture, pH and acidity. Mineral content (iron, zinc and calcium), phytate and bioavailability of minerals (phytate(Phy) molar ratios: Phy:Fe, Phy:Ca, Phy:Zn and Phy:Ca:Zn). Lactobacillus count. Organoleptic characteristics (color, aroma, texture and flavor) of fermented quinoa flour roasted before and after fermentation. 	<ul style="list-style-type: none"> <i>L. plantarum</i> and <i>L. brevis</i> are the most abundant within the genus <i>Lactobacillus</i>. <i>L. plantarum</i> CRL1905 and <i>Leuconostoc mesenteroides</i> CRL1907 have potential for use in starter culture 	Ruiz Rodriguez et al. (2016)
To assess the effect of fermentation and dry roasting on the biochemical composition and organoleptic characteristics of quinoa.	<ul style="list-style-type: none"> Quinoa flour and demineralized water (1:2) with <i>L.plantarum</i> 299v fermenting at 30°C for 10 or 4h. Oven drying at 60°C for 4h and milling. Process 1: roasted and ground quinoa seeds fermented with <i>L.plantarum</i> 299v for 10h. Process 2a: quinoa seeds as in process 1 mixed with wheat phytase and fermented for 10 hours. Processes 2b and 2c: activated quinoa phytase, 10 and 50g, respectively. Process 3a and 3b: raw quinoa flour fermented for 10 and 4h, respectively. Dry roasting process: Whole quinoa seeds 5min at 120°C. Grinding to size < 500µm. Quinoa flour 3 minutes at 120°C. BAL cells inoculated at log density. 7.0 cfu/g dough. Quinoa dough: quinoa flour and tap water with a yield of 160. Fermentation: 16h at 30°C. Paste: yield 130, 77g flour + 23g water. Pasta control (WP): 77g wheat semolina + 23g water. Quinoa paste (QP): 61.6g semolina + 15.4g quinoa flour + 23g water. Fermented quinoa paste (FQP): 61.6g semolina + 24.64g fermented quinoa mass + 13.76g water. 	<ul style="list-style-type: none"> Hydration, optimum time (OCT) and cooking loss and water absorption. Total titratable acidity (TTA), ash, protein, peptides and free amino acids (FAA), lipids, total dietary fiber (TDF), total starch, total phenols, antioxidant activity (AA) and moisture. Texture, L (gloss), a (red-green), b (yellow-blue). In vitro protein digestibility (IVPD), total amino acids of the digested protein fraction, protein score (CS), essential amino acid index (EAAI), biological value (BV), protein efficiency ratio (PER), nutritional index (NI), starch hydrolysis index and glycemic index. 	<ul style="list-style-type: none"> <i>Lactobacillus plantarum</i> T6B10. <i>Lactobacillus rossiae</i> T0A16. 	Lorusso et al. (2018)
To assess the effect of spontaneous fermentation and fermentation with <i>Lactobacillus plantarum</i> 299v [®] affects phytate in pseudocereal seeds and flours and the bioavailability of minerals in pseudocereal flours before and after fermentation.	<ul style="list-style-type: none"> Spontaneous fermentation: suspension of grains or flour with demineralized water (1:2) 48h at 30°C. Fermentation with <i>Lactobacillus plantarum</i> 299v[®] (Lp299v[®]): inoculation with 7.35 Log10 CFU Lp299v[®] g⁻¹ DM. Drying 4h at 60°C and grinding to size < 500µm. Phytase activity of Lp299v[®] phytase during fermentation: roasting of quinoa seeds 5min at 120°C, grinding and inoculation with 7.35 Log10 CFU Lp299v[®] g⁻¹ DM and fermentation 10h at 30°C. Fermentation of flours with Lp299v[®]: up to pH ≤ 4, 24h for quinoa and 12h for canihua and amaranth. 	<ul style="list-style-type: none"> Moisture, pH and total acidity. pH, lactic acid, phytate and minerals before and after fermentation. Accessibility and bioavailability of Fe, Zn and Ca. 	<ul style="list-style-type: none"> <i>Lactobacillus plantarum</i> 299v[®]. 	Castro-Alba et al. (2019)

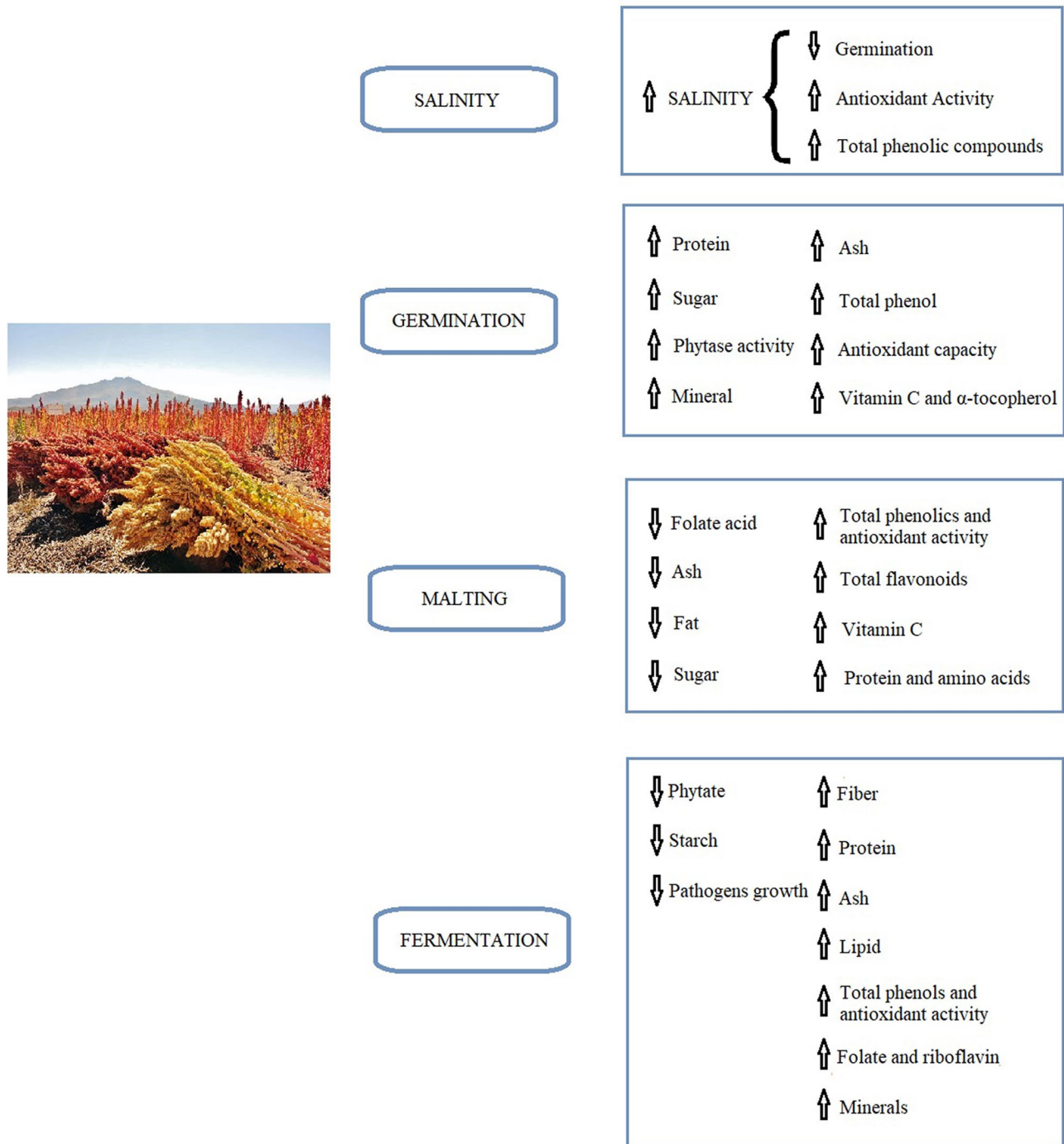


Figure 1. Summary infographic on the effect of salinity, germination and fermentation on quinoa quality.

resilience makes it suitable for climate change. Quinoa, cultivated since ancient times in the Andes region, is spreading to other areas and could become a global staple food due to its excellent nutritional properties; it has a high biological value protein, is gluten-free and has a low glycemic index, which makes it suitable for all kind of people.

Conclusions

Quinoa is a facultative halophilic plant, resistant to various abiotic stresses such as salinity. The higher the salt

concentration, the lower the germination and consequently the lower the yield of the crop. However, in some varieties there was an increase in antioxidant activity and total phenol content. The ecotype is therefore a determinant of resistance to salt stress. The use of pretreatments with NO-donor (SNP), O₂-reactive (H₂O₂) and Ca²⁺-source (CaCl₂) substances reduces the effects of salinity, thus increasing germination and crop yield.

Factors such as germination temperature, humidity, time and ecotype used influence germination. Germination leads to an increase in α -amylase activity. Consequently starch

content decreases and reducing sugar content increases. It also leads to an increase in protein content and total phenols (especially vanillic acid and ferulic acid) due to increased enzyme activity, release of wall-bound compounds and neosynthesis of phenolic compounds and proteins. Ash and mineral content also raise. The latter is due to an increase in phytase activity and a decrease in anti-nutritional compounds, which results in an increase in bioavailability. The use of germinated quinoa flour decreases the phytic acid content, increases the protein and mineral content, ash, total phenols and antioxidant activity. The higher the proportion of sprouted flour used, the higher the content, although in most cases this means a decrease in the quality of the pasta, especially in terms of consumer acceptability.

Malting increases the content of amino acids (especially essential amino acids), proteins, minerals, phenols and total flavonoids. This increase in polyphenolic compounds translates into an increase in the antioxidant activity of quinoa. Factors such as seed color, ecotype and germination time influence the content of protein, saponins and phytic acid. The use of malted quinoa flour in the production of gluten-free products results in increased firmness and chewiness. Further studies would be necessary to find the ideal proportion of malted quinoa flour to be added to improve the nutritional quality of these products without significantly affecting the sensory parameters of the product.

Fermentation with LAB can be suitable for the production of gluten-free fermented functional products enriched in different nutrients. These LAB are used to obtain starter cultures for quinoa sourdough, notably *L. rhamnosus* (produces folate and riboflavin and has phytase and amylolytic activity), *L. plantarum* CRL1905 and *Leuc. mesenteroides* CRL1907. The latter two due to their acidification power, activity against *Aspergillus* and *Bacillus* and no resistance to antibiotics. Fermentation produces modifications in the nutritional and sensory characteristics of the seeds, such as a decrease in phytate and an increase in the bioavailability of minerals. Inoculation with *L. plantarum* 299v[®] has been shown to produce a greater increase than if the fermentation is done spontaneously.

Quinoa undergoing processes such as fermentation, malting and germination can be used in the production of functional products, which are in great demand today by health-conscious consumers. Further studies would be necessary to see how much germinated, malted or fermented quinoa flour should be added to achieve better nutritional properties without losing the technological characteristics of the product, especially in gluten-free products. In addition, conditions of temperature, salt concentration, humidity, quinoa ecotype, etc., are the most suitable to improve the performance of both the crop and the nutritional, functional, technological and sensory characteristics of the quinoa seeds.

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