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Germination as a bio-technological process to enhance the use of quinoa (*Chenopodium quinoa* Willd.) in Cereal-based products

[Scientific field AGR/15]

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Summary

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledonous plant belonging to the *Chenopodiaceae* family and is widespread in Latin America, particularly in South America where this crop had its origin 5000 years ago (González et al., 2015) on the present Peruvian and Bolivian border near Titicaca lake. In ancient times, native South American populations used this grain in their daily diet as their main food. Over the last few years, there has been a global re-evaluation of this crop, in light of numerous traits that make quinoa a sustainable and healthy grain: in fact, the National Academy of Sciences of the United States includes quinoa as one of the best foods of vegetal origin for human consumption (Carrasco & Soto, 2010). Moreover, the 66th session of the General Assembly of the United Nations declared 2013 as the International Year of Quinoa, citing the potentially significant contribution of quinoa in the fight against hunger and malnutrition. Indeed, quinoa is one of the best alternatives to the global need to increase the dietary intake of plant proteins with high nutritional value for greater sustainability, safety and nutritional benefits (Scanlin & Lewis, 2017).

Awareness of the health benefits of quinoa, reflected in the growing number of gluten-free and vegetarian and vegan dieters, might account for the on-going global expansion of quinoa production, up 65% from 2013 to 2014 (FAO; http://faostat3.fao.org/compare/E). Moreover, the last few years have been characterized by a proliferation of research on quinoa from various perspectives (e.g. agriculture, environmental impact, nutrition, food production, etc.). A systematic review of the scientific literature of the last 10 years using “quinoa” as a search term resulted in the identification of about 930 scientific papers (Figure 1A). It is worth mentioning that the number of contributions has doubled in the last five years, highlighting the growing interest in this topic. Almost 50% of the contributions (Figure 1B) fall into the “food science/chemistry/nutrition” categories of research, with about 40% of them dealing with agricultural and agronomic aspects of quinoa. Most of the reviews concern agronomic and nutritional aspects of the “golden grain”, while, others are dedicated to the development of food products, including bread, pasta, snacks and cookies, enriched with quinoa to improve their nutritional properties. However, in South America it’s the whole seed of quinoa that is mainly used and generally cooked like rice to be used in soups, salads, and stews (Valencia-Chamorro, 2016).

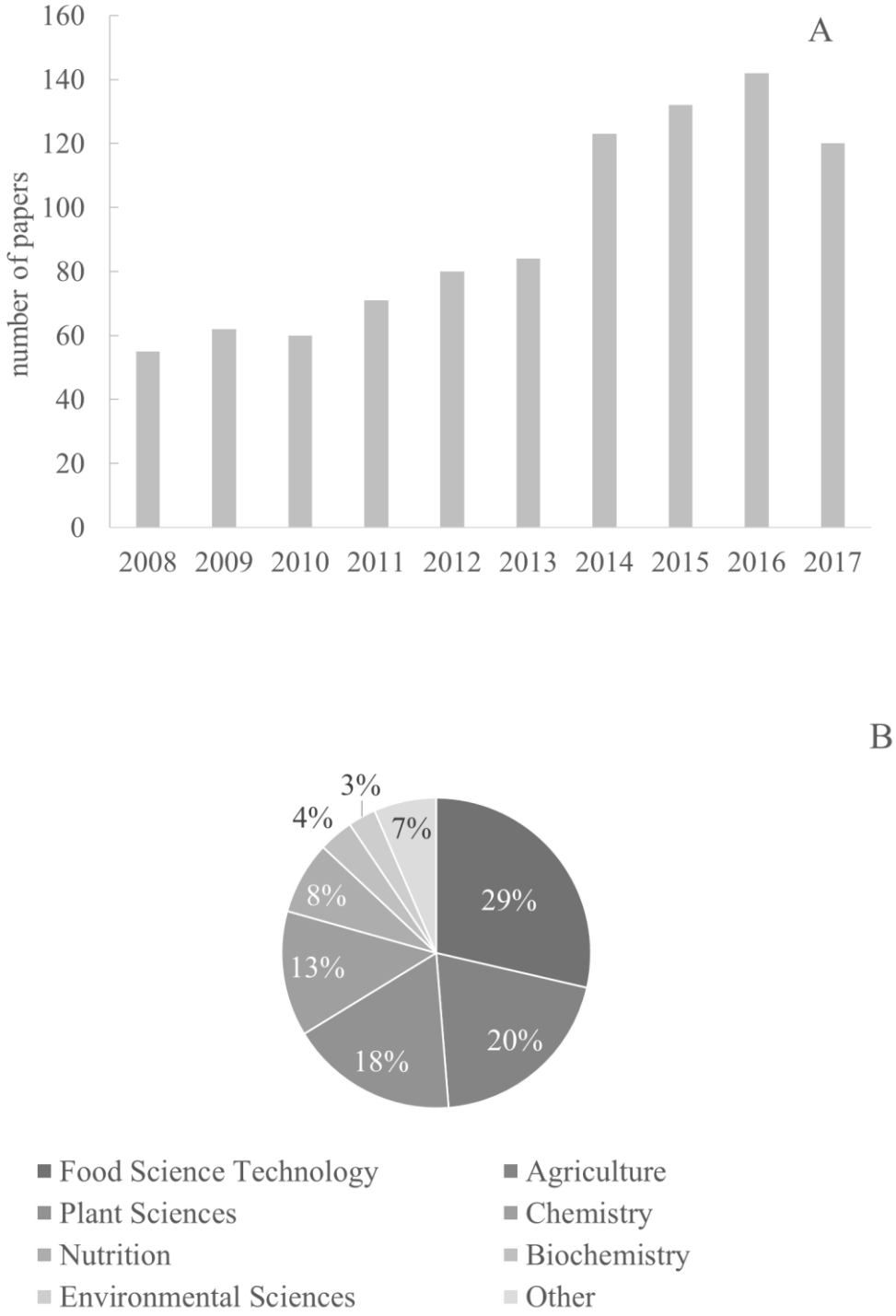

Figure 1. Papers on quinoa (A) and the related distribution in the main research areas (B) (source: Web of Science; 2008-2017; updated to August 31th, 2017).

Producing quinoa-enriched wheat or gluten-free based products alters several quality attributes according to Wang & Zhu (2016). Among these, sensory acceptance is the most critical factor in ensuring the consumption of quinoa and its successful use in food products. In this context, the presence of bitter compounds in quinoa limits its consumption, despite its numerous nutritional benefits. Developing processes to decrease or modify the bitterness of quinoa serve to enhance its

palatability. Such processing involves washing, pearling, and biotechnological treatments. This PhD thesis will propose sprouting as a suitable processing for enhancing both technological and sensory traits of quinoa seeds and quinoa-enriched bread.

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2. Rationale and aims

Awareness of the several agronomic, environmental, and health benefits of quinoa has led to a constant increase in its production and consumption not only in South America - where it is a native crop – but also in Europe and the United States. However, producing wheat or gluten-free based products enriched with quinoa alters some quality characteristics, including sensory acceptance. Several anti-nutritional factors such as saponins are concentrated in the grain pericarp. These bitter and astringent substances may interfere with the digestion and absorption of various food components. Developing processes to decrease or modify the bitterness of quinoa can enhance palatability and thus consumption of quinoa. In addition to the production of sweet varieties of quinoa, other processes have been proposed. Some of them (i.e. washing, pearling and the combination of the two) have a direct effect on saponins, either by solubilisation and/or the mechanical removal of seed layers. Others, such as fermentation, are able to mask the bitterness with aroma compounds and/or sugar formation.

The overall aim of this PhD thesis was to investigate the effects of sprouting on the molecular, functional and sensory properties of quinoa, in order to enhance the use of sprouted seeds as a new ingredient in food formulation, with particular interest in bread-making applications.

Thus, the present thesis has been divided into four main parts. In the first part (section 3.1), a review of the literature has been carried out to highlight the major sources of the undesirable sensory attributes of quinoa and various ways of counteracting the negative characteristics of quinoa and enhance its use in human nutrition. Since in cereals and pulses, sprouting has been proposed as a suitable process to enhance the nutritional and sensory properties of grains, attention was paid to the effects of sprouting on quinoa characteristics. In this context, the second part of the work (section 3.2) focused on the effects of sprouting time on starch and protein features and their impact on the functional properties of quinoa seeds and their related flour. The impact of sprouting time on the sensory traits of quinoa seeds before and after cooking were assessed in section 3.3, in which the decrease in bitterness due to sprouting was related to the potential changes of specific compounds, including saponins. Finally, in section 3.4, sprouted quinoa was used as an ingredient in wheat-based bread in order to improve the technological performance of quinoa-enriched baked products.

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3. Results and Discussion

3.1 Quinoa bitterness: causes and solutions for improving product acceptability

3.1.1 Abstract

Awareness of the several agronomic, environmental, and health benefits of quinoa has led to a constant increase in its production and consumption not only in South America - where it is a native crop – but also in Europe and the United States. However, producing wheat or gluten-free based products enriched with quinoa alters some quality characteristics, including sensory acceptance. Several anti-nutritional factors such as saponins are concentrated in the grain pericarp. These bitter and astringent substances may interfere with the digestion and absorption of various food components. Developing processes to decrease or modify the bitterness of quinoa can enhance palatability and thus its consumption. In addition to the production of sweet varieties of quinoa, other processes have been proposed. Some of them (i.e. washing, pearling and the combination of the two) have a direct effect on saponins, either by solubilisation and/or the mechanical removal of seed layers. Others, such as fermentation or germination, are able to mask the bitterness with aromatic compounds and/or sugar formation. This review presents the major sources of the undesirable sensory attributes of quinoa and various ways of counteracting the negative characteristics of quinoa.

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3.1.2 Introduction

From a botanical and agronomic standpoint, quinoa can be characterized using the terms “biodiversity” and “sustainability”, two keywords of the 21st century denoting qualities that make this crop one of the best alternative and resistant grains with respect to current climate change. Its environmental adaptability and efficient water utilization make it an excellent substitute for traditional cereals, especially in marginal areas (Zurita-Silva et al., 2014; Algosaibi et al., 2017). Despite its mountain origin, research indicates that quinoa can be grown from sea level to altitudes over 4000 meters with large yield ranges (from 0.32 to 9.83 t ha^{-1}) (Bhargava et al., 2007). Moreover, the quinoa

plant is able to grow under stress conditions of temperature (from -5 °C to 38 °C, with optimal temperatures ranging from 15 °C to 20 °C), relative humidity (40% - 88%), drought and water availability (from 50 mm up to 2000 mm/year of precipitation), soil salinity, aridity and pH (from 4.8 to 9.5) (Jacobsen et al., 2003). Quinoa's genetic diversity, its exceptional tolerance to drought and salinity, and the crop's ecological advantages have been extensively reviewed by Ruiz et al., (2014).

Concerning biodiversity, quinoa presents a wide genetic variability in terms of size, color and grain composition. Originally quinoa classification was made according to the color of the plant and fruits, in fact seed color can range from white to grey and black, but varieties exhibiting a yellow, rose, red, purple or violet color are also found; sometimes, with several of them present on the same panicule. Betalains are the most relevant phytochemicals present in quinoa grains and are responsible for their color. They are classified into yellow betaxanthins and violet betacyanins; the joint presence of both types of pigments makes the orange and red shades that coexist in nature with the pure yellow and violet colors. The presence of betalains is correlated with high antioxidant and free radical scavenging activities (Escribano et al., 2017; Abderrahim et al., 2015). Violet, red and yellow quinoa grain extracts show remarkable antioxidant activity in comparison with the white and black. The highest activity was observed in the red-violet varieties containing both betacyanins and betaxanthins, with remarkable activity also in the yellow varieties, where dopaxanthin is a significant constituent (Escribano et al., 2017).

The potential health benefits of quinoa have been extensively reviewed in recent years. It was reported that one serving of quinoa (about 40 g) meets an important part of daily requirements for essential nutrients and health-improving compounds (Graf et al., 2015). In particular, the high amount of lysine – the limiting amino acid in all cereals – makes quinoa unique among grains (Maradini-Filho, 2017a; Mota et al., 2016). It can be used not only as a highly nutritious source of proteins but also as source of minerals and antioxidants, such as phenolic compounds. High dietary fiber and stable polyunsaturated fatty acids increase its potential to treat obesity, hypercholesterolemia and cardiovascular disorders (Tang & Tsao, 2017; Navruz-Varli & Sanlier, 2016). Quinoa is tolerable and acceptable to people with celiac disease and/or gluten intolerance. Indeed, although several varieties (Ayacuchana, Pasankalla, LP-4B and Witulla) have celiac-toxic prolamine epitopes (Zevallos et al., 2012), the maximum amount detected (2.56 mg kg^{-1}) is considerably lower than the level required for gluten-free products (20 mg kg^{-1}) (The European Commission, 2014). Finally, it has been suggested that quinoa could contain a significant amount of rapidly digestible starch fraction (Li et al., 2016), likely due to smaller starch granules (1.2 to 2.66 µm), indicating that careful formulation and processing of quinoa products would be needed for glycemic index management. However, to the best of our knowledge, the available information on enzymatic susceptibility of quinoa starch refers to pure starch or to uncooked samples, neglecting

the role of other components and/or cooking processes on starch hydrolysis kinetics. *In-vivo* studies showed that about one cup of cooked quinoa (or 150 g) has a glycemic index score of 53, which is considered low (Singh et al., 2016). *In-vitro* studies on gluten-free bread demonstrated that quinoa-enriched products had a significantly lower glycemic index than white wheat bread due to its lower content of total available carbohydrates (Wolter et al., 2014a). However, gluten-free bread made with quinoa indicated higher starch digestibility compared to bread from other gluten-free grains (i.e. buckwheat, sorghum and teff) (Wolter et al., 2014a), although these findings need to be confirmed by *in vivo* studies.

3.1.3 Sensory properties and acceptability of quinoa food products

As already mentioned, the boom in gluten-free, vegan and vegetarian diets reflects the increase in quinoa consumption even outside producer countries. In the Occident, quinoa seeds are mainly used as in salads, whereas quinoa flour is mixed with other gluten-free grains for making bread, pasta, and cookies (Wang & Zhu, 2016). The following section will summarize consumer perception of its sensory attributes and consumer acceptance of quinoa-containing foods in the past 10 years.

3.1.3.1 Grains

We know of only one study dealing with the descriptive analysis of quinoa grains (Wu et al., 2017). The results of this study showed a wide range of sensorial characteristics. For example, a grassy aroma or a firm and crunchy texture were considered as positive qualities, whereas attributes such as pasty, sticky and cohesive were negative. Preference seemed to be influenced not only by the sensory properties of the grain but also by the consumer's familiarity with quinoa. Those whose diets consisted of 75 to 100% of organic foods scored significantly higher for all quinoa varieties than those who consumed 0 to 25% (Wu et al., 2017).

3.1.3.2 Bread

Quinoa in varying amounts has mainly been used in bread-making as a partial substitute for wheat or rice flour. Quinoa-enriched bread has typically been prepared using whole quinoa seeds, flakes or flour.

Despite a slightly bitter taste, wheat-based bread with up to 20% of dehulled and washed quinoa seeds were judged to be fully acceptable to the taste, with a very pleasant aroma and flavour (Stikic et al., 2012). These positive results were subsequently confirmed for bread with higher percentages (30 and 40%) of similarly treated quinoa seeds (Demin et al., 2013).

Using quinoa flakes in bread-making has also been investigated and no significant differences were revealed for appearance, colour, texture, flavour, taste, porosity and overall acceptability when up to 20% of quinoa had been added (Gewehr et al., 2017). As regards its aroma profile, a pea-like odour was the outstanding note of the crumb for 100% quinoa bread, unlike the negative results for cooked potato and mould aromas (Hager et al., 2012). Using sourdough fermented with *Lactobacillus plantarum* (Wolter et al., 2014b) or *Weissella cibaria* (Wolter et al., 2014c) did not improve the sensory characteristics of quinoa bread. On the other hand, another study (Rizzello et al., 2016) demonstrated that the addition of 20% quinoa sourdough made with autochthonous selected lactic acid bacteria (*Lactobacillus plantarum* T6B10 and *Lactobacillus rossiae* T0A16) resulted in a wheat bread with improved crust and crumb colour, saltiness, acid flavour and taste, and overall positive taste attributes. Bread with good palatability and overall acceptable taste were obtained with sourdough fermentation of quinoa flour, also when blended with flours from other pseudo-cereals (i.e. amaranth and buckwheat) and pulses (i.e. chickpea) (Coda et al., 2010).

The 6% substitution of wheat flour with quinoa flour for bread was considered acceptable by a group of 103 consumers. Moreover, the quinoa bread demonstrated good commercial potential, since 85% of the participants liked the taste and the potential benefits to their health (Calderelli et al., 2010). Adding texturing ingredients, such as whey, was efficacious in guaranteeing the acceptability of wheat bread fortified with 15% quinoa flour. On the contrary, a 20% addition of quinoa made the bread less acceptable, due to its slight bitterness (Salazar et al., 2017). Another study demonstrated that acceptability significantly decreased for samples with 50 and 100% additions of quinoa flour, even if the quinoa grains were washed before milling (Rosell et al., 2009).

Quinoa flour can also replace rice flour in gluten-free formulations. Substitution levels in the range of 30% - 100% increased acceptability in terms of crust and crumb color and appearance, in comparison with acceptance scores for 100% rice flour reference bread (Burešová et al., 2017). Overall, a substitution level equal to 30% was considered the maximum addition of quinoa flour possible to avoid negative aroma and taste effects (Burešová et al., 2017; Turkut et al., 2016). Conversely, other studies showed that 50% quinoa flour increased crumb softness and cohesiveness of rice-based breads, without adversely affecting sensory properties (Alvarez-Jubete et al., 2010). As expected, the removal of bran components largely decreased bitterness and off-flavour in white quinoa breads, compared to whole quinoa samples (Elgeti et al., 2014). Indeed, as mentioned elsewhere, saponins – which are responsible for quinoa bitterness – are located in the bran. Therefore, only 10% of quinoa bran can be added to rice and corn bran to improve appearance, and reduce crumb firmness (Föste et al., 2014).

3.1.3.3 Pasta

Information regarding the effect of quinoa on the sensory properties of pasta products is scarce. Corn-based pasta with a 10% addition of quinoa flour was moderately liked, so that 70% of consumers declared they would probably or certainly buy the product (Caperuto et al., 2000). A similar quinoa enrichment resulted in a product with lower firmness but similar adhesiveness and bulkiness than the control (100% amaranth) (Chillo et al., 2008). A higher percentage of quinoa (25%) in a gluten-free formulation received lower liking scores than wheat noodles, for the attributes evaluated before (i.e. surface smoothness, appearance, and colour) or after (i.e. taste, odour, colour and overall acceptability) cooking (Bilgiçli et al., 2013). However, the acceptability of the quinoa-based product was high when containing chick-pea or soy flour compared to other gluten-free formulations (Mastromatteo et al., 2012).

3.1.3.4 Cookies

Several studies reported the impact of quinoa on cookie acceptability, however with contrasting results, whether for wheat-based or gluten-free products. As expected, low quinoa enrichment levels (< 10%) did not affect the sensory acceptability of cookies made primarily from wheat flour, but a slightly higher substitution level (15%) reduced flavour, taste and overall acceptability (Watanabe et al., 2014). However, quinoa cookies were still acceptable, and similar results were observed by Pagamunici et al., (2014). In gluten-free formulations, the presence of quinoa positively affected overall acceptance and purchase intention (Brito et al., 2015).

3.1.4 Bitter compounds in quinoa

Various compounds with diverse structures (i.e. amino acids and peptides, esters and lactones, phenols and polyphenols, flavonoids and terpenes) are responsible for bitterness in foods and multiple mechanisms have been described for the perception of bitterness (Drewnowski et al., 2001). The most common bitter compounds in quinoa and the key mechanisms leading to bitterness are summarized in Table 1 and described in the following section.

The bitterness of quinoa has always been associated with the presence of saponins in quantities higher than 1.1 mg g^{-1}, corresponding to the amount proposed by Koziol, (1991) as the threshold for human perception of bitterness. Very little work has focused on the role of polyphenols and other compounds on the bitter taste or aftertaste of quinoa seeds and its products.

Table 1. Hypothesis of key mechanisms leading to bitterness in quinoa (adapted from Heiniö et al., 2016)

Compound	Mechanism	References
Saponins	Molecule properties	Kuljanabhagavad & Wink 2009
Phenolic compounds	Release of unbound flavour-active phenolic compounds	Challacombe et al.,2012 Heiniö et al., 2008 Kobue-Lekalake et al., 2007 Soares et al., 2013
Peptides/aminoacids	Proteolysis of the albumins and proteolysis of globulins forming bitter peptides	Jiang & Peterson, 2013 Brijs et al., 1999 Heiniö et al., 2012

3.1.4.1 Saponins

Saponins are a class of natural compounds produced by some plants for protection against harmful microorganisms, birds and insects (Singh & Kaur, 2018). Saponins are present in legumes (such as soybeans, broad beans, chickpeas, peas, etc.) (Osbourn, 2003; Shi et al., 2004) and some vegetables (as spinach, lettuce, cauliflower, mustard, asparagus) (Negi et al., 2004; Gupta et al., 1988). Regarding grains, only oats (Osbourn, 2003) and quinoa exhibit detectable amounts of saponins (Taylor et al., 2002). In quinoa, these compounds are mainly located in the husk and the quantity therein – which is greatly influenced by the environment, climate conditions and genotype (Zurita-Silva et al., 2014; De Santis et al., 2016a, Jarvis et al., 2017) - varies from 0.01% to about 3% (Quiroga et al., 2013). Indeed, “bitter” varieties (with a saponin content higher than 0.11%), are more resistant to pests than “sweet” varieties (Zurita-Silva et al., 2014; San Martin et al., 2008). The bitter taste is recognizable in samples having an amount of saponin greater than 0.11% (Koziol et al., 1991).

Saponin molecules are characterized by the presence of a non-polar aglycone (or sapogenin), bonded to one or more carbohydrate chains (Kuljanabhagavad & Wink, 2009; Güçlü-Ustündağ et al., 2007). Quinoa contains only triterpene saponins, (Woldemichael & Wink, 2001; Madl et al., 2006; Kuljanabhagavad et al., 2008) which can be classified according to the number of carbohydrate chains linked to aglycone (Kuljanabhagavad et al., 2009). The saccharide chains of saponins assure high hydrophilic properties, whereas the sapogenins (formed only by the triterpene fraction) exhibit lipophilic traits. Hence, the amphiphilic properties of saponins assure high solubility both in polar and non-polar solvents.

Detailed information about the chemical and structural characteristics of quinoa saponins are presented in comprehensive reviews by Güçlü-Ustündağ & Mazza (2007) and Kuljanabhagavad & Wink (2009).

Although the majority of studies report around 20 saponins in quinoa (Kuljanabhagavad et al., 2008), Madl *et al*., (2006) refer to 87 triterpene saponins. More recently, Jarvis et al., (2017) identified 43 different saponins in a variety used as a reference.

Several studies have focused on the chemical and biological properties of saponins (Kuljanabhagavad & Wink, 2009; Güçlü-Ustündağ & Mazza, 2007; Singh et al., 2017), highlighting their complexity and controversial biological role. Indeed, quinoa extracts containing saponins have been exploited in numerous traditional and industrial applications for their foaming and bioactive properties but, usually, saponins in foods have traditionally been considered as anti-nutritional factors, as stated by Güçlü-Ustündağ and Mazza (2007). However, the consequences of long term consumption of saponins for human health are still unknown (Gee et al., 1993). The anti-nutritional properties of saponins have been investigated in several studies (Vega-Gálvez et al., 2010; Maradini-Filho et al., 2017b). The main negative effects associated with consumption of foods rich in saponins are the decrease in mineral and vitamin bioavailability (Southon et al., 1988; Cheeke, 2000), the damage to small intestine mucous cells due to the alteration of their membrane permeability, and the decrease in food conversion efficiency (Gee et al., 1993). The chemical structure of quinoa saponins strictly influences their biological activities (Kuljanabhagavad & Wink, 2009), e.g. the carbohydrate chain attached at C3 of the terpenic fraction is usually critical for both membrane permeabilization and antifungal properties (Kuljanabhagavad & Wink, 2009; Kuljanabhagavad et al., 2008).

Nowadays, saponins are considered bioactive, health-promoting compounds, with many interesting nutritional characteristics as a result of their hypocholesterolemic, analgesic, antiallergic and antioxidant activities (Güçlü-Ustündağ & Mazza, 2007; Kuljanabhagavad et al., 2008). In any case, as already mentioned, the bitter taste associated with saponins greatly limits the use of quinoa as food.

3.1.4.2 Phenolic compounds

Phenolic compounds constitute a group of important components to bitterness in cereal products (Heiniö et al., 2008). Free phenolic compounds are the most flavour-active because they adhere to taste receptors (Heiniö et al., 2008). However, studies on bread and crackers suggest that the bound fraction of phenolic acids may also contribute to taste and flavour properties of wholegrain products (Challacombe et al., 2012). In this context, the authors hypothesized that during mastication the bound phenolic acids might be freed by salivary enzymes, allowing them to interact with taste

receptors and other compounds inside the mouth (Challacombe et al., 2012). Moreover, it has been shown that lower-molecular-weight phenolic compounds tend to be bitter, whereas higher-molecular-weight polymers are more likely to be responsible for food astringency (Noble, 1994). In addition, the impact of free phenolic compounds on flavour is greater than that of bound compounds (Heiniö et al., 2016).

Phenolic compounds are mainly located in the outer layers of the grain, and therefore have a high wholegrain and bran content (Heiniö et al., 2006; Hemalatha et al., 2016; Rocchetti et al., 2017). Various strategies have been proposed to increase the bioaccessibility and bioavailability of phenolic compounds, in baked products because of the health benefits associated with them (Gordillo-Bastidas et al., 2016; Asao & Watanabe 2010; Angelino et al., 2017). A comprehensive review of phytochemicals in quinoa grains and their potential health benefits have been proposed by Tang and Tsao (2017). Quinoa contained lower levels of phenolic acids compared with common cereals like wheat and rye, but they were of the same magnitude (250–600 mg kg^{-1}) as in other cereals (Mattila et al., 2005; Repo-Carrasco-Valencia et al., 2010). The majority of phenolic compounds found in quinoa were phenolic acids consisting of vanillic acid, ferulic acid and their derivatives (132-250 mg kg^{-1}), along with flavonoids quercetin, kaempferol and their glycosides (36.2-144.3 mg kg^{-1}) (Repo-Carrasco-Valencia et al., 2010); also tannins have been reported with concentrations of up to 5.3 g kg^{-1} (Rizzello et al., 2016; Chauhan & Tkachuk, 1992).

The perceived bitterness of rye results from pinoresinol and syringic acid in particular (Heiniö et al., 2008), whereas ferulic acid was identified as the most abundant phenolic acid in wheat bread crust and crumb (Jensen et al., 2011). On the contrary, phenolic compounds responsible for bitter taste have not been adequately determined in whole grain foods (Bin & Peterson, 2016). To the best of our knowledge, no information is available regarding quinoa seeds. Thus, further efforts should be directed to identifying the major phenolic compounds responsible for the bitterness of quinoa seeds.

3.1.4.3 Peptides

Bitter peptides occur to a varying extent after protein hydrolysis (Brijs et al., 1999). Although small molecular weight peptides are deemed responsible for the bitter taste in rye (Heiniö et al., 2012), the amino acid composition of peptides has been considered to be a more important determinant of bitterness than peptide size (Lemieux & Simard, 1992). The role of peptides and amino acids in the perceived flavour of cereal products, including quinoa, remains, however, largely unknown (Heiniö et al., 2016).

3.1.5 Approaches to decrease bitterness in quinoa

Attempts to introduce quinoa as an ingredient in food products all over the world have proved difficult because of the presence of saponins which are responsible for lowering product acceptability due to their bitter taste and/or aftertaste. To this end, several strategies have been proposed to remove saponins or to hide their bitterness. The effects of the main processing together with their advantages and disadvantages are summarized in Table 2 and discussed in the following sections.

Table 2. Approaches to decrease bitterness in quinoa

Approach	Type of effect	Advantages	Disadvantages
Washing	Direct effect: saponin solubilisation from the seed layers	Low investment Efficiency	Drying cost Water contamination Possibility of grain germination
Pearling	Direct effect: Mechanical removal of seed layers which contain saponins	No drying costs No water need and contamination	Limited efficiency Loss in bioactive compounds
Pearling and washing	Direct effect: Mechanical removal of seed layers which contain saponins and saponin solubilisation from the seed layers	Low washing and drying time cost Low water need Low amount of broken seeds High efficiency	Water contamination Loss in bioactive compounds
Fermentation	Indirect effect: masking of bitterness by aroma compounds and sugar formation	Widespread knowledge Side advantages (nutritional, technological and sensory characteristics) No/limited equipment costs	Refreshment required Time-consuming
Germination	Indirect effect: masking of bitterness by sugar formation	Widespread knowledge Side advantages (nutritional, technological and sensory characteristics) No/limited equipment costs	Standardization Possibility of mold growth
Breeding	Direct effect: Development of sweet cultivars	Low environmental impact	Limited number of varieties

3.1.5.1 Washing

Washing is the most common way to remove saponins from the seeds, due to the high water solubility of these compounds. American pre-Hispanic populations, such as the Incas, Cañaris and others used to wash quinoa in rivers and lakes (Ridoult. et al., 1991). Traditionally, in rural areas, washing is done by hand in water - placed in rudimentary tanks (Quispe-Fuentes et al., 2013;

Quiroga et al., 2013) - which sometimes could be alkaline to enhance saponin extraction (Valencia-Chamorro et al., 2016; Quiroga et al., 2013) or in river water. (Quiroga et al., 2013). The large amount of water used and contaminated with saponins constitutes a health hazard for cold-blooded animals (Ruales et al., 1993a) and creates economic and ecological concerns. Moreover, wet seeds need to be dried immediately to inhibit their high germinating power (De Santis et al., 2016a; Repo-Carrasco et al., 2003) as well as mold growth (Pappier et al., 2008).

Washing is also used a commercial scale by using tanks equipped with rotating blades for turbulence washing (Scanlin & Burnett, 2010). Heating treatment in a tunnel completes the drying process.

Quispe-Fuentes *et al.,* (2013) have proposed an efficient, industrial scale mathematical model to reduce cost, energy waste and optimize water flow rate when leaching saponins from quinoa seeds by means of a continuous washing process. Saponins leach out very rapidly at the beginning of the washing process and the total concentration of saponins inside quinoa seeds tend to have an asymptotic value. High temperatures accelerate saponin leaching, in fact leaching at 70 °C was more effective than at 20 °C (Quispe-Fuentes et al., 2013). However, since starch gelatinization begins at 50 ºC for most quinoa varieties (Li et al., 2016), this treatment could cause the quinoa perisperm to swell, thus facilitating embryo separation.

Another consideration is that valuable nutrients including vitamins and minerals may also be lost during these washing procedures (Ruales & Nair, 1993b).

3.1.5.2 Pearling

Dry polishing techniques (i.e. pearling) apply abrasion to separate the external layers and allow the intact seeds to be recovered and processed in successive stages. Pearling is a well-established technology in the processing of covered cereals, such as rice and barley (Dexter & Wood, 1996). Nowadays, pearling is also used on wheat to reduce microbial contamination, as most of the microorganisms present can be found on the surface of the kernel (Bottega et al., 2009). More recently, pearling has proven to be an effective way to recover the phenolic compounds in the external layers of grains (Angelino et al., 2017).

As regards quinoa seeds, the pearling process has been successfully used to decrease the amount of saponins, located in the external layers of the seed (Ridout et al., 1991; Gomez-Caravaca et al., 2014). An abrasion degree of 30% reduced saponin levels by more than 70%, compared with the initial content in whole quinoa, reaching a level below 1.1 mg g^{-1} for several varieties, which is the threshold for the detection of bitterness and astringency in quinoa based products (Koziol, 1991).

Pearling is a more environmental-friendly process compared to washing because no water is needed, no thermal treatment to dry the seeds is required, and no environmental contamination is produced (Gomez-Caravaca et al., 2014). Other advantages of the abrasion process include the reduction of time and energy consumption. Pearled by-products – which comprise from 8% to 12% of the grain weight and contain from 20% to 30% of saponins (Stuardo & San Martín, 2008) - can be used for medical purposes, detergents, and pesticides.

On the other hand, as the degree of abrasion increases, the content of fiber and phenolic compounds decreases (Stikic et al., 2012; Gomez-Caravaca et al., 2014). However, the loss of phenolic compounds in quinoa after pearling is lower than in cereals. Gómez-Caravaca *et al*., (2014) found that after intense pearling (30%) in order to obtain a sweet product, the quantity of freed and bound phenolic compounds decreased by only 21.5% and 35.2%, respectively. Fiber and mineral content, especially calcium, sodium, potassium and manganese, also decreased after pearling (Stikic et al., 2012; Chauhan et al., 1992; Konishi et al., 2004).

Pearling and washing can be performed separately or combined to enhance the effects on saponin removal, and lower the negative impact of each individual process (Table 2).

3.1.5.3 Other methods

Other methods have been proposed such as the combination of washing and heat treatments in different conditions (i.e. toasting, cooking at atmospheric pressure, cooking under pressure) (Nickel et al., 2016). However, none of them resulted in a higher loss of saponin content than just washing (Nickel et al., 2016).

3.1.5.3.1 Bioprocessing

Sourdough fermentation is a biotechnological process that transforms complex molecules into simpler ones through the enzymatic activity of microorganisms, such as yeasts and lactic acid bacteria. The positive effects of grain fermentation include the degradation of anti-nutritional compounds, such as phytates, and the formation of bioactive and/or antifungal compounds (Dallagnol et al., 2015; Poutanen et al., 2009). Moreover, sourdough fermentation improves the sensory quality of products, due to the production of organic acids and the development of new aromatic compounds (Poutanen et al., 2009). In particular, adding quinoa sourdough to wheat enhances the sensory traits of wheat bread, resulting in higher acidity, a salty taste and less sweetness (Rizzello et al., 2016). However, it is not clear if this new sensory profile masks the bitterness of quinoa.

Sprouting (or germination) is a natural process that decreases the anti-nutrient compounds in cereals, pseudocereals and pulses while substantially increasing micronutrient bioavailability and improving sensory properties (Omary et al., 2012; Marengo et al., 2016). Germinated grains are characterized by a sweet taste, due to the formation of simple sugars that may mask the bitter taste in whole wheat bread (Richter et al., 2014). However, no information about the effect of germination on quinoa saponins and, consequently, on its bitter taste or aftertaste has been reported. Nevertheless, the effectiveness of germination in decreasing saponin content in bitter quinoa varieties might be a hoped-for result, given the precedent of the positive results observed in sprouted chickpeas (El-Adawy, 2002) and huazontle (Lazo-Vélez et al., 2016) (*Chenopodium berlandieri spp.)*, closely related to quinoa.

3.1.5.3.2 Breeding

Several bio-technological approaches have been proposed to decrease the amount of saponins. Although effective, they are costly and impact negatively on the environment. Therefore, the possibility of selecting “sweet” genotypes with low saponin content for direct consumption without any grain pre-treatments are being explored: this approach would facilitate the expansion of quinoa production and utilization, above all, beyond the Andean regions (Ward, 2000).

Quinoa, in fact, is still an under-utilized crop and breeding efforts to improve its agronomic traits (length of growing season; crop yield) are required to expand its production worldwide, especially at higher latitudes where some lines are characterized by poor yields (Ward, 2001). There is a general consensus that development of sweet cultivars with little or no saponin is one of the most important breeding objectives for the future (Bhargava et al., 2006; Spehar et al., 2010), not only to improve crops in South American countries but also in Mediterranean environments (De Santis et al., 2016b). However, breeding this trait into quinoa varieties is still a challenge due to the difficulty of measuring saponin levels prior to anthesis and fixing appropriate alleles (Masterbroek et al., 2000). Jarvis et al., (2017) recently sequenced the genome of a Chilean coastal variety of quinoa along with the genomes of additional *Chenopodium* species to characterize the genetic diversity of quinoa. They also proposed the pathway for saponin biosynthesis, indicating the enzymes involved in each step and the genes encoding each enzyme. Interestingly, these scientists discovered that only one key gene is implicated in the regulation of saponin production. The authors suggest using the identified genetic markers to develop non-bitter or sweet commercial quinoa varieties with lower saponin levels. These findings would provide the scientific bases for accelerating the genetic improvement of quinoa, to enhance global food security for a growing world population.

3.1.6 Conclusions

The presence of bitter compounds - mainly saponins - highly affect sensory acceptance of quinoa; consequently, the consumption of this pseudocereal as whole grain and/or as a valuable nutritive ingredient in composite flours for wheat or gluten-free products has to carefully consider this aspect. Presently, decreasing or modifying the bitterness of quinoa is achieved applying washing and/or mechanical pearling. Although they are widely used, these processes present critical aspects, namely low environment-sustainability, energy and specific equipment requirements, that force researchers to find other approaches. Besides the breeding studies that might select new “sweet” varieties with low or no saponin content and with high adaptability to different climatic environments, bio-technological and not-expensive processes have to be developed. Indeed, germination could not only enhance important nutritional traits of grains, but also represent a valid tool for decreasing bitterness in quinoa, due to sugar formation. Our efforts are addressed to set up processing conditions that could be easily adopted also in low-incoming countries.

3.1.7 References

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3.2 Effects of sprouting on functional properties of quinoa (*Chenopodium quinoa* Willd.)

3.2.1 Abstract

Sprouting has been proposed as a suitable process to enhance the nutritional traits of grains, including quinoa. Whereas, there is a lack of information about the effects of sprouting time on starch and protein features and their impact on the functional properties of quinoa seeds and the related flour. The present work aimed at understanding the relation among sprouting time, molecular changes and flour functionality. Whole quinoa was sprouted for 12, 24, 48 and 72 h at 22 °C and 90% of relative humidity and dried at 55 °C for 6 h. The development of amylases and proteases promoted changes in both starch and protein features. After 48 h sprouting, starch granules were less effective in absorbing water and gelatinizing during heating, and less prompted to re-associate in a more ordered structure during cooling. As regards proteins, the process mostly affected albumin/globulin fractions and, once again, the main events were observed after 48 h of sprouting. Such molecular changes affected flour functionality, by: (1) decreasing the ability of absorbing and retaining water (as shown by WAI_{90} and WSI indices); (2) lowering syneresis during freeze-thawing; (3) decreasing foaming capacity but improving the stability of the foam.

Overall, the results might suggest a potential use of flour from sprouted quinoa in the production of frozen foods as thickener agent (especially after 72 h of sprouting) and in baked products as improver (especially after 48 h of sprouting).

I am grateful to Dr. Mauro Marengo for protein characterization and to Professor Stefania Iametti for data interpretation.

3.2.2 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledonous plant belonging to the Amaranthaceae family widespread in South-American countries. In recent years, its production and consumption have constantly been increasing around the world due to the re-valorization of its sustainable traits and nutritional properties, including its better balance of essential amino acids than cereals, low glycemic index and its content of unsaturated fatty acids, vitamins and minerals (Bastidas et al., 2016). Despite that, the main limitation for quinoa consumption as a whole grain is its bitterness, caused by bitter components, such as saponins, that are accumulated in the external layers of the seed. Suárez-Estrella et al., (2018) reported lights and shadows of the main approaches - washing

and pearling - used to decrease the amount of saponins and improve quinoa acceptability. Washing is the simplest and most ancient process, still employed in the Andean countries, but it is associated with water consumption and pollution. Nowadays, pearling is the preferred approach, especially in the Western Countries, but it causes significant decreases in minerals, fiber, and bioactive compounds (Konishi et al., 2004; Chahuan et al., 1992; Gomez-Caravaca et al., 2014).

Sprouting or germination has been widely carried out on grains to enhance both sensory and nutritional profiles, through the decrease in antinutritional compounds (namely, phytic acid) and the increase in compounds with antioxidant activity and micronutrient bioavailability (Erba et al., 2019; Marengo et al., 2016; Morita et al., 2013). Moreover, when processing conditions were accurately controlled, positive effects have been also reported on the technological performance of flours, thanks to the balanced accumulation of enzymatic activities (Marti et al., 2017; Grassi et al., 2018; Marti et al., 2018). Development of amylolytic and proteolytic activities have been followed also during quinoa sprouting (Hager et al., 2014; Mäkinen et al., 2013; 2014), but there is a lack of information about the effects of sprouting time on starch and protein features and their impact on the functional properties of seeds and the related flour, in order to enhance the consumption of this pseudocereal. In this context, the present work aimed at understanding the relation among sprouting time, molecular changes and functionality. Both seeds and flour physical properties were considered. Indeed, quinoa is widely used as boiled seeds, especially in South America; in addition, the absence of gluten proteins makes it a suitable raw material in gluten-free products, including baked goods. The nature and the intensity of grain modifications upon sprouting might promote new functional and technological properties, encouraging the use of quinoa flour as ingredient in food formulations.

3.2.3 Materials and methods

3.2.3.1 Raw materials

Whole quinoa and pearled quinoa seeds (*Chenopodium quinoa* Willd. var. *Titicaca)* were purchased from Quinoa Marche s.r.l. (Ancona, Italy). Whole quinoa was sprouted at laboratory scale (Constant climate chamber HPP, Memmert GmbH+Co. KG, Schwabach, Germany), as reported in Figure 1. Briefly, five aliquots of seeds (1 kg each) were soaked in water (1:1 w/w) for 14 h, at 22 °C and 90% of relative humidity. After draining the water, an aliquot was dried at 55 °C for 6 h (namely “soaked sample”) and the remaining seeds were sprouted for 12, 24, 48 and 72 h at 22 °C and 90% of relative humidity. After sprouting, samples were dried at 55 °C for 6 h (Self Cooking Center, Rational International AG, Landsberg am Lech, Germany). An aliquot (50 g) was lyophilized for enzymatic analysis only.

Whole (native) and pearled seeds were considered as references. In fact, the comparison with the first sample type was necessary to understand the type and the entity of sprouting-related changes.

While, the pearled seeds represent the way the product is currently on the market for food consumption.

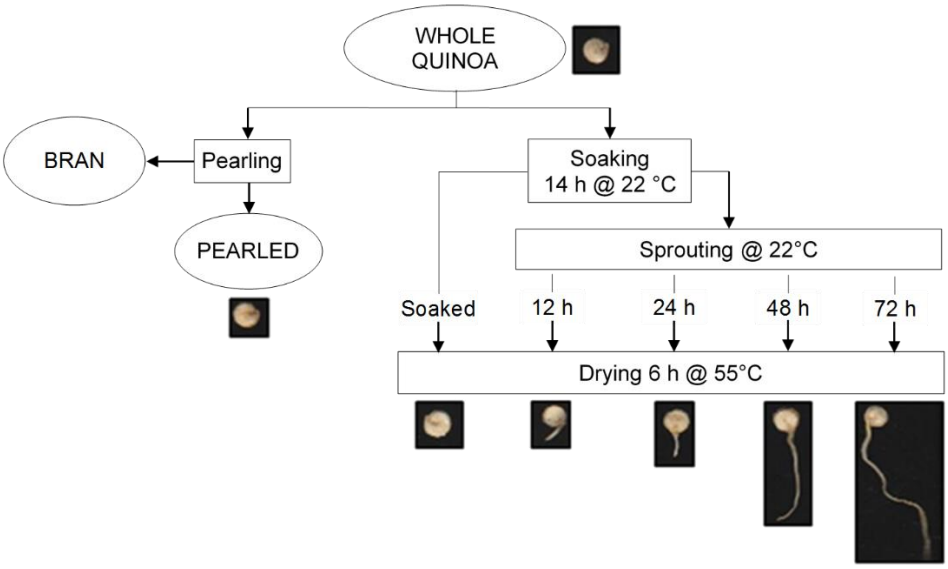

Figure 1. Quinoa samples and processing conditions.

Samples were used as seeds or flours after milling to particle sizes lower than 250µm (99%) in a laboratory scale mill (Cyclotec 1093 Foss Sample Mill, Höganäs, Sweden). The moisture was measured at 130 ºC in a thermo-balance (Radwag MA 50/1.R, Radom, Poland). After drying the moisture content ranged from 7.0% to 9.9% for the samples sprouted for 24 h and 48 h, respectively.

3.2.3.2 Characterization of quinoa seeds

Hectolitric weight was measured (n=5) on the basis of the mass of quinoa seed contained in 100 mL and converting the amount into kg/hL. Thousand kernel weight was calculated (n=10) from the average weight of fifty seeds. Seed diameter was measured by image analysis (ImagePro Plus 6.0) from the images of fifty seeds using a scanner set at 600 dpi (Canoscan Lide 200; Canon Inc., Tokyo, Japan).

Water absorption index (WAI) was measured (n=3) in boiling water at 5, 10, 15, and 20 min. The last sampling time corresponded to the complete gelatinization of the seeds (data not shown). Seeds (2 g) were added to distilled water (25 mL) at 90 °C in a covered beaker to avoid water losses due to evaporation. After each sampling time, samples were sifted and then weighed. The WAI was expressed as the increase in weight per gram of sample (db) (Anderson et al., 1969).

3.2.3.3 Chemical indices

3.2.3.3.1 Enzymatic activities

The enzymatic activities were measured on freeze-dried samples, to avoid enzymes inactivation during drying. Alpha-amylase activity (n = 3) was measured according to the AACC method 22-02.01 (AACCI, 2001), whereas β-amylase activity (n = 3) was determined by Megazyme kit (K-BETA3, Megazyme International Ireland Ltd., Wicklow, Ireland). Briefly, for the enzyme extraction 0.5 g of sample were diluted in test tubes containing 5.0 mL of extraction buffer for 1 h at room temperature and vortexed each 10 min. Test tubes were centrifuged at 2000 x g for 10 min. Supernatants (0.2 mL) were added to assay buffer (4.0 mL). Then, 0.2 mL of each extract were added to 0.2 mL Betamyl-3 subtrate solution and incubated at 40 ºC for 10 min. Stopping reagent was added (3 mL) and the absorbances were read at 400 nm against distilled water.

Proteolytic activity (n = 3) was determined by the method of Arnon (1970) using azocasein (Sigma Chemical Co., St Louis, MO, USA) as the substrate.

3.2.3.3.2 Protein, starch, and sugar content

Total nitrogen content was determined (n=2) by AACC 46-12.01 method (AACCI, 2001) using 6.25 as a conversion factor for proteins. Total starch content (n=3) were measured according to the AACC 76-13.01 method (AACCI, 2001). Maltose, sucrose and D-glucose contents were measured (n=3) by Megazyme assay kit (K-MASUG) (Megazyme International Ireland Ltd., Wicklow, Ireland) with a slight variation during the extraction phase to avoid the interference of enzymatic activities. Briefly, an aliquot of 1 g of sample was diluted in 100 mL of distilled water. Then, a two-stage extraction was applied with constant agitation: 20 min at 75 ºC and 40 min while temperature decreased until 25ºC. The extracts were centrifuged at 1400 x g for 10 min. The enzymes and reagents were added according to the assay procedure (https://secure.megazyme.com/Maltose-Sucrose-D-Glucose-Assay-Kit).

3.2.3.3.3 Protein features

Studies on protein aggregation events during sprouting were carried out by measuring soluble proteins in various buffers as described by Marengo et al., (2015). Protein aggregation studies were carried out by suspending 0.15 g of various flour in 5 ml of 50 mM phosphate buffer, 0.1 M NaCl, pH 7.0, in the presence of 6 M urea or of 6 M urea and 10 mM dithiothreitol (DTT) when indicated (Marengo et al., 2015). Suspensions were stirred for 60 minutes at room temperature. After centrifugation at 10000 x g for 20 min, the amount of protein in the supernatant was determined by a dye-binding method (Bradford 1976). Results are expressed as mg proteins/g flour.

Proteins solubilized from flour were treated with denaturing buffer (0.125 M Tris-HCl, pH 6.8, 50% (v/v) glycerol, 1.7% SDS; 0.01 % Bromophenol Blue), containing 1% (v/v) 2-mercaptoethanol when indicated, and boiled for 10 min. SDS-PAGE was carried out in a MiniProtein apparatus (BioRad, Richmond, VA, USA), by loading a volume of each sample corresponding to 0.015 mg protein per lane (Iametti et al., 2006). Gels were stained with Coomassie Brilliant Blue. Accessible thiols (expressed as micromol thiols g^{-1} flour) were determined by suspending 0.15 g of finely ground samples in 5 ml of 0.05 M sodium phosphate, pH 7.0, containing 0.1 M NaCl and 0.2 mM 5,5′-dithiobis-(2-nitrobenzoate) (DTNB), in the presence/absence of 6 M urea. After 1 h stirring at room temperature, samples were centrifuged (~5000 x g, 30 min, 25 °C), and the supernatant absorbance was read at 412 nm (Bonomi et al., 2012; Marengo et al., 2015).

3.2.3.4 Characterization of quinoa flours

3.2.3.4.1 Hydration properties

Water absorption index (WAI_{20}) of flours at 20 °C was measured according to the AACC 56-20.01 method (AACCI, 2001) with slight modifications. Briefly, 0.5 g d.b. of samples were added to 10 mL of distilled water at 20 ºC, vortexed and left to rest for 10 min. After centrifuging for 15 min at 1000 x g, the supernatant liquid was drained and the test tubes were weighed. Finally, WAI_{20} (g/g) was calculated as the ratio between the weight of the precipitate and the weight of the flour.

Hydration properties of quinoa flour were also assessed (n=3) in water at 90 ºC for 5, 10, 15 and 20 min. Water absorption index was assessed at 90 ºC (WAI_{90}) according to Steffolani et al., (2016) whilst water solubility index (WSI) was measured according to Li & Zhu, (2017). The test was performed with 5 mL of a solution of quinoa flour in water (5% db w/v). Samples were added to hot water and vortexed to avoid foam development. At the prefixed times, the test tubes were cooled in ice for 5 min and centrifuged for 10 min at 1400 x g. The precipitate was weighed and the supernatant was dried at 105 ºC for 12 h. Then, the weight of the solubilized sample was registered. For the calculation, the following equations were applied:

$$WAI_{90}\ (g/g) = \frac{precipitate\ weight - (initial\ weight - solubilized\ sample\ weight)}{initial\ weight\ db}$$

$$WSI(g/g) = \frac{solubilized\ sample\ weight}{initial\ weight\ db}$$

3.2.3.4.2 Freeze-thaw stability

The freeze-thaw stability of flours was determined (n=3) according to Ahamed et al., (1996). Test tubes containing the precipitate from hydration properties assessment (i.e. initial weight) were frozen for 18 h and then thawed for 6 h at room temperature. The test tubes were centrifuged for 10 min at

1400 x g. The supernatant was removed and the test tubes weighed. Three cycles of freezing and thawing were applied after one, two, and three days of storage. Then, the following formula was applied for each cycle.

$$Freeze-thaw\ stability\ (\%) = \frac{initial\ \ weight - sample\ weight\ after\ 1, 2, and\ 3\ cycles}{initial\ weight} * 100$$

3.2.3.4.3 Foaming capacity and stability

Foaming capacity and stability were assessed (n=3) as reported by Coffman & García, (1977) with modifications. An aliquot of 2 g of each flour sample was added to 50 mL of distilled water in a cylinder. The cylinder was closed and vortexed for 1 min, vigorously agitated from top to bottom for 30 s by hand and the level of foam produced was measured. The foaming stability was based on the remaining height after 1 h. Image ProPlus v.6.0 software (Media Cybernetics Inc., Rockville, USA) was used to measure each height. Foaming capacity and stability were expressed as a percentage. For their determination the following formulas were applied:

$$Foaming\ capacity\ (\%) = \frac{foam\ height}{initial\ height} * 100$$

$$Foaming\ stability\ (\%) = \frac{foam\ height\ after\ 1\ h}{initial\ foam\ height} * 100$$

3.2.3.4.4 Pasting properties

The pasting profiles of flours were assessed (n=3) using a Micro Visco-Amylo-Graph Brabender (Brabender OHG, Duisburg, Germany). An aliquot of 12 g of sample was dispersed in 100 mL of distilled water, scaling both flour and water weight on 14% flour moisture basis. The suspensions were assessed at 250 rpm of speed. Time and temperature profiles included 3 min of pre-treatment at 30 °C, heating up to 95 °C, holding for 20 min, cooling to 30 °C and holding for 1 min. Heating and cooling phases were carried out at 3 °C·min $^{-1}$. The parameters determined were the temperature at the beginning of gelatinization, maximum viscosity, final viscosity and setback using the software provided with the device (Viscograph Data correlation. Version 4.2.0. Brabender OHG, Duisburg, Germany).

3.2.3.5 Statistics

Analysis of variance (ANOVA) was performed by Statgraphics Plus 5.1 (StatPoint Inc., Warrenton, VA, USA). Different treatments and sprouting times were considered as factors. When a factor effect was identified as significant ($p < 0.05$), the significant differences among the samples were determined using Tukey Honest Significant Difference (HSD) test.

3.2.4 Results and discussion

3.2.4.1 Seed characteristics

3.2.4.1.1 Physical characteristics

Sprouting promoted relevant changes in quinoa seeds as regards size and weight, according to time (Table 1). Indeed, the hectolitric weight (also known as test weight), the thousand kernel weight, and the diameter significantly decreased already after 12 h of sprouting, with no more changes after 48 h of treatment. The differences between whole (native, unsprouted) and pearled seeds and the sprouted samples might be related to the drying step that was necessary to remove the excess of water in sprouted grains. The effect of sprouting on the physical characteristics of seeds was clear when comparing the sprouted seeds with the soaked sample.

Table 1. Physical characteristics of quinoa seeds.

		Test weight (kg/hL)	Thousand kernel weight (g)	Diameter (mm)
Whole		73.1±0.6^{e}	2.77±0.13^{c}	2.23±0.19^{b}
Soaked		71.6±0.7^{d}	2.63±0.13^{bc}	2.21±0.20^{b}
Sprouted	12 h	65.6±0.5^{c}	2.59±0.14^{b}	2.09±0.15^{a}
	24 h	55.6±1.1^{b}	2.58±0.11^{b}	2.06±0.10^{a}
	48 h	48.8±1.1^{a}	2.55±0.11^{b}	2.05±0.17^{a}
	72 h	48.7±0.3^{a}	2.39±0.14^{a}	2.01±0.16^{a}
Pearled		77.7±0.5^{f}	2.58±0.10^{b}	2.09±0.19^{a}

Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$). Test weight (n=5); thousand kernel weight (n=10); diameter (n=50).

Changes in physical characteristics of seeds were probably related to the biochemical changes occurring during sprouting, as discussed in section 3.2.1. However, the test weight of unsprouted seeds were in the range found by other authors for whole seeds (63.6 - 72.5 kg/hL) (Eisa et al., 2018; Naneli et al., 2017). Moreover, the physical characteristics (in terms of thousand kernel weight and diameter) of 48 h sprouted sample were similar to those of pearled quinoa. As mentioned above,

due to the high saponin level, quinoa is currently consumed solely after pearling. Compared to whole seed, this process promoted an increase in hectolitric weight and a decrease in both thousand kernel weight and seed diameter as a consequence of the removal of the external layers of seeds (Naneli et al., 2017 and Reichert et al., 1986).

3.2.4.1.2 Hydration properties of seeds

Quinoa is mainly consumed as grains, thus monitoring the hydration kinetics during cooking might elucidate the effect of sprouting on cooking performance. During cooking, sprouted samples showed a faster capacity to absorb water than the control samples (i.e. whole and pearled seeds), as measured by the WAI (Fig. 2). The higher WAI of sprouted samples compared to that of whole seeds might be related to the loss of compactness of seeds, as highlighted by the decrease in test weight and thousand kernel weight indices (Table 1).

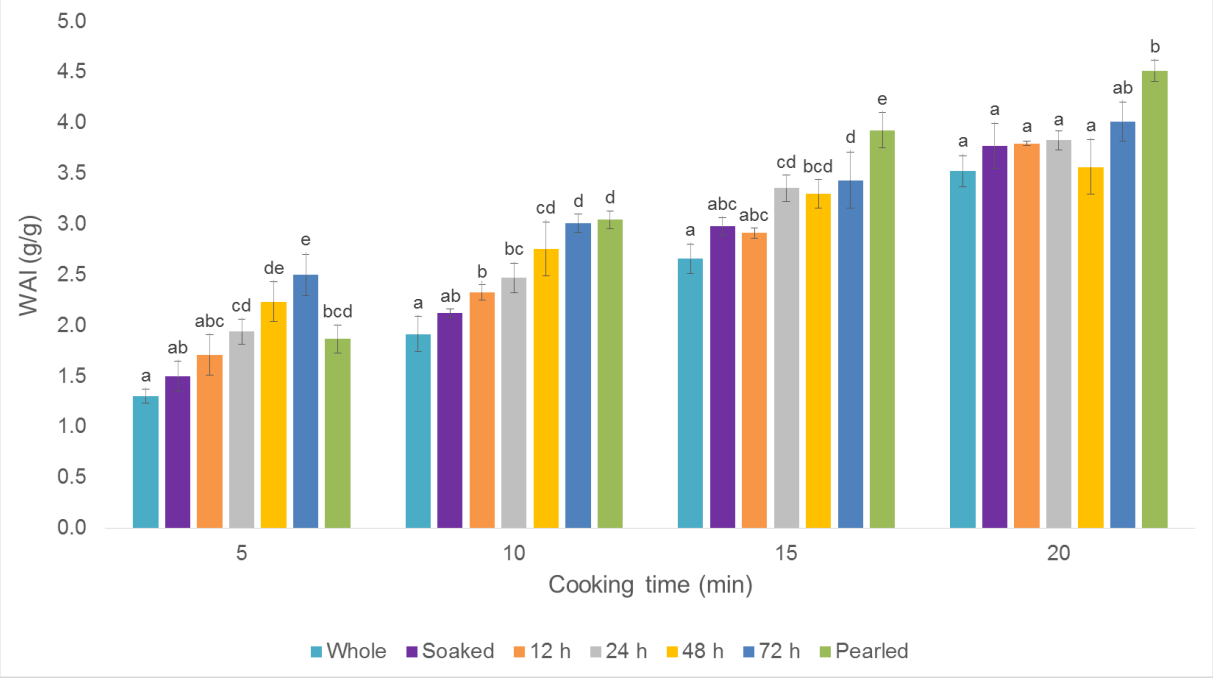

Figure 2. Water absorption kinetics of quinoa seeds.

Different letters at the same cooking time indicate significant differences (Tukey HSD; $p<0.05$; n=3).

The most significant changes occurred after 24 h of process up to 15 min of cooking. Longer cooking time (i.e. 20 min, corresponding to the optimal cooking time) decreased the differences among the samples, due to the maximum extent of starch gelatinization. The only exception is the pearled seeds

that kept absorbing water even after 20 min of cooking, due to differences in chemical composition as discussed in section 3.2.4.2.

3.2.4.2 Chemical composition

3.2.4.2.1 Enzymatic activities

Enzymatic activities were measured in freeze-dried samples to avoid the loss of enzymatic activity in sprouted samples, as a consequence of the temperature applied during the drying phase. In fact, no enzymatic activity was detected in the dried samples (data not shown). Whole and pearled seeds showed low enzymatic activities (Table 2). No changes were observed after pearling, since the process removed only a small percentage of external layers (data not shown).

As regards the sprouting process, an increase in enzymatic activities was observed even after soaking. Previous studies reported that quinoa sprouting could starts at 4 or 6 h of soaking (Moreno et al., 2017). In the present study, significant changes in α – amylase activity were observed after 12 h of treatment. The maximum accumulation of α – amylase activity in the entire seed (embryo + perisperm) occurred within 48 h of sprouting.

Table 2. Enzymatic activities in quinoa seeds.

		α – amylase (U/g db)	β – amylase (U/g db)	Protease activity (U/g db)
Whole		0.22±0.03^{a}	1.65±0.12^{a}	1.4 ±0.4^{a}
Soaked		0.65±0.22^{a}	2.08±0.09^{b}	1.7±0.4^{ab}
Sprouted	12 h	2.34±0.39^{b}	2.07±0.16^{b}	1.9 ±0.2^{b}
	24 h	3.87±0.77^{c}	2.02±0.14^{b}	2.9±0.5^{c}
	48 h	5.89±0.79^{d}	1.99±0.12^{b}	2.9±0.4^{c}
	72 h	5.34±0.51^{d}	2.00±0.06^{b}	2.9±0.6^{c}
Pearled		0.21±0.01^{a}	1.56±0.11^{a}	1.6±0.2^{ab}

Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; n=3).

Hager et al., (2014) followed the changes in α – amylase activity in embryo and perisperm separately. While the enzymatic activity continuously increased up to 72 h in the perisperm, it reached its maximum level at 48 h in the embryo, suggesting a migration of enzymes from the embryo to the starchy perisperm upon sprouting time (Hager et al., 2014).

The main increase in β – amylase activity was detected already after 14 h of soaking, with no more changes upon sprouting time (Table 2). A similar trend has been described during wheat sprouting

(Grassi et al., 2018) and attributed to the release of β – amylases in the free form due to proteases that hydrolyzed the bond form present in the unsprouted grain (Ziegler, 1995).

Makinen et al., (2013) showed no changes in amylolytic activities before and after sprouting carried out for 24 h at 15 °C. Differences among studies might be related to differences in both varieties and processing conditions.

The endogenous proteolytic activity increased after 24 h of sprouting and remained the same when prolonging sprouting time (Table 2). It can be noted that the protease activity detected after 24 h of sprouting is two-fold higher that detected in the soaked sample. The protease activity detected in the sprouted samples can be involved in the formation of peptides as pointed out in the protein characterization section.

3.2.4.2.2 Protein, starch, and sugars content

Sprouting promoted a significant decrease in starch content after 48 h of process (Table 3), being the primary source of energy required during germination and seedling growth. No further changes were observed up to 72 h (Table 3). Consequently, the percentage of proteins increased.

Table 3. Chemical composition of quinoa samples.

		Proteins	Total starch	Sucrose	D-Glucose	Maltose
Whole		14.6±0.1^{a}	60.6±1.7^{b}	2.33±0.06^{b}	0.42±0.05^{a}	0.31±0.06^{a}
Soaked		14.4±0.2^{a}	62.0±1.8^{b}	1.41±0.10^{a}	1.61±0.06^{b}	0.31±0.04^{a}
Sprouted	12 h	14.5±0.3^{a}	60.6±1.9^{b}	1.43±0.11^{a}	1.75±0.04^{b}	0.36±0.06^{a}
	24 h	14.2±0.0^{a}	59.1±2.4^{b}	2.06±0.04^{b}	2.06±0.15^{c}	0.41±0.07^{a}
	48 h	16.5±0.1^{b}	52.6±1.4^{a}	2.18±0.12^{b}	2.20±0.13^{c}	0.61±0.08^{b}
	72 h	16.4±0.1^{b}	50.7±1.9^{a}	3.09±0.21^{c}	2.23±0.09^{c}	0.60±0.11^{b}
Pearled		14.7±0.1^{a}	66.3±1.6^{c}	2.04±0.10^{b}	0.50±0.07^{a}	0.23±0.04^{a}

All data are expressed as g/100 g db. Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; n=2 for proteins; n = 3 for starch and sugars).

Starch hydrolysis by amylase activities promoted an increase in glucose and maltose content, in agreement with Hager et al., (2014). Sucrose content decreased during the first 24 h of sprouting, since it is used from the seeds as energy source (Hager et al., 2014), and then it increased up to 72 h. Previous studies related the increase in sucrose content after sprouting to the high levels of enzymes able to synthetize sucrose (Rosa *et al*., 2004).

The removal of the external layers during pearling accounted for the increase in starch content in pearled quinoa. As expected, pearling did not change either sugar distribution or content (Table 3).

3.2.4.3 *Protein features*

3.2.4.3.1 Protein aggregates

Information on the nature of the inter-protein interactions in cereals (Iametti et al., 2006; Bonomi et al., 2012; Marengo et al., 2015), pseudocereal (Cabrera-Chávez et al., 2012) and legumes (Marengo et al., 2016) can be provided by detecting the amount of proteins solubilized in different media. Since drying process likely affects protein solubility, the effect of sprouting on protein solubility was assessed by using the soaked sample as reference.

As shown in Figure 3, regardless of the type of media, sprouting resulted in a significant decrease in soluble proteins only after 48 and 72 h of treatment. Proteins solubilized in phosphate buffer (i.e. albumins and globulins) were the most degraded, reaching a residual content of 47.5% and 55.7% after 48 and 72 h, respectively, compared with the amount in soaked seeds. In the presence of denaturant agent (such as urea), the amount of soluble proteins increased in all the samples, and no difference was observed by adding to the denaturant media a reducing agent (such as DTT), suggesting that the presence of disulfide bonds in the stabilization of protein association was very low (Iametti et al., 2006). In this context, it can be noted that the sprouting mostly affected albumin/globulin fractions (solubilized in the phosphate buffer) rather than protein aggregates stabilized by hydrophobic or disulfide bonds. In particular, the amount of proteins stabilized by these kinds of interactions decreased only by 30% after 48 and 72 h of sprouting (Fig. 3).

Despite the protease activity developed during sprouting, we did not observe an increase in soluble proteins in sprouted samples, due to the protein quantification method (e.g. Bradford assay), that is not sensitive to small peptides with molecular weight lower than 4 kDa. This observation has to be applied mainly to data obtained for samples sprouted 48 and 72 h where an increase in peptides has been confirmed by analysis of the supernatant from TCA precipitation (data not shown).

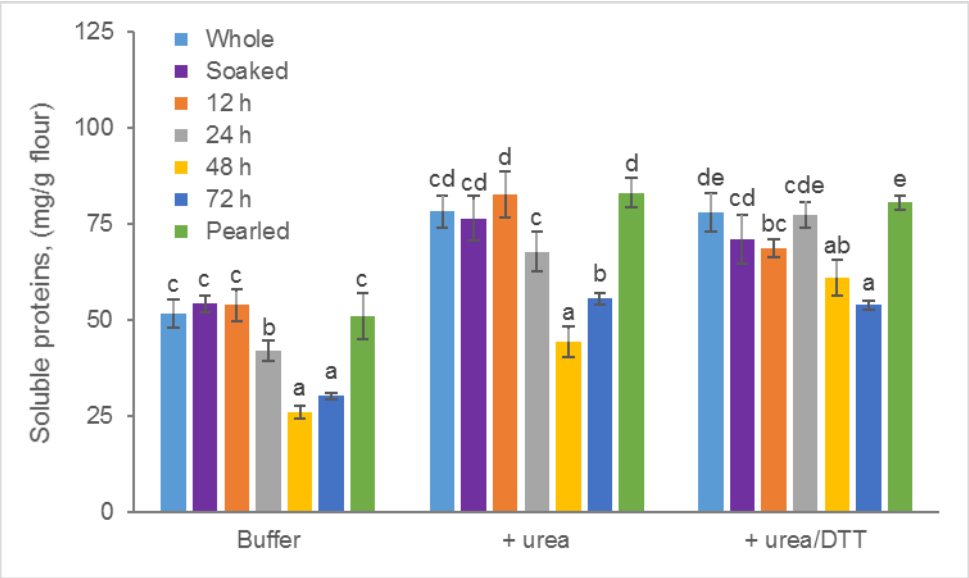

Figure 3. Amount of quinoa proteins solubilized in various media.

Different letters indicate significant differences for each solvent (Tukey HSD; $p<0.05$; n=3)

3.2.4.3.2 SDS-PAGE

The electrophoretic profile of proteins and large peptides extracted in media of various dissociating ability and analyzed in the presence of disulfide-reducing agents are shown in Figure 4. The protein patterns indicated that sprouting-related changes involving proteins were most evident from 48 h of sprouting, confirming the observation provided by solubilization studies (Fig. 3). However, a release of a 60 kDa component in the buffer soluble material - similar to a component that was only urea-soluble in the un-sprouted materials - was detected after 24 h. This component was not degraded at longer sprouting times. The disappearance of high molecular mass species, that are present in the original flour, is evident in protein pattern of 48 h sprouting sample (Fig. 4).

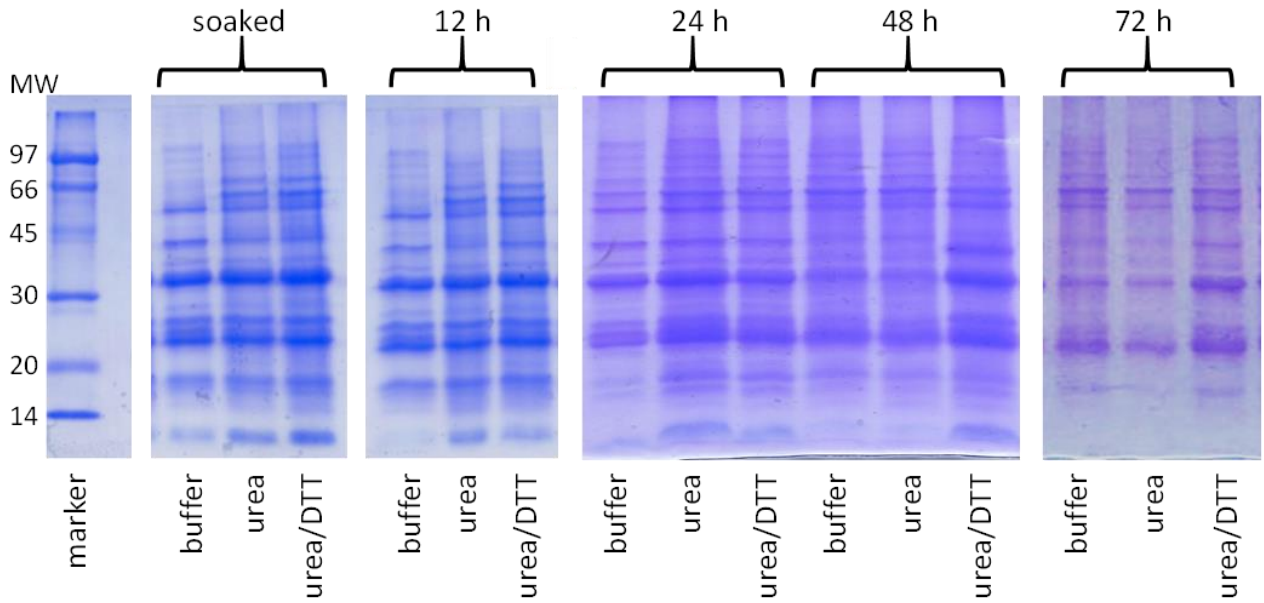

Figure 4. SDS-PAGE of quinoa proteins solubilized in various media.

The main events observed at 48 h sprouting relate to extensive breakdown of components at 48 and 25 kDa, and to the appearance of a component at 35 kDa. This latter species appears to be a transient proteolytic product, as it is no longer evident at longer sprouting times.

The breakdown of high molecular weight proteins at 48 and 72 h sprouting seems to be more extensive when the involved proteins are not participating to a disulfide-stabilized network (i.e. proteins mainly solubilized in buffer and urea). Proteolysis of the non-crosslinked proteins is clearly time-progressive.

Small peptides (escaping detection by the dye-binding method used here, and not evident in the SDS-PAGE gels in Fig. 4) were possibly the most abundant products of any sprouting-induced protein breakdown. This has been confirmed by analysis of the supernatants from TCA precipitation of samples as a whole (not shown).

3.2.4.3.3 Accessible and total thiols

Sulfhydryls (-SH) and disulfides (-S-S-) have a fundamental role in defining the technological properties of flours, since the formation of a proper protein network in dough has been shown to depend on the presence and/or formation of intra- and intermolecular S-S bonds among involved proteins. Sprouting did not significantly affect the content of readily-accessible thiols (i.e., reacting with DTNB in the absence of denaturants), that remained in the range from 3.2 to 2.7 micromol g^{-1} flour, with no clear-cut dependence on the treatment. Treatment with urea increased the content in accessible thiols to 5.3±1.5 micromol g^{-1} flour in the non–sprouted quinoa. The presence of urea-

extracted colored materials made these determinations difficult, and poise some questions as for any possible time-dependence. Thus, although the sprouting treatment may imply a loosening of the compact structure of storage proteins, this does not result in increasing the number of protein thiols accessible upon protein unfolding. This new structural organization could facilitate the incorporation of these proteins into the protein network relevant in baked foods, but the contribution of thiols to improving the network-forming ability in dough containing mixtures of flour from different sources appears questionable.

3.2.4.4 Functional properties of flours

Nowadays, the use of quinoa flour as ingredient in cereal-based products is increasing thanks to its nutritional profile. To identify potential uses of new food ingredients, it is important to assess their functional properties, suitable for predicting the quality of the final product. In this context, WAI and WSI are useful in formulating dough-type food applications because of their hydrophobic/ hydrophilic nature (Kadan et al., 2003). Moreover, starch changes during a heating process might be followed by assessing the pasting properties (Marti et al., 2011), whereas, the freeze-thaw stability provides information about the use of a new ingredient in frozen foods (Williams et al., 2009). Finally, the emulsifying characteristics are determinant in each type of bakery products (Kohajdová et al., 2009).

3.2.4.4.1 Hydration properties

Water absorption and solubility indices (WAI and WSI) are widely used to provide information about the starch/protein-water affinity that might affect flour behavior during technological processing (i.e. mixing and baking/cooking). Sprouting did not significantly affect the capacity of quinoa flour to absorb water at room temperature (data shown in the appendix, Table A1), suggesting no changes in protein/starch-water interactions. This result could be interesting for bread-making processes where the water absorption of flour is important for dough development and productivity yield. On the contrary, pearling changed the hydration properties (by decreasing WAI_{20}) due to the removal of the external layer that is rich in fiber.

WAI was also determined at 90 °C at different times (5, 10, 15, and 20 min), together with WSI, and the related data were reported in the appendix (Table A2). As expected, flour from both whole and pearled quinoa showed an increase in WAI_{90} as heating time increased from 5 min to 10 - 15 min. On the other hand, no significant effect of heating time was detected in the sprouted samples. For this reason, only the results after 20 min of heating, corresponding to the maximum starch gelatinization extent - were reported in Figure 5.

Sprouting significantly decreased WAI_{90} after 48 h (Fig. 5), due to the effect of enzymatic hydrolysis on starch macromolecules. This parameter decreased not only because the total starch content decreased upon sprouting, but also because soluble sugars and/or hydrolyzed starch are less effective in absorbing water than native starch (Tömösközi et al., 2011). As WAI_{90} decreased, WSI increased reaching the maximum extent after 48 h of sprouting. Indeed, this trend is related to the release of soluble molecules both from starch and protein hydrolysis (Table. 3 and Fig. 3).

As regards the pearled sample, the decrease in WAI_{90} was likely due to the removal of non-starch-polysaccharides of teguments, able to absorb water, with no significant effect on WSI_{90}.

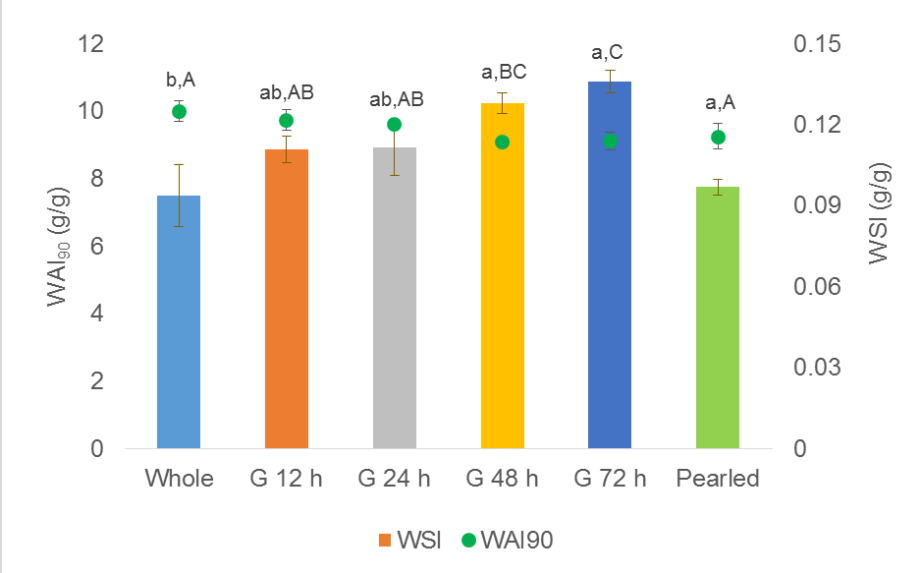

Figure 5. Water absorption index (WAI_{90}; circle) and water solubility index (WSI; bars) of flours after 20 min at 90 °C.

Different letters indicate significant differences (Tukey HSD; $p<0.05$; $n=3$): lowercase letters refer to WAI_{90}; capital letters refer to WSI.

3.2.4.4.2 Freeze-thaw stability

The stability of starch gels during freeze-thaw cycling enhances its potential use in food products. Traditionally, this index has been measured by the amount of water separated from a gel after freezing and thawing has occurred. When a cooked starch paste is frozen, phase separation (namely syneresis) occurs due to the retrogradation of glucan chains. The test has been carried out on cooked flour for 5, 10, 15 and 20 min and data are reported in the appendix (Table A3). A similar trend has been observed at each heating time, thus Table 4 summarizes the results at 10 min, which is a reasonable time for processing.

Sprouting did not significantly affect the freeze-thaw stability of the gel. Overall quinoa samples showed low syneresis evaluated as high percentage of precipitate after centrifugation or as low amount of water separated from the precipitate (Table 4). 72 h-sprouted samples showed a slightly higher syneresis after 1 cycle. These results were in agreement with a decreased ability of absorbing and retaining water, as shown by WAI_{90} and WSI indices (Fig. 5).

The extent of syneresis apparently increased with additional cycles, in a manner that varied with sample type. The better freeze-thaw stability over cycles was observed in 72 h sprouted samples. No differences were observed after two and three cycles of freeze-thawing. Generally, differences in freeze-thaw stability among starches may be due to a variety of factors, including the length of the starch chains and the degree of association between starch components (Vamadevan & Bertoft., 2015). Overall, the results suggest a potential application of sprouted quinoa as thickener for frozen foods.

Table 4. Freeze-thaw stability of quinoa pastes.

		Cycle 1 (%)	Cycle 2 (%)	Cycle 3 (%)
Whole		76.1	68.4	64.9
Sprouted	24 h	74.4	67.2	64.2
	72 h	69.7	67.0	64.5
Pearled		78.3	70.5	68.3

3.2.4.4.3 Foaming capacity and stability

Foaming capacity and stability generally depend on the interfacial properties of proteins, which maintain the air bubbles in suspension and slow down the rate of coalescence. Foaming properties are dependent on the balance of hydrophobic/hydrophilic aminoacids in proteins and on some other components, such as carbohydrates, that are present in the flours (Seerama, Sashikala, Pratape, & Singh, 2012).

Foaming capacity of quinoa flour significantly decreased after 48 h of sprouting, with no further changes till 72 h (Table 5). A similar effect was measured in sprouted soybeans (Mostafa et al., 1987), as a consequence of the overall modification on protein fractions. The foaming capacity of

flours might be attributed to albumin and globulin fractions, whose amount decreased starting from 24 h of sprouting (Fig. 3). Also the decrease in foaming capacity after pearling can be attributed to the removal of albumins and globulins present in the external layers. As foaming capacity decreased upon sprouting, the stability of the foam increased. However, the effect of sprouting on foaming stability was significant even after 24 h of processing. This effect might be ascribed to the variation in distribution of protein molecular weight, as previously described. In particular, the low molecular weight proteins and/or peptides accumulated during sprouting could be more prompted to assure foam stabilization.

Table 5. Foaming capacity and stability of quinoa flours.

		Foaming capacity (%)	Foaming stability (%)
Whole		30.5±1.3^{c}	46.9±2.0^{ab}
Soaked		18.3±0.4^{a}	35.1±2.9^{a}
Sprouted	12 h	26.6±3.2^{ab}	42.4±4.8^{ab}
	24 h	24.5±3.5^{b}	62.9±7.3^{cd}
	48 h	18.3±0.5^{a}	55.4±2.2^{bc}
	72 h	18.6±0.7^{a}	74.1±3.9^{e}
Pearled		17.3±1.7^{a}	53.8±6.8^{bc}

Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; n=3).

Besides proteins, also saponins are responsible for the formation of foam in quinoa seeds. The results suggest that the effects of sprouting on these components are worthy of interest.

3.2.4.4.4 Pasting properties

Pasting profiles of quinoa flour are reported in Figure 6 and the related indices in Table 6. Sprouting did not affect the temperature of the beginning of starch gelatinization (Table 6), that ranged from 65 to 67 °C, typical of quinoa which is characterized by small starch granules (Puncha-arnon et al., 2008).

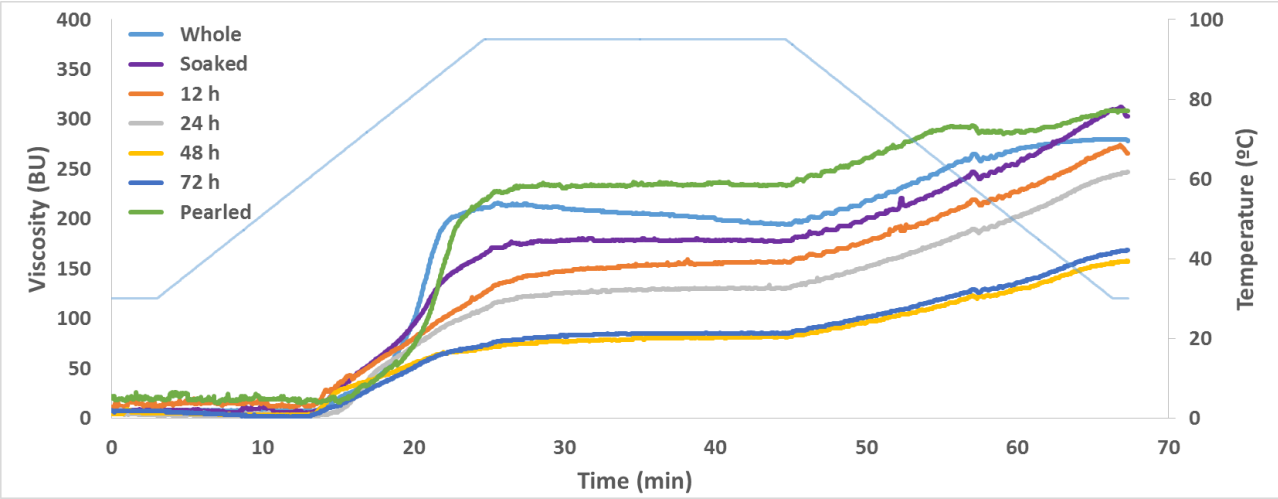

Figure. 6. Pasting properties of quinoa flours.

Table 6. Indices obtained from the pasting profiles of quinoa flours.

		Beginning of gelatinization (°C)	Maximum viscosity (BU)	Final viscosity (BU)	Setback (BU)
Whole		67.3±1.2^{ab}	218.8±6.5^{e}	277.8±4.7^{c}	80.0±8.6^{a}
Soaked		66.4±1.3^{ab}	186.0±5.3^{d}	310.7±7.5^{d}	127.0±3.5^{c}
Sprouted	12 h	65.7±0.5^{ab}	160.0±1.7^{c}	267.7±1.5^{c}	109.0±1.0^{b}
	24 h	66.1±0.6^{ab}	135.0±3.6^{b}	244.7±4.9^{b}	114.7±7.1^{bc}
	48 h	65.2±0.2^{a}	80.7±6.1^{a}	155.0±3.0^{a}	76.3±2.1^{a}
	72 h	65.7±1.5^{ab}	84.7±2.3^{a}	166.3±3.1^{a}	81.0±6.1^{a}
Pearled		68.2±1.1^{b}	235.0±1.7^{f}	305.7±6.8^{d}	73.7±5.5^{a}

Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; n=3).

On the contrary, sprouting was responsible for the decrease in maximum and final viscosity up to 48 h of processing. Longer sprouting (i.e. 72 h) did not further decrease the hot and cold viscosity.

Maximum viscosity decrease could be related to starch hydrolysis into small molecules (see Table 3 and Fig. 3) that are less effective in absorbing water, gelatinizing, and assuring viscosity. Indeed, when enzymatic inhibitor ($AgNO_3$) was used as solvent instead of water, no significant differences in the pasting profile were found (data shown in the appendix, Fig. A1). This result suggested that the enzymatic activity developed during sprouting was inactivated by the drying step at 55 °C and the profile seen in Figure 6 was the consequence of sprouting. During cooling, hydrolyzed starch was less prompted to reassociate in a more ordered structure. Indeed, sprouting decreased the retrogradation tendency of flours, as shown by the decrease in setback values (Table 6) and confirming the results of the freeze-thaw stability (Table 4). In sprouted wheat, the decrease in setback values was responsible for a slower staling rate in bread (Marti et al., 2017; 2018). The effects of sprouting on pasting profile limit the use of quinoa as thickening agent in sauces and in baby-foods. On the contrary, the positive effects of sprouting on decreasing viscosity during cooling might be exploited in the formulation of gluten-free bakery products that are usually characterized by low shelf-life.

3.2.5 Conclusions

Quinoa seeds underwent relevant physical changes during sprouting, that can be appreciated at a macroscopic level and reach their maximum intensity after 48 h processing at controlled moisture and temperature conditions. Those modifications might be considered the signal of significant phenomena induced by several enzymes, responsible for the starch and protein hydrolysis and the formation of soluble compounds, such as sugars and peptides.

Consequently, sprouting processes might enlarge the use of this pseudocereal as flour with new and interesting functional and technological properties. The increased foaming stability and the low tendency to retrograde of sprouted flours could favor the addition of quinoa in baked goods (both from gluten or gluten-free mixtures). In fact, the presence of sprouted quinoa could promote crumb softness and extend the shelf-life of the final baked products due to staling slowdown. Nevertheless, flours from sprouted quinoa could successfully be used also in frozen food due to their interesting freeze-thaw stability. Finally, the lower foaming capacity observed in quinoa after 48-72 h sprouting could be associated with a decrease in saponin content. This trend has to be better investigated; if confirmed, sprouting could represent an easy and economic bio-process suitable to remove the anti-nutritional compounds present in the native seeds, promoting food products with high nutritive traits (richness in fiber and in proteins of high biological value).

3.2.6 References

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Appendix

Table A1. Water absorption capacity of quinoa flour at 20 ºC.

		Water absorption capacity	
		(w/w db)	(%)
Whole		2.55±0.05[b]	155.50
Sprouted	24 h	2.55±0.03[b]	155.49
	72 h	2.65±0.03[b]	165.23
Pearled		2.39±0.02[a]	139.05

Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; n=3)

Table A2. Hydration kinetics of quinoa flours.

Cooking time (min)	Samples		WAC (g of water / g flour db)	WSI (% db)
5	Whole		9.2±0.1$^{ab; A}$	7.8±1.1$^{a; A}$
	Sprouted	12 h	8.9±0.3$^{ab; A}$	8.0±0.3$^{a; A}$
		24 h	9.6±0.7$^{b; A}$	8.4±2.0$^{ab; A}$
		48 h	9.0±0.5$^{ab; A}$	9.5±1.2$^{ab; A}$
		72 h	8.7±0.2$^{ab; A}$	11.1±0.4$^{b; A}$
	Pearled		8.2±0.4$^{a; A}$	8.4±0.2$^{ab; A}$
10	Whole		10.0±0.2$^{b; B}$	7.5±0.4$^{a; A}$
	Sprouted	12 h	9.4±0.2$^{ab; A}$	9.1±1.4$^{a; AB}$
		24 h	9.9±0.4$^{ab; A}$	8.5±1.8$^{a; A}$
		48 h	8.9±0.3$^{ab; A}$	9.6±0.9$^{a; A}$
		72 h	8.7±0.7$^{a; A}$	10.6±1.8$^{a; A}$
	Pearled		9.1±0.6$^{ab; AB}$	8.0±0.1$^{a; A}$
15	Whole		10.3±0.2$^{b; B}$	9.2±0.00$^{a; A}$
	Sprouted	12 h	9.5±0.5$^{ab; A}$	9.2±0.9$^{a; AB}$
		24 h	9.8±0.1$^{ab; A}$	10.2±0.1$^{ab; A}$
		48 h	8.8±0.7$^{a; A}$	11.5±1.2$^{b; AB}$
		72 h	9.3±0.4$^{ab; A}$	12.1±1.0$^{b; AB}$
	Pearled		9.5±0.2$^{ab; B}$	8.8±0.7$^{a; AB}$
20	Whole		10.0±0.3$^{b; B}$	9.4±1.2$^{a; A}$
	Sprouted	12 h	9.7±0.3$^{ab; A}$	11.1±0.5$^{ab; B}$
		24 h	9.6±0.0$^{ab; A}$	11.1±1.0$^{ab; A}$
		48 h	9.1±0.1$^{a; A}$	11.7±0.4$^{bc; B}$
		72 h	9.1±0.3$^{a; A}$	13.6±0.4$^{c; B}$
	Pearled		9.3±0.4$^{a; AB}$	9.7±0.3$^{a; B}$

Different lowercase letters in the same column indicate significant differences between treatments at the same cooking times (Tukey HSD; $p<0.05$; n=3). Different capital letters in the same column indicate significant differences for each sample at different cooking times (Tukey HSD; $p<0.05$; n=3).

Table A3. Freeze-thaw stability of quinoa flour at different cooking time.

		Cooking time (min)	Cycle 1 (%)	Cycle 2 (%)	Cycle 3 (%)
Whole		5	80.39±4.10	71.11±3.05	68.33±4.29
		10	76.14±2.64	68.38±4.00	64.92±4.71
		15	76.61±0.62	67.30±2.36	61.60±0.97
		20	79.03±5.26	71.76±5.82	63.94±3.39
Sprouted	24 h	5	75.66±4.33	65.77±0.38	63.94±3.64
		10	74.40±3.41	67.16±2.98	64.21±3.54
		15	77.26±1.52	68.35±1.45	65.97±3.93
		20	76.15±1.98	71.12±7.20	74.27±4.29
	72 h	5	73.80±7.95	66.79±6.32	63.60±5.00
		10	69.72±7.74	66.95±8.36	64.49±6.16
		15	70.99±5.36	64.51±5.26	60.58±4.52
		20	78.48±2.99	69.36±2.99	64.50±3.51
Pearled		5	81.11±1.35	76.96±4.97	71.81±3.69
		10	78.28±2.78	70.53±3.41	68.34±3.58
		15	77.93±2.04	68.06±0.70	64.61±2.24
		20	75.56±0.92	72.95±4.36	66.39±2.12

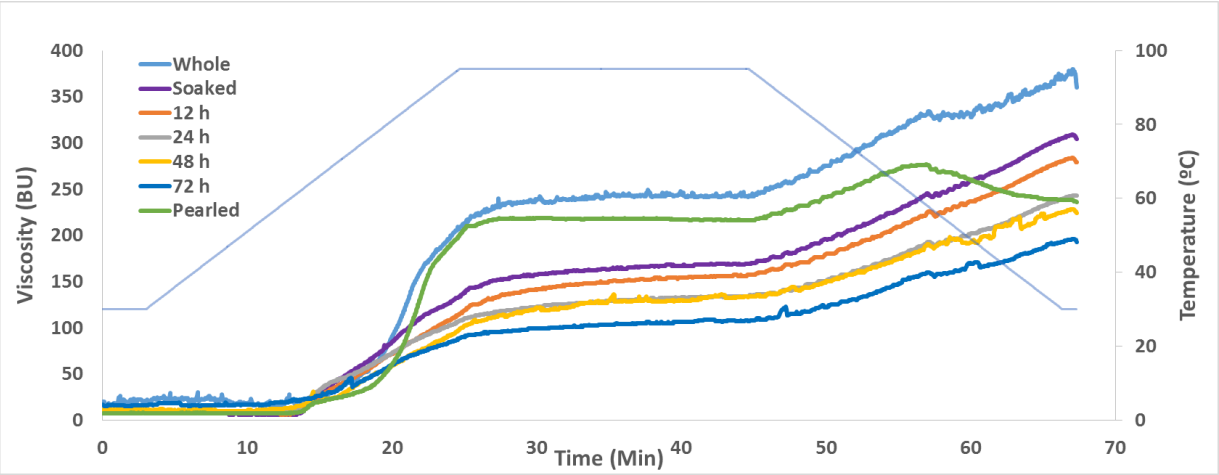

Figure A1. Pasting profile of quinoa flours in presence of $AgNO_3$.

3.3 Understanding the effects of sprouting on quinoa (*Chenopodium quinoa* Willd.) sensory traits

3.3.1 Abstract

Sprouting has been proposed as a suitable process to enhance the nutritional and functional properties of grains, including quinoa. However, there is a lack of information about the effects of sprouting time on the sensory traits of the seeds. In this context, the present study aimed at addressing the effect of sprouting time on the sensory traits of quinoa seeds before and after cooking, and to describe the potential changes to specific compounds. Whole quinoa was sprouted for 12, 24, 48 and 72 h at 22 °C and 90% of relative humidity and dried at 55 °C for 6 h. Sensory traits were assessed by electronic tongue and related to total titratable acidity and pH, total, free and bound phenolic and flavonoid compounds. Finally, the effect of sprouting time on saponin content was assessed by different approaches: afrosimetric method, spectrophotometric method, Thin Layer Chromatography (TLC), and Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HR-MS/MS). Sprouting determined an increase in sourness in agreement with the increase in total titratable acidity and the decrease in pH. Interestingly, quinoa was perceived as less bitter after 48 h of sprouting, as the consequence of the decrease in the amount of saponins measured by TLC and LC-HR-MS/MS, and confirming the decrease in foaming capacity as measured by the afrosimetric method, which is widely used in South America as a fast and simple method to provide information about saponin content in quinoa seeds.

I am grateful to: Professor Susanna Buratti and Dr. Simona Benedetti for e-sensing assessment; Dr. Gigliola Borgonovo for the analysis on phenols and saponin by spectrophotometric approach; Professor Pasquale Ferranti for saponin determination by TLC and LC-HR-MS/MS.

3.3.2 Introduction

Quinoa (*Chenopodium quinoa* Willd.) is one of the best alternatives for the fight against hunger and malnutrition in the world due to its high nutritional, safety, and sustainable profiles, including its great capacity to grow in stress conditions (Scanlin & Lewis, 2017). These characteristics – together with growing consumer interest in healthy foods - have triggered a continuous increase in quinoa production and consumption. In fact, starting in 2009 the fastest rise in world production has been reported, peaking in 2015 at 193,822 tons, more than 2.5 times the world yield in 2009 (FAO; www.fao.org).

Quinoa is mainly consumed as boiled seeds, especially in South America, whereas, in the other Western countries, there is a growing interest in using quinoa flour as an ingredient in cereal-based products. Nevertheless, the presence of bitter substances, which are mainly located in the external layers of quinoa, limits its consumption. Even though quinoa bitterness has always been associated with saponin content, other compounds such as phenolics or peptides or their interaction might play a role in determining quinoa bitterness (Suárez-Estrella et al., 2018). Pearling and washing are the two main industrial processes used, sometimes together, to remove saponins from seeds. Nevertheless, many compounds – interesting from a nutritional standpoint - are also lost during these processes (Gómez-Caravaca et al., 2004). Furthermore, washing requires high amounts of tap water, resulting in high environmental impact and pollution. More recently, sourdough fermentation of quinoa has been proposed as a tool to enhance the sensory traits of quinoa-enriched wheat bread, resulting in an acid, salty taste, with a decrease in sweetness (Rizzello et al., 2016), due to the enzymatic activities developed during the process. In this frame, the endogenous enzymatic activities developed during sprouting (or germination) might be a valid alternative to sourdough fermentation, thanks to the formation of simple sugars from starch hydrolysis (see section 3.1).

Sprouting is a natural process that decreases anti-nutrient compounds such as phytates in grains while substantially increasing the amount of components with antioxidant activity and the bioavailability of micronutrients (Lemmens et al., 2019; Benincasa et al., 2019). From a sensory standpoint, sprouted grains and their related products are characterized by a sweet taste, likely due to the formation of simple sugars (Richter et al., 2014). However, no information about the effect of sprouting on quinoa's bitter taste or aftertaste has been reported. Nevertheless, the effectiveness of sprouting in decreasing quinoa bitterness might be a hoped-for result, given the precedent of the positive results observed in other pseudocereals such as chickpeas (El-Adawy, 2002) and huazontle (Chenopodium berlandieri spp.) (Lazo-Vélez et al., 2016), which is botanically closely related to quinoa.

In this context, the aim of this study was to evaluate the effects of sprouting time on the main factors related to quinoa taste. In a previous study (see section 3.1), changes in chemical, physical, and functional properties after quinoa sprouting were reported. Understanding the effects of sprouting on sensory characteristics of both quinoa before (as flour) and after cooking might enhance the use of this pseudocereal as an ingredient in several food applications. In particular, using electronic tongue (e-tongue) will allow to evaluate the role of different chemical species in determining tastes in quinoa seeds and related flour. In the present study, the objective analysis of sensory traits through electronic senses was integrated with the quantification of the main components responsible for quinoa acceptability, namely saponins and phenolic compounds.

3.3.3 Materials and methods

3.3.3.1 Raw materials and sample preparation

Whole and pearled quinoa seeds (*Chenopodium quinoa* Willd. var. *Titicaca)* as well as the pearling by-product (bran) were provided by Quinoa Marche s.r.l. (Jesi, Italy). Whole quinoa was sprouted for 12, 24, 48 and 72 h as described in section 3.1. Samples were analyzed as flour after milling to particle size lower than 250µm in a lab scale mill (Cyclotec 1093 Foss Sample Mill, Höganäs, Sweden). The seeds (20 g) were cooked in 250 mL of boiling distilled water for 20 min (previously determined as the necessary time for complete starch gelatinization of the seeds). After draining the water, samples were frozen, freeze-dried and ground.

3.3.3.2 *Chemicals*

Only chemicals of analytical grade were used. Chemicals, solvents and reagents were acquired from diverse suppliers: liquid nitrogen from Air Liquid (Paris, France); selenium tabs from Thermo Fisher Scientific (Waltham, MA, USA); Tashiro indicator from Riedel-de Haen (Seelze, Germany); Folin Ciocalteau reagent from Carlo Erba (Milan, Italy); extra pure gallic acid from Merck (Darmstadt, Germany). All the other reagents as well as oleanolic acid were acquired from Sigma Aldrich (St. Louis, MO, USA).

3.3.3.3 Electronic tongue assessment

Analyses were performed with the Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Atsugi, Japan), namely, Electronic Tongue (e-tongue). The e-tongue is an analytical device that mimics the taste-sensing mechanism of the human gustatory system; it consists of comprises two sensor arrays that are specific for liquids and are able to evaluate tastes such as sourness, saltiness, bitterness, umami and astringency. The detecting part of the system consists of 8 sensors whose surface is attached with artificial lipid membranes having different response properties to chemical substances on the basis of their taste, as reported in Table 1.

The measurement principle of the e-tongue is based on the capability of taste compounds to change the potential of detecting sensors through electrostatic or hydrophobic interaction with the hydrophilic and hydrophobic groups of the lipid membranes. The response of each sensor, recorded as the difference between the potential detected by the sensor and the potential of the reference electrode, was elaborated by a computer and processed via a pattern recognition system.

The detecting sensors and reference electrodes were dipped into the reference solution (30 mM potassium chloride and 0.3 mM tartaric acid). The electric potential measured for each sensor was recorded (Vr). Then the sensors were dipped for 30 s into the sample solution and the potential was measured (Vs). For each sensor the “relative value” (Rv) was the difference between the potential

of the sample and the reference solution (Vs-Vr). Then, sensors were rinsed for 6 s and dipped into the reference solution again. The new potential of the reference solution was recorded (Vr'). The difference between the potential measured in the reference solution before and after measurement (Vr'-Vr) was the change of membrane potential caused by absorption "CPA value" (CPAv) and it corresponds to the electronic tongue "aftertastes". Before a new measurement cycle started, electrodes were rinsed for 90 s with a washing solution and then for 180 s with the reference solution.

Table 1. Characteristics of the detecting sensors of electronic tongue.

Sensor name	Application	Electrical charge	Taste
AAE	Food	Blended	Umami/Richness
CT0	Food	Blended	Saltiness
CA0	Food	Blended	Sourness
C00	Food/Pharmacology	Plus	Bitterness/Aftertaste bitterness
AE1	Food/Pharmacology	Plus	Astringency/Aftertaste astringency
GL1	Food	Plus	Sweetness
AC0	Pharmacology	Minus	Bitterness 1
AN0	Pharmacology	Minus	Bitterness 2

For the present work, five detecting sensors (AAE; CT0; CAO; C00; AE1) specific for food products and two reference electrodes, separated in two arrays according to membrane charge, were used.

Starting from the sensor outputs (Rv and CPAv), the "taste values", expressing the information regarding tastes, were calculated by multiplying them for appropriate coefficients based on Weber–Fechner's law: an empirical law which states that the intensity or strength that people feel is proportional to the magnitude of a physical stimulus. Based on Weber-Fechner's Law, a person can distinguish taste with a relative change in concentration of approximately 20% (Weber's ratio), a change in taste can be sensed when the relative change in concentration is 1.2 times. Hence, all taste information calculated from the sensor output is scaled to one unit of 20% relative change in concentration. In particular, the "taste values" were estimated as: Sourness = 0.3316 x Rv (CA0); Saltiness = - 0.252 x Rv (CT0); Bitterness = - 0.140 x Rv (C00) + 0.084 x Rv (CT0); Aftertaste-Bitterness = - 0.210 x CPAv (C00); Astringency = - 0.1575 x Rv (AE1) + 0.1575 Rv (CT0); Aftertaste-Astringency= - 0.252 x CPAv (AE1); Umami = - 0.1575 x Rv (AAE); Richness = - 0.420 x CPAv (AAE).

Both uncooked and cooked seeds (20 g) were suspended in 250 mL of distilled water. Solutions were vortexed at 10,000 rpm for about 5 min and centrifuged at 4000 x g for 10 min at 20° C. After

centrifugation, the supernatants were filtered. The filtered solutions were tested (n=3) and the sensor outputs were converted into taste information.

The sensory traits of quinoa samples were compared to those of quinoa bran, saponin extract, and oleanolic acid, which is one of the most common aglycones reported in quinoa.

Saponins are chemical compounds structured by an aglycone linked to one or more glycosidic fractions (Kuljanabhagavad et al., 2008). Quinoa saponins were extracted from quinoa bran according to Ng et al., (1994). Briefly, 1 g db of bran was extracted three times for 1 h each with 90 mL of methanol in a round-bottomed flask with reflux. The extracts were combined and evaporated in a rotavapor under reduced pressure until dry. The extract was diluted again with 10 mL of distilled water. To defat the extract, 10 mL of hexane were added and maintained with constant agitation for 5 min, and then a funnel was used to separate the hexanic fraction. The defatting phase was repeated three times. Afterwards, the aqueous fraction, containing the saponin was evaporated in a rotavapor as previously described.

3.3.3.4 Acidity and pH

Total titratable acidity and pH of raw quinoa were measured (n=3) according to Marengo et al., (2015), with slight modifications. For the extraction phase, 10 g (db) of each sample were diluted in 90 mL of distilled water and agitated at room temperature for 1 h. Then, the samples were centrifuged at 2500 x g for 10 min. Aliquots of the supernatants were maintained in darkness at 4ºC until analysis.

For the measurement of acidity and pH of cooked samples, 10 g (db) of each sample were cooked in a covered beaker with 125 mL of distilled water at boiling temperature for 20 min. After draining the water, the samples were frozen and freeze-dried. An aliquot of freeze-dried cooked quinoa seeds (5 g) was diluted in 100 mL of distilled water and agitated for 1 h at room temperature. The extracts were centrifuged at 2500 x g for 10 min and aliquots of the supernatants were maintained in darkness at 4ºC until analysis.

The acidity and pH of both raw and cooked samples were measured using a titrator T50 (Mettler-Toledo AG, Greifensee, Switzerland) equipped with a pH sensor (Mettler Toledo DGi 115-SC). Data acquisition was performed by LabX titration software (Mettler-Toledo Int. Inc.). Aliquots of the samples were analyzed, the pH values were measured before adding the titrant (NaOH 0.25N). The volume of titrant necessary to obtain pH 8.5 was measured. The results were reported as the volume of NaOH 0.25N necessary to achieve pH 8.5 in a solution of 10 g of dry sample diluted in 100 mL of distilled water.

3.3.3.5 Phenolic compounds and flavonoids

Total phenolic compounds and total flavonoids were extracted according to Carciochi et al., (2015), with slight modifications. An aliquot of sample (1 g db) was dispersed in 20 mL of ethanol : water solution (80:20 v/v) acidified with 1% HCl at 60 ºC in darkness for 1 h. Samples were filtered and completed to volume, then maintained at -28 ºC until analysis. Extraction for free phenolic and free flavonoid compounds was performed in the same conditions but at pH 6.9 instead of pH 1.5. The content of bound phenolic and bound flavonoid compounds amounted to the difference between the total and free contents of each one. Extraction was applied in duplicate for each sample, and two measurements in duplicate were performed from each extract, for a total of 8 replicates.

Phenolic and flavonoid contents were determined according to Carciochi et al., (2015) with slight modifications. For phenolics, 200 µL of extracted samples were diluted in 7.8 mL of water in a test tube, 0.5 mL of Folin-Ciocalteau reagent (2N) were added and the test tubes were vortexed. Then, 1.5 mL of sodium carbonate (20%) were added and the test tubes were vortexed again. The samples were left to rest for 2 h in darkness. The absorbance was measured at 760 nm with a spectrophotometer Lambda 2 (PerkinElmer, Inc., Waltham, MA, USA). To calculate the phenolic content a calibration curve was used (data not shown) and the results were expressed in mg of Gallic acid equivalent (GAE) per g of quinoa flour (db).

For the measurement of flavonoid content, 250 µL of extracted samples were diluted in test tubes with 2 mL of distilled water and 150 µL of sodium nitrite solution (5%). After 5 min, 150 µL of aluminum chloride solution (10%) were added, the test tubes were vortexed and left to rest for 6 min. Then, 1 mL of sodium hydroxide solution (1 M) and 1.2 mL of distilled water were added. The test tubes were vortexed again and the absorbance of the samples was measured at 415 nm. A calibration curve was used to determine the flavonoid content (data not shown) and the results were expressed in mg of Quercetin equivalent (QE) per g (db) of quinoa flour.

3.3.3.6 Saponin content

Four approaches were applied to quantify the amount of saponins in uncooked quinoa seeds (i.e. flour): afrosimetric method, spectrophotometric method, Thin Layer Chromatography (TLC), and Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HR-MS/MS).

3.3.3.6.1 Afrosimetric method

The afrosimetric method is an indirect approach for saponin quantification; based on the foaming capacity of quinoa saponins, it is the official method applied in Ecuador (NTE. INEN 1672, 1988). Briefly, an aliquot of sample (0.5 g) was vigorously agitated by hand with 5 mL of distilled water in a test tube for 30 s. This agitation was repeated three times with 30 min of interval between them. After 5 min from the third agitation, the foam height was measured (in centimeters) by Image Pro

Plus v. 6.0 (Media Cybernetics Inc., Rockville, USA). The afrosimetric method was applied in triplicate. The range of applicability for this method is between 0.2 and 3.0 cm of foam height (0.05 – 3.7 mg g^{-1} of saponin) (NTE. INEN 1672, 1988; Koziol, 1991). For saponin content determination, the following formula was applied and the saponin content expressed as mg g^{-1} (db).

$$Saponin\ content = \frac{(0.646) * (foam\ height) - 0.104}{(sample\ weight)}$$

3.3.3.6.2 Spectrophotometric method

The spectrophotometric approach was applied according to Nickel et al., (2016) with modifications. Quinoa seeds were submerged in liquid nitrogen in a mortar and then crushed to facilitate saponin extraction, which was carried out in triplicate. Then, milling was performed for 90 s in a lab scale mill (M 20 Universal mill, IKA®-Werke GmbH & Co. KG, Staufen, Germany). Samples (1 g db) were extracted in 10 mL of 50% methanol solution at room temperature for 72 h. Subsequently, samples were filtered (filter discs 4/N, Munktell & Filtrak GmbH, Bärenstein, Germany) into 10 mL volumetric flasks and the volume was completed with 50% methanol. Extracted samples were maintained at -28 ºC until analysis. For analysis, 3.5 mL of Liebermann–Burchard reagent were added to 1 mL of diluted sample (1:20), vortexed and left to stand for 30 min. Absorbance (n =2) was read with a spectrophotometer Lambda 2 at 528 nm (PerkinElmer, Inc., Waltham, MA, USA) and quantified with a calibration curve (17.5 – 1000 µg/mL) previously obtained with oleanolic acid ≥ 97% (data not shown).

3.3.3.6.3 Thin-Layer Chromatography (TLC) analysis

Quinoa extracts for TLC analysis were obtained according to Stuardo et al., (2008). Briefly, samples were extracted using a dilution of sample in distilled water (1:10) for 6 h at room temperature and with constant agitation. Then, samples were filtered (Whatman filter paper #2).

Each sample extract (4 µL) was directly applied on silica gel TLC plates with a micropipette and slightly dried using a slight air flow. TLC was performed using silica gel on aluminum foil with fluorescent indicator 254 nm (Sigma-Aldrich) 20 x 10 cm plates, layer thickness 250 µm. The mobile phase used for eluition was a mixture of chloroform/methanol/water 65/35/10 (Krishnamurthy et al., 2012).

At the end of chromatography the plates were dried at 100°C for 10 min to completely evaporate the mobile phase. Saponin dots were visualized by spraying the plates with a solution of sulfuric acid 10% after being heated at 115 °C. The results were scanned using a Scanjet 5370c scanner (Hewlett-Packard Company, USA).

3.3.3.6.4 Liquid chromatography coupled to high resolution mass spectrometry (LC-HR-MS/MS)

Mass spectrometry analysis was performed on the extract using a Q Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA), online coupled with an Ultimate 3000 ultra-high performance liquid chromatography (UHPLC) instrument (Thermo Scientific). Samples were suspended in 0.1% (v/v) formic acid solution, loaded through a 5 mm long, 300 µm id pre-column (LC Packings, USA) and separated by an EASY-Spray™ PepMap C_{18} column (2 µm, 15 cm x 75 µm) 3 µm particles, 100 Å pore size (Thermo Scientific). Eluent A was 0.1% formic acid (v/v) in Milli-Q water; eluent B was 0.1% formic acid (v/v) in acetonitrile. The column was equilibrated at 5% B. Saponins were separated by applying a 4–40% gradient of B over 40 min. The flow rate was 300 nL min^{-1}. The mass spectrometer operated in data-dependent mode and all MS1 spectra were acquired in the positive ionization mode with an *m/z* scan range of 100 to 1600. Up to 10 of the most intense ions in MS1 were selected for fragmentation in MS/MS mode. A resolving power of 70000 full width at half maximum (FWHM), an automatic gain control (AGC) target of $1x10^6$ ions and a maximum ion injection time (IT) of 256 ms were set to generate precursor spectra. MS/MS fragmentation spectra were obtained at a resolving power of 17,500 FWHM. In order to prevent repeated fragmentation of the most abundant ions, a dynamic exclusion of 10s was applied. Spectra were processed using the Xcalibur Software 3.1 version (Thermo Scientific). An example of a profile is reported in the Figure A1 in the appendix.

3.3.3.7 Statistics

The data were subjected to analysis of variance (ANOVA) to determine significant differences among the samples ($p \leq 0.05$). ANOVA analysis was performed by Statgraphics Plus 5.1 (StatPoint Inc., Warrenton, VA, USA). Different treatments and treatment times were considered as factors. When a factor effect was identified as significant ($p \leq 0.05$), the significant differences among the samples were determined using Tukey Honest Significant Difference (HSD) test.

The taste values collected by electronic tongue were elaborated by Principal Component Analysis (PCA) using MINITAB 14 (v.12.0) software package. PCA was used for explorative data analysis in order to achieve a partial visualization of the data set in a reduced dimension. PCA identifies orthogonal directions of maximum variance in the original dataset in decreasing order and projects the data onto a lower-dimensionality space formed by a subset of the highest-variance components. The orthogonal directions are linear combinations of the original variables and each component explains in turn a part of the total variance of the data; in particular, the first principal component (PC1) explains the largest percentage of the total variance, the second one (PC2), the second largest percentage, and so forth. PCA was performed in correlation (the variables were scaled). From the elaboration, two figures were collected: a PCA-score plot representing the relationship among the

samples, and a PCA-loading plot showing the relationship among the variables and how they influence the system.

3.3.4. Results and discussion

3.3.4.1 Electronic tongue assessment

The sensory traits obtained from e-tongue measurements and elaborated through the Principal Component Analysis (PCA) are shown in Figure 1. The two main components accounted for 73.8% of total variance. The score plot (Figure 1a) showed that quinoa type (uncooked *vs* cooked seeds) is mainly discriminated on PC1 (54.8% of total variance); while PC2 discriminated the samples (19% of total variance) according to the sprouting time.

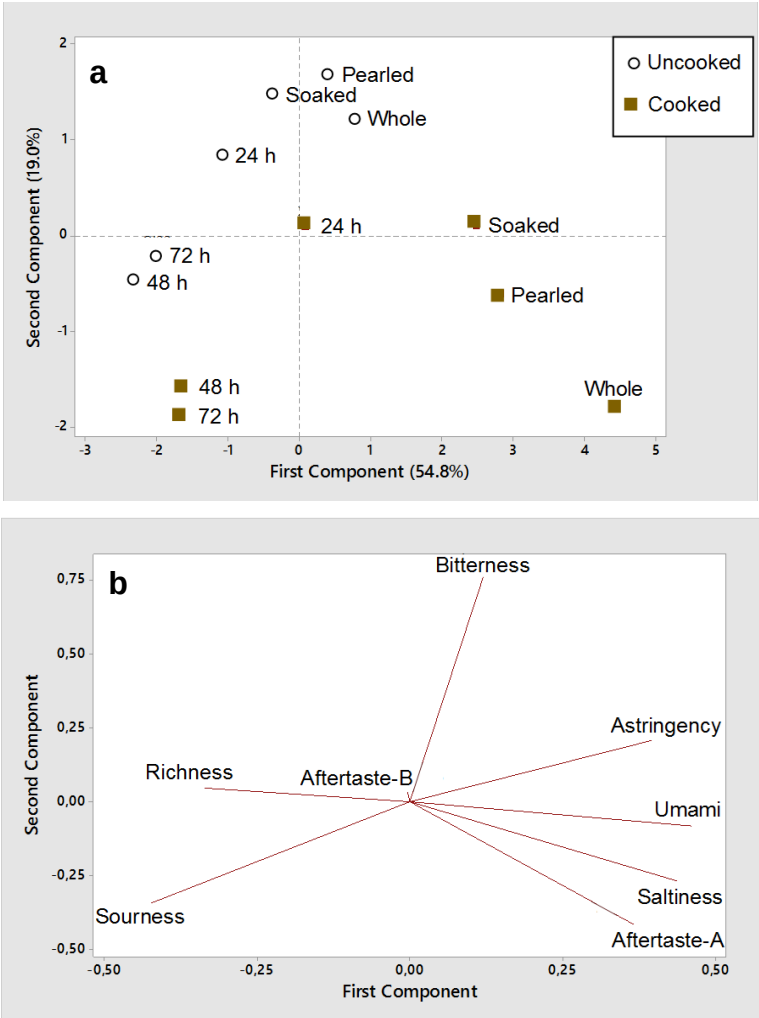

Figure 1. Score plot (a) and loading plot (b) from e-tongue PCA of quinoa before (circles) and after (squares) cooking. Aftertaste-A: Aftertaste-Astringency; Aftertaste-B: Aftertaste-Bitterness.

In particular, whole and pearled seeds (i.e. unsprouted samples) are mainly located on the right side (positive) of PC1. The loading plot (Figure 1b) highlighted the tendency to bitterness for the uncooked samples, whereas the cooked samples are characterized by astringency, umami, saltiness and aftertaste-Astringency taste. Sprouted samples are located on the left side (negative) of PC1, evidencing that sprouting increased the sourness and richness of uncooked and cooked seeds.

Sample grouping along PC2 highlights the effect of cooking in reducing bitterness in whole, pearled, and sprouted samples. On the other hand, cooking increased the astringency, umami, saltiness and aftertaste-Astringency tastes in unsprouted samples (both whole and pearled quinoa), while increasing the sourness in sprouted seeds. As regards the effect of sprouting, the PC2 evidenced a progressive decrease in bitterness and an increase in richness and sourness as the sprouting time was carried out for 48-72 h.

To better understand the role of saponins in determining the sensory traits of quinoa, e-tongue measurements were also carried out on quinoa bran, the saponins extracted from the bran, and the aglycone. The elaborated data are reported in Figure 2.

The PC2 evidenced that bran (where saponins are mainly located) and the extracted saponins have a high tendency to both bitter (Aftertaste-Bitterness) and astringent (Aftertaste-Astringency) aftertastes. Bran and saponins are discriminated along PC1, suggesting that bitter compounds other than saponins are present in the bran.

There is a lack of information about the effects of sprouting on the sensory traits of quinoa. Moreover, it is difficult to discuss our results in relation to the literature, since the effects of sprouting on chemical, physical and sensory properties strongly depend on the type of grains and sprouting conditions (namely time). To the best of our knowledge, some studies focused on the sensory profile of sprouts or sprout-enriched bread, with a different objective from our work. The latter indeed aimed at investigating the effects of sprouting on the sensory traits of quinoa seeds before and after cooking.

A previous study was carried out in our group using sprouted cowpea in rice-based breakfast cereals, highlighting the positive effects of sprouting in decreasing both astringency and bitterness (Marengo et al., 2017). On the other hand, the products enriched in sprouted cowpea showed an increase in sourness compared to the control (Marengo et al., 2017).

To better understand the factors responsible for the changes in sensory traits, the effects of sprouting on acidity and on the compounds involved in quinoa bitterness will be discussed in the next section.

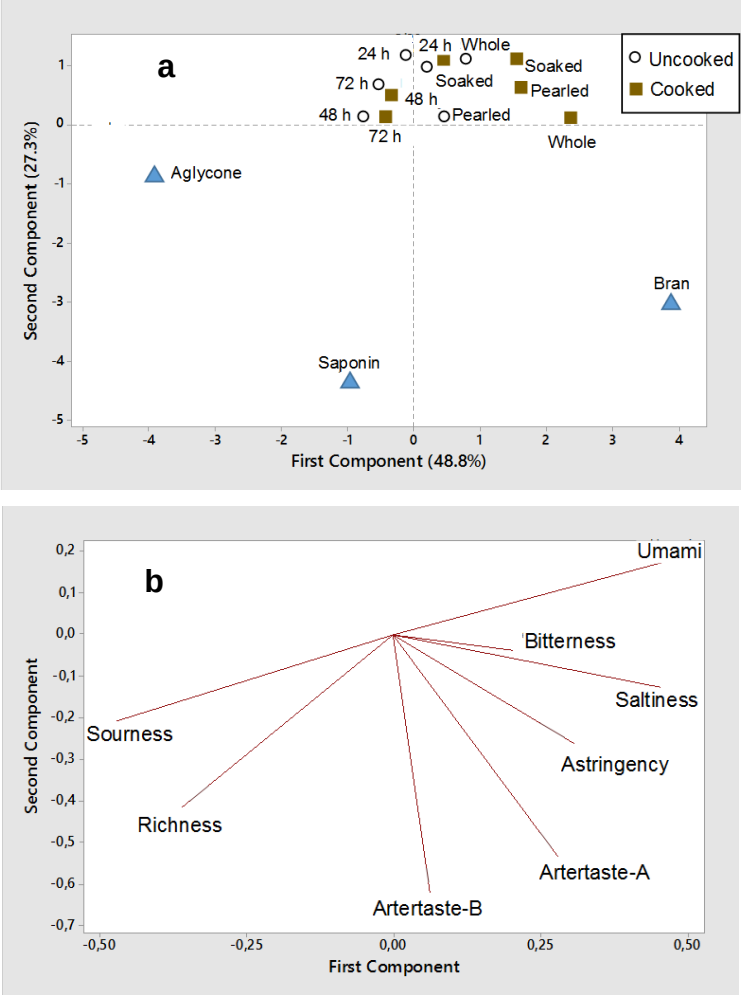

Figure 2. Score plot (a) and loading plot (b) from e-tongue PCA of quinoa before (circles) and after (squares) cooking and standards (triangles). Aftertaste-A: Aftertaste-Astringency; Aftertaste-B: Aftertaste-Bitterness.

3.3.4.2 Total Titratable Acidity (TTA) and pH

Total titratable acidity increased upon sprouting, showing significant differences from 48 h till the last sampling time considered in this study (Table 2). As expected, as acidity increased, pH values significantly decreased. The latter parameter was more sensitive to sprouting than acidity, since significant differences were observed already at early stages of sprouting (i.e. 12 h).

A similar trend in TTA and pH changes was observed after cooking. However, the values for cooked samples were lower in comparison with the values observed in the uncooked samples, probably due to the leaching of acid compounds in the boiling water. The solubility of organic acid was confirmed by the decrease in acidity after soaking (Table 2).

Pearling significantly changed the TTA and pH values with respect to the whole sample. Nevertheless, no significant differences were evidenced between them after cooking.

The changes in acidity and pH upon sprouting were in agreement with the increase in sourness highlighted by the e-tongue measurements (Figure 1), and they could be partially responsible for the decrease in perception of the bitter taste (Drewnowski, 2001).

Table 2. Effects of sprouting on total titratable acidity and pH.

		Total titratable acidity (mL NaOH 0.25N/10 g db)		pH	
		Uncooked	Cooked	Uncooked	Cooked
Whole		11.95±0.1^{d}	2.41±0.07^{a}	6.28±0.01^{f}	6.66±0.05^{d}
Soaked		9.10±0.2^{a}	2.76±0.12^{b}	6.36±0.01^{g}	6.40±0.06^{c}
Spouted	12 h	11.18±0.2^{c}	3.05±0.05^{c}	6.02±0.02^{d}	6.23±0.01^{bc}
	24 h	12.41±0.1^{d}	4.13±0.02^{d}	5.82±0.02^{c}	6.15±0.02^{b}
	48 h	19.28±0.2^{e}	5.40±0.19^{e}	5.33±0.01^{b}	5.74±0.21^{a}
	72 h	21.46±0.2^{f}	6.32±0.02^{f}	5.17±0.01^{a}	5.73±0.01^{a}
Pearled		10.35±0.1^{b}	2.27±0.01^{a}	6.23±0.01^{e}	6.72±0.02^{d}

Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; $n=3$).

3.3.4.3 *Phenolic compounds and flavonoids*

Polyphenols are inherently bitter compounds, thus one of the factors determining the perception of bitterness and astringency in wholegrains (Heiniö et al., 2016). Thus, in this section the evolution of both phenolic and flavonoid compounds was assessed during sprouting in order to provide

information about their potential role in affecting the sensory traits detected by e-tongue measurements.

Total phenolic content (TPC) progressively increased during sprouting, peaking at 48 h of process (Table 3). The steady increase in these compounds could be due to their synthesis as a consequence of the biochemical phenomena occurring during seed sprouting (Singh et al., 2015). Nevertheless, it could also be attributed to the easier extractability of phenolic compounds from the kernel structures, as shown in sprouted oats (Kaukovirta–Norja et al., 2004). This result is in agreement with the increase in free phenolic fraction and with the decrease in bound phenolic compounds (Table 3), probably as a result of the action of endogenous esterases synthesized during sprouting (Carciochi et al., 2016). The hydrolytic action also seems to be responsible for the changes in the ratio of free and bound flavonoids (Table 3). However, sprouting did not positively affect the Total flavonoid content (TFC). Indeed, 72 h of sprouting caused a significant decrease in both total and bound fractions and a significant increase in free flavonoids.

Table 3. Effects of sprouting on phenolic compound and flavonoid content.

		Phenolic compounds (mg GAE/g db)			Flavonoids (mg QE/g db)		
		Total	Bound	Free	Total	Bound	Free
Whole		3.04±0.05^{ab}	0.94±0.09^{ab}	2.10±0.05^{bc}	5.78±0.07^{d}	2.52±0.05^{c}	3.26±0.12^{ab}
Soaked		3.19±0.02^{bc}	0.95±0.03^{ab}	2.23±0.05^{c}	4.33±0.02^{ab}	0.22±0.03^{a}	4.12±0.05^{de}
Sprouted	12 h	3.29±0.05^{c}	1.36±0.04^{c}	1.93±0.08^{ab}	4.27±0.09^{ab}	1.10±0.17^{b}	3.17±0.09^{a}
	24 h	3.61±0.04^{d}	1.40±0.08^{c}	2.21±0.04^{c}	4.51±0.01^{b}	0.59±0.10^{a}	3.92±0.11^{cd}
	48 h	4.39±0.04^{f}	0.78±0.02^{a}	3.61±0.06^{e}	4.47±0.02^{b}	0.45±0.08^{a}	4.02±0.10^{de}
	72 h	3.92±0.02^{e}	0.72±0.09^{a}	3.21±0.07^{d}	4.75±0.03^{c}	0.37±0.07^{a}	4.38±0.11^{e}
Pearled		2.97±0.05^{a}	1.08±0.05^{b}	1.89±0.01^{a}	5.74±0.05^{d}	2.15±0.10^{c}	3.59±0.05^{bc}

Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; n=4).

Pearling did not affect the TPC while promoting a significant decrease in free phenolic fraction (Table 4). Indeed, free phenolic compounds are mainly located in the pericarp of cereal seeds, while bounds are mainly located in the cell wall of the seeds (Carciochi et al., 2016). Pearling did not significantly

modify the content of total, free or bound flavonoids with respect to the whole sample. On the contrary, Hemalatha et al., (2016) reported an increase in TFC after pearling. Such differences might be due to differences in the pearling process and in the amount of bran removed from the kernels.

Overall, the effects of sprouting on phenolic and flavonoid compounds are not consistent. Indeed, some authors reported an increase (Carciochi et al., 2014; Alvarez-Jubete et al., 2010; Laus et al., 2017), while others reported a decrease (Paśko et al., 2008), likely due to differences in internal and external conditions such as cultivar (Bois et al., 2006), sprouting conditions (Carciochi et al., 2016) and methods used for measuring phenolic and flavonoid compounds.

The results highlighted that the changes in polyphenols did not account for the changes in sensory traits detected due to sprouting.

3.3.4.4 Saponin content

The bitterness of quinoa has always been associated with the presence of saponins in quantities higher than 1.1 mg g^{-1}, corresponding to the amount proposed by Koziol, (1991) as the threshold for human perception of bitterness. In this section, the effect of sprouting on saponin content was assessed by different analytical approaches of different complexity.

3.3.4.4.1 Quantification of saponins by afrosimetric method

The afrosimetric approach is the official method widely used in South America for the indirect determination of saponin content in quinoa seeds. This fast, simple and cheap test is based on the measurement of the foam formed after vigorously shaking of quinoa seeds in water.

A significant and progressive decrease in foam height was evidenced in sprouted samples (Figure 3). The application of the formula developed by Koziol, (1991) allows to indirectly quantify the amount of saponins that decreased as sprouting increased until 48 h (Table 4).

It is worth mentioning that the formula is valid only for the development of foam heights ranging between 0.2 cm and 3.0 cm (Koziol, 1991), which correspond to values from 0.05 to 3.67 mg g^{-1} (wb) of saponins, respectively. Foam heights out of this range do not correspond with the linearity of the equation. Consequently, it was not possible to quantify the saponin content in both whole and 12 h-sprouted samples. Besides saponins, the decrease in albumin and globulin might also have contributed to the decrease in foaming capacity in quinoa (see chapter 2), in agreement with Koziol (1991).

Even if sprouting promoted a relevant decrease in saponin content, this biotechnological process was not as effective as pearling (Table 4). Moreover, the very low saponin content measured in the pearled sample suggests that its bitterness (Figure 1) is not related to saponin content, but likely to phenols (Table 3).

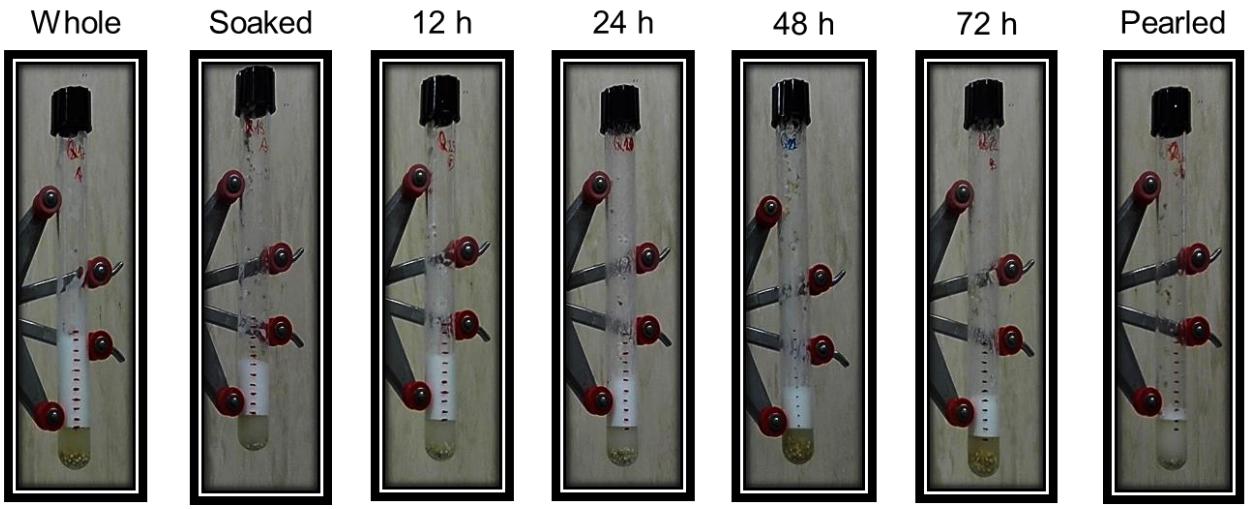

Figure 3. Developed foam during the saponin content evaluation using the afrosimetric approach (Method NTE INEN 1672).

Table 4. Effects of sprouting on saponin content, measured by different approaches.

		Afrosimetric method		Spectrophotometry (mg/g db)
		Foam height (cm)	Saponin content (mg/g db)	
Whole		4.8±0.1^{e}*	≥3.7	5.3±0.4^{c}
Soaked		2.7±0.3^{c}	3.3±0.3^{c}	4.3±0.4^{ab}
Sprouted	12 h	3.5±0.1^{d}*	≥3.7	4.3±0.5^{ab}
	24 h	2.5±0.2^{bc}	3.0±0.3^{c}	4.6±0.5^{bc}
	48 h	2.2±0.1^{b}	2.6±0.1^{b}	6.6±0.4^{d}
	72 h	2.1±0.1^{b}	2.5±0.1^{b}	7.3±0.3^{d}
Pearled		0.6±0.1^{a}	0.6±0.1^{a}	3.7±0.4^{a}

* The concentration of saponin is linearly correlated with foam heights between 0.2 and 3.0 cm. Different letters in the same column indicate significant differences (Tukey HSD; $p<0.05$; n=3).

3.3.4.4.2 Quantification of saponins by spectrophotometric method

The effect of sprouting on saponin content was also evaluated by a colorimetric test, which represents the most used methods in the literature. The test is based on the Liebermann–Burchard (LB) reaction, which is an oxidant reagent (16.7% of acetic anhydride in concentrated sulfuric acid) that develops a green-blue color from pentacyclic cations (Xiong et al., 2007).

Results showed a decrease in saponin content in soaked, 12 h- and 24 h-sprouted quinoa (Table 4). On the other hand, prolonged sprouting (from 48 h until 72 h) unexpectedly promoted a significant increase in these components. Early stage sprouting was as effective as pearling in removing the total amount of saponins.

The effects of sprouting on saponin content were not consistent in the literature. Indeed, decreases in these components have been reported in huazontle (Lazo-Vélez et al., 2016) - a quinoa-like grain - and in some pulses, including chickpea (El-Adawy, 2002), pigeon pea (Duhan et al., 2001), black gram (Jood et al., 1986) and kidney bean (Shimelis & Rakshit, 2007). On the other hand, other studies reported an increase in saponins in soybean (Shimoyamada & Okubo, 1991; Bau et al., 2000) and lentils (Ayet et al., 1997) during sprouting. Finally, no changes in lentils and chickpeas have been also reported (Ruíz et al., 1996). Once again, the type of grain, sprouting conditions, and analytical approach might account for the differences of the results among the studies.

3.3.4.4.3 Semi-quantification of saponins by Thin Layer Chromatography (TLC)

The semi-quantitative TLC analysis, based on the intensity of bands, showed a decrease in saponin content as sprouting proceeds (Figure 4). Indeed, the intensity of the bands associated with saponins decreased. This phenomenon was more and more effective as sprouting time proceeded. At the end of the process (72 h), it was not possible to detect any bands in the saponin area, suggesting the significant effect of sprouting on decreasing the amount of saponins. This result is in agreement with the afrosimetric method (Table 4) and the decrease in bitterness detected by the e-tongue (Figure 1).

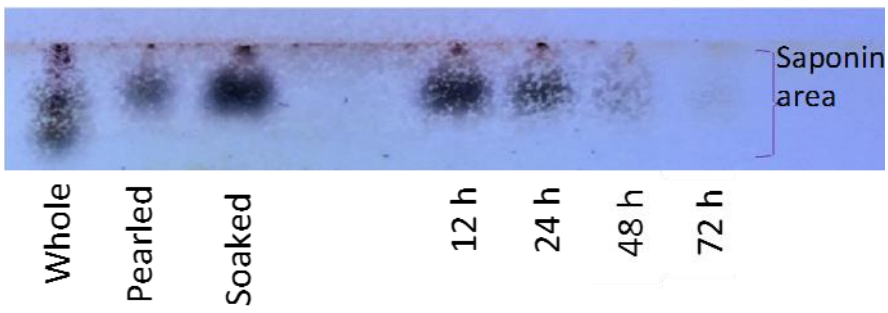

Figure 4. TLC of quinoa extracts.

3.3.4.4.4 Quantification of saponins by Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HR-MS/MS).

Each TLC spot was analyzed by LC-HR-MS/MS to quantify the total amount of saponins, in order to confirm the role of sprouting on decreasing the amount of saponins in quinoa flour. Data are summarized in Figure 5.

As the sprouting time progressed, the native saponins diminished from the quantitative point of view and degradation products were produced starting from 24-48 h. These products are derived from the deglycosylation and dehydration of serjanic acid and phytolaccagenic acid, identified by MS/MS. This data could explain the discrepancies observed between the spectrophotometric and the afrosimetric methods.

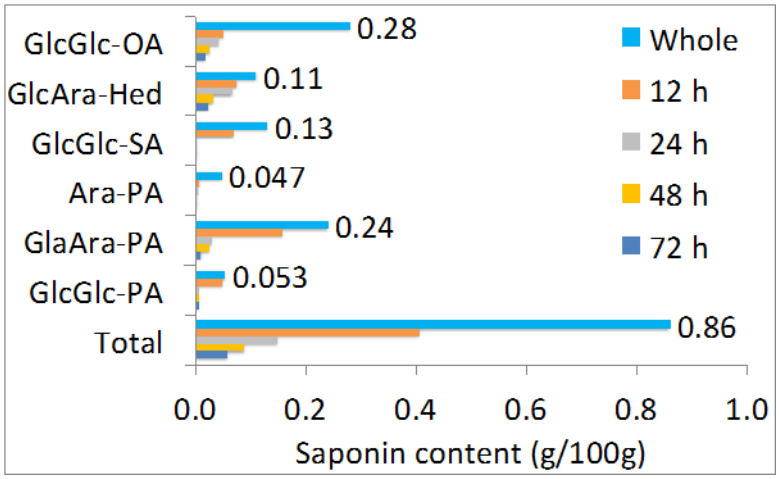

Figure 5. Effect of sprouting on saponin content. Glycosidic fraction: Glc, Glucose; Ara, Arabinose; Gla, glucuronic acid. Aglycone: OA, Oleanolic acid; Hed, Hederagenin; SA, Serjanic acid; PA, Phytolaccagenic acid

3.3.5 Conclusions

Besides the positive effects on nutritional and technological properties, sprouting enhances the sensory profile of quinoa before and after cooking, suggesting a potential use of sprouted quinoa not only as seeds but also as an ingredient in food formulation. The increase in the sweet note has been showed in previous studies and attributed to the formation of simple sugars during sprouting as a consequence of starch hydrolysis by amylases. The present study showed that sprouting determined an increase in sourness and a decrease in bitterness for both flour and cooked seeds. Mapping the

changes in acidity, pH, phenols and saponins during sprouting time allowed to better understand the role of each component/factor in determining the sensory traits of sprouted samples. The accumulation of acids during sprouting needs to be confirmed as potentially responsible for the increase in acidity and thus in the perception of sourness. Changes in polyphenols due to sprouting did not account for the improvement in the sensory traits; however, the quantification of specific phenolic acids might provide further insight into the potential role of these compounds in defining the sensory characteristics of sprouted samples. Interestingly, sprouting can be proposed as an alternative method to washing or pearling to decrease the amount of saponins, starting from 24-48h of process, while increasing the nutritional value of the product.

3.3.6 References

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Appendix

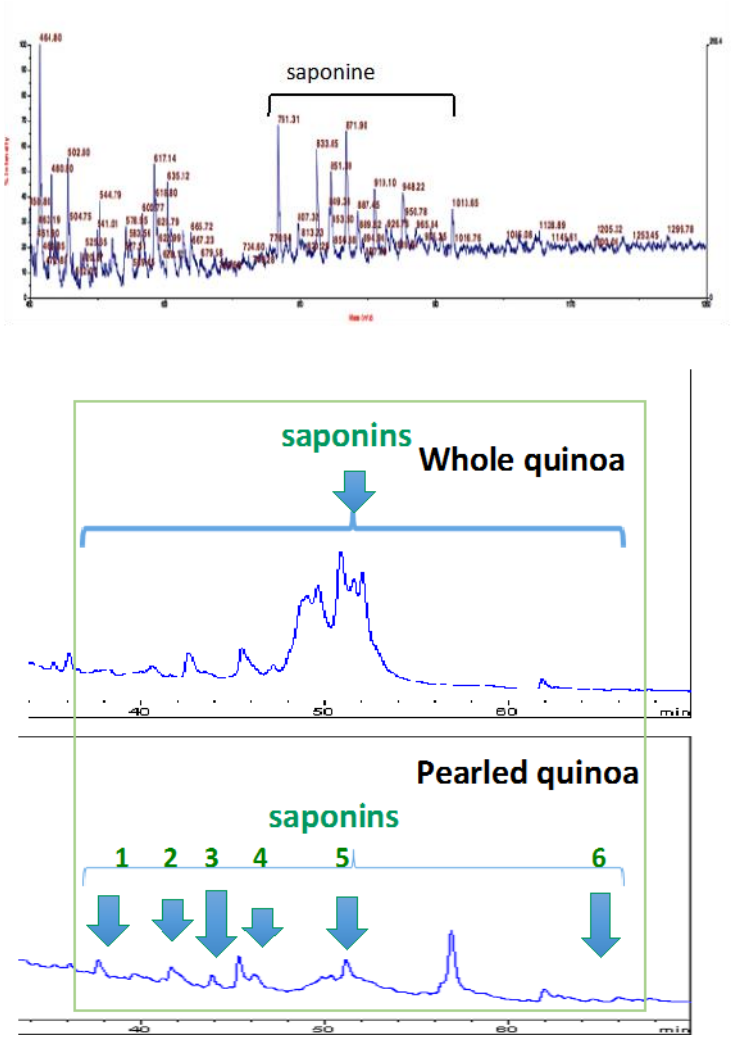

Figure A1. Examples of quinoa water extract (Λ= 220 nm)

3.4 Technological and sensory performance of sprouted quinoa flour in bread-making

3.4.1 Abstract

In the first part of this study, whole flour from sprouted quinoa was added to wheat at 10, 20, or 30% and their rheological properties and baking performance were assessed.

The 20% enrichment level was able to assure a good gluten aggregation suitable for the formation of dough able to maintain its structure during leavening and retain a high percentage of gas. These dough characteristics allow bread to reach the highest specific volume and the lowest crumb firmness up to three days of storage.

The behavior of sprouted quinoa enriched-bread was compared with that of pearled quinoa-enriched bread at the same percentage (20%). Results showed that sprouting can be preferred to pearling, which is the most common pre-treatment for allowing the use of quinoa in bread-making. Indeed, sprouted quinoa-enriched bread showed the best results in terms of physical (volume, softness) and sensory (decrease in bitterness) traits.

I am grateful to Professor Susanna Buratti and Dr. Simona Benedetti for e-sensing assessment.

3.4.2 Introduction

Quinoa is an interesting raw material in food formulation thanks to its potential health benefits that have been extensively reviewed (Tang & Tsao, 2017). In particular, the high amount of lysine – the limiting amino acid in all cereals – makes quinoa unique among grains. Moreover, quinoa is a good source of minerals and antioxidants, such as phenolic compounds. High dietary fiber and stable polyunsaturated fatty acids increase its potential to treat various diseases (Tang & Tsao, 2017). These characteristics are the driving force for enhancing the consumption of quinoa not only as seeds but also as ingredient in various food applications, including cereal-based products.

Despite the well known nutritional features of quinoa, its consumption is limited by the bitter and astringent taste, typical of whole seeds and caused by the presence of saponin compounds that are mainly located in the external layers of the grain. Washing and/or pearling are the main processes currently applied to quinoa for improving its acceptability. However, results in section 3.3 showed that quinoa sprouting was successful in decreasing the amount of saponins and thus improving quinoa sensory traits. Moreover, our previous research (see section 3.2) described the changes in functional properties after quinoa sprouting, suggesting a potential exploitation of sprouted quinoa

in stabilizing foam and decreasing starch retrogradation in baked products (see section 3.2). Besides improving the nutritional value of cereals and pulses (Lemmens et al., 2019), sprouting also enhanced their bread-making performance (Marti et al., 2017; Marti et al., 2018; Marengo et al., 2017a). However, no information is available about using sprouted quinoa in bread formulation in comparison with the use of this grain after pearling. Indeed, up to now, quinoa has been proposed in bread-making only as flour from washed and/or pearled grains (Rosell et al., 2009; Chauhan et al., 1992; Lorenz and Coulter, 1991). Therefore, the aim of this study was to investigate the effects of enrichment in sprouted quinoa (from 10 to 30%) on the features of wheat-based dough and bread.

3.4.3. Materials and methods

3.4.3.1 Materials

Flours from pearled and 48h-sprouted quinoa were prepared as described in section 3.2. Commercial wheat flour (WF; protein: 12.3%; W: $290*10^{-4}$ J) was provided by Molino Quaglia S.p.A. (Vighizzolo D'Este, Italy) and used for preparing the blends with 10% (10S), 20% (20S), and 30% (30S) of sprouted quinoa and 20% of pearled quinoa (20P). All samples were stored at 4 ºC until analysis. Moisture was measured at 130 ºC (n=2) by a thermo-balance (Radwag MA 50/1.R, Radom, Poland).

3.4.3.2 Gluten aggregation properties

Gluten aggregation properties (n=3) were measured by means of GlutoPeak® device (Brabender GmbH & Co. KG, Duisburg, Germany). An aliquot of 9 g of sample was dispersed in 9 mL of water, scaling its weight on a 14% flour moisture basis. Testing was performed at 35 ºC and the paddle speed was set at 2750 rpm. The main indices assessed by the software (Brabender GlutoPeak v.2.2.2) were: the maximum torque (peak evidenced when gluten aggregation occurs), peak maximum time (time at maximum torque) and the aggregation energy (area under the curve from 15 s before to 15 s after peak).

3.4.3.3 Mixing properties

Mixing properties (n=3) of WF and mixtures were measured by using the Farinograph-E® (Brabender GmbH & Co. KG, Duisburg, Germany) equipped with a 50 g mixing bowl according to ICC 115/1 Approved Method (ICC, 1992). The parameters measured using the device software (Brabender ® Farinograph Version 4.1.0) were: water absorption, dough development time, dough stability, and degree of softening.

3.4.3.4 Leavening properties

Dough for leavening properties (n=3) was prepared with commercial baker's yeast (AB Mauri Italy S.p.A., Casteggio, Pavia, Italy) (2.5 g/100 g flour) and salt (1.5 g/100 g flour). The amounts of water used and the mixing times were previously determined through the farinographic test. Samples were kneaded with a lab mixer (Artisan 5KSM150PS KitchenAid, St. Joseph, Michigan, USA) equipped with a hook. An aliquot of 315 g was placed in the Chopin Rheofermentometer F4 device (Chopin, Tripette & Renaud, Villeneuve La Garenne Cedex, France) for 3 h at 30 °C, to measure dough development and gas production during the leavening phase.

3.4.3.5 Pasting properties

Pasting properties (n=3) were assessed using the Micro Visco-AmyloGraph (MVAG; Brabender OHG, Duisburg, Germany) as reported in section 3.2. The following parameters were considered using the Viscograph software (Version 4.3.0. Brabender OHG, Duisburg, Germany): the temperature at the beginning of gelatinization, peak viscosity, breakdown, final viscosity and setback.

3.4.3.6 Bread-making

Sample doughs were prepared in the same conditions as those for evaluating leavening properties. After mixing, the dough was left to rest for 10 min at room temperature, divided in portions of 250 g each, molded into cylindrical shapes and placed into baking pans (8x15x5 cm) previously sprayed with a food grade commercial non-stick agent (rapeseed oil, Alsa brandxx) to facilitate loaf removal from the pan after cooking. Doughs were leavened in a laboratory scale oven (Self Cooking Center, Rational International AG, Landsberg am Lech, Germany) at 30 ºC up to 10 cm of height. The time necessary for leavening varied from 75 to 85 min, according to the type of sample. Samples were baked at 220 ºC for 25 min. Two hours after baking, bread samples were characterized in terms of specific volume, whereas crumb moisture, water activity and firmness were measured after 2 h, 24 h and 72 h. Finally, weight loss was determined 24 h and 72 h after baking. For the storage studies, samples were stored in polypropylene bags Three baking tests were performed for each sample and three loaves were obtained from each baking test.

3.4.3.7 Bread characterization

3.4.3.7.1 Specific volume

Loaf volume (n=9) was measured by using the rapeseed displacement method with sesame seeds. Loaf weight (n=9) was determined by using a technical scale. After that, specific volume was calculated by dividing the loaf volume by its weight.

3.4.3.7.2 Crumb moisture and water activity

Moisture and water activity (n=3) were determined on the central area of the central slice of each bread loaf. Moisture was measured in a moisture analyzer MA 210.R, Radwag (Wagi Elektroniczne, Poland), using 3g of sample and drying at 130 ºC until the weight did not change by 1 mg for 10 s. Water activity (a_w) was evaluated by an electronic hygrometer Acqua Lab, CX-2 (Decagon Devices, Pullman, WA, USA).

3.4.3.7.3 Crumb firmness

Crumb firmness (n=9) was evaluated according to the AACCI 74-09.01 method by means of a Texture analyzer device TA-TX plusC (Stable Micro Systems, Godalming, Surrey, UK) equipped with a 100 N load cell. Three central slices (25 mm thick) were compressed at 40% of deformation using a 36 mm diameter cylindrical plunger (SMS P/36R). The compression force value was measured at 25% deformation and expressed in Newtons.

3.4.3.8 Electronic tongue assessment

Electronic-tongue (e-tongue) assessment was performed (n=3) on breads from either 20% sprouted or 20% pearled quinoa, as well as on crusts and crumbs. Bread were freeze-dried and milled in a lab scale mill (IKA M20, Staufen, Germany). Analyses were performed with the Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Atsugi, Japan) as described in section 3.3.

3.4.3.9 Statistics

All data were elaborated by means of Statgraphics Plus 5.1 (StatPoint Inc., Warrenton, VA, USA). Analysis of variance (one-way ANOVA) was performed to determine significant differences among the wheat and sprouted samples ($p<0.05$). The significant differences among the samples were determined using Tukey Honest Significant Difference (HSD) test. Data from e-tongue measurements were elaborated by Principal Component Analysis as described in section 3.3. A two-sample t-test was used for compare pearled with sprouted bread.

3.4.4. Results and discussion

In the first part of this study, whole flour from sprouted quinoa was added to wheat at different levels: 10, 20, and 30%. The rheological properties and the baking performance of these blends were assessed to identify the highest quinoa enrichment level associated with an increase in protein content, without compromising the overall quality of bread. In this context, the discussion will be focused on the effects of quinoa levels rather than the effects of quinoa addition to wheat. Indeed, the expected worsening of dough properties in the presence of gluten-free flours has already been

discussed in previous studies (Wang et al., 2015; Park & Morita, 2005) as well as for wheat based breads (Tömösközi et al., 2011).

Once the best enrichment level was established, the behavior of sprouted quinoa enriched-bread was compared with that of pearled quinoa-enriched bread at the same percentage. Currently, quinoa can be used in foods only as pearled seeds, namely after removing the external layers that are rich in saponins. Indeed, these compounds are associated with quinoa bitterness and thus with its low sensory acceptability.

3.4.4.1 Rheological properties

3.4.4.1.1 Gluten aggregation kinetics

The GlutoPeak ® is a new test used to evaluate the gluten quality in wheat (Marti et al., 2014b; Malegori et al., 2018).

The gluten aggregation kinetics of quinoa-enriched flours is reported in Figure 1, while the data are summarized in Table A1 in the appendix. Adding 10% or 20% of sprouted quinoa to wheat did not compromise the gluten aggregation capacity. Maximum torque and peak maximum time slightly (but significantly) decreased in the presence of 20% and 10% quinoa, respectively.

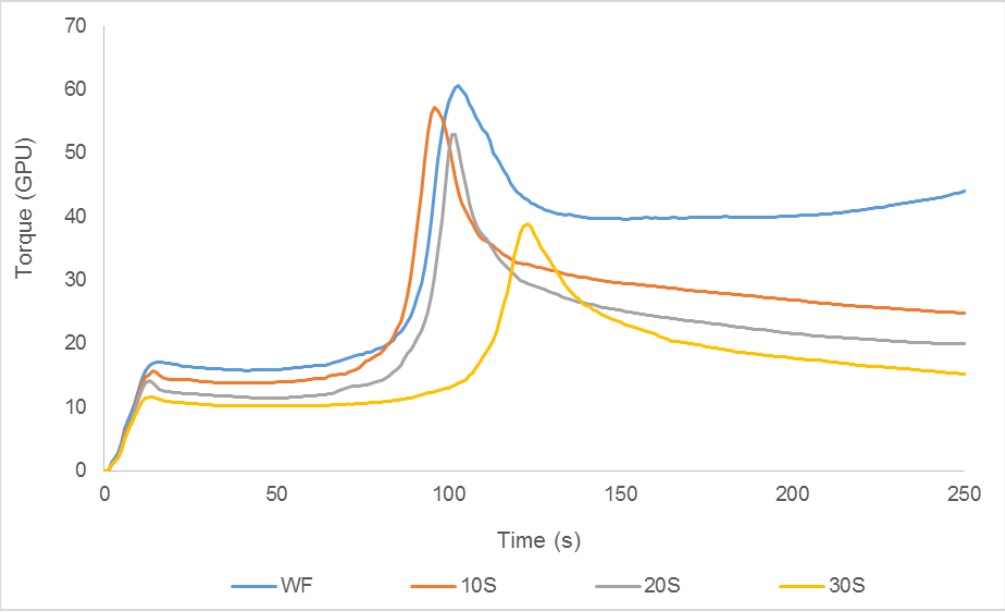

Figure 1. Gluten aggregation properties of sprouted quinoa-enriched wheat flours.

The decrease in maximum torque was likely due to the dilution of gluten proteins, as reported in previous studies (Marti et al., 2015a; Quayson et al., 2016). Moreover, the GlutoPeak profile of quinoa-enriched flours showed a sharper peak compared to wheat (Figure 1), showing great loss of

consistency after reaching the maximum torque (i.e. the gluten formation) which suggested weakening of the gluten network (Marti et al., 2014a). The decrease in energy values (see Table A1) – which is proportional to the enrichment level - confirmed gluten weakening as the amount of quinoa increased. Increasing the amount of sprouted quinoa from 20 to 30% dramatically affected the gluten aggregation of the mixture, suggesting that this blend might not be suitable for bread-making. The worsening in gluten aggregation properties - in terms of decrease in maximum torque and aggregation energy and increase in peak maximum time - was also observed when high amounts of fiber were included in wheat-based products (Marti et al., 2014b; Marti et al., 2015b).

3.4.4.1.2 Mixing properties

The mixing properties of quinoa-enriched flours are reported in Figure 2, and the related indices are summarized in Table A1 in the appendix. Regardless of the enrichment level, adding sprouted quinoa to wheat significantly increased the amount of water needed to reach optimal consistency (500 FU). A relevant decrease in dough stability was observed increasing the quinoa level from 10 to 20%, with no further worsening in the case of 30%. Nevertheless, prolonged mixing of dough highlighted the further worsening in mixing properties (evaluated by the degree of softening) when the highest level of quinoa was considered. The results can be explained by taking into consideration that as the percentages of quinoa increased, the levels of fiber and non-gluten proteins increased too.

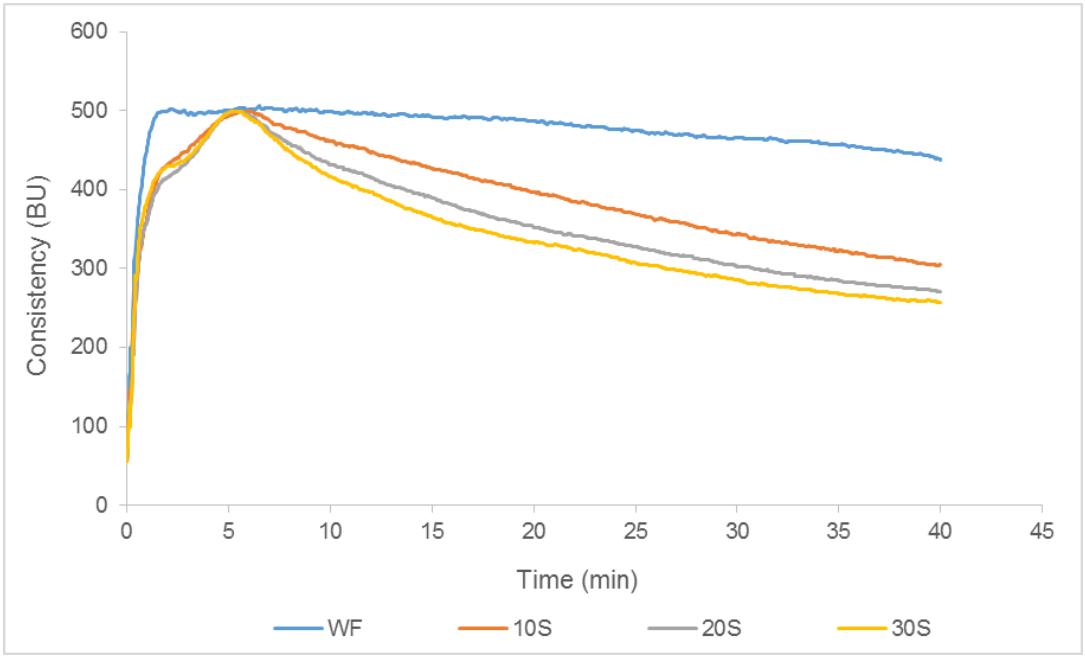

Figure 2. Mixing properties of sprouted quinoa-enriched dough.

3.4.4.1.3 Leavening properties

Leavening performances are reported in Figure 3, while the indices are summarized in Table A1 in the appendix. At the beginning of the leavening phase (up to 1 h), the sprouted quinoa-enriched samples exhibited a rapid dough development, regardless of the amount of added quinoa. This behavior was likely due to the presence of fermentable sugars in sprouted flours (see section 3.2), as was also reported for sprouted wheat (Marti et al., 2017; Marti et al., 2018). At longer leavening time (>1.5 h), either dough with 20 or 30% sprouted quinoa were not able to maintain their own structure, resulting in a decrease in dough height as a consequence of the weakening of the gluten network (see Figure 2). However, dough weakening was less dramatic in 10% enriched-sample and therefore no loss in maximum height was detected. The production of fermentable sugars during sprouting also accounted for the increase in total gas production, with no differences according to quinoa level (Figure 4). Despite the differences in gluten matrix, all samples were able to retain the same amount of gas (Figure 4), which was lower compared to wheat dough.

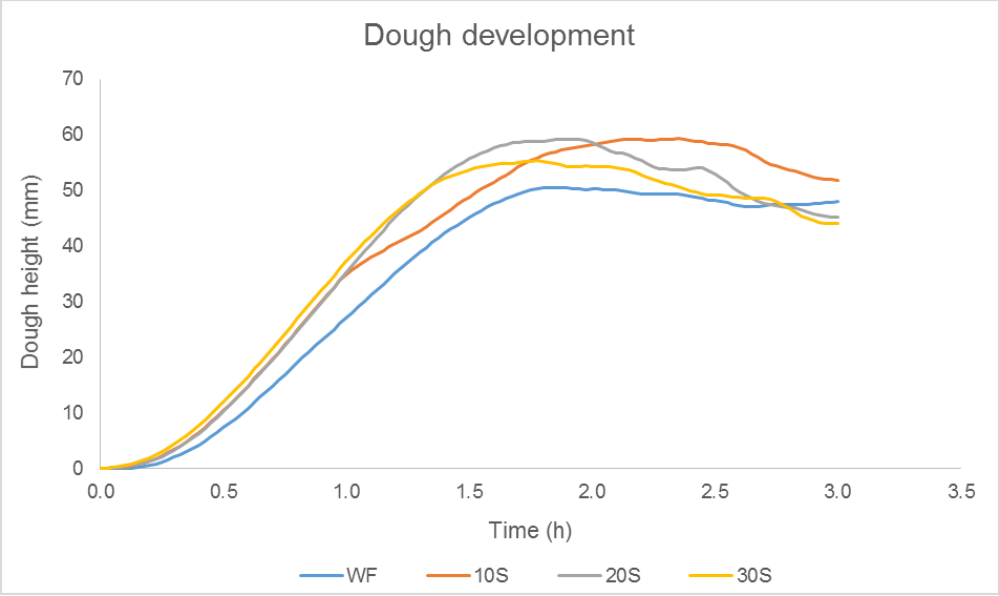

Figure 3. Height development of sprouted quinoa-enriched dough.

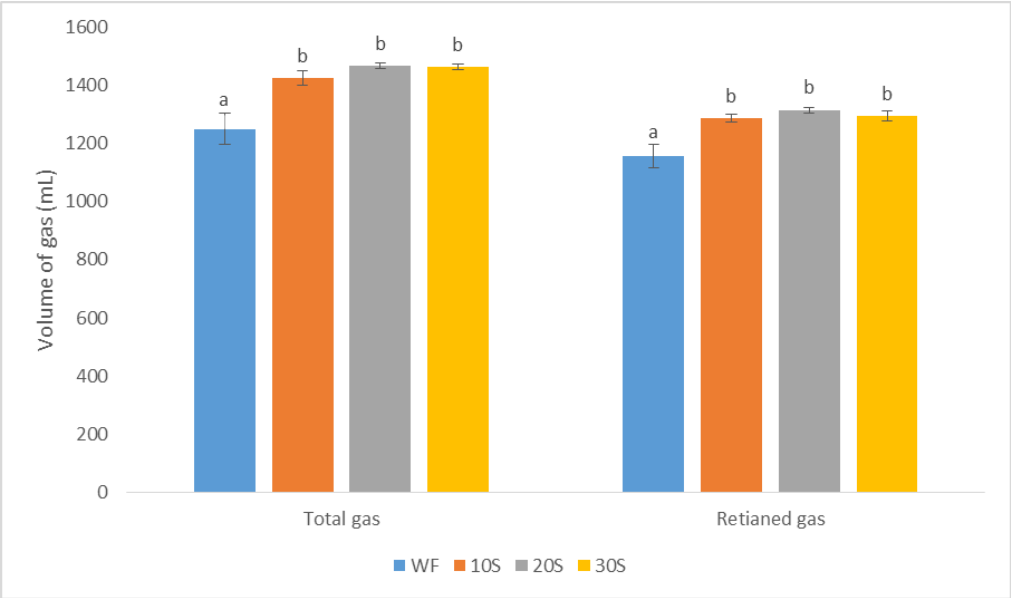

Figure 4. Gas production and retention in sprouted quinoa-enriched dough.

Different letters indicate significant differences for produced and retained gas (Tukey HSD; $p<0.05$; $n=3$).

3.4.4.1.4 Pasting properties

Pasting profiles of the blends were assessed in order to provide information on the effects of baking on starch gelatinization and to predict starch retrogradation during bread storage, which is responsible for bread staling. Changes in starch gelatinization and retrogradation that are shown in Figure 5 were due to modifications in starch structure during sprouting, as discussed in section 3.2. The low retrogradation tendency of blends with 20 and 30% sprouted quinoa (see setback values in Table A1 in the appendix) might result in the decrease in bread staling, thereby maintaining crumb softness during storage.

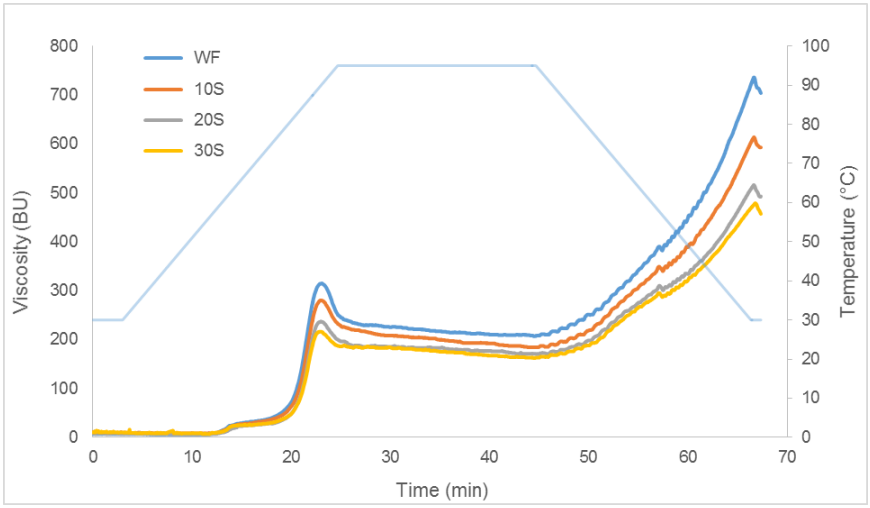

Figure 5. Pasting profiles of sprouted quinoa-enriched blends.

3.4.4.2 Bread characterization

The best baking performance in terms of specific volume was reached by the sample made from 20% sprouted quinoa, whose volume was even higher compared with wheat bread (Figure 6). This result is in agreement with the effect of 20% sprouted quinoa enrichment on gluten aggregation (Figure 1), mixing (Figure 2) and leavening (Figure 3 and 4) properties.

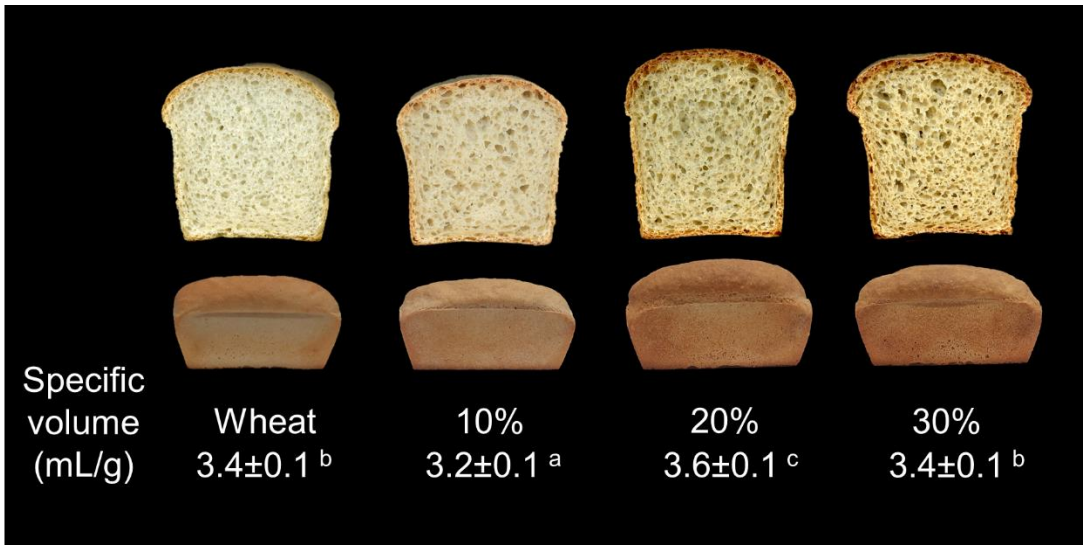

Figure 6. Characteristics of sprouted quinoa-enriched bread.

Adding sprouted quinoa at high percentages (20-30%) significantly decreased crumb firmness, contributing high crumb softness (Figure 7). Differences among samples were not related to differences in moisture or water activity (as shown in Table A2 in the appendix), but to the high specific volume of bread (Figure 6). These samples (i.e. 20% and 30% quinoa enriched breads) were characterized by high degree of softness even up to 72 h of storage, in agreement with the low starch retrogradation tendency observed in Figure 5, as a consequence of the α-amylase activity developed during sprouting (see section 3.2).

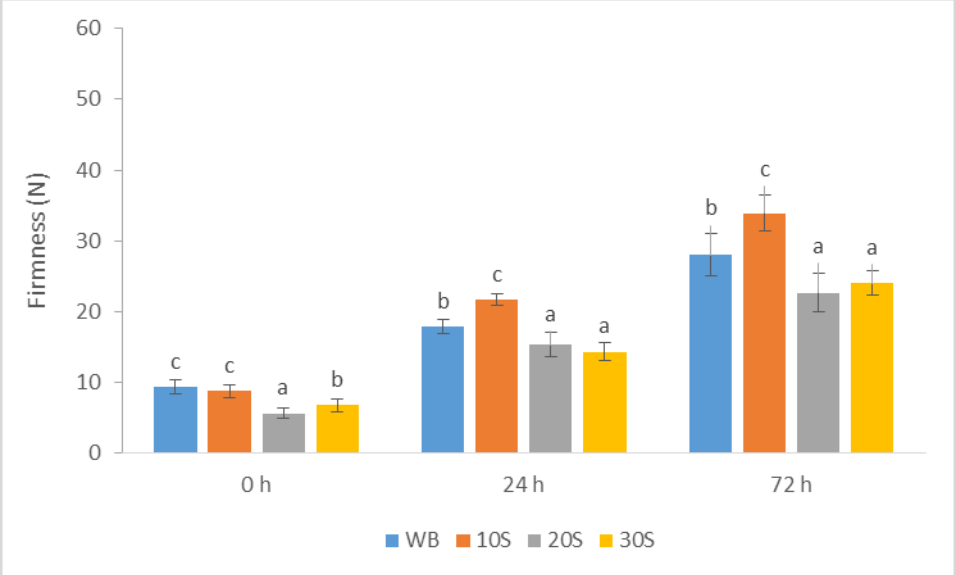

Figure 7. Crumb firmness of sprouted quinoa-enriched bread.

Different letters indicate significant differences between samples after 0h, 24h and 72h of storage (Tukey HSD; $p<0.05$; n=3)

3.4.4.3 Characterization of sprouted and pearled dough and bread

The results about dough rheological properties and bread characteristics highlighted that the blend containing 20% sprouted quinoa showed the most interesting performances. For this reason, the second part of the study focused on the comparison of dough and bread properties prepared with sprouted or pearled quinoa, both of them used at 20% enrichment level.

The comparison of dough properties between pearled and sprouted quinoa highlighted higher water absorption when the latter was used (Table 1). This result is in agreement with the increased water absorption index after sprouting, as shown in section 3.2.

Table 1. Mixing and leavening properties of sprouted and pearled quinoa-enriched dough

		Sprouted quinoa	Pearled quinoa
Mixing properties	Water absorption (%)	58.0±0.1	56.3±0.1***
	Development Time (min)	5.6±0.1	7.0±0.1***
	Stability (min)	3.5±0.1	6.8±0.7**
Leavening properties	Maximum dough height (mm)	60±1	49±2***
	Maximum height time (h)	1.9±0.1	2.0±0.2 n.s.
	Final dough height (mm)	45±2	40±1**
	Maximum height (mm)	82±2	84±2 n.s.
	Porosity time (h)	1.4±0.1	1.0±0.1***
	Total CO_2 (mL)	1468±10	1900±20***
	Retained CO_2 (mL)	1315±9	1475±15***
	Released CO_2 (mL)	153±1	424±13***
	CO_2 retention coefficient (%)	90±1	78±1***

* indicate significant differences at the same row (**, p<0.01; ***, p<0.001; n.s: not significant; t-Test. n=3).

The differences between the dough samples were likely due to the high content in proteins, fiber and sugars in sprouted quinoa, as discussed in section 3.2. The higher amount of fiber was also responsible for the lower dough stability in sprouted quinoa compared to the control (Figure 8 and Table 1).

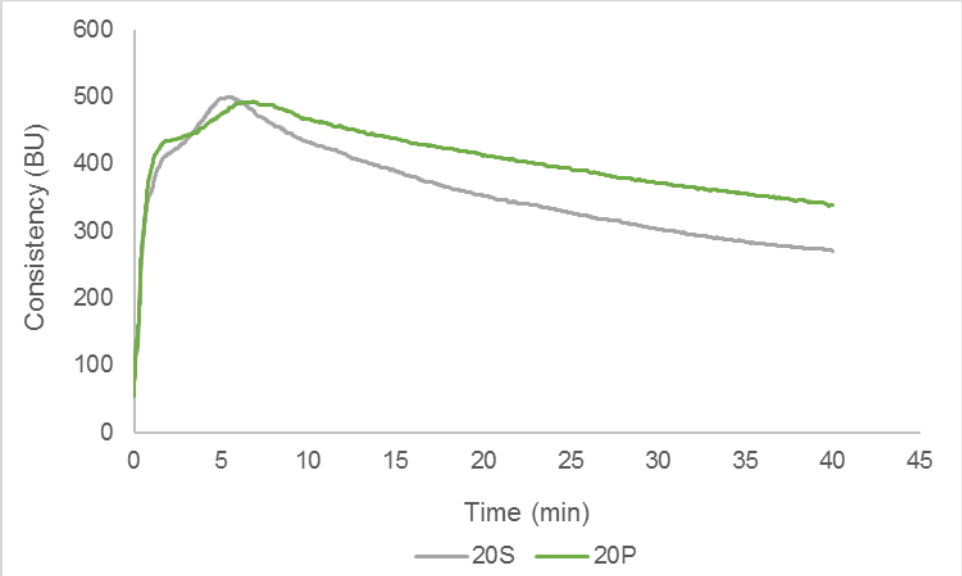

Figure 8. Mixing properties of sprouted and pearled quinoa-enriched dough.

As regards dough performance during leavening, sprouted quinoa showed the best behavior in terms of maximum dough development and retention capacity (Table 1). The best dough leavening performance in sprouted quinoa was due to the higher amount of sugars (i.e. glucose and maltose) in sprouted quinoa compared to pearled sample.

The best leavening performance accounted for the highest specific volume of sprouted quinoa enriched bread (Figure 9 and Table 2), likely due to the improved foaming stability in flour from sprouted quinoa as discussed in section 3.2. The improved bread-making performance upon sprouting has been also reported in wheat (Marti et al., 2017, 2018). Compared to the sample with pearled quinoa, using sprouted quinoa improved crumb softness not only of fresh bread (two hours after baking) but also during storage (Table 2). As an example, crumb firmness of fresh bread with pearled quinoa was similar to that of bread with sprouted quinoa after one day of storage. Moreover, bread crumb with sprouted quinoa after three days was as firm as bread enriched with pearled quinoa after one day of shelf-life. This behavior was not influenced by either crumb moisture or activity water but only to a different crumb structure.

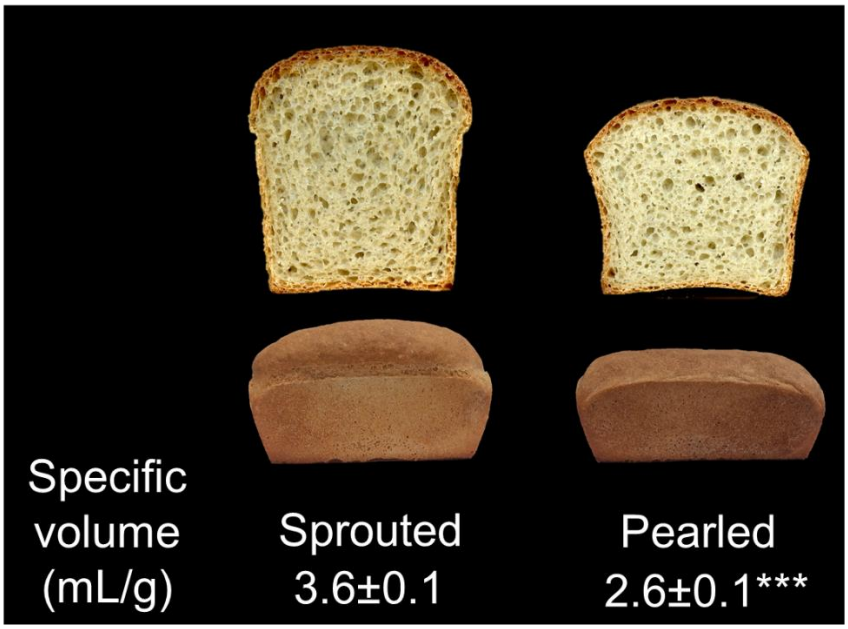

Figure 9. Characteristics of bread enriched with sprouted of pearled quinoa

Table 4. Properties of bread with sprouted and pearled quinoa at 20% of substitution

		Sprouted quinoa	Pearled quinoa
Volume (ml)	2 h	768±18	566±19***
Weight (g)	2 h	211±1	214±2***
Specific Volume (ml/g)	2 h	3.61±0.11	2.62±0.05***
Crumb moisture (%)	2 h	43.8±0.1	43.7±0.1 n.s.
	24 h	43.6±0.3	43.7±0.1 n.s.
	72 h	42.9±0.3	42.9±0.2 n.s.
Crumb water activity	2 h	0.914±0.005	0.913±0.004 n.s.
	24 h	0.914±0.004	0.913±0.003 n.s.
	72 h	0.909±0.004	0.910±0.004 n.s.
Crumb firmness (N)	2 h	5.6±0.7	13.1±1.8***
	24 h	15.3±1.7	25.3±2.8***
	72 h	22.7±2.8	47.6±6.4***

Asterisk indicate significant differences at the same row (***: p<0.001; n.s.: not significant differences between samples; t-Test. n=3).

3.4.4.3.1 Electronic tongue assessment

The sensory traits of quinoa-enriched bread obtained from e-tongue measurement and elaborated through the Principal Component Analysis (PCA) are shown in Figure 10. The two main components accounted for 81.5% of the total variance. As shown in the score plot (Figure 10a), samples were clearly discriminated on PC1 (48.9% of the total variance) based on the treatment applied to seeds before milling (pearling vs sprouting). In fact, the sprouted samples were located on the right side (positive) of PC1. The loading plot in Figure 10b evidenced the tendency of bread with sprouted quinoa to umami, richness, sourness, astringency and bitterness aftertastes. On the contrary, samples with pearled quinoa were located on the left side (negative) of PC1, evidencing their tendency to saltiness, bitterness and astringency. These results are in agreement with the changes promoted by sprouting on the sensory traits of both flour and cooked seeds already discussed in section 3.2. Moreover, the pearling process here applied was not sufficient in removing the bitterness that is likely due to both saponin and phenol compounds. The bitter taste in bread with pearled quinoa was also assessed by a panel test in the study of Stikic et al., (2012). Therefore, sprouting can be considered a biotechnological approach suitable for decreasing the bitterness perception in quinoa-enriched bread. A similar trend has been reported in bread from sprouted whole wheat (Richter et al., 2014). The positive effects of sprouting in enhancing the sensory traits of the products have been also showed in the case of cowpea-enriched breakfast cereals (Marengo et al., 2017b).

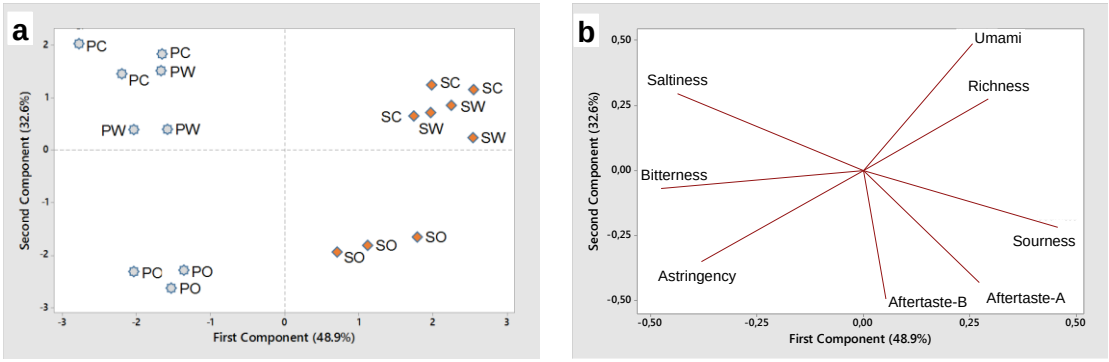

Figure 10. Score plot (a) and loading plot (b) from e-tongue PCA of bread with pearled (cyan) or sprouted (orange) quinoa. P: Pearled; S: Sprouted; W: whole bread; C: crumb; O: crust. Aftertaste-A: aftertaste-astringency; Aftertaste-B: aftertaste-bitterness.

PC2 discriminated the samples (32.6% of the total of the variance) according to the assessed bread sections (whole bread, crumb, or crust). In particular, whole bread as well as crumb were located on the upper level (positive), without great differences between them. Indeed, crumb represents more than 90% of the whole bread (data not shown). Whereas, bread crust was located on the lower level (negative) of PC2. The variables affecting the discrimination between crust and crumb were: astringency, aftertaste-bitterness and aftertaste-astringency. Maillard reaction in crust might account for the results.

3.4.5 Conclusions

With respect to the growing interest and need for the consumption of products rich in fiber and protein with high biological value, this study demonstrated that spouting is a suitable strategy for producing quinoa-enriched bread, while keeping high the acceptability of the products even during storage. The 20% enrichment level allows the production of bread with interesting nutritional characteristics and high volume and crumb softness. Comparing this bread-making performance with that of pearled quinoa-enriched bread - at the same percentage - showed that sprouting is more effective than pearling in decreasing bread bitterness while keeping a soft crumb up to three days of storage. Thus, sprouting is a valid alternative to pearling that is at the moment the only applied method in order to allow the use of quinoa in bread-making.

3.4.6 References

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Appendix

Table A1. Rheological properties of sprouted quinoa-enriched dough.

		Wheat	Sprouted quinoa		
			10%	20%	30%
Pasting properties	Beginning of gelatinization (°C)	62.2±1.2^{a}	64.0±0.1^{a}	64.7±1.5^{a}	63.6±1.2^{a}
	Peak viscosity (BU)	320±5^{d}	278±5^{c}	238±3^{b}	217±2^{a}
	Breakdown (BU)	128±8^{c}	93±5^{b}	71±6^{a}	56±4^{a}
	Final viscosity (BU)	712±15^{d}	578±13^{c}	493±7^{b}	449±11^{a}
	Setback (BU)	505±11^{d}	393±14^{c}	326±5^{b}	288±7^{a}
Aggregation properties	Peak Maximum Time (s)	104±3^{b}	96±2^{a}	102±1^{b}	123±2^{c}
	Maximum Torque (GPU)	61.1±0.3^{c}	60.1±0.6^{c}	53.3±2.3^{b}	39.6±1.3^{a}
	Aggregation Energy (GPE)	1480±4^{d}	1239±25^{c}	1105±34^{b}	916±16^{a}
Mixing properties	Water absorption (%)	55.5±0.4^{a}	57.2±0.1^{b}	58.0±0.1^{c}	58.2±0.2^{c}
	Development Time (min)	6.8±0.3^{c}	6.1±0.1^{b}	5.6±0.1^{ab}	5.5±0.3^{a}
	Stability Time (min)	23.8±1.2^{c}	5.6±0.2^{b}	3.5±0.1^{a}	3.3±0.2^{a}
	Degree of Softening (FU)	17±2^{a}	93±4^{b}	132±5^{c}	152±3^{d}
Leavening properties	Maximum dough height (mm)	51±1^{a}	60±1^{c}	60±1^{c}	56±1^{b}
	Maximum height time (h)	2.3±0.1^{a}	2.3±0.1^{a}	1.9±0.1^{a}	1.9±0.1^{a}
	Final dough height (mm)	48±2^{ab}	52±3^{b}	45±2^{a}	44±4^{a}
	Maximum height (mm)	63±5^{a}	79±5^{b}	82±2^{b}	82±2^{b}
	Porosity time (h)	1.5±0.1^{b}	1.1±0.2^{a}	1.4±0.1^{ab}	1.2±0.1^{a}
	Total CO_2 (ml)	1250±54^{a}	1426±26^{b}	1469±10^{b}	1464±9^{b}
	CO_2 retained (ml)	1157±41^{a}	1286±14^{b}	1315±9^{b}	1294±17^{b}
	CO_2 released (ml)	93±13^{a}	141±11^{b}	153±1^{bc}	169±8^{c}
	CO_2 retention coefficient (%)	93±1^{c}	90±1^{b}	90±1^{ab}	88±1^{a}

Different letters in the same row indicate significant differences (Tukey HSD; $p<0.05$; n=3). Maximum dough height: maximum height achieved during the test; Final dough height: height at the end of the test; Leavening time: time required for maximum dough development; Maximum height: maximum height of gaseous production; Porosity time: time when the porosity of the dough developed; Total CO_2: total production of CO_2; CO_2 retained: amount of CO_2 retained in the dough during the test; CO_2 released: amount of CO_2 released during the test; CO_2 retention coefficient: ratio between CO_2 retained and total CO_2.

Table A2. Characteristics of sprouted quinoa-enriched bread

	Wheat	Sprouted quinoa		
		10%	20%	30%
Volume mL (mL)	712±25^{a}	688±23^{a}	768±18^{b}	712±19^{a}
Weight (g)	212.0±1.3^{ab}	212.3± 1.3^{b}	211.2±1.1^{a}	210.8±2.0^{ab}
Specific Volume (mL/g)	3.37±0.11^{b}	3.18±0.04^{a}	3.61±0.11^{c}	3.38±0.10^{b}
	43.7±0.5^{ab}	44.1±0.1^{b}	43.8±0.1^{ab}	43.6±0.4^{a}
Crumb Moisture	43.7±0.2^{a}	43.8±0.2^{a}	43.6±0.3^{a}	43.5±0.4^{a}
	42.1±0.6^{ab}	43.0±0.4^{b}	42.9±0.3^{b}	41.9±0.7^{a}
	0.916±0.007^{a}	0.915±0.004^{a}	0.914±0.005^{a}	0.913±0.003^{a}
Crumb Water activity	0.919±0.003^{b}	0.915±0.002^{ab}	0.914±0.004^{ab}	0.910±0.005^{a}
	0.915±0.002^{b}	0.913±0.005^{ab}	0.909±0.004^{ab}	0.908±0.002^{a}
	9.4±1.0^{c}	8.8±0.9^{c}	5.6±0.7^{a}	6.8±0.9^{b}
Crumb firmness (N)	17.9±1.0^{b}	21.7±0.8^{c}	15.3±1.7^{a}	14.3±1.2^{a}
	28.0±3.0^{b}	33.9±2.6^{c}	22.7±2.8^{a}	24.0±1.7^{a}

Different letters in the same row indicate significant differences (Tukey HSD; $p<0.05$; n=3).

4. General conclusions

The presence of bitter compounds - mainly saponins - greatly affect the sensory acceptance of quinoa; consequently, the consumption of this pseudocereal as a whole grain and/or as a valuable nutritive ingredient in composite flours for wheat or gluten-free products has to carefully consider this aspect. Presently, decreasing or modifying the bitterness of quinoa is achieved by washing and/or mechanical pearling. Although their wide use, these processes present critical aspects, namely low environment-sustainability, energy and specific equipment requirements that force researchers to find other approaches. Besides, for the breeding studies that might select new “sweet” varieties with low or no saponin content and with high adaptability to different climatic environments, bio-technological and not-expensive processes have to be developed. In this context, sprouting has been proposed as a suitable process to enhance the nutritional and sensory characteristics of pulses, while no information is available on the properties of sprouted quinoa. Overall, the results of this PhD thesis highlighted that:

- quinoa seeds underwent relevant physical changes during sprouting, reaching their maximum intensity after 48 h of processing at controlled moisture and temperature conditions. Molecular changes on starch and protein upon sprouting affected the functional properties of the related flours, suggesting new potential applications of sprouted quinoa in food formulations, including frozen-foods and baked products;
- sprouting can be proposed as an alternative method to washing or pearling to decrease the amount of saponins starting from 24-48h of process, while decreasing bitterness in both flour and cooked seeds;
- sprouted quinoa can be used as a new ingredient in cereal-based products thanks to its capacity of increasing bread volume and crumb softness even during storage, without using additives.

5. Products

5.1 Publications

Review

SCI

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Quinoa bitterness: causes and solutions for improving product acceptability

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Abstract

Awareness of the several agronomic, environmental, and health benefits of quinoa has led to a constant increase in its production and consumption not only in South America, where it is a native crop, but also in Europe and the USA. However, producing wheat or gluten-free based products enriched with quinoa alters some quality characteristics, including sensory acceptance. Several anti-nutritional factors such as saponins are concentrated in the grain pericarp. These bitter and astringent substances may interfere with the digestion and absorption of various nutrients. Developing processes to decrease or modify the bitterness of quinoa can enhance palatability, and thus consumption, of quinoa. In addition to the production of sweet varieties of quinoa, other processes have been proposed. Some of them (i.e. washing, pearling and the combination of the two) have a direct effect on saponins, either by solubilization and/or the mechanical removal of seed layers. Others, such as fermentation or germination, are able to mask the bitterness with aroma compounds and/or sugar formation. This review presents the major sources of the undesirable sensory attributes of quinoa, including bitterness, and various ways of counteracting the negative characteristics of quinoa.

Keywords: quinoa; bitterness; saponins; washing; pearling

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a dicotyledonous plant belonging to the Chenopodiaceae family and is widespread in Latin America, particularly in South America where the crop had its origin 5000 years ago,[1] on the present Peruvian and Bolivian border near Lake Titicaca. In ancient times, native South American populations used this grain in their daily diet as their main food. In 1989, the US National Academy of Sciences includes quinoa as one of the best sources of protein in the vegetal kingdom.[2] Moreover, in the last few years, there has been a global re-evaluation of this crop, in light of numerous traits that make quinoa a sustainable and healthy grain. In fact, the 66th session of the General Assembly to the United Nations declared 2013 as the International Year of Quinoa, citing the potentially significant contribution of quinoa in the fight against hunger and malnutrition. Indeed, quinoa is one of the best alternatives to the global need to increase the dietary intake of plant proteins with high nutritional value for greater sustainability, safety and nutritional benefits.[3]

Awareness of the health benefits of quinoa, reflected in the growing number of gluten-free and vegetarian/vegan dieters, might account for the ongoing global expansion of quinoa production, which increased by 60% from 2013 to 2014 (FAO; www.fao.org). Moreover, the last few years have been characterized by a proliferation of research on quinoa from various perspectives (e.g. agriculture, environmental impact, nutrition, food production). A systematic review of the scientific literature of the last 10 years using 'quinoa' as a search term resulted in the identification of about 930 scientific papers (Fig. 1A). It is worth mentioning that the number of contributions has doubled in the last 5 years, highlighting the growing interest in this topic. Almost 50% of the contributions (Fig. 1B) fall into the 'food science/chemistry/nutrition' categories of research, with about 40% of them dealing with agricultural and agronomic aspects of quinoa. Fourteen of the articles are reviews containing 'quinoa' in their titles, and a tentative classification according to their particular research area and topic is summarized in Table 1. Most concern agronomic and nutritional aspects of the 'golden grain', while others are dedicated to the development of food products, including bread, pasta, snacks and cookies, enriched with quinoa to improve their nutritional properties. However, in South America it is the whole seed of quinoa that is mainly used and generally cooked like rice to be used in soups, salads and stews.[1,4]

Producing quinoa-enriched wheat- or gluten-free-based products alters several quality attributes according to Wang and Zhu.[5] Among these, sensory acceptance is the most critical factor in ensuring the consumption of quinoa and its successful use in food products. In this context, the presence of bitter compounds in quinoa limits its consumption, despite its numerous nutritional benefits. Developing processes to decrease or modify the bitterness of quinoa serve to enhance palatability. Such processing involves washing, pearling and biotechnological treatments.

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5.1 Conference's abstracts

21st Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Portici, September 14th-16th, 2016

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Cereal-based products from Andean grains

This PhD project is aimed to obtain cereal-based products with and without gluten, enriched in Andean grains, mainly quinoa (*Chenopodium quinoa* Willd.). The project proposes the application of bio-technological processes such as fermentation and/or sprouting for decreasing the anti-nutritional factors (mainly saponins) on quinoa seeds, whereas enhancing their nutritional characteristics and uses in food formulations around the world.

Prodotti a base di cereali da grani andini

Questo progetto di dottorato si propone di ottenere prodotti a base di cereali, con e senza glutine, arricchiti con cereali andini, principalmente quinoa (*Chenopodium quinoa* Willd.), applicando alcuni processi bio-tecnologici, come la fermentazione e/o la germinazione, per diminuire i fattori antinutrizionali presenti nella quinoa, e, contemporaneamente, migliorarne le loro proprietà nutrizionali in formulazioni che possano soddisfare le esigenze dei consumatori non solo sud-americani ma anche di paesi occidentali.

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Effect of bio-technological processes on saponins amount of quinoa (*Chenopodium quinoa* Willd)

Quinoa (*Chenopodium quinoa* Willd.) is an antique Andean grain classified as Amaranthaceae. In the last decade, quinoa consumption is constantly increasing due to high interest in the consumption of enhanced nutritional foods. Indeed, the chemical composition of quinoa is superior compared to other cereals. On the other hand, in the grain pericarp are concentrated some anti-nutritional factors as saponins, a bitter and astringent substance (from 0.01% to 5.0%), and may interfere the digestion and the absorption of various food components. Up to now washing, soaking, air flowing and pearling have been applied for decreasing the quinoa saponin content. Several laboratory techniques as Thin Layer Chromatography (TLC) and spectrophotometry have been applied to evaluate the efficiency for saponin decrease from quinoa of these methods. However, the processes proposed until now do not appear the most appropriate in terms of sustainability and feasibility. Thus, more information on novel biotechnological approaches for decreasing the saponins content is required. In fact, the effect of biotechnological approaches as fermentation and controlled germination at several germination times could be assessed.

A second relevant barrier to the use of quinoa in cereal-based products relates to the absence in these grains of proteins that allow creating the viscoelastic network, which is fundamental for bread making, and is commonly provided by gluten proteins in wheat-based foods. However, the quinoa proteins have demonstrated interesting foaming properties, which might be exploited in bread-making for the production of wheat-based or gluten-free products.

22nd Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology

Bozen, September 20th-22nd, 2017

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Cereal-based products from Andean grains

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Controlled germination (12, 24, 48 and 72 h @ 22°C) was applied to quinoa (Titicaca cv.) to study the effects of this process on chemical, physical, and technological properties. Modifications induced by germination were assessed on whole seeds and the related flour. Particular attention was paid to saponin decrease. A pearled sample from the same cultivar and a US commercial germinated quinoa were used as control.

Prodotti a basi di cereali da grani andini

Sui semi di quinoa (cv. Titicaca) sono stati germinati in condizioni controllate (12, 24, 48 e 72 h @ 22°C). Le modificazioni indotte dalla germinazione sono state rilevate sia sui semi che sulla relativa farina ottenuta dopo macinazione, mediante approcci chimici, fisici e reologici. Particolare attenzione è stata dedicata alla possibilità di abbassare il contenuto di saponine presenti nel seme. Come controllo sono stati utilizzati il campione della stessa cultivar sottoposto a decorticazione e un campione commerciale germinato reperito sul mercato USA.

11° CONVEGNO AISTEC
"I cereali per un sistema agroalimentare di qualità"
Roma, November 22nd – 24th, 2017

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Effetto della germinazione controllata sulle caratteristiche fisiche e tecnologiche della quinoa (*Chenopodium quinoa* Willd.)

La quinoa (*Chenopodium Quinoa* Willd.) è un antico seme andino della famiglia delle Amaranthaceae. Questo pseudocereale, coltivato fin dall'epoca dell'impero Inca, oggi è ampiamente diffuso e apprezzato a libello globale.

Al pari di numerosi cereali, anche per gli pseudocereali viene frequentemente consigliato l'applicazione del processo di germinazione che alcuni studi indicano associato a numerosi effetti positivi di carattere nutrizionale. In questo lavoro, gli effetti della germinazione condotta in condizioni controllate per 24 e 48 ore sono stati valutati sia sulle caratteristiche fisiche e chimiche del seme che sulle proprietà reologiche degli sfarinati integrali.

In particolare, sui semi di quinoa - prima e dopo germinazione - sono stati controllati i parametri di colore, peso ettolitrico, peso di 1000 semi e gli indici morfologici. E' stato anche monitorato il contenuto di saponine – composti con proprietà antinutrizionali – mediante un test indiretto basato sullo sviluppo di schiuma dopo agitazione vigorosa in presenza di acqua.

I risultati indicano che il colore, il diametro e il peso ettolitrico variano significativamente dopo germinazione rispetto al controllo, così come l'attività alfa-amilasica. Al contrario, il processo non introduce modificazioni importanti per l'indice peso di 1000 chicchi.

La modificazioni associate alla germinazione sono state completate da controlli delle proprietà reologiche degli sfarinati sottoposti a diversi tempi di cottura. Sono state infatti valutati i parametri relativi all'assorbimento (WAI) e alla solubilità in acqua (WSI), swelling power, stabilità al congelamento – scongelamento e gli indici relativi alle pasting properties determinate mediante il MicroViscoAmilografo di Brabender.

17th European Young Cereal Scientists and Technologists Workshop
Varsaw, April 18th – 20th 2018

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Effects of germination on physical and technological characteristics of quinoa (*Chenopodium quinoa* Willd.)

- Effects of germination under controlled conditions on physical characteristics of quinoa.
- Germination affected saponin, phenolic content and taste.
- The main changes associated with germination occurred within 48h.

Quinoa is a pseudocereal belonging to the *Chenopodiaceae* family; it is widespread in South America countries where is mainly consumed as cooked seed. Quinoa is rich in saponins, that are bitter compounds responsible for the decrease in product acceptability. They are mainly located in the external layers. In order to remove saponins, quinoa is generally washed and/or pearled before its consumption. However, washing contaminates water whilst pearling lowers the nutritional properties of seeds. In this study, controlled germination has been explored as an alternative treatment to decrease quinoa bitterness, avoiding polluting processes and loss of nutrients. Seeds were germinated at 22 ºC for 12, 24, 48 and 72 hours, then dried at 55ºC for 6 hours and maintained at 4 ºC until analysis. Untreated and pearled quinoa were used as references. Chemical, physical, technological and sensory changes were assessed in both seeds and flours.

Germination was associated with a decrease in seed diameter, test weight, and thousand-kernel weight, as a result of macromolecular hydrolysis occurring during germination.

Germination process seemed to promote a significant decrease in saponin content (from about 4 mg/g in native seeds to 2.61 mg/g after 48 h), evaluated by the official Ecuadorian method NTE INEN 1672, an indirect approach, based on the foam height developed after vigorous agitation of seeds in water. Nevertheless, this trend is opposed to that shown by the spectrophotometric method: the latter exhibited an increase in saponin content during germination, from 5.3 to 6.6 mg/g db after 48 h. Moreover, other potential bitter compounds, as phenols and flavonoids, increased from 2.06 to 3.57 mg GAE/g db and from 3.18 to 4.09 mg QE/g db, respectively. Nevertheless, germination enhanced sugar formation, likely responsible for masking the perception of bitter taste, as shown by e-tongue results. The main effects occurred during the first 48 hours of the process.

The relevant increase in amylase activity during germination highly affected the starch content, its pasting properties and water affinity. Foaming capacity of quinoa flour decreased from 30.5 to 18.4 %v/v, nevertheless, its stability increased from 47.0 to 54.1 %. On the other hand, no changes in freeze-thaw stability were observed.

Information about the effects of germination on technological properties of quinoa flour will be useful for the development quinoa-enriched products. In this context, the bread-making performance of flour from germinated quinoa will be evaluated, taking into account both gluten and gluten-free formulations.

SIB Advanced School on "Food Proteins"
Bergamo, May 2nd – 4th, 2018

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Exploring quinoa germination

Quinoa (*Chenopodium quinoa* Willd.), a pseudocereal belonging to the Chenopodiaceae family, is one of the best sources of protein in the vegetal kingdom. Indeed, 40 g of quinoa covers an important part of daily requirements of essential amino acids. Moreover, quinoa is a gluten-free grain and an important source of minerals, fiber and phenolic compounds. On the other hand, acceptability of quinoa is low due to the presence of saponins in its external layers. Besides being antinutritional compounds, saponins are well known for giving bitterness. In order to remove saponins, washing and/or pearling are carried out before quinoa consumption. However, washing contaminates water whilst pearling lowers its nutritional properties.

Germination improves the technological, nutritional and sensory characteristics of both cereals and pseudocereals when process conditions are properly controlled. In this study, controlled germination has been explored as an alternative treatment to decrease quinoa bitterness, avoiding polluting processes and nutrient losses. Seeds were germinated at 22 ºC up to 72 hours and then gently dried.

Germination decreased total starch content from 61.3 to 52.3 % db. It increased the protein amount from 14.6 to 16.4 % db after 48 h. Currently, changes in protein hydrophobicity is under evaluation. The content of bitter compounds such as saponins, phenols and flavonoids increased from 5.3 to 6.6 mg/g db, 2.06 to 3.57 mg GAE/g db and from 3.18 to 4.09 mg QE/g db, respectively. Nevertheless, germination decreased the perception of bitter after-taste, as shown by e-tongue results, probably as a consequence of sugar accumulation, masking the bitter taste. The main effects occurred during the first 48 hours of the process.

1st Congreso Internacional de Cereales, Leguminosas y Afines (CICLA I)
Cuenca, July 25th – 28th 2018

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Controlled germination applied to quinoa: physical and technological effects

Quinoa (*Chenopodium quinoa* Willd.), a pseudocereal belonging to the Chenopodiaceae family, is an important source of proteins, minerals, fiber and phenolic compounds. On the other hand, the presence of bitter compounds such as saponins in its external layers decreases quinoa acceptability. In order to remove saponins, washing and/or pearling are generally applied. However, washing contaminates water whilst pearling lowers its nutritional properties.

In this study, controlled germination was explored as an alternative approach to improve quinoa sensory profile. Seeds were germinated up to 72 hours at 22°C. Chemical, physical, technological and sensory changes were assessed.

Controlled germination promoted the decrease in hectoliter weight, thousand kernel weight and seed diameter. Increasing in enzymatic activity affected starch content, pasting properties and water affinity of quinoa flour. Foaming capacity of quinoa flour decreased from 30.5 to 18.4 %v/v, nevertheless, its stability increased from 47.0 to 54.1 %.

After 48 h germination, a decrease in total starch content (from 61.3 to 52.3 % db) and an increase in protein amount (from 14.6 to 16.4 % db) was observed. At the same time, according to the official Ecuadorian method NTE INEN 1672, the saponin content decreased from about 4 mg/g in native seeds to 2.61 mg/g; this test is an indirect approach based on the foam height developed after vigorous agitation of seeds in water. In fact, when a spectrophotometric approach was applied, an increase in saponin content was detected (from 5.3 to 6.6 mg/g db after 48 h). Moreover, germination increased extractability of other potential bitter compounds, as phenols and flavonoids passing from 2.06 to 3.57 mg GAE/g db and from 3.18 to 4.09 mg QE/g db, respectively. Further studies about their bioavailability are necessary. Anyway, probably as a consequence of sugar accumulation, the germinated seeds were perceived less bitter by e-tongue results.

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Chemical and physical properties of quinoa (*Chenopodium quinoa* Willd.) as affected by germination

Awareness of the several agronomic, environmental, and health benefits of quinoa has led to a constant increase in its production and consumption not only in South America - where it is a native crop – but also in Europe and the United States. Producing quinoa-enriched products alters some quality characteristics, including sensory acceptance, due to the presence of bitter and astringent compounds (i.e. saponins). Developing processes to decrease or modify the bitterness of quinoa can enhance palatability and thus consumption of quinoa. In addition to the production of sweet varieties of quinoa, other processes have been proposed. Some of them (i.e. washing, pearling and the combination of the two) have a direct effect on saponins, either by solubilisation and/or the mechanical removal of seed layers. Others, such as fermentation, are able to mask the bitterness with aroma compounds and/or sugar formation. In this study, controlled germination has been explored as an alternative treatment to decrease quinoa bitterness and improve technological performance of the related flour. Seeds were germinated at 22 ºC for 12, 24, 48 and 72 hours, then dried at 55 ºC for 6 hours. Untreated and pearled quinoa were used as references. Chemical, physical, technological and sensory changes were assessed in both seeds and flours. Enzymatic activity developed during germination highly affected the starch content, its pasting properties and water affinity. Foaming capacity of quinoa flour decreased from 30.5 to 18.4 %v/v, nevertheless, its stability increased from 47.0 to 54.1 %. Germination process promoted a significant decrease in saponin content (from about 4 mg/g in native seeds to 2.61 mg/g after 48 h), evaluated by the official Ecuadorian method NTE INEN 1672, an indirect approach, based on the foam height developed after vigorous agitation of seeds in water. Nevertheless, this trend is opposed to that shown by the spectrophotometric method: the latter exhibited an increase in saponin content during germination, from 5.3 to 6.6 mg/g db after 48 h. Moreover, other potential bitter compounds, as phenols and flavonoids, increased from 2.06 to 3.57 mg GAE/g db and from 3.18 to 4.09 mg QE/g db,

respectively. Nevertheless, germination enhanced sugar formation, likely responsible for masking the perception of bitter taste, as shown by e-tongue results. The main effects occurred during the first 48 hours of the process. Information about the effects of germination on technological properties of quinoa flour will be useful for the development quinoa-enriched products.

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