Quinoa bitterness: causes and solutions for improving product acceptability

Running title: Quinoa bitterness: causes and solutions

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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 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 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 solubilisation 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, included 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 Titicaca lake. In ancient times, native South American populations used this grain in their daily diet as their main food. In 1989, the National Academy of Sciences of the United States 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 on-going global expansion of quinoa production, that 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, 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. Fourteen of the articles are reviews containing “quinoa” in their titles, and a

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's the whole seed of quinoa that is mainly used and generally cooked like rice to be used in soups, salads, and stews.[4]

Producing quinoa-enriched wheat- or gluten-free based products alters several quality attributes according to Wang and Zhu.[17] 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. This review presents the major sources of the undesirable sensory attributes of quinoa; the various approaches for counteracting the negative perception of quinoa consumption are also discussed.

AGRONOMIC, COMPOSITIONAL AND NUTRITIONAL BENEFITS

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 grain 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.[5,19,20] Despite its mountain origin, research indicates it can be grown from sea level to altitudes over 4000 meters with large yield ranges (from 0.32 to 9.83 t ha $^{-1}$).[21] 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^{-}

diversity, its exceptional tolerance to drought and salinity, and the crop's ecological advantages have been extensively reviewed by Ruiz *et al*.[9]

Concerning biodiversity, quinoa presents a wide genetic variability in terms of forms, 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.[23,24] Violet, red and yellow quinoa grain extracts show remarkable antioxidant activity in comparison with the white and black one. 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.[23]

The potential health benefits of quinoa have been extensively reviewed in recent years (Table 1). 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.[16] In particular, the high amount of lysine - the limiting amino acid in all cereals - makes quinoa unique among grains.[13,25] 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.[11,12] 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-

considerably lower than the level required for gluten-free products (20 mg kg^{-1}).[27] Finally, it has been suggested that quinoa could contain a significant amount of rapidly digestible starch fraction,[28] 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.[29] *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.[30] 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),[30] although these findings need to be confirmed by *in vivo* studies.

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 in salads, whereas quinoa flour is mixed with other gluten-free grains for making bread, pasta, and cookies.[17] The following section will summarize consumer perception of its sensory attributes and consumer acceptance of quinoa-containing foods in the past 10 years.

Grains

We know of only one study dealing with the sensory analysis of quinoa grains.[31] The results of this study showed a wide range of sensorial characteristics. For example, a

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 750 g kg^{-1} to 1000 g kg^{-1} of organic foods scored significantly higher for all quinoa varieties than those who consumed 0 to 250 g kg^{-1}.[31]

Bread

Quinoa has been used in bread-making as a partial substitute for wheat or rice flour in varying amounts. 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 200 g kg^{-1} of dehulled and washed quinoa seeds were judged to be fully acceptable to the taste, with a very pleasant aroma and flavour.[32] These positive results were subsequently confirmed for bread with higher levels (300 and 400 g kg^{-1}) of similarly treated quinoa seeds.[33]

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 200 g kg^{-1} of quinoa had been added.[34]

Although the positive results found using quinoa seeds and flakes as bread ingredients, using quinoa flour often opposed sensory problems.

The 60 g kg^{-1} substitution of wheat flour with quinoa flour for bread was considered acceptable.[35] Adding texturing ingredients, such as whey, was efficacious in guaranteeing the acceptability of wheat bread fortified with 150 g kg^{-1} quinoa flour. On the contrary, adding 200 g kg^{-1} of quinoa made the bread less acceptable, due to its slight bitterness.[36] Another study demonstrated that acceptability significantly decreased for samples with 500 and 1000 g kg^{-1} of quinoa flour, even if the quinoa

kg^{-1} quinoa flour induced the perception of a strong pea-like odour and of cooked potato and mould aromas, mainly responsible for moderate overall disliking.[38] Using sourdough fermented with *Lactobacillus plantarum*[39] or *Weissella cibaria*[40] did not improve the sensory characteristics of quinoa bread, while with *Lactobacillus plantarum* T6B10 and *Lactobacillus rossiae* T0A16 a wheat bread with improved crust and crumb colour, saltiness, acid flavour and taste, and overall positive taste attributes was made[41]. 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).[42]

Quinoa flour can also replace rice flour in gluten-free formulations. Substitution levels in the range of 300 – 1000 g kg^{-1} increased acceptability in terms of crust and crumb color and appearance, in comparison with acceptance scores for 1000 g kg^{-1} rice flour reference bread.[43] Overall, a substitution level equal to 300 g kg $^{-1}$ of quinoa flour was considered suitable to avoid negative aroma and taste and guarantee an overall acceptability comparable to that obtained for the control rice bread.[43,44] Conversely, other studies showed that 500 g kg^{-1} quinoa flour increased crumb softness and cohesiveness of rice-based breads, without adversely affecting sensory properties.[45] As expected, the removal of bran components largely decreased bitterness and off-flavour in white quinoa breads, compared to whole quinoa samples.[46] Indeed, as mentioned elsewhere, saponins – which are responsible for quinoa bitterness – are mainly located in the bran. Therefore, only addition of 100 g kg^{-1} of quinoa bran to rice and corn bran resulted in improved appearance, and reduced crumb firmness, without compromising taste, whereas higher quantities (200, 300 and 400 g kg^{-1}) increased bitterness and off-flavours.[47]

Pasta

Information regarding the effect of quinoa on the sensory properties of pasta products

that 70% of consumers declared they would probably or certainly buy the product.[48] A similar quinoa enrichment resulted in a product with lower firmness but similar adhesiveness and bulkiness than the control (1000 g kg^{-1} amaranth).[49] A higher percentage of quinoa (250 g kg^{-1}) 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.[50] However, the acceptability of the quinoa-based product was high when containing chick-pea or soy flour compared to other gluten-free formulations.[51]

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 (< 100 g kg^{-1}) did not affect the sensory acceptability of cookies made primarily from wheat flour, but a slightly higher substitution level (150 g kg^{-1}) reduced flavour, taste and overall acceptability.[52] However, quinoa cookies were still acceptable, and similar results were observed by Pagamunici *et al*.[53] In gluten-free formulations, the presence of quinoa positively affected overall acceptance and purchase intention.[54]

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.[55] The most common bitter compounds in quinoa and the key mechanisms leading to bitterness are summarized in Table 2 and described in the following section. The bitterness of quinoa has always been associated with the presence of saponins in

187 the threshold for human perception of bitterness. Very little work has focused on the
188 role of polyphenols and other compounds on the bitter taste or aftertaste of quinoa
189 seeds and its products.

190 ***Saponins***

191 Saponins are a class of natural compounds produced by some plants for protection
192 against harmful microorganisms, birds and insects.[66] Saponins are present in legumes
193 (such as soybeans, broad beans, chickpeas, peas, etc.)[67,68] and some vegetables (as
194 spinach, lettuce, cauliflower, mustard, asparagus).[69,70] Regarding grains, only oats[67]
195 and quinoa exhibit detectable amounts of saponins.[71] In quinoa, these compounds are
196 mainly located in the husk and the quantity therein – which is greatly influenced by the
197 environment, climate conditions and genotype[5,72,73] - varies from 0.1 mg g^{-1} to about 50
198 mg g^{-1}.[74] Indeed, "bitter" varieties (with a saponin content higher than 1.1 mg g^{-1}), are
199 more resistant to pests than "sweet" varieties.[5,75] As will be extensively discussed later
200 on in this paper, the bitter taste is recognizable in samples having an amount of
201 saponin greater than 1.1 mg g^{-1}.[56]

202 Saponin molecules are characterized by the presence of a non-polar aglycone (or
203 sapogenin), bonded to one or more carbohydrate chains.[58,76] Quinoa contains only
204 triterpene saponins,[77-79] which can be classified according to the number of
205 carbohydrate chains linked to aglycone.[58] The saccharide chains of saponins assure
206 high hydrophilic properties, whereas the sapogenins (formed only by the triterpene
207 fraction) exhibit lipophilic traits. Hence, the amphiphilic properties of saponins assure
208 high solubility both in polar and non-polar solvents.

209 Detailed information about the chemical and structural characteristics of quinoa
210 saponins are presented in comprehensive reviews.[58,76,80]

211 Although the majority of studies report around 20 saponins in quinoa,[78,79] Madl *et al*.[78]
212 refer to 87 triterpene saponins. More recently, Jarvis *et al*.[73] identified 43 different
213 saponins in a variety used as a reference.

214 Several studies have focused on the chemical and biological properties of
215 saponins,[58,76,81] highlighting their complexity and controversial biological role. Indeed,
216 quinoa extracts containing saponins have been exploited in numerous traditional and
217 industrial applications for their foaming and bioactive properties but, usually, saponins
218 in foods have traditionally been considered as anti-nutritional factors, as stated by
219 Güçlü-Ustündağ and Mazza.[76] However, the consequences of long term consumption
220 of saponins for human health are still unknown.[82]
221 The anti-nutritional properties of saponins have been investigated in several
222 studies.[14,83,] The main negative effects associated with consumption of foods rich in
223 saponins are the decrease in mineral and vitamin bioavailability,[84-86] the damage to
224 small intestine mucous cells due to the alteration of their membrane permeability, and
225 the decrease in food conversion efficiency.[82] The chemical structure of quinoa
226 saponins strictly influences their biological activities,[58] e.g. the carbohydrate chain
227 attached at C3 of the terpenic fraction is usually critical for both membrane
228 permeabilization and antifungal properties[58,79] and their toxicity depends on the saponin
229 type and on the sensitivity of the recipient organism, [83]
230 Nowadays, saponins are considered bioactive, health-promoting compounds, with
231 many interesting nutritional characteristics as a result of their hypocholesterolemic,
232 analgesic, antiallergic and antioxidant activities.[76,79] In any case, as already mentioned,
233 the bitter taste associated with saponins greatly limits the use of quinoa as food.

234 ***Phenolic compounds***

235 Phenolic compounds constitute a group of important components to bitterness in cereal
236 products.[60] Free phenolic compounds are the most flavour-active because they adhere
237 to taste receptors.[60] However, studies on bread and crackers suggest that the bound
238 fraction of phenolic acids may also contribute to taste and flavour properties of
239 wholegrain products.[59] In this context, the authors hypothesized that during mastication

241 with taste receptors and other compounds inside the mouth.[59] Moreover, it has been
242 shown that lower-molecular-weight phenolic compounds tend to be bitter, whereas
243 higher-molecular-weight polymers are more likely to be responsible for food
244 astringency.[87] In addition, the impact of free phenolic compounds on flavour is greater
245 than that of bound compounds.[57]

246 Phenolic compounds are mainly located in the outer layers of the grain, and therefore
247 highly found in wholegrain and bran content.[88-90] Various strategies have been
248 proposed to increase the bioaccessibility and bioavailability of phenolic compounds, in
249 baked products because of the health benefits associated with them.[91-93] A
250 comprehensive review of phytochemicals in quinoa grains and their potential health
251 benefits have been proposed by Tang and Tsao.[11] Quinoa contained lower levels of
252 phenolic acids compared with common cereals like wheat and rye, but they were of the
253 same magnitude (250–600 mg kg $^{-1}$) as in other cereals.[94,95] The majority of phenolic
254 compounds found in quinoa were phenolic acids consisting of vanillic acid, ferulic acid
255 and their derivatives (303-597 mg kg^{-1}), along with flavonoids quercetin, kaempferol
256 and their glycosides (36.2-72.6 mg kg^{-1});[95] also tannins have been reported with
257 concentrations of up to 5.3 g kg^{-1}.[41,96]

258 The perceived bitterness of rye results from pinoresinol and syringic acid in particular,[60]
259 whereas ferulic acid was identified as the most abundant phenolic acid in wheat bread
260 crust and crumb.[97] On the contrary, phenolic compounds responsible for bitter taste
261 have not been adequately determined in whole grain foods.[98] To the best of our
262 knowledge, no information is available regarding quinoa seeds. Thus, further efforts
263 should be directed to identifying the major phenolic compounds responsible for the
264 bitterness of quinoa seeds.

265 ***Peptides***

Bitter peptides occur to a varying extent after protein hydrolysis.[64] Although small molecular weight peptides are deemed responsible for the bitter taste in rye,[65] the amino acid composition of peptides has been considered to be a more important determinant of bitterness than peptide size.[99] The role of peptides and amino acids in the perceived flavour of cereal products, including quinoa, remains, however, largely unknown.[57]

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 3 and discussed in the following sections.

Washing

Washing is the most common way to remove saponins from the seeds at the household level, 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.[100] Traditionally, in rural areas, washing is done by hand in water - placed in rudimentary tanks[101,102] - which sometimes could be alkaline to enhance saponin extraction[4,102] or in river water.[102] The large amount of water used and contaminated with saponins constitutes a health hazard for cold-blooded animals[103] and creates economic and ecological concerns. Moreover, wet seeds need to be dried immediately to inhibit their high germinating power[72,104] as well as mold growth.[105]

Washing is also used on a commercial scale by using tanks equipped with rotating blades for turbulence washing.[106] Heat treatment in a tunnel completes the drying

292 Quispe-Fuentes *et al*.[101] have proposed an efficient, industrial scale mathematical
293 model to reduce cost, energy waste and optimize water flow rate when leaching
294 saponins from quinoa seeds by means of a continuous washing process. Saponins
295 leach out very rapidly at the beginning of the washing process and the total
296 concentration of saponins inside quinoa seeds tend to have an asymptotic value. High
297 temperatures accelerate saponin leaching, in fact leaching at 70 °C was more effective
298 than at 20 °C.[101] However, since starch gelatinization begins at 50 ºC for most quinoa
299 varieties,[28] this treatment could cause the quinoa perisperm to swell, thus facilitating
300 embryo separation.

301 Another consideration is that valuable nutrients including vitamins and minerals may
302 also be lost during these washing procedures.[85]

303 ***Pearling***

304 Dry polishing techniques (i.e. pearling) apply abrasion to separate the external layers
305 and allow the intact seeds to be recovered and processed in successive stages.
306 Pearling is a well-established technology in the processing of covered cereals, such as
307 rice and barley.[107] Nowadays, pearling is also used on wheat to reduce microbial
308 contamination, as most of the microorganisms present can be found on the surface of
309 the kernel.[108] More recently, pearling has proven to be an effective way to recover the
310 phenolic compounds in the external layers of grains.[93]

311 As regards quinoa seeds, the pearling process has been successfully used to
312 decrease the amount of saponins, located in the external layers of the seed.[100,109] An
313 abrasion degree of 30% reduced saponin levels by more than 70%, compared with the
314 initial content in whole quinoa, reaching a level below 1.1 mg g^{-1} for several varieties,
315 which is the threshold for the detection of bitterness and astringency in quinoa based
316 products.[56]

317 Pearling is a more environmental-friendly process compared to washing because no
318 water is needed, no thermal treatment to dry the seeds is required, and no
319 environmental contamination is produced.[109] Other advantages of the abrasion process
320 include the reduction of time and energy consumption. Pearled by-products – which
321 comprise from 8% to 12% of the grain weight and contain from 200 to 300 g kg $^{-1}$ of
322 saponins[74] – can be used for medical purposes, detergents, and pesticides.

323 On the other hand, as the degree of abrasion increases, the content of fiber and
324 phenolic compounds decreases.[32,109] However, the loss of phenolic compounds in
325 quinoa after pearling is lower than in cereals. Gómez-Caravaca *et al.*[109] found that after
326 intense pearling (30%) in order to obtain a sweet product, the quantity of freed and
327 bound phenolic compounds decreased by 35%. Fiber and mineral content, especially
328 calcium, sodium, potassium and manganese, also decreased after pearling.[34,96,110]

329 Pearling and washing can be performed separately or combined to enhance the effects
330 on saponin removal, and lower the negative impact of each individual process (Table
331 3).

332 ***Other methods***

333 Other methods have been proposed such as the combination of washing and heat
334 treatments in different conditions (i.e. toasting, cooking at atmospheric pressure,
335 cooking under pressure).[111] However, none of them resulted in a higher loss of saponin
336 content than just washing.[111]

337 ***Bioprocessing***

338 Sourdough fermentation is a biotechnological process that transforms complex
339 molecules into simpler ones through the enzymatic activity of microorganisms, such as
340 yeasts and lactic acid bacteria. The positive effects of grain fermentation include the

342 bioactive and/or antifungal compounds.[112,113] Moreover, sourdough fermentation
343 improves the sensory quality of products, due to the production of organic acids and
344 the development of new aromatic compounds.[113] In particular, adding quinoa
345 sourdough to wheat enhances the sensory traits of wheat bread, resulting in higher
346 acidity, a salty taste and less sweetness.[41] However, it is not clear if this new sensory
347 profile masks the bitterness of quinoa.

348 Sprouting (or germination) is a natural process that decreases the anti-nutrient
349 compounds in cereals, pseudocereals and pulses while substantially increasing
350 micronutrient bioavailability and improving sensory properties.[114,115] Germinated grains
351 are characterized by a sweet taste, due to the formation of simple sugars, that may
352 mask the bitter taste in whole wheat bread.[116] However, no information about the effect
353 of germination on quinoa saponins and, consequently, on its bitter taste or aftertaste
354 has been reported. Nevertheless, the effectiveness of germination in decreasing
355 saponin content in bitter quinoa varieties might be a hoped-for result, given the
356 precedent of the positive results observed in sprouted chickpeas[117] and huazontle[118]
357 (*Chenopodium berlandieri spp.)*, closely related to quinoa.

358 ***Breeding***

359 Several bio-technological approaches have been proposed to decrease the amount of
360 saponins. Although effective, they are costly and impact negatively on the environment.
361 Therefore, the possibility of selecting “sweet” genotypes with low saponin content for
362 direct consumption without any grain pre-treatments are being explored: this approach
363 would facilitate the expansion of quinoa production and utilization, above all, beyond
364 the Andean regions.[119]

365 Quinoa, in fact, is still an under-utilized crop and breeding efforts to improve its
366 agronomic traits (length of growing season; crop yield) are required to expand its
367 production worldwide, especially at higher latitudes where some lines are characterized

with little or no saponin is one of the most important breeding objectives for the future,[121,122] not only to improve crops in South American countries but also in Mediterranean environments.[123] 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.[124] Jarvis *et al*.[73] 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 by means of the marker assisted selection. These findings would provide the scientific bases for accelerating the genetic improvement of quinoa, to enhance global food security for a growing world population.

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.

ACKNOWLEDGEMENTS

Diego Suárez is grateful recipient of a PhD fellowship from Secretaría de Educación Superior, Ciencia, Tecnología e Innovación (SENESCYT), Ecuador.

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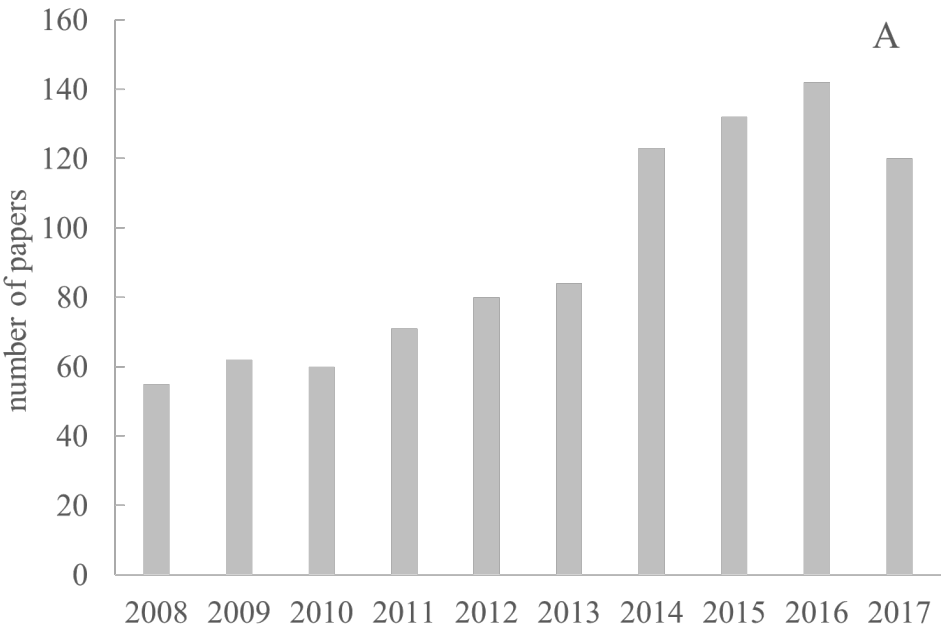

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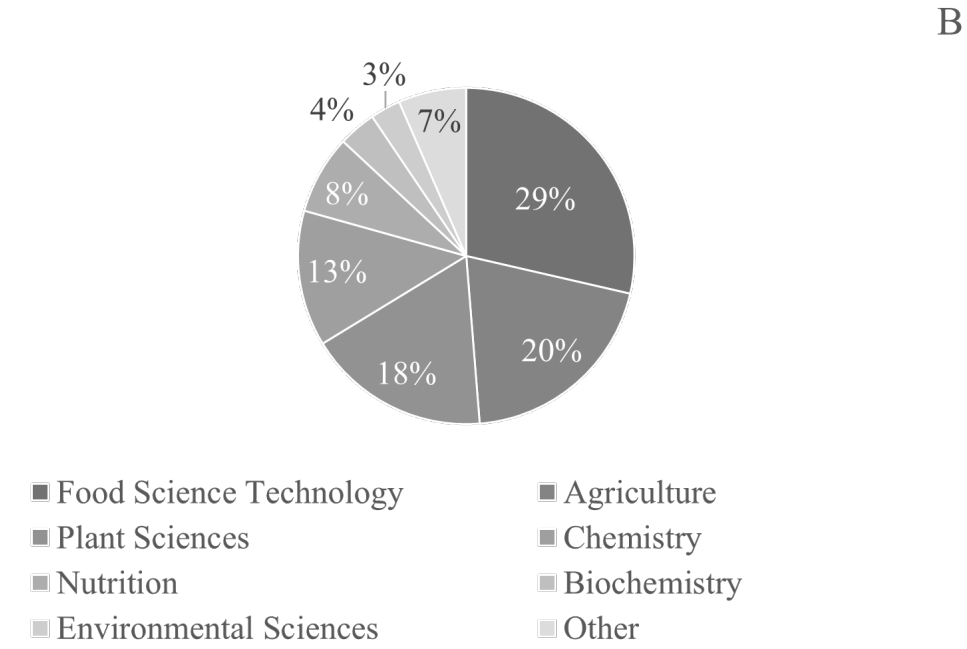

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769 Figure 1. Papers on quinoa (A) and the related distribution in the main research areas

770 (B) (source: Web of Science; 2008-2017; updated to August 31th, 2017).

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772 Table 1. Topics of the main reviews published on quinoa (source: Web of Science;

773 2008-2017; updated to August 31th, 2017)

Research area	Topic	References
Agriculture/Agronomy	Breeding	Zurita-Silva *et al*.[5]
	Structure	Burrieza *et al.*[6]
	Cultivation	Bazile *et al*.[7]
	Sustainability	Choukr-Allah *et al.*[8] Ruiz *et al*.[9]
Nutrition/Health benefits	Weight gain	Simnadis *et al.*[10]
	Lipid profile	Simnadis *et al*.[10]
	Antioxidant activities	Simnadis *et al.*[10] Tang & Tsao[11]
	Anti-inflammatory activities	Tang & Tsao[11]
	Anti-obesity and anti-diabetic activities	Tang & Tsao[11] Navruz-Varli & Sanlier[12]
	Cardiovascular disease and other chronic diseases	Tang & Tsao[11] Navruz-Varli & Sanlier[12]
	Celiac disease safety	Tang & Tsao[11]
Food Science and Technology	Compositional, nutritional and functional aspects	Navruz-Varli & Sanlier[12] Maradini-Filho *et al*.[13] Vega-Galvez *et al.*[14] Jancurová *et al.*[15]
	Product development	Graf *et al.*[16] Wang & Zhu[17]
	Protein functionality	Janssen *et al*.[18]

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776 Table 2. Hypothesis of key mechanisms leading to bitterness in quinoa (adapted from
777 Heiniö *et al.*[57])

Compound	Mechanism	References
Saponins	Molecule properties	Kuljanabhagavad & Wink[58]
Phenolic compounds	Release of unbound flavour-active phenolic compounds	Challacombe *et al*.[59] Heiniö *et al*.[60] Kobue-Lekalake *et al*.[61] Soares *et al*.[62]
Peptides/aminoacids	Proteolysis of the albumins and proteolysis of globulins forming bitter peptides	Jiang & Peterson[63] Brijs *et al*.[64] Heiniö *et al*.[65]

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780 Table 3. 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

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