Sprouting of quinoa (*Chenopodium quinoa* Willd.): effect on saponin content and relation to the sensory characteristics assessed by e-tongue

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Abstract

This study aimed at addressing the effect of sprouting on the sensory traits of quinoa and to relate such changes in saponins which are considered the main responsible for quinoa bitterness and astringency. Whole quinoa was sprouted up to 72 h at 22 °C and dried at 55 °C for 6 h. Sensory traits were assessed by electronic tongue and mainly related to the amount of saponins that were assessed by thin layer chromatography (TLC) and mass spectrometry (MS). Sprouting decreased quinoa bitterness and astringency, with the main changes occurring within the 48 h of treatment. Both semi-quantitative (by TLC) and quantitative (by MS) analysis revealed that sensory enhancement upon sprouting was mainly related to the decrease in the amount of saponins, reaching the value of 0.086 mg/100 g after 48 h of treatment. Finally, the quantification of phenolic compounds showed an increase in free phenols and free flavonoids upon sprouting, suggesting that the sprouting-related changes in regard to phenolic compounds play a minor role in affecting the sensory traits of sprouted quinoa. Further studies will focus on the assessment of consumer acceptability for sprouted quinoa, together with the analysis of the polyphenol composition in sprouted quinoa.

Keywords: quinoa; bitterness; saponins; germination

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is one of the best alternatives for the fight against hunger and malnutrition 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 all over the world.

Quinoa is mainly consumed as boiled seeds, especially in South America, whereas, in the Western countries, it is milled to flour and used as an ingredient in cereal-based products. Nevertheless, the presence of bitter compounds (including saponins and phenolic acids), which are mainly located in the external layers of the seeds, limits the consumption of quinoa (Suárez-Estrella, Torri, Pagani, & Marti, 2018). Nowadays, pearling is the main process used at industrial level to improve quinoa acceptability. Unfortunately, many compounds with high nutritional value - such as fiber, phenolic acids, minerals and vitamins - are lost during pearling (Gómez-Caravaca, Iafelice, Verardo, Marconi, & Caboni, 2014). More recently, quinoa fermentation has been proposed as an alternative process to enhance the sensory traits of quinoa-enriched wheat bread, resulting in an acid, salty taste, with a decrease in sweetness, due to the enzymatic activities developed during the process (Rizzello, Lorusso, Montemurro, & Gobbetti, 2016). In this frame, the effects of the endogenous enzymatic activities developed during sprouting on the sensory traits of quinoa have not been addressed yet.

Sprouting (or germination) is a natural process that decreases anti-nutrient compounds such as phytates in grains while substantially increase the amount of components with antioxidant activity and the bioavailability of micronutrients (Lemmens et al., 2019). In the case of quinoa, important changes in chemical, physical, functional, and nutritional properties were reported as effect of sprouting (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Suárez-Estrella, Bresciani, Iametti, Marengo, Pagani, & Marti, 2020a). Specifically, the endogenous proteases have an impact on the capacity of proteins to interact with nutritionally relevant metals, including copper and zinc,

thus likely improving their bioavailability. The increase in α-amylase activity occurring in the early stages of sprouting affects starch, making it less prompt to gelatinize upon heating. Changes upon sprouting resulted in improved foam stability, but in impaired foaming capacity, and in decreased starch retrogradation (Suárez-Estrella et al., 2020a). Using sprouted quinoa in wheat formulation (at 20:80 replacement level) led to bread with enhanced volume and crumb softness (Suárez-Estrella, Cardone, Buratti, Pagani, & Marti, 2020b). However, the effects of sprouting on quinoa saponin content has been not yet addressed. Understanding the effects of sprouting on sensory characteristics of quinoa seeds might enhance the use of this crop as an ingredient in several food applications. Thus, the aim of this study was to evaluate the effects of sprouting on the main factors related to quinoa taste. In this context, the analysis of sensory traits by electronic tongue was integrated with the quantification of saponins, as the main components responsible for quinoa acceptability.

2. Materials and Methods

2.1 Materials

Whole quinoa seeds (*Chenopodium quinoa* Willd. var. Titicaca) were provided by Quinoa Marche s.r.l. (Ancona, Italy), as well as the pearled seeds. Seeds (5 kg) were soaked, sprouted in a chamber at 22 °C for 12, 24, 48, and 72 h (Memmert GmbH Co. KG, Schwabach, Germany), and dried at 55 °C for 6h (Self Cooking oven, Rational International AG, Mestre, Italy), as previously reported by Suárez-Estrella et al. (2020a). Samples were used as flour (particle size < 250 μm using a Cyclotec 1093, Foss Sample Mill, Höganäs, Sweden) or cooked seeds. Seeds (20 g) were cooked in boiling distilled water (250 mL) for 20 min. The cooking time was previously determined as the necessary time for complete starch gelatinization of the seeds, evinced by the complete disappearance of their white cores. After cooking, samples were freeze-dried (-80°C for 72h; Alpha 1-2 LD plus; Deltek s.r.l., Naples, Italy) and milled in a lab scale mill (IKA M20, Staufen, Germany). All the samples were maintained at 4 ˚C until analysis.

70 *2.2 Methods*

71 *2.2.1 Electronic tongue assessment*

72 Electronic-tongue (e-tongue) assessment was performed in triplicate on both uncooked and cooked
73 seeds. Analyses were performed with the Taste-Sensing System SA 402B (Intelligent Sensor
74 Technology Co. Ltd, Atsugi, Japan). For this study 5 detecting sensors and 2 reference electrodes
75 were used, separated in 2 arrays according to the membrane charge: hybrid (AAE; CT0; CAO) and
76 positive (C00; AE1). Twenty grams of flour from cooked seeds were suspended in 250 mL of
77 distilled water, centrifuged at 5000 x g for 10 min at 20 °C and the supernatants were tested
78 according to Marengo et al. (2017). Each sample was analysed in triplicate and sensor outputs were
79 converted to taste information. The "taste values" were calculated by multiplying sensor outputs for
80 appropriate coefficients based on the Weber–Fechner law, which gives the intensity of sensation
81 considering the sensor property for tastes (Kobayashi, Habara, Ikezazki, Chen, Naito, & Toko,
82 2010; Buratti, Casiraghi, Minghetti, & Giovanelli, 2013).

83 *2.2.2 Saponin content*

84 Saponin extracts were prepared according to Stuardo & San Martín (2008). Briefly, 1 g quinoa flour
85 were added with 10 g H_2O, kept for 360 min at 25°C and filtered on #2 Whatman paper.
86 Preliminary semi-quantitative Thin Layer Chromatography (TLC) analysis was carried out
87 according to the method of Krishnamurthy, Tsukamoto, Yang, Lee, & Chung (2012).
88 Mass spectrometry analysis was performed on the quinoa water extract using a Dionex UltiMate
89 3000 nano-UHPLC system coupled with nano-ESI-linear ion trap (LIT) Thermo XL mass
90 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were resuspended in 0.1%
91 (v/v) formic acid solution, loaded through a 5 mm long, 300 µm in pre-column (LC Packings, USA)
92 and separated by an Acclaim™ PepMap™ C18 column (150 mm × 75 µm, 3µm). Flow rate: 0.200
93 µL/min. Eluent A was 0.1% formic acid (v/v) in Milli-Q water; eluent B was 0.1% formic acid (v/v)
94 in acetonitrile. The column was equilibrated at 5% B. Analytes were separated applying a 4–40%

gradient of B over 40 min. LC–MS analysis was performed operating in both (continuum) MS mode and in MS/ MS mode for data dependent acquisition (DDA) of saponin fragmentation spectra, according to Madl, Sterk, Mittelbach, & Rechberger (2006). DDA MS/MS spectra were collected from the five most abundant precursor ions upon fragmentation (charge state 1; isolated width of 1 Da; min. signal required: 500) using CID activation with 35.0% normalized collision energy, activation Q of 0.25, and activation time of 30 ms. MS data was obtained from 200 to 2000 m/z mass range. Nitrogen was used as nebulizer gas (6 l/min, 260 °C). The intensity of the protonated molecular ions (MH^+) and of the fragment corresponding to loss of the 28-O linked glucpyranose residue of each saponin (Madl et al., 2006) were used for compound quantification. A standard of oleanolic acid (Sigma, Italy) was used as reference for method setting. Spectra were processed using the Xcalibur Software 3.1 version (Thermo Scientific).

Phenolic compounds

2.2.3.1 Extract Preparation

Sample extraction was performed according to Carciochi, Manrique & Dimitrov (2015).Specifically, the solid-solvent ratio was 1:20 and the extraction was carried out in 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 was carried out at pH 1.5 (for total phenolic and total flavonoid compounds determination) and at pH 6.9 (for free phenolic and total flavonoid compounds determination). 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.

2.2.3.2 Total, free and bound phenols

The Folin-Ciocalteau assay was carried out as reported by Carciochi et al. (2015). Specifically, 200 µL of extracted samples were diluted in 7.8 mL of water, 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). The calibration curve for gallic acid was used to express the results as milligrams of gallic acid equivalent (GAE) per gram of the sample on a dry weight basis. Two measurements in duplicate were performed from each extract, for a total of eight replicates.

2.2.3.3 Total, free and bound flavonoids

The flavonoid content was measured as reported by Carciochi et al. (2015). Specifically, 250 µL of extracted samples were diluted in 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. The calibration curve for quercetin was used to express the results as milligrams of quercetin equivalent (QE) per gram of the sample on a dry weight basis. Two measurements in duplicate were performed from each extract, for a total of eight replicates.

2.2.3 Total titratable acidity and pH

Total titratable acidity and pH of both uncooked and cooked seeds were measured in triplicate using a titrator T50 (Mettler-Toledo AG, Greifensee, Switzerland) equipped with a pH sensor (Mettler Toledo DGi 115-SC), as reported by Marengo, Bonomi, Marti, Pagani, Elkhalifa, & Iametti (2015), with slight modifications. Specifically, samples (10 g) were diluted in distilled water (90 mL) and agitated at room temperature for 1 h. Then, the samples were centrifuged at 2500 x g for 10 min.

2.2.4 Statistics

Analysis of variance (one-way ANOVA; α=0.05) was assessed by Statgraphics Plus 5.1 (StatPoint Inc., Warrenton, USA) using the samples as factors. The significant differences ($p \leq 0.05$) were determined by using Tukey HSD test. Data from e-tongue measurements were elaborated by

144 Principal Component Analysis (PCA) using MINITAB 14 (v.12.0; Minitab Inc, State College,
145 USA) software package.

146 **3. Results**

147 *3.1 Sensory characteristics by electronic tongue assessment*

148 The sensory traits of quinoa seeds before and after cooking, obtained from e-tongue measurement
149 and elaborated through the Principal Component Analysis (PCA), are shown in Figure 1. The first
150 two Principal Components (PC1 and PC2) accounted for 73.8% of the total variance. As shown in
151 the score plot (Figure 1a), samples were clearly discriminated on PC1 (54.8% of the total variance)
152 based on the applied treatments. In fact, the untreated (i.e., whole quinoa), pearled and soaked seeds
153 were located on the positive part of PC1; whereas the sprouted samples were located on the
154 negative part of PC1. Such discrimination was similar in both uncooked and cooked samples. On
155 PC2 (19% of total variance) samples were discriminated according to the seed status (uncooked or
156 cooked samples) and sprouting time.
157 The loading plot (Figure 1b) evidenced the tendency of uncooked samples to bitterness, while the
158 cooked seeds were more characterized by astringency, umami, saltiness and aftertaste-astringency.
159 Sprouted samples, located on the negative part of PC1, were discriminated by sourness and were
160 perceived less bitter and astringent.

161 *3.2 Quantification of saponins by Liquid Chromatography coupled to High Resolution Mass*
162 *Spectrometry (LC-HR-MS/MS).*

163 The semi-quantitative TLC analysis based on the intensity of the bands shows a decrease in the
164 intensity of the bands associated with saponins in correspondence of the sprouted samples (Figure
165 2). This phenomenon was more and more effective as sprouting time proceeded. At the end of the
166 process (72 h), it was visible only a very faint band in the saponin area, suggesting the significant
167 effect of sprouting on decreasing the amount of saponins. This result agrees with the decrease in
168 bitterness detected by the e-tongue (Figure 1).

169 Quinoa water extracts were then analyzed by LC-HR-MS/MS to quantify the single and total
170 amount of saponins, in order to confirm the positive role of sprouting on decreasing the
171 concentration of these compounds in quinoa flour. The main saponin components were identified
172 on the basis of the characteristic MS/MS fragmentation spectrum and quantified by measuring the
173 intensity the relative molecular ion intensity and of the fragment at -162 Da corresponding to loss of
174 the 28-O linked glucopyranose moiety (Madl et al., 2006). Data are summarized in Figure 3 and in
175 Supplementary Table 1. Analysis of MS showed that, as the sprouting time progressed, the amount
176 of native saponins decreased, from about 0.4 g/100g after 12 h sprouting, to 0.05 g/100g in the
177 seeds sprouted for 72 h (Figure3). At the same time, degradation products were observed starting
178 from 24-48 h. These products derived from the deglycosylation and dehydration of serjanic acid and
179 phytolaccagenic acid, identified by MS/MS.

180 *3.3 Phenolic compounds*

181 Total, free and bound phenols and flavonoids of whole and treated seeds are shown in Table 1. The
182 amount of total phenols progressively increased during sprouting. Specifically, upon sprouting, the
183 free phenolic fraction increased at the expense of the phenolic compounds that are bound to the cell
184 walls. Similarly, the free flavonoids increased, whereas the bound fraction decreased as sprouting
185 duration increased. Such changes resulted in an overall decrease in the content of total flavonoids in
186 sprouted seeds. The greatest effect of sprouting on phenol and flavonoid content was observed after
187 48 h and 72 h, respectively.

188 Pearling promoted only a significant decrease in free phenolic fraction (Table 1). Indeed, such
189 compounds are mainly located in the pericarp of cereal seeds, while bound phenols are mainly
190 located in the cell wall of the seeds (Carciochi, Galván-D'Alessandro, Vandendriessche, & Chollet,
191 2016b). Pearling did not significantly modify the content of total, free or bound flavonoids with
192 respect to the whole sample. On the contrary, Hemalatha, Bomzan, Rao, & Sreerama (2016)

reported an increase in flavonoids after pearling. Such differences might be due to differences in the pearling process and in the amount of bran removed from the kernels.

3.4 Total Titratable Acidity and pH

The acidity and pH values of whole and treated seeds are shown in Table 2. The acidity increased upon sprouting, showing significant differences after 48 h till 72 h. As expected, as acidity increased, pH values significantly decreased. The pH parameter seemed to be more sensitive to sprouting than acidity, since significant differences were observed already at early stages of sprouting (i.e., 12 h). The effect of sprouting duration on acidity was also evident in the cooked seeds, although the latter showed lower values in comparison with those found for the uncooked samples. This result might be to the leaching of acid compounds in the cooking water. A similar behaviour was found when seeds were soaked in water prior to germination.

4. Discussion

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 USA. However, using quinoa in food formulations alters some quality characteristics of the final product, including its sensory acceptance (Suárez-Estrella et al., 2018). This is due to the presence of bitter and astringent compounds that are mainly located in the pericarp. Applying suitable processes – such as pearling - to separate the external layers of the seeds and thus decreasing their bitterness/astringency would enhance the sensory attributes of quinoa, thus its consumption. On the other hand, pearling also caused a decrease in the content of bioactive compounds such as fibre, vitamins, minerals, and phenolic compounds (Gómez-Caravaca et al., 2014).

Sprouting has been proposed as a suitable process to enhance the nutritional and sensory properties of cereals and pulses grains. Thus, in this study the attention was paid to the effects of sprouting on the sensory traits of quinoa seeds before and after cooking. Since the scores from a panel test are

often far from being univocal, there is a great interest in using electronic senses (e.g., e-tongue) for objective analysis of sensory traits. Indeed, electronic senses allow to evaluate the contribution of different chemical species in determining aroma and tastes in food products (Sliwinska, Wisniewska, Dymerski, Namiesnik, & Wardencki, 2014). Based on the e-tongue measurement (Figure 1), bitterness, sourness, astringency, umami and astringency aftertaste resulted the sensory traits most affecting the separation between whole and pearled seeds from the sprouted ones along the first two principal components of the PCA score plot. The location of 48 h and 72 h samples suggested that the taste changes promoted by sprouting reached the maximum intensity already after 48 h, with no further modifications within the 72 h.

After cooking, sprouted seeds were perceived sourer and less astringent and salty than unsprouted seeds. Specifically, sprouting seemed to increase the sourness. Changes in acidity and pH upon sprouting (Table 2) agreed 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). Except for sourness, sprouting seemed to decrease all the other attributes. These results agreed with a previous study on bread highlighting the suitability of sprouting process to decrease the bitter perception in quinoa-enriched bread (Suárez-Estrella et al., 2020b). Similarly, sprouting enhanced the sensory traits of cowpea-based breakfast cereals, decreasing both astringency and bitterness (Marengo et al., 2017). Moreover, the products enriched in sprouted cowpea showed an increase in sourness compared to the control (Marengo et al., 2017).

To understand what the factors are responsible for the changes in the sensory traits, the effect of sprouting on amount of the compounds involved in bitterness/astringency traits were assessed in the present study.

The bitterness of quinoa has always been associated with the presence of saponins in amounts higher than 1.1 mg g^{-1}, corresponding to the amount proposed by Koziol (1991) as the threshold for human perception of quinoa bitterness. Semiquantitative TLC and quantitative HPLC MS analysis were effective in measuring the saponin levels in the samples. TLC allowed rapid detection of the

effects of treatments on quinoa levels, while MS analysis (Escribano et al., 2017) allowed sensitive and accurate quantification of the single components and of their changes upon treatments.

The two methods were in good agreement in defining that quinoa seed germination decreases saponin levels drastically within 24-48h. Interestingly, while the native saponin levels decreased upon sprouting time, formation of novel derivatives was detected, whose levels increased starting from 24 h. These products were derived from dehydration and deglycosylation of phytolaccagenic and serjanic acid, as determined by MSMS analysis, although a careful investigation of their structure was outside the scope of the present study. These findings are in agreement with the report of Brady, Ho, Rosen, Sang, & Karwe (2007), which described similar compounds in quinoa samples by MS analysis, including a dominating one at 481 m/z, which was also detected in this study. The possible sensory impact of these compounds would deserve investigation in further studies.

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, Guajardo-Flores, Mata-Ramírez, Gutiérrez-Uribe, & Serna-Saldivar, 2016) - a quinoa-like grain - and in some pulses, including chickpea (El-Adawy, 2002), pigeon pea (Duhan, Khetarpaul, & Bishnoi, 2001), black gram (Jood, Chauhan, & Kapoor, 1986) and kidney bean (Shimelis & Rakshit, 2007). On the other hand, other studies reported an increase in saponins in soybean (Bau, Villaume, & Mejean, 2000) and lentils (Ayet et al., 1997) during sprouting. Finally, no changes in lentils and chickpeas have been also reported (Ruiz, Price, Rose, Rhodes, & Fenwick, 1996). Once again, the type of grain, sprouting conditions, and analytical approach might account for the differences of the results among the studies.

Besides saponins, also polyphenols are responsible for bitterness and astringency in grains (Heiniö et al., 2016). Specifically, free phenolic compounds are the most flavour active because they interact with taste receptors (Heiniö, Liukkonen, Myllymäki, Pihlava, Adlercreutz, & Heinonen,

2008). However, during mastication the bound phenolic acids might be converted into the free from by the salivary enzymes, allowing them to interact with taste receptors (Challacombe, Abdel-Aal, Seetharaman, & Duizer, 2012). Considering the findings above, the evolution of both phenols and flavonoids was assessed during sprouting in order to provide information about their potential role in affecting the sensory traits detected by e-tongue measurements. Results suggested that changes in phenols monitored during sprouting didn't account for the changes in sensory profile. Indeed, despite the increase in phenol content (Table 1), the bitterness and astringency of seeds decreased (Figure 1). The steady increase in total and free phenols might be due to their synthesis because of the biochemical phenomena occurring during seed sprouting (Singh, Rehal, Kaur, & Jyot, 2015). Nevertheless, it could also be attributed to the easier extractability of phenolic compounds from the cell walls, as previously observed in sprouted oats (Kaukovirta–Norja, Wilhemson, & Poutanen, 2004), probably as a result of the action of endogenous esterases synthesized during sprouting (Carciochi et al., 2016b). Alvarez-Jubete et al (2010) also found an increase in the polyphenol content in sprouted quinoa. Specifically, kaempferol and quercetin glycosides in quinoa sprouts reached 56.0 and 66.6 μmol/100 g compared with 36.7 and 43.4 μmol/100 g in quinoa seeds (Alvarez-Jubete et al., 2010).

On the other hand, total flavonoids decreased upon sprouting (Table 1). Overall, the effects of sprouting on phenols and flavonoids described in literature are not consistent. Indeed, some authors reported their increase (Carciochi, Manrique, & Dimitrov, 2014; Laus et al., 2017), while others their decrease (Paśko, Sajewicz, Gorinstein, & Zachwieja, 2008), likely due to differences in cultivar (Bois, Winkel, Lhomme, Raffaillac, & Rocheteau, 2006), sprouting conditions (Carciochi, Dimitrov, & Galván, 2016a) and methods used for measuring these compounds.

5. Conclusions

Besides the positive effects on nutritional and technological properties, sprouting enhanced the sensory profile of quinoa seeds before and after cooking, suggesting a potential use of sprouted

quinoa not only as seeds but also as ingredient in food formulations. Sprouting determined an increase in sourness and a decrease in bitterness/astringency. Mapping the changes in acidity, pH, phenols, flavonoids and saponins during sprouting duration allowed to better understand the role of each component/factor in determining the sensory traits of sprouted seeds. The accumulation of organic acids during sprouting needs to be confirmed as potentially responsible for the increase in acidity and thus in the perception of sourness. Changes in phenolic compounds 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. On the other hand, the decrease in saponins is responsible for the decrease in bitterness. These findings provide the molecular basis of the improvement of taste in quinoa by processes such as sprouting. The same MS-based methodology can be applied to investigate similar structural-sensory relationship in quinoa as well as in other crops.

Declarations of interest: none.

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Figure Captions

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.

Figure 2. Thin Layer Chromatography of quinoa extracts.

Figure 3. Effect of sprouting on saponin content.

Glycosidic fraction: Glc, Glucose; Ara, Arabinose. Aglycone: OA, Oleanolic acid; Hed, Hederagenin; SA, Serjanic acid; PA, Phytolaccagenic acid

Table 1. Effects of sprouting on phenolic compound and flavonoid content

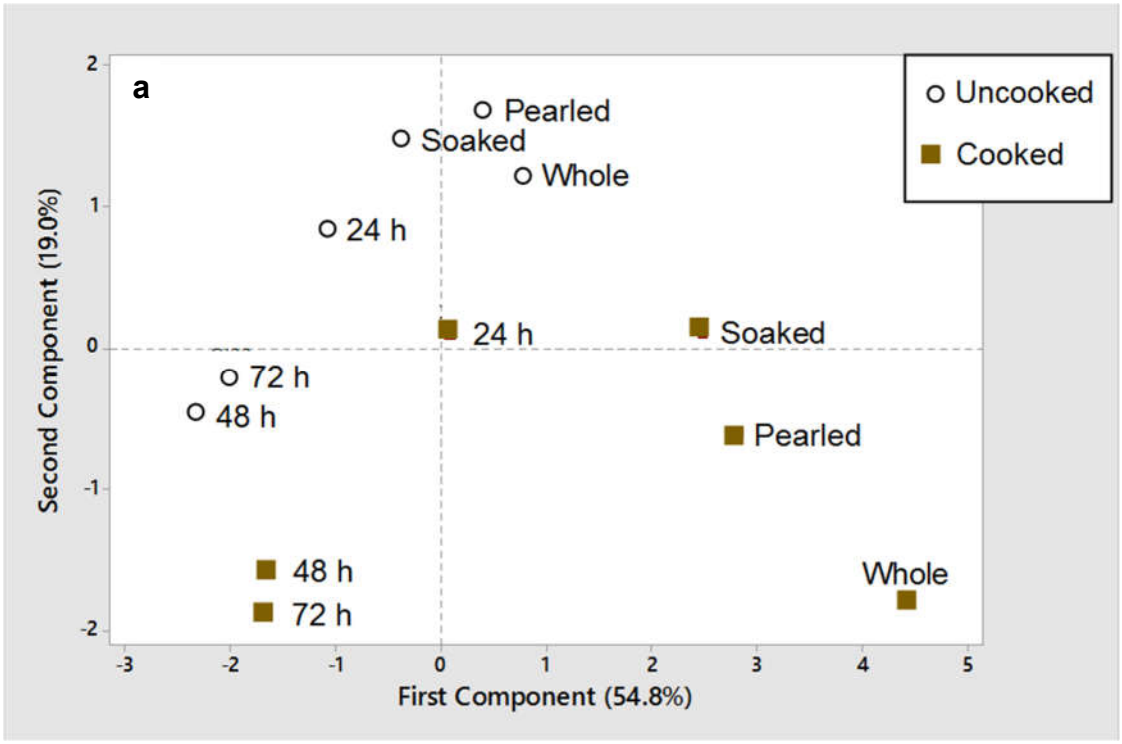
		Phenolic compounds (mg GAE/g db)			Flavonoids (mg QE/g db)		
		Total	Bound	Free	Total	Bound	Free
Whole		3.04±0.05^{ab}	0.94±0.09^{ab}	2.10±0.05^{bc}	5.78±0.07^{d}	2.52±0.05^{c}	3.26±0.12^{ab}
Soaked		3.19±0.02^{bc}	0.95±0.03^{ab}	2.23±0.05^{c}	4.33±0.02^{ab}	0.22±0.03^{a}	4.12±0.05^{de}
Sprouted	12 h	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; $p<0.05$; $n=8$).

Table 2. Total titratable acidity and pH of quinoa seeds

		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]
Sprouted	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).


a
Uncooked
Cooked
Pearled
Soaked
Whole
24 h
24 h
Soaked
72 h
48 h
Pearled
48 h
72 h
Whole
Second Component (19.0%)
First Component (54.8%)
2
1
0
-1
-2
-3
-2
-1
0
1
2
3
4
5

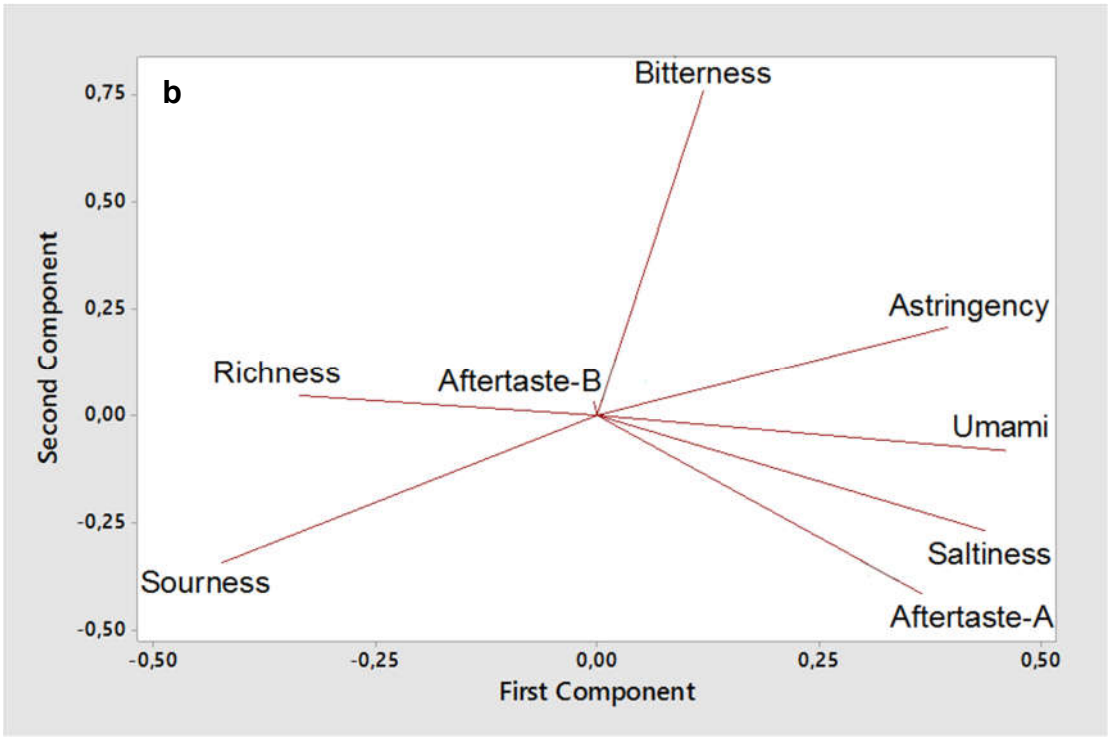

b
Bitterness
Astringency
Richness
Aftertaste-B
Umami
Saltiness
Sourness
Aftertaste-A
Second Component
First Component
0,75
0,50
0,25
0,00
-0,25
-0,50
-0,50
-0,25
0,00
0,25
0,50

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

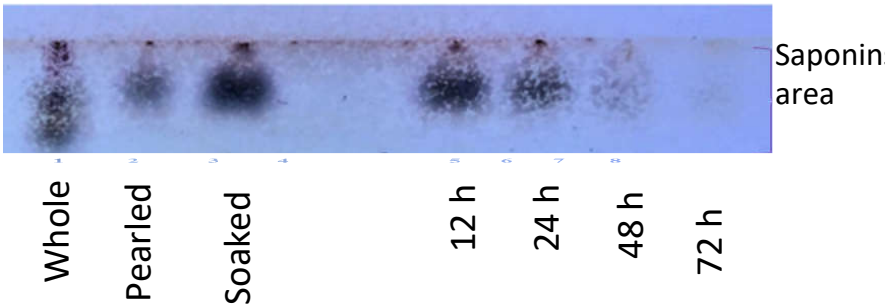


Figure 2. Thin Layer Chromatography of quinoa extracts.

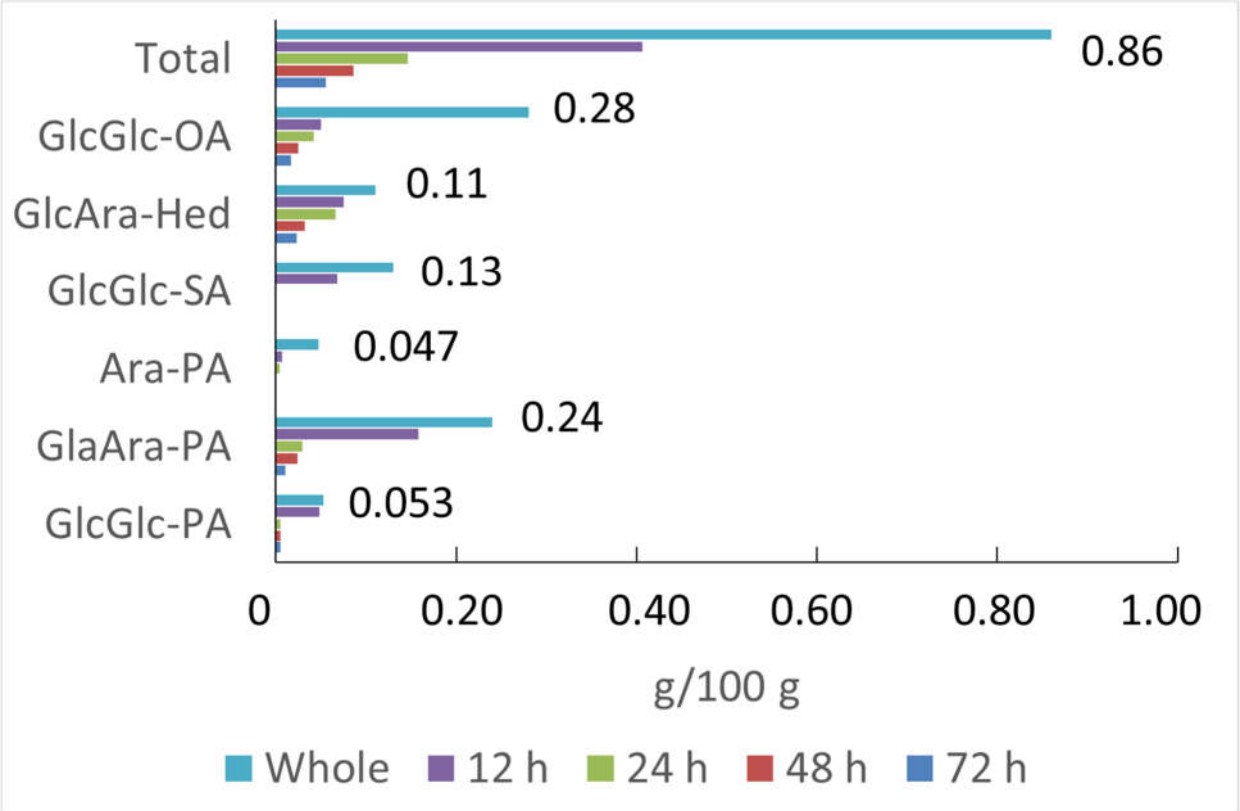


Figure 3. Effect of sprouting on saponin content. Glycosidic fraction: Glc, Glucose; Ara, Arabinose. Aglycone: OA, Oleanolic acid; Hed, Hederagenin; SA, Serjanic acid; PA, Phytolaccagenic acid