Effects of germination and kilning on the phenolic compounds and nutritional properties of quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthus caudatus*)

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ABSTRACT

Quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthus caudatus*) are nutritious pseudocereals that originate from the Andean region. The aim of this research was to study the effect of germination and the subsequent kilning on the phenolic compounds and proximate composition in selected Peruvian varieties of quinoa ("Chullpi") and kiwicha ("Oscar Blanco"). The germination process was carried out for 24, 48 and 72 h at 22°C, and the kilning was performed with samples germinated for 72 h by drying the seeds at 90°C for 5 min. Both processes increased the protein content of the samples. However, lipid content was reduced during germination. On the other hand, germination and kilning clearly increased the concentration of total phenolic compounds in both quinoa and kiwicha. Germination for 72 h either with or without kilning process resulted in a significant ($p < 0.05$) increase in the total content of phenolics compared to untreated materials, which was especially due to coumaric acid and a kaempferol tri-glycoside in quinoa and caffeoylquinic acid in kiwicha. Based on the results, germination and kilning may improve the nutritional quality of the Andean grains, encouraging the usage of the processed grains as ingredients in functional products for people with special gluten-free or vegetarian diets.

Keywords: *liquid chromatography; mass spectrometry; plant protein; processing*

1. Introduction

Pseudocereals quinoa (*Chenopodium quinoa*) and kiwicha (*Amaranthus caudatus*) originate from the high Andean regions of South America, ranging from Ecuador to southern Chile. Both crops demonstrate a large biodiversity in Peru owing to the various agro-ecological zones in the country. Quinoa and kiwicha have been consumed for hundreds of years as cooked and roasted whole grains or flours in traditional foods such as cooked dishes, puddings, and salads. Currently there is a large selection of quinoa-based products available on the market. These include beverages, breakfast cereals, pastries, snacks, pastry products, chocolates, gluten-free products, dietary supplements, baby porridge, and emulsifier stabilizers (Pellegrini et al. 2018).

Seeds of quinoa and kiwicha have a high nutritional value. They surpass the common cereals in terms of the content of lipids, proteins, dietary fiber, vitamins B1, B2, B6, C, and E and many minerals (calcium, phosphorus, iron and zinc). They also possess a good essential amino acid composition, lysine and tryptophan typically being the limiting amino acids in common cereals. From the point of view of digestibility, bioavailability, available lysine, and net utilization, pseudocereal proteins are often better option compared than cereal proteins (Repo-Carrasco et al. 2003). The grains do not contain gluten, which allows a greater supply and variety of nutritious food products for individuals with celiac disease (Nowak et al. 2016). In addition, quinoa and kiwicha are rich in phenolic compounds that are potentially responsible for a wide range of biological and physiological functions. Major phenolic compounds in quinoa include vanillic acid, ferulic acid (together with its derivatives), and certain flavonoids such as kaempferol and quercetin (Hemalatha et al. 2016; Tang et al. 2015). In kiwicha, the major phenolic compounds are caffeic acid, *p*-hydroxybenzoic acid, and ferulic acid (Paucar-Menacho et al. 2017).

Both chemical composition and nutritional value of the grains are highly influenced by germination process. As studied previously, the germination of cereal and pseudocereal grains results in a general increase in nutritional value and antioxidant properties of the grains, which possibly exerts health promoting effects and reduces the risk of various diseases. During germination, numerous biochemical changes take place that generate mobilization, accumulation, and metabolism of nutrients and other

phytochemicals (Gawlik-Dziki et al. 2013). Paśko et al. (2009) and Perales-Sánchez et al. (2014) studied some *Amaranthus* species (*A. hyponcondriacus* and *A. cruentus)* and found increasing contents of protein, dietary fibers, and phenolic compounds during germination. The content of secondary metabolites is diminished or increased depending on the conditions (such as temperature and time) of germination. The kilning process after germination includes heat treatment to stop metabolic processes and to develop flavor and aroma (Kaukovirta-Norja et al. 2004).

Germination and kilning processes have been studied in cereals such as wheat, sorghum, rice, and barley. However, there are very few studies focused on evaluating the effect of the processes on phenolic and nutritional compounds in pseudocereals such as quinoa and kiwicha, and even less in Peruvian varieties. In addition, most of the studies have not identified the phenolic compounds generated during the germination and subsequent kilning process. This investigation aimed to study the effect of germination and kilning on the proximate composition and phenolic compounds in selected varieties of quinoa and amaranth.

2. Materials and methods

2.1. Samples

Quinoa (*C. quinoa* var. Chullpi) and kiwicha seeds (*A. caudatus* var. Oscar Blanco) were purchased from the city of Puno and Arequipa, Peru, respectively. The samples were stored in polyethylene bags at 4°C.

2.2. Chemicals

Solvents of LC and MS grade, such as methanol, acetonitrile, acetic acid and formic acid, were purchased from VWR International Oy (Espoo, Finland). Reference standars of quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside were purchased from Extrasynthese (Genay, France). Chlorogenic acid, kaempferol- 3-*O*-glucuronide, *p*-coumaric acid, *trans*-ferulic acid and vanillic acid were purchased from Sigma-Aldrich Co. (St. Louis, U.S.A.).

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84 2.3. Procedure of germination and kilning

85 Quinoa and kiwicha seeds (30 g of each) were first soaked in a 70% (v/v) ethanol
86 solution for 30 min for disinfection purposes. Then, they were rinsed and soaked in
87 distilled water (seeds: water; 1: 20) for 24 h at room temperature. Subsequently, the water
88 was drained and the seeds were placed on the grids of the germinator (bioSnacky®,
89 A.Vogel, Canada) for 72 h at a temperature of 22°C and relative humidity of 95%. All
90 seeds were watered with distilled water (10 ml) every 12 h. The germinated samples of
91 24, 48 and 72 h were collected and dried in the oven (Metos Futura MSR 104, Milan,
92 Italy) at 40°C for 60 min. Half of the 72 h germinated samples were kilned in a coffee
93 roaster at a temperature of 90°C for 5 minutes, after which the radicles were removed.
94 Germination and kilning were performed once for each sample.

95 Both raw materials and germinated/kilned samples were ground into fine powders by
96 using a grain grinder (Mill AT320A, Kenwood, United Kingdom), and then sieved
97 (0.5mm) with steel strainer. The powders were collected, stored at 4°C, and kept in dark
98 until later use.

99

100 2.4. Proximate composition

101 International standard methods (AOAC 2005) were used to determine moisture
102 (AOAC 925.09), protein content (AOAC – 960.52, micro Kjeldahl method), lipids
103 (AOAC – 2003.05, soxhlet extraction), ash (AOAC – 923.03) and crude fiber (AOAC
104 978.10). Carbohydrates were calculated based on other measurements. All the analyses
105 were performed three times.

106

107 2.5. Phenolic compounds

108 2.5.1. Extraction of phenolic compounds

109 The extraction method of phenolic compounds was based on the method of Tian et al.
110 (2017) with a slight modification. Approximately 5 g of each powder sample was

weighed and dissolved in 15 ml of acidic aqueous methanol (methanol: water: acetic acid, 70/30/0.1, v/v/v). The samples were mixed (Vortex Genie 2, G560, Scientific Industries, U.S.A.) for 3 min followed by centrifugation for 30 min (4420 × g). After the supernatant was collected, the residue was re-extracted twice with same extraction solvents. The solvent from combined supernatants from the total three-time extractions was evaporated to complete dryness with a vacuum rotary evaporator at 40°C (pressure set at 50 mbar), and the residue was dissolved in 1mL of methanol. Each three-step extraction was performed in triplicate and each of the combined extracts analyzed separately.

2.5.2. Identification of phenolic compounds using UPLC-DAD-ESI-MS

A Waters Acquity ultra performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA, U.S.A.) was applied in the mass spectrometric analysis of the extracts of quinoa and kiwicha. The equipment consisted of Waters Quattro Premier tandem quadrupole mass spectrometer (Waters Corp) utilizing electrospray ionization (ESI) as well as of type 2996 diode array detector (DAD; Waters Corp) (used here for identification only). Both positive and negative ion modes were utilized to collect data; the conditions of MS and MS/MS methods were the same as described previously by Tian et al. (2017).

The liquid chromatographic separation was modified based on previous research (Tian et al. 2019). Briefly, a Phenomenex Aeris peptide XB-C18 column (150 × 4.6 mm, 3.6 µm, Torrance, CA, U.S.A.) was applied in the analysis of phenolic compounds. The mobile phase consisted of formic acid/water as solvent A (0.1/99.9, v/v) and formic acid/acetonitrile as solvent B (0.1/99.9, v/v). The LC gradient program was optimized as the following: 0−15 min with 5−8% solvent B, 15−20 min with 8−10% B, 20−25 min with 10−13% B, 25−30 min with 13−15% B, 30−35 min with 15% B, 35−40 min with 15−20% B, 40−45 min with 20−25% B, 45−50 min with 25−30% B, 50−55 min with 30−60% B, 55−60 min with 60−5% B, and 60−63 min with 5% B. An aliquot of 10 µL of the extracts were injected into LC system at 25°C with a total flow rate of 1.0 mL/min. LC chromatograph were recorded at the wavelengths of 280, 320, and 360 nm for hydroxybenzoic acids, hydroxycinnamic acids, and flavonols, respectively.

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143 2.5.3 Quantification of phenolic compounds using HPLC-DAD

144 Quantitative analysis was performed on a Shimadzu LC-30AD liquid chromatograph
145 system, including a SIL-30AC auto-sampler, a CTO-20AC column oven, and a SPD-
146 M20A photodiode array detector. The chromatographic conditions were as same as that
147 described in the UPLC-DAD-ESI-MS method. HPLC analysis was performed from
148 triplicate samples. All identified compounds were quantified by the calibration curves of
149 compounds with closest structures. For example, all quercetin derivatives were quantified
150 by the calibration curve of quercetin 3-*O*-rutinoside ($y = 6 \times 10^{-8} x - 0.0009$, $R^2 =$
151 0.9999). The detailed information on external standards is described in **Supplemental**
152 **Table 1**.

153

154 2.6. Statistical analysis

155 Quantitative results were expressed as mean value ± standard deviation (SD).
156 Statistical analysis was performed with Statistica 13.0 software (Stat Soft Inc., Tulsa, OK,
157 U.S.A.) and significant differences were established at $p < 0.05$. Difference in chemical
158 composition among samples was analyzed using a one-way ANOVA with Tukey's post-
159 hoc significance test. Bartlett and Shapiro -Wilks tests were applied to assess equality of
160 variance and normal distribution, respectively.

161

162 **3. Results and discussion**

163 3.1. Proximate composition of untreated and processed samples

164 **Table 1** shows the changes of proximate composition between raw and
165 germinated/kilned grains. The total content of protein in quinoa was increased during 72
166 h germination from 9.6 to 26.0 g / 100 g dry weight (dw). This increasing trend was also
167 observed in kiwicha samples, where the protein content increased from 15.4 to 23.7 g /
168 100 g dw during 72 h germination. The increasing content of protein during germination
169 can be explained by the generation and mobilization of reserve nutrients in grains. Bewley
170 et al. (2013) state that in dicotyledons, such as pseudocereals, the content of free amino
171 acids increases during the first three days of germination in seeds, favoring the increase

of total protein. The germination took place with pure water without additional nitrogen source, so the absolute protein content is not expected to change. Xu et al. (2019) suggest that the relative, dry-weight based increase in crude protein may be due to the loss of total dry weight during germination resulting from metabolic loss and shoot snap; thus, the absolute protein content does not change. Similar results were reported by Fouad and Rehab (2015) with a 5% increase in protein content in sprouted lentil seeds that was attributed to weight loss mainly due to carbohydrates in respiration during germination. The results by Chavan et al. (1989) indicate that germination increases proteolytic activity, leading to degradation of prolamins and consequent release of glutamic acid and proline, which provides nitrogen for the synthesis of the limiting amino acid lysine, leading into improvement of protein quality.

Surprisingly, in our study, kilning process consequent to 72 h germination caused a slight decrease in total protein. In quinoa, total content of protein decreased by 7.3 g/100g dw and in kiwicha by 7.4 g/100g dw. Aguilar et al. (2019) reported a slight decrease of 12.5 % and 8.7 % in total protein after kilning process (48 h of germination followed by drying at 55°C for 24 h) in Peruvian quinoa varieties "INIA Salcedo" and "Pasankalla Roja", respectively, but an increase in variety "Negra Collana". Decrease in total protein content – compared with total protein after germination – is logical due to the fact that the final product of catabolism during the germination is sucrose that is translocated bound with proteins and amino acids from the embryo to the radicles. The radicles are lost as a result of deculming (radicle removing step) during kilning, causing a decrease in the total protein content (Bewley et al. 2013).

Although the lipid content of the grains in the current research is high compared to the earlier published literature, earlier studies on the varieties of quinoa and kiwicha investigated in this study have been scarce. Repo-Carrasco (2011) reported 10.15% fat in kiwicha var. Oscar Blanco, which is relatively high for this pseudocereal. Genetic factors and environmental conditions such as water stress, salinity, and light conditions are the main sources of variation of the nutrients (Aguilar et al. 2019; Fischer et al. 2017). Contrary to protein, content of lipids was decreased significantly during the process of germination. At the end of germination (72 h), the total content of lipids was decreased from 15.2 to 7.6 g / 100g dw in quinoa and from 13.7 to 5.4 g / 100g dw in kiwicha. This may have been due to the biochemical changes in the conversion of lipids to sucrose

during germination stage of grains. Generally, 25% of lipids in grain seeds can be hydrolyzed in order to promote respiratory activity and meet energy requirement during germination process (Bewley et al. 2013). The reduction of lipid content was also observed in a study by Park and Morita (2004), who found a slight decrease of 2% in quinoa grown in Peru, and in a study by Colmenares and Bressani (1990), who observed a 3.2% decrease in kiwicha grown in Peru. Both studies utilized samples germinated for 72 h. However, in the study of Pachari et al. (2019) who investigated native Peruvian quinoa varieties "Blanca de Juli", "Roja Pasankalla" and "Negra Collana", a slight increase in lipid content of 1.2%, 1.6%, and 1.1%, respectively, was observed after 72 hours of germination.

The relatively high ash content in seeds of quinoa in the current study can be affected by the soil composition of the growing location (Bewley et al. 2013). A significant decrease was found between untreated and germinated/kilned seeds of quinoa. The lowest concentration (2.6 g/100g dw) was observed after the first 24 hours of germination, after which the concentration increased slowly to 4.5 g/100g dw. For kiwicha seeds, the effect of germination on ash content was not clear; however, the kilned samples contained 2.5 g/100g dw of ashes, which was 1.5 times lower than the content in raw seeds. In studies in kiwicha germinated for 48 h (Gamel et al. 2006) or 72 h (Colmenares and Bressani 1990), no significant changes were observed in the total ash content. Bewley et al. (2013) suggest that the loss of mineral content can be due to lixiviation in water during soaking and due to utilization of minerals as coenzymes for carbohydrate and protein catalysis during germination, leading into their relocation to the radicles that are later removed in the deculming during kilning.

A slight decrease in carbohydrate content was observed from untreated quinoa seeds (64.6 g/100 g dw) to seeds germinated for 72 h (56.7 g/100 g dw.) In kiwicha, slight variations in carbohydrates were observed, and the lowest value was observed in the untreated sample. In our study, there were no significant differences in the crude fiber content in the grains. Similar results were reported by Colmenares and Bressani (1990) in kiwicha germinated for 72 h.

3.2. Phenolic composition of the quinoa and kiwicha samples

Phenolic compounds in both raw and treated samples were characterized by comparing UV spectra, LC retention time, and typical MS ions/fragments with reference standards and previous literature. Altogether, twenty-one phenolic compounds, mainly hydroxybenzoic acids, hydroxycinnamic acids, and flavonols, were identified in the samples. The characterization of phenolic compounds and LC chromatograms of samples are given in **Table 2** and **Fig. 1**.

As shown in **Table 2**, flavonols were the major group of phenolic compounds identified in the seed extracts of quinoa. The positive fragments at *m/z* 303 and 287 in MS spectra indicated the presence of quercetin and kaempferol, respectively. These two flavonols were represented primarily as derivatives with tri- and disaccharides as sugar moieties. The identified sugars consist of *β-D*-galactopyranose, *α-L*-rhamnopyranose, *β-D*-apiofuranose, and glucuronide (Gómez-Caravaca et al. 2011 and 2014). Based on the study of Repo-Carrasco-Valencia et al. (2010), some varieties of Peruvian quinoa also contain other flavonols, such as myricetin and isorhamnetin; however, none of these compounds were detected in this study. Hydroxycinnamic acids identified from quinoa seeds mainly contained derivatives of coumaric and ferulic acids. Ferulic acid was present conjugated with glucose, whereas coumaric acid was detected as both free and glycosylated forms. It was not *p*-coumaric acid, but the isomeric form could not be verified due to lack of reference standards other than *p*-coumaric acid. Hemalatha et al. (2016) and Tang et al. (2015) quantified hydroxybenzoic acid derivatives from the extracts of (white, red and black) quinoa grains at high levels; these included gallic acid, *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, vanillic acid, and vanillic acid 4-glucoside. Nevertheless, in the present study, 4-glucosylated vanillic acid was the only hydroxybenzoic acid identified.

The extracts of kiwicha demonstrate a simple phenolic composition (**Table 2**). Hydroxycinnamic acids formed the dominant groups of phenolic compounds, which was in agreement with previous results (Paucar-Menacho et al. 2017). The major sub-groups of hydroxycinnamic acids were caffeic, coumaric, and ferulic acids. Unlike quinoa, these acids were present primarily as esters with quinic acids, by showing high intensity of typical $[M+H]^+$ ions at *m/z* 355 (caffeoylquinic acid), 339 (coumaroylquinic acid), and 369 (feruloylquinic acid) in MS spectra. The tentative identification was accomplished by the secondary fragment ions at *m/z* 179 (caffeic acid), 163 (coumaric acid) and 193

(ferulic acid), respectively. Quercetin 3-*O*-rutinoside was the only flavonol compound found in kiwicha extracts.

The contents of phenolic compounds identified in quinoa and kiwicha samples are shown in **Table 3** and **Table 4**, respectively. **Table 3** shows that during germination and subsequent kilning process the phenolic compounds in quinoa increased their content by 100% (from 2319.1 to 4656.9 μg/g dw). Similar behavior was reported in quinoa seeds by Alvarez-Jubete et al (2010) after 82 hours of germination. In our study, the most abundant phenolics after 72 h germination were coumaric acid (1346.4 μg/g dw) and kaempferol-deoxyhexosido-deoxyhexosido-hexoside (725.8 μg/g dw) although the content of the latter compound decreased during the germination process. A decrease of compounds such as acacetin / questin / apigenin-7-methylether was observed during the germination process of 72 h (from 311.5 to 83.0 μg/g dw). Similar results were reported in a study by Carciochi et al. (2016) where the most abundant compounds after 72 h germination of white quinoa were *p*-coumaric acid and vanillic acid (19.7 and 8.8 mg/kg dw, respectively). Alvarez-Jubete et al. (2010) demonstrated a high quantity of quercetin glycosides (43.4 μmol/100g dw) and kaempferol glycosides (36.7 μmol/100g dw) in samples of quinoa (grown in Bolivia) germinated for 82 h. In the present study, these compounds had low concentrations.

The content of non-flavonoid compounds increased by 62% in kilned samples compared with untreated quinoa. On the contrary, a decrease of 38% of flavonoid compounds was observed in kilned samples. Similar behavior was reported by Carciochi et al. (2016) who demonstrated an increase in non-flavonoids (33%) and a decrease in flavonoid compounds (10%) after kilning. Also, Paucar-Menacho et al. (2018) reported in quinoa "Pasankalla" germinated at 20 °C for 42 h (optimal conditions to increase phenolic content) an increase of 32% in non-flavonoid phenolic compounds and, on contrary to our results, a 44% increase in flavonoid compounds.

Table 4 demonstrates the increase of total phenolic compounds in kiwicha from 41.3 to 4504.6 μg/g dw. Similar results were reported by Paucar-Menacho et al. (2017) after 63 h of germination of kiwicha var. Centenario (from 0.01 to 1.08 mg/g dw). In our study, the most abundant compound after 72 h of germination was caffeoylquinic acid (2700 μg/g dw), but in the study on Centenario the compound was not detected after 63 h of

germination. In our study, in untreated kiwicha samples, only an unknown compound was detected. Repo-Carrasco-Valencia et al. (2010) detected ferulic acid (0.07 mg/g dw) in untreated kiwicha; however, in the present study, it was only detected (as glucose conjugate) starting at 48 h after germination. The presence of non-flavonoid compounds in kiwicha was approximately 99% of the total phenolic compounds, and the amount increased during germination and kilning. Flavonoid compounds were only detected after 72 h of germination and were maintained during kilning. Alvarez-Jubete et al. (2010) did not detect flavonoids in germinated samples of kiwicha, the only non-flavonoid compound detected was protocatechuic acid(14 μmol / 100 g).

4. Conclusion

This research provides new compositional information on the scarcely studied varieties of quinoa and kiwicha, "Chullpi" and "Oscar Blanco", respectively. Based on our results, germination can improve the nutritional composition of quinoa and kiwicha by increasing total content of protein. In addition, our study suggests that germination and kilning processes favor the accumulation of phenolic compounds in the grains. The results encourage the application of germinated and kilned quinoa and kiwicha as potential ingredients for the development of innovative and nutritious products.

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Declaration of competing interest

The authors declare no conflict of interest.

Appendix. Supplementary data

Supporting information is provided: Information of external standards applied in quantification of phenolic compounds (**Supplemental Table 1**).

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Figure captions

Fig. 1. LC chromatograms of phenolic compounds in samples of quinoa (a) and kiwicha (b). Peaks in chromatograms are: 1. acacetin/questin/apigenin-7-methylether; 2. vanillic acid 4-glucoside; 3. unknown compound; 4. coumaric acid-hexoside; 5. ferulic acid 4-glucoside; 6. coumaric acid; 7. quercetin 3-*O*-(2,6-di-*α*-*L*-rhamnopyranosyl)-*β*-*D*-galactopyranoside); 8. quercetin-deoxyhexoside-pentoside-hexoside; 9. kaempferol 3-*O*-(2,6-di-*α*-*L*-rhamnopyranosyl)-*β*-*D*-galactopyranoside); 10. kaempferol 3-*O*-(*β*-*D*-apiofuranosyl-*α*-*L*-rhamnopyranosyl)-*β*-*D*-galactopyranoside; 11. quercetin 3-*O*-glucuronide; 12. kaempferol-pentoside-hexoside; 13. kaempferol-deoxyhexoside-hexoside; 14. kaempferol-pentoside-glucuronide; 15. kaempferol 3-*O*-glucuronide; 1'.

438 unknown compound; 2'. ferulic acid 4-glucoside; 3'. caffeoylquinic acid; 4'. ferulic acid-
439 hexoside-hexoside; 5'. *trans*-ferulic acid; 6'. coumaroylquinic acid; 7'. feruloylquinic
440 acid; 8'. quercetin 3-*O*-rutinoside.

441

442

Table 1
Proximate composition of Andean grains untreated and processed samples (g/100g dry weight)

Samples		Moisture	Protein	Lipids	Crude fiber	Ash	Carbohydrates
Quinoa	**Untreated**	$10.7 \pm 0.3^{a,b}$	9.6 ± 0.4^{d}	15.2 ± 1.1^{a}	6.2 ± 0.1^{a}	5.5 ± 0.4^{a}	$64.6 \pm 0.2^{a,b}$
Chullpi	**24 h**	10.7 ± 0.2^{a}	15.6 ± 0.4^{c}	10.4 ± 0.7^{b}	6.2 ± 0.5^{a}	2.6 ± 0.4^{c}	66.0 ± 0.5^{a}
	48 h	8.5 ± 0.1^{b}	18.6 ± 0.2^{b}	7.4 ± 0.1^{c}	6.2 ± 0.7^{a}	3.0 ± 0.2^{c}	$64.4 \pm 0.6^{a,b}$
	72 h	5.4 ± 0.2^{c}	26.0 ± 0.7^{a}	7.6 ± 1.0^{c}	7.4 ± 0.7^{a}	4.5 ± 0.4^{b}	56.7 ± 0.3^{c}
	72 h kilned	$9.1 \pm 0.2^{a,b}$	18.7 ± 0.2^{b}	10.5 ± 0.4^{b}	7.3 ± 0.8^{a}	$3.6 \pm 0.0^{b,c}$	$61.7 \pm 1.0^{a,b}$
Kiwicha	**Untreated**	6.9 ± 0.3^{c}	15.4 ± 0.2^{c}	13.7 ± 0.5^{a}	7.5 ± 0.4^{a}	3.8 ± 0.3^{a}	57.1 ± 0.7^{a}
Oscar Blanco	**24 h**	11.4 ± 0.1^{a}	$17.4 \pm 0.1^{b,c}$	9.3 ± 0.5^{b}	7.3 ± 0.1^{a}	$3.4 \pm 0.2^{a,b}$	$61.1 \pm 0.5^{b,c}$
	48 h	$5.1 \pm 0.1^{c,d}$	20.2 ± 0.8^{b}	7.9 ± 0.3^{c}	7.2 ± 0.5^{a}	$3.1 \pm 0.4^{a,b}$	62.6 ± 1.2^{c}
	72 h	4.6 ± 0.1^{d}	23.7 ± 0.6^{a}	5.4 ± 0.4^{d}	7.5 ± 0.6^{a}	3.7 ± 0.1^{a}	$58.3 \pm 0.1^{a,b}$
	72 h kilned	9.3 ± 0.2^{bc}	16.3 ± 0.2^{c}	$8.5 \pm 0.3^{b,c}$	7.7 ± 1.2^{a}	2.5 ± 0.2^{b}	62.6 ± 0.1^{c}

Values are expressed as mean ± SD ($n = 3$). Different letters in the same column means indicate significant difference ($p < 0.05$) among samples.

Table 2
Identification of phenolic compounds in quinoa and kiwicha by UPLC-DAD-MS/MS

No.	Tentative identification	UV λ_{max} (*nm*)	$[M+H]^+/[M-H]^-$ (*m/z*)	$[A+H]^+/[A-H]^-$ and other ions (*m/z*)	daughter ions of $[M+H]^+/[M+Na]^+$ (m/z)
Quinoa					
1	acacetin/questin/apigenin-7-methylether	234,262	285/283	569,450,328,207,166/ 851,567,332,183,151,137	285→153,136,133,115
2	vanillic acid 4-glucoside	253,290	331/329	353,169/659,167,137	331→186,169,125
3	unknown compound	278	205/203	409,188/407	205→188,170,159,146,132,118
4	coumaric acid-hexoside	295(sh),314	327/325	349,165/163	
5	ferulic acid 4-glucoside	295(sh),330	357/355	195/193	
6	coumaric acid	295(sh),312	165/163		
7	quercetin 3-*O*-(2,6-di-*α*-*L*-rhamnopyranosyl)-*β*-*D*-galactopyranoside)	254,265(sh),353	757/755		757→611,465,449,303
8	quercetin-deoxyhexoside-pentoside-hexoside	255,265(sh),352	743/741	-/303	743→611,597,465,303
9	kaempferol 3-*O*-(2,6-di-*α*-*L*-rhamnopyranosyl)-*β*-*D*-galactopyranoside)	264,344	741/739	595,449,287/-	741→595,449,433,287
10	kaempferol 3-*O*-(*β*-*D*-apiofuranosyl-*α*-*L*-rhamnopyranosyl)-*β*-*D*-galactopyranoside	263,338	727/725	595,287/593	727→595,581,449,287
11	quercetin 3-*O*-glucuronide	254,265(sh),350	479/477	303/301	
12	kaempferol-pentoside-hexoside	264,338	581/579	449,287/-	581→449,287
13	kaempferol-deoxyhexoside-hexoside	264,345	595/593		595→449,287
14	kaempferol-pentoside-glucuronide	264,344	595/593		595→463,287
15	kaempferol 3-*O*-glucuronide	264,344	463/461	287/285	463→287
Kiwicha					
1’	unknown compound	278	205/203	409,188/407	205→188,170,159,146,132,118
2’	ferulic acid 4-glucoside	295(sh),329	357/355	379,195/193	379→217,185
3’	caffeoylquinic acid	298(sh),329	355/353	709,163/707,191	355→163
4’	ferulic acid-hexoside-hexoside	298(sh),319	519/157	357/355	541→379,185
5’	*trans*-ferulic acid	298(sh),322	195/193		
6’	coumaroylquinic acid	295(sh),313	339/337	361,147/173	339→147
7’	feruloylquinic acid	298(sh),328	369/367	391,177/173	369→177,145
8’	quercetin 3-*O*-rutinoside	254,265(sh),350	611/609		611→465,303

Table 3
Quantity of phenolic compounds in untreated, germinated and kilned quinoa

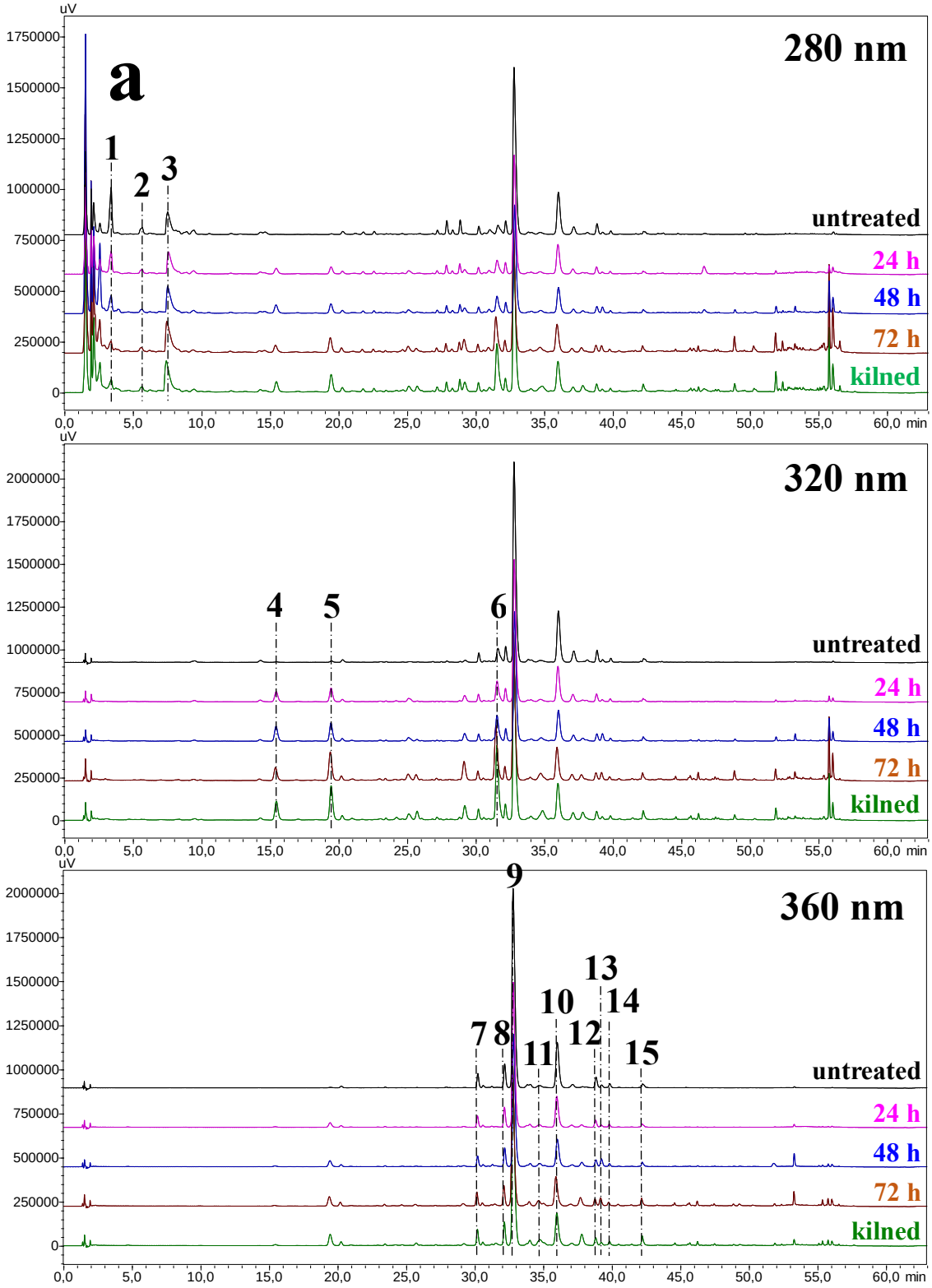
No.	Phenolic compounds (µg/g dw)	Untreated	24 h	48 h	72 h	72 h kilned
1	acacetin/questin/apigenin-7-methylether	311.5 ± 10.7^{a}	149.2 ± 9.6^{b}	136.2 ± 27.8^{b,c}	83.0 ± 13.9^{c}	82.8 ± 16.0^{c}
2	vanillic acid 4-glucoside	18.5 ± 2.3^{c}	2.6 ± 1.4^{d}	22.5 ± 4.8^{b,c}	36.4 ± 5.1^{a}	33.4 ± 0.4^{a,b}
3	unknown compound	252.7 ± 2.8^{a}	264.9 ± 11.8^{a}	401.1 ± 56.1^{b}	382.4 ± 36.8^{b}	408.6 ± 12.0^{b}
4	coumaric acid-hexoside	n.d.	330.1 ± 57.5^{a}	382.0 ± 28.2^{a}	410.3 ± 72.1^{a}	771.0 ± 25.5^{b}
5	ferulic acid 4-glucoside	n.d.	35.2 ± 0.6^{a}	45.2 ± 28.4^{a}	70.5 ± 2.7^{a}	74.2 ± 6.5^{a}
6	coumaric acid	n.d.	508.7 ± 18.8^{a}	1031.3 ± 57.7^{b}	1346.4 ± 40.5^{c}	1608.0 ± 28.4^{d}
7	quercetin-deoxyhexoside-deoxyhexoside-hexoside	65.9 ± 1.0^{a}	52.6 ± 2.8^{a}	52.4 ± 11.0^{a}	64.2 ± 6.5^{a}	67.0 ± 7.2^{a}
8	quercetin-deoxyhexoside-pentoside-hexoside	113.5 ± 3.4^{a}	92.3 ± 4.4^{a}	84.6 ± 14.5^{a}	94.5 ± 10.7^{a}	93.0 ± 14.6^{a}
9	kaempferol-deoxyhexoside-deoxyhexoside-hexoside	1055.8 ± 9.4^{a}	737.7 ± 31.4^{b}	767.7 ± 45.0^{b}	725.8 ± 53.0^{b}	773.5 ± 62.4^{b}
10	kaempferol-deoxyhexoside-pentoside-hexoside	270.1 ± 5.0^{a}	182.5 ± 11.4^{b}	167.6 ± 26.6^{b}	172.0 ± 9.2^{b}	180.2 ± 9.5^{b}
11	quercetin 3-*O*-glucuronide	n.d.	15.0 ± 1.5^{a}	26.8 ± 13.9^{a,b}	50.4 ± 11.8^{a,b}	52.1 ± 13.8^{b}
12	kaempferol-pentoside-hexoside	42.1 ± 0.8^{a}	39.0 ± 2.0^{a}	40.1 ± 3.2^{a}	38.1 ± 2.1^{a}	40.4 ± 2.4^{a}
13	kaempferol-deoxyhexoside-hexoside	9.3 ± 0.2^{a}	7.6 ± 0.7^{a}	39.1 ± 20.7^{a}	35.0 ± 4.6^{a}	25.6 ± 22.0^{a}
14	kaempferol-pentoside-glucuronide	12.9 ± 0.2^{a}	8.1 ± 0.6^{b}	10.1 ± 1.9^{a,b}	12.9 ± 2.5^{a}	11.7 ± 0.4^{a,b}
15	kaempferol 3-*O*-glucuronide	166.8 ± 4.9^{a}	155.8 ± 5.1^{a}	198.8 ± 73.8^{a}	403.8 ± 51.5^{b}	435.1 ± 44.8^{b}
	Total non-flavonoids[1]	**271.2 ± 4.3^{a} (12%)**	**1141.5 ±108.2^{b} (44%)**	**1882.1 ± 176.5^{c} (55%)**	**2245.9 ± 155.6^{d} (57%)**	**2895.5 ± 44.7^{e} (62%)**
	Total flavonoids[2]	**2047.9 ± 32.0^{a} (88%)**	**1439.8 ± 83.0^{b} (56%)**	**1522.7 ± 167.7^{b} (45%)**	**1679.7 ± 191.6^{a,b} (43%)**	**1761.4 ± 181.4^{a,b} (38%)**
	Total phenolics	**2319.1 ± 28.9^{a}**	**2575.1 ± 163.2^{a}**	**3405.0 ± 210.1^{b}**	**3925.6 ± 204.2^{b}**	**4656.9 ± 197.8^{c}**

Values are expressed as mean ± SD ($n = 3$). Different letters in the same row indicate significant difference ($p < 0.05$) among samples. n.d. means not detected. [1]Total content of non-flavonoid compounds was calculated based on the contents of compounds 2-6 (the percentage value means the proportion to total content of phenolics).
[2]Total content of flavonoids was calculated based on the contents of compounds 1 and 7-15 (the percentage value means the proportion to total content of phenolics).

Table 4
Quantity of phenolic compounds in untreated, germinated and kilned kiwicha

No	Phenolic Compounds (µg/g dw)	Untreated	24 h	48 h	72 h	72 h kilned
1’	unknown compound	41.3 ± 3.8^{a}	399.6 ± 10.8^{a}	476.1 ± 103.1^{a,b}	1264.9 ± 172.4^{c}	897.0 ± 258.2^{b,c}
2’	ferulic acid 4-glucoside	n.d.	n.d.	3.5 ± 2.6^{a}	8.3 ± 0.8^{a}	13.6 ± 5.1^{a}
3’	caffeoylquinic acid	n.d.	83.3 ± 45.5^{a}	233.1 ± 399.8^{a}	2700.1 ± 655.7^{a,b}	3313.5 ± 1481.0^{b}
4’	ferulic acid-hexoside-hexoside	n.d.	4.5 ± 1.0^{a}	8.5 ± 2.0^{a,b}	8.1 ± 2.6^{a,b}	12.2 ± 1.2^{b}
5’	***trans*-ferulic acid**	**n.d.**	**7.5 ± 1.6^{a}**	**11.7 ± 1.3^{a}**	**52.5 ± 1.3^{b}**	**30.3 ± 15.3^{a,b}**
6’	coumaroylquinic acid	n.d.	46.6 ± 10.6^{a}	57.1 ± 24.6^{a}	149.2 ± 22.8^{b}	140.1 ± 15.8^{b}
7’	feruloylquinic acid	n.d.	6.5 ± 1.8^{a}	8.2 ± 7.3^{a}	104.7 ± 12.9^{b}	82.4 ± 8.7^{b}
8’	quercetin 3-*O*-rutinoside	n.d.	n.d.	n.d.	26.2 ± 4.3^{a}	15.4 ± 2.2^{b}
	Total Non-flavonoids	**41.3 ± 3.8^{a}**	**547.9 ± 48.0^{a}**	**798.3 ± 435.5^{a}**	**4287.8 ± 985.1^{b} (99%)**	**4489.1±1608.2^{b} (99%)**
	Total Flavonoids	**n.d.**	**n.d**	**n.d**	**26.2 ± 4.3^{a} (1%)**	**15.4 ± 2.2^{b} (1%)**
	Total	**41.3 ± 3.8^{a}**	**547.9 ± 48.0^{a}**	**798.3 ± 435.5^{a}**	**4313.9 ± 806.0^{b}**	**4504.6 ± 1314.7^{b}**

Values are expressed as mean ± SD ($n = 3$). Different letters in the same row indicate significant difference ($p < 0.05$) among samples. n.d. means not detected. [1]Total content of non-flavonoid compounds was calculated based on the contents of compounds 1’-7’ (the percentage value means the proportion to total content of phenolics). [2]Total content of flavonoids was calculated based on the contents of compound 8’ (the percentage value means the proportion to total content of phenolics).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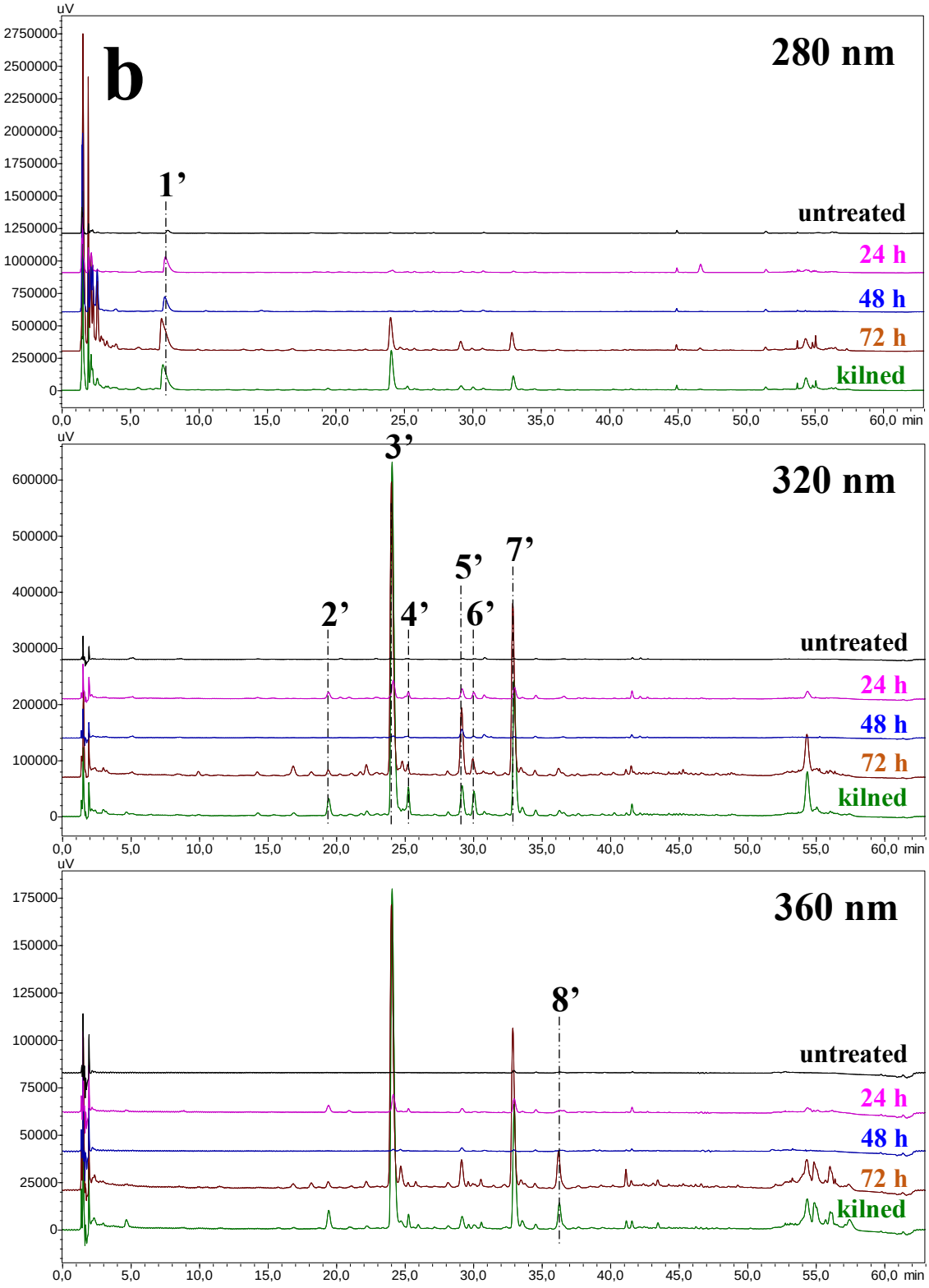
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Fig. 1.