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Simultaneous Determination of Phenolic Compounds and Saponins in Quinoa (*Chenopodium quinoa* Willd) by a Liquid Chromatography−Diode Array Detection−Electrospray Ionization−Time-of-Flight Mass Spectrometry Methodology

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ABSTRACT: A new liquid chromatography methodology coupled to a diode array detector and a time-of-flight mass spectrometer has been developed for the simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quinoa* Willd). This method has allowed the simultaneous determination of these two families of compounds with the same analytical method for the first time. A fused-core column C18 has been used, and the analysis has been performed in less than 27 min. Both chromatographic and electrospray ionization time-of-flight mass spectrometry parameters have been optimized to improve the sensitivity and to maximize the number of compounds detected. A validation of the method has also been carried out, and free and bound polar fractions of quinoa have been studied. Twenty-five compounds have been tentatively identified and quantified in the free polar fraction, while five compounds have been tentatively identified and quantified in the bound polar fraction. It is important to highlight that 1-*O*-galloyl-*β*-D-glucoside, acacetin, protocatechuic acid 4-*O*-glucoside, penstebioside, ethyl-*m*-digallate, (epi)-gallocatechin, and canthoside have been tentatively identified for the first time in quinoa. Free phenolic compounds have been found to be in the range of 2.746−3.803 g/kg of quinoa, while bound phenolic compounds were present in a concentration that varies from 0.139 and 0.164 g/kg. Indeed, saponins have been found to be in a concentration that ranged from 5.6 to 7.5% of the total composition of whole quinoa flour.

KEYWORDS: Free and bound phenolic compounds, saponins, quinoa, *Chenopodium quinoa*, HPLC-DAD-ESI-TOF-MS

INTRODUCTION

Quinoa is among the oldest cultivated plant species. It has been cultivated in the Andean region for more than 7000 years,[1] and it is one of the main grain crops supplying highly nutritious food to poor farmers in the Andes. Besides maize and potatoes, it was a staple crop in the Pre-Colombian cultures in Latin America. After the Spanish conquest, quinoa consumption and cultivation were suppressed principally due to different religious beliefs. Thereafter, its growing was only continued on a small scale. However, in Northern Europe, the interest in its cultivation has grown in the past few years.[2−4] One of the principal reasons of the renewed interest in this crop is the important nutritional value of quinoa. It contains proteins with a high level of essential amino acids, and among these, lysine is present in a high percentage.[5,6] Furthermore, it contains dietary fiber, lipids rich in unsaturated fats,[7] important amounts of micronutrients such as phosphorus, magnesium, and iron,[8] vitamin C, and bioactive compounds like saponins, phytosterols, squalene, fagopyritols, and polyphenols.[9,10] In addition, quinoa is also naturally gluten-free, and it is emerging as an alternative in gluten-free diets for patients with celiac disease.[12,13]

Chenopodium genus belongs to *Caryophyllales*. Because of that, quinoa (*Chenopodium quinoa*) is dicotyledonous, unlike cereals, which are monocotyledonous. However, quinoa is referred as a pseudocereal because its starchy seeds can be used similarly to cereal crops (they are able to be used in the elaboration of flour, bread, noodles, etc.).

The polyphenol fraction of food has been widely studied in the last years because of the healthy benefits that these compounds have proven to provide. Its consumption has been linked to the decrease of the risk of diseases associated with oxidative stress, such as cancer and cardiovascular diseases.[14] Nevertheless, only a few articles concerning the study of the individual phenolic compounds of quinoa whole flour have been carried out, and moreover, nearly none of them has performed a quantitative study.[15]

As reported in the bibliography, polyphenolic compounds from quinoa have been mostly studied by spectrophotometric methods,[16−18] and only a few research studies have been performed using high-performance liquid chromatography (HPLC)-UV−vis, HPLC−diode array detection (DAD),[11,15,19,20] and NMR.[21,22] Besides, analyses performed by HPLC were time-consuming since the analysis times were up to 1 h.

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Quinoa is especially rich in saponins. These compounds have been reported to have adverse properties for nutrition, but it has been demonstrated that they can also act as immunological and adsorption adjuvants to enhance immune responses and mucosal responses.[23]

Because of that, the aim of this work was to develop a LC-DAD-electrospray ionization (ESI)-time-of-flight (TOF)-MS method to identify and quantify the individual phenolic compounds and saponins of quinoa. The hyphenation of the DAD and TOF-MS to HPLC allowed the identification of the phenolic compounds and saponins with a high reliability. Furthermore, special attention was paid to the analysis time, and a fused-core column was used to optimize the HPLC method and to reduce the analysis time.

MATERIALS AND METHODS

Chemicals and Reagents. HPLC-grade acetonitrile and water, methanol, acetone, acetic acid, ethyl acetate, *n*-hexane, diethyl ether, NaOH, and HCl were purchased from Merck KGaA (Darmstadt, Germany). Ferulic acid, rutin, and oleanolic acid were from Sigma-Aldrich (St. Louis, MO).

Samples. Two genotypes of quinoa were used to carry out the optimization of the methodology: Kancolla and Witulla. These varieties of quinoa were grown in Perú under the same agronomic conditions. Quinoa grains were briefly milled before extraction for 3 min at 15 °C to a particle size <0.6 mm using a laboratory mill (IKA A10-IKAWERKE GmbH &Co. KG, Staufen, Germany).

Extraction of the Free Polar Fraction of Quinoa. To isolate the free polar fraction, the modified protocol of Hirose et al.[24] was used. Briefly, quinoa flour (2 g) was in an ultrasonic bath (20 min) with 30 mL of a solution of methanol/water (4:1 v/v) with 0.1% acetic acid. After centrifugation at 1000*g* for 10 min, the supernatant was removed, and the extraction was repeated once more. The supernatants were collected, evaporated, and reconstituted in 2 mL of methanol/water (1:1 v/v). The final extracts were filtered through 0.22 μm PTFE syringe filters and stored at −18 °C until the analyses.

Extraction of the Bound Phenolic Fraction of Quinoa. Once extraction with aqueous methanol was performed to discharge free phenolic compounds, bound phenols were collected by alkaline hydrolysis following the method proposed by Bonoli et al.[25] with some modifications. Two grams of whole flour was digested with 100 mL of 2 M NaOH at room temperature for 20 h by shaking under nitrogen gas. The mixture was then brought to pH 2−3 by adding 10 M hydrochloric acid in a cooling ice bath and extracted with 500 mL of hexane to remove the lipids. The final solution was extracted three times with 100 mL of 1/1 diethyl ether/ethyl acetate (v/v). The organic fractions were pooled and evaporated to dryness. The phenolic compounds were reconstituted in 2 mL of methanol/water (1:1 v/v). The final extracts were filtered through 0.22 μm PTFE syringe filters and stored at −18 °C until the analyses.

HPLC-DAD-ESI-TOF-MS Analysis. LC analyses were made with an Agilent 1200 series rapid resolution LC system (Agilent Technologies, Palo Alto, CA) equipped with a degasser, a binary pump, an autosampler, a column heater, and a diode array detector (DAD). Separation was carried out on a fused core type column Kinetex C18 (100 mm × 4.6 mm, 2.6 μm) (Phenomenex, St. Torrance, United States). A gradient elution was programmed using as a mobile phase A, acidified water (1% acetic acid), and a mobile phase B, 60% phase A and 40% acetonitrile. The program was as follows: an isocratic step, 2% phase B from 0 to 3.5 min; from 2 to 6% B, 3.5−4.5 min; from 6 to 10% B, 4.5−6 min; from 10 to 17% B, 6−7.5 min; from 17 to 36% B, 7.5−13 min; from 36 to 38.5% B, 13−14 min; from 38.5 to 60% B, 14−19 min, from 60 to 100% B, 19−24 min; 100% B, 24−30 min; and from 100 to 2% B in 2 min. The flow rate was constant at 0.8 mL/min, and the column temperature was maintained at 25 °C. The injection volume was 10 μL, and UV spectra were recorded from 200 to 600 nm, whereas the chromatograms were registered at 240, 280, and 330 nm. The effluent from the HPLC column was split using a T type phase separator before introducing it into the mass spectrometer (split ratio 1:3). Therefore, the flow that arrived to the ESI-TOF-MS detector was 0.2 mL/min.

The RP-HPLC system was coupled to a microTOF (Bruker Daltonics, Bremen, Germany), an orthogonal-accelerated TOF mass spectrometer (oaTOF-MS), equipped with an ESI interface. Parameters for analysis were set using negative ion mode with spectra acquired over a mass range from m/z 50 to 1500. The optimum values of the ESI-TOF-MS parameters were as follows: capillary voltage, +4.5 kV; drying gas temperature, 190 °C; drying gas flow, 9.0 L/min; and nebulizing gas pressure, 2 bar.

The accurate mass data of the molecular ions were processed through the software DataAnalysis 3.4 (Bruker Daltonik GmgH, Bremen, Germany), which provided a list of possible elemental formulas by using the GenerateMolecularFormula (GMF) Editor. The GenerateMolecularFormula Editor uses a CHNO algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration, and ring-plus double bonds equivalents, as well as a sophisticated comparison of the theoretical with the measured isotopic pattern (Sigma-Value) for increased confidence in the suggested molecular formula (Bruker Daltonics Technical Note 008, Molecular formula determination under automation). Even with very high mass accuracy (<1 ppm), many chemically possible formulas are obtained depending on the mass regions considered. Hence, high mass accuracy (<1 ppm) alone is not enough to exclude enough candidates with complex elemental compositions. The use of isotopic abundance patterns as a single further constraint removes >95% of false candidates. This orthogonal filter can condense several thousand candidates down to only a small number of molecular formulas. The widely accepted accuracy threshold for confirmation of elemental compositions has been established at 5 ppm.[26,27]

All of the spectra were calibrated prior to compound identification. The calibrant was a sodium formate cluster containing 5 mM sodium hydroxide and 0.2% formic acid in water:isopropanol (1:1, v/v), injected at the beginning of each run with a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL) directly connected to the interface.

RESULTS AND DISCUSSION

Optimization of the HPLC Method. The optimization of a new method for the determination of phenolic compounds and saponins in quinoa samples has been carried out. To perform this optimization, the selection of the mobile phase was done on the basis of previous experiments reported in the bibliography,[28] which show that acetonitrile presents a system back pressure much lower than other solvents such as methanol. This fact allows us to work at higher flow rates and to obtain faster analysis times.

On the basis of this fact, several trial-and-error experiments were carried out to obtain the optimum gradient of the method. Once an optimum separation of the compounds under study was obtained, the attention was focused on reducing the analysis time. For this purpose, temperature and flow rate were also optimized before arriving to the final conditions described in the section "HPLC-DAD-ESI-TOF-MS Analysis". Quinoa extracts were used to optimize the method.

The increase of the temperature allows reduction of the viscosity of the solvents used as mobile phases, which gives higher velocities of the mobile phases. Thus, the change of the temperature

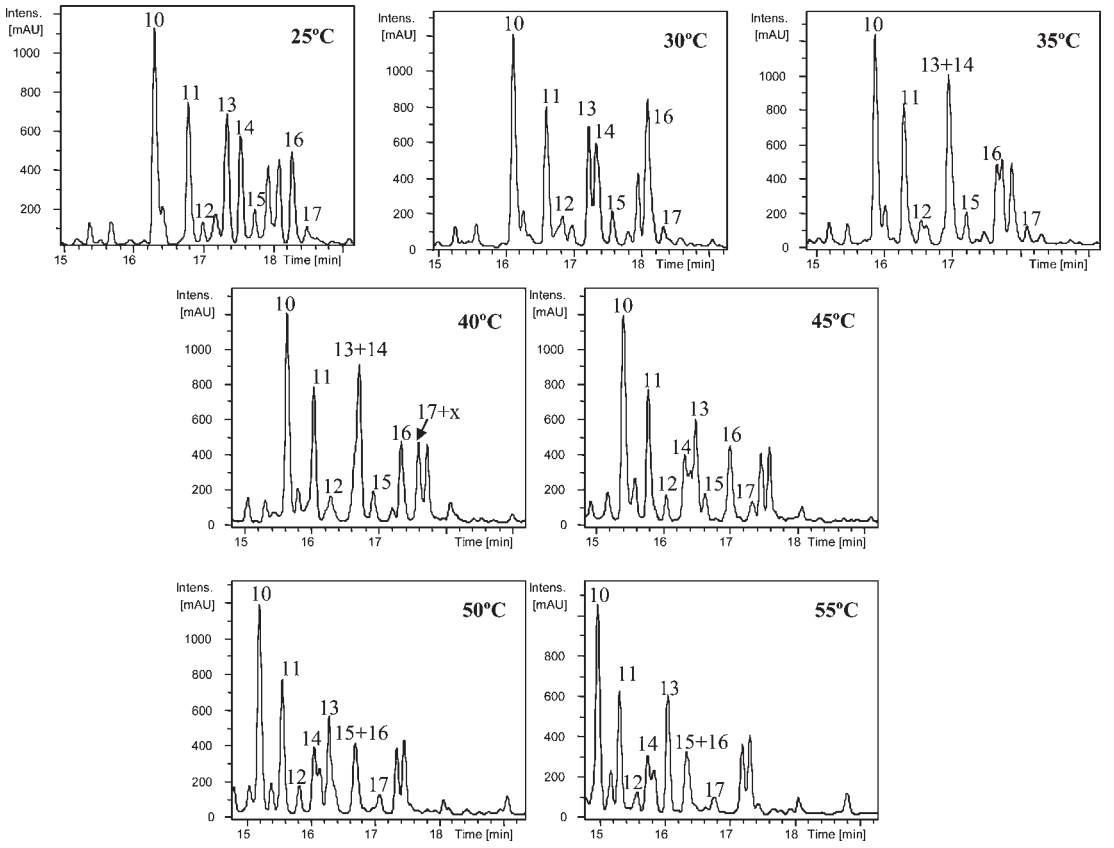

Figure 1. Effect of temperature on the separation of main phenolic compounds of quinoa (Kancolla variety). See Table 1 for peak identification numbers.

is a very useful tool to reduce analysis times. To optimize this parameter, temperatures from 25 to 55 °C in steps of 5 °C were tested. Temperatures higher than 55 °C were not checked because the column maximum operating temperature is 60 °C and compounds can decompose at high temperatures.

The change of the temperature did not cause a considerable decrease of analysis time. Phenolic acids and flavonoids showed a high variation in retention time with temperature (around 2 min from 25 to 55 °C), while saponins did not show any variation in their retention time. Moreover, the changes in retention time were different depending on the compounds, which led to an overlapping or changes in the elution order of some of them. Figure 1 shows the effect of the temperature on the separation of some of the compounds of this fraction (numbers refer to the peaks that have been identified; see the section below).

As it can be seen, at 25 °C, all of the peaks are well separated. When the temperature increases, only a small decrease in analysis time can be observed. Furthermore, the increment of temperature makes separation worse. From 30 °C, it can be observed that peak 12 overlapped with the peak that is next to it. Besides, between 35 and 40 °C, peaks 13 and 14 overlapped, and peaks 16 and 17 changed their elution order with regard to their initial position. Temperatures even higher, between 50 and 55 °C, gave cause to the overlapping of peaks 15 and 16 and to the change of the elution order of peaks 13 and 14.

Because of the no difference in the total analysis time with temperature and the overlapping produced at higher temperatures, 25 °C was chosen as the optimum temperature of analysis.

Afterward, different flow rates were tested to shorten analysis time. The flow rate was increased from 0.8 to 1.4 mL/min in 0.2 mL/min intervals. It could be observed that higher flow rates caused worse resolutions, and again, the decrease of the analysis time did not justify the choice of higher flow rates. Because of that, 0.8 mL/min was chosen as the flow rate that allowed the best compromise between resolution and analysis time.

Characterization of the Free Polar Fraction of Quinoa. The adapted method showed itself as a valuable tool for the analysis of the polar fraction in quinoa. A good peak resolution was obtained in less than 27 min, and three main families of compounds could be tentatively identified: phenolic acid derivatives, flavonoid derivatives, and saponins.

To obtain more information, a microTOF analyzer was used. Variables involved in the procedure such as capillary voltage, drying gas flow rate, drying gas temperature, and nebulizing gas pressure were optimized to improve sensitivity of TOF analysis. The optimal conditions are reported in the Materials and Methods.

Phenolic compounds were tentatively identified using the UV and MS data, while saponins were tentatively identified only using the mass spectra data because saponins have no absorption. Figure 2 shows the UV chromatogram at λ = 280 nm of the free polar fraction of a quinoa extract obtained under the optimum conditions of the method. The three families of the studied compounds have been highlighted. Furthermore, in this figure, the phenolic compounds identified in the free polar fraction can be seen.

Figure 3 presents the base peak chromatogram (BPC) and the extract ion chromatograms (EICs) of the tentatively identified compounds by TOF-MS.

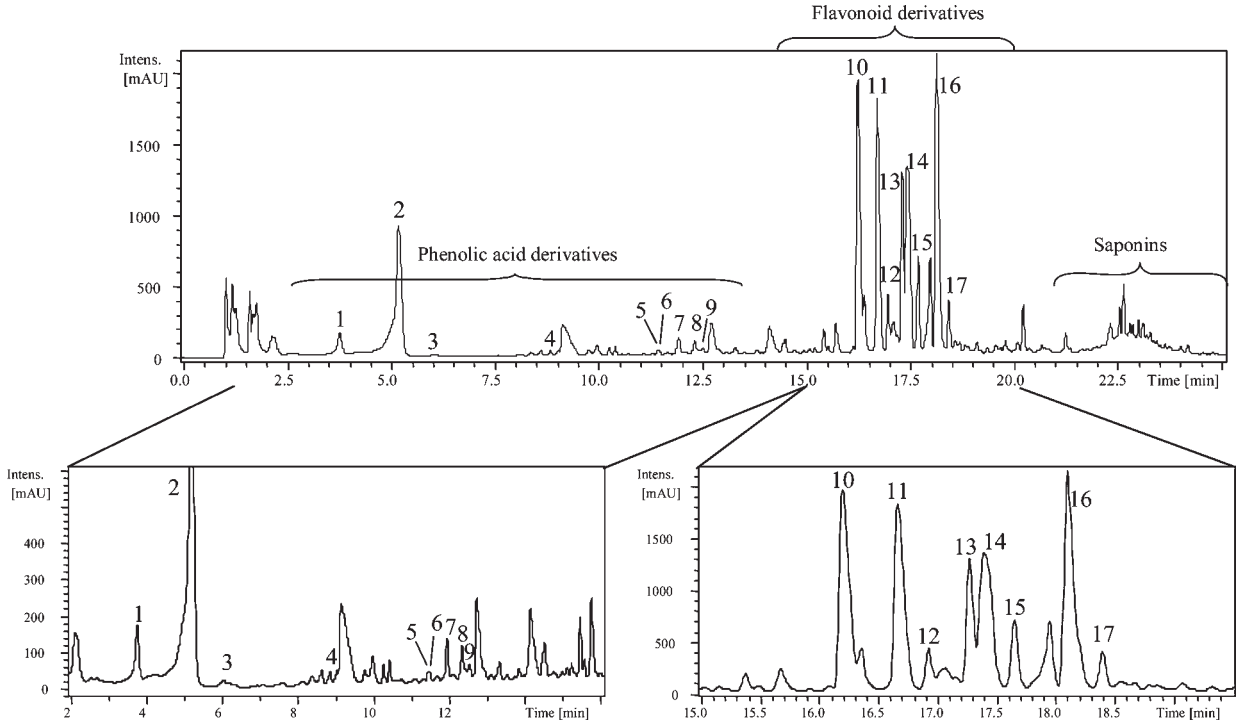

Figure 2. Chromatogram of the free polar fraction of quinoa at λ = 280 nm. See Table 1 for identification of phenolic compounds (Kancolla variety).

As it can be observed, 17 phenolic compounds could be tentatively identified by the interpretation of the information of the generated molecular formulas provided by TOF-MS analysis and studying their respective fragments.

All of the free phenolic compounds studied are summarized in Table 1. This table includes molecular formula, calculated and experimental m/z, fragments, error, σ value, tolerance (ppm) in generated molecular formula, retention time, and possible compounds.

Peak 1 at retention time 4.6 min with m/z 331.0710 was tentatively identified as 1-*O*-galloyl-β-D-glucoside. This compound, as far as we are concerned, has not been identified in quinoa previously.

Compound at retention time 6.5 min and m/z 283.0612 (peak 2) has been tentatively identified as acacetin, questin, or apigenin-7-methylether, since all of them are structural isomers.

Peak 3 migrated at time 7.1 min and presented m/z 315.0722. This compound was tentatively identified as protocatechuic acid 4-*O*-glucoside. This is the first time that this compound has been described in quinoa. However, its aglycone form had been previously reported by Alvarez-Jubete et al.[11]

Vanillic acid glucoside (peak 4), m/z 329.0878, was detected at retention time 9.1 min. A fragment with m/z 167, which corresponds to the loss of the glucose moiety, was also found. The presence of this compound in quinoa has already been reported by Dini et al.[22]

Another new compound in quinoa was tentatively identified at retention time 11.7 and m/z 605.2451 (peak 5). According to Boros and Stermitz,[29] it was tentatively identified as penstebioside.

Peak 6 was also tentatively identified for the first time in quinoa. It eluted at 11.8 min and presented an m/z 445.1350. It was identified as canthoside A as reported by Kanchanapoom et al.[30] Ferulic acid 4-*O*-glucoside (peak 7) at m/z 335.1035, and its aglycone fragment at m/z 167 was detected at 12.1 min.[19,20]

Peaks 8 and 9, at m/z 349.0565 and 305.0667, respectively, were also tentatively identified in this matrix for the first time as ethyl-*m*-digallate[31] and (epi)-gallocatechin, respectively.

As reported by other authors, different flavonoid derivatives were detected in the polar fraction of quinoa. According to Dini et al.[22] and Zhu et al.,[21] peaks 10, 11, 12, 13, and 14 have been tentatively identified (see Table 1). These compounds appeared as the most abundant in the polar fraction of quinoa.

Peak 15 at m/z 609.1461 and 17.6 min confirmed the presence of rutin. This fact agrees with information found in the bibliography by Pasko et al.[17] Peaks 16 and 17 were tentatively identified as quercetin glucuronide and quercetin 3-*O*-glucoside as previously reported by Alvarez-Jubete et al.[11] As far as we are concerned, it is the first time that compounds **1**−**3**, **5**, **6**, **8**, and **9** have been tentatively identified in quinoa samples.

Regarding saponins, Figure 4 shows the BPC of the saponin fraction and the EICs of the identified saponins in quinoa extracts. Simultaneous detection of different families of phenolic compounds and saponins could be achieved by this optimized methodology.

Twelve compounds could be tentatively identified in the saponin fraction according to the respective m/z values of their parent and product ions (see Table 1). The fragmentation pattern of these compounds and their presence in quinoa were described by Madl et al. and Kuljanabhagavad and Wink.[32,33]

Table 1 also summarizes the information obtained by Generate Molecular Formula after carrying out mass spectra of the saponin fraction of quinoa.

Thus, the proposed method is able to detect and identify 29 compounds in the same run. Furthermore, all detected compounds observed in Table 1 exhibited good σ values (less than 0.05) and mass accuracy (ppm) as indicated by the error values.

Characterization of the Bound Phenolic Fraction of Quinoa. The study of the bound phenolic fraction was performed

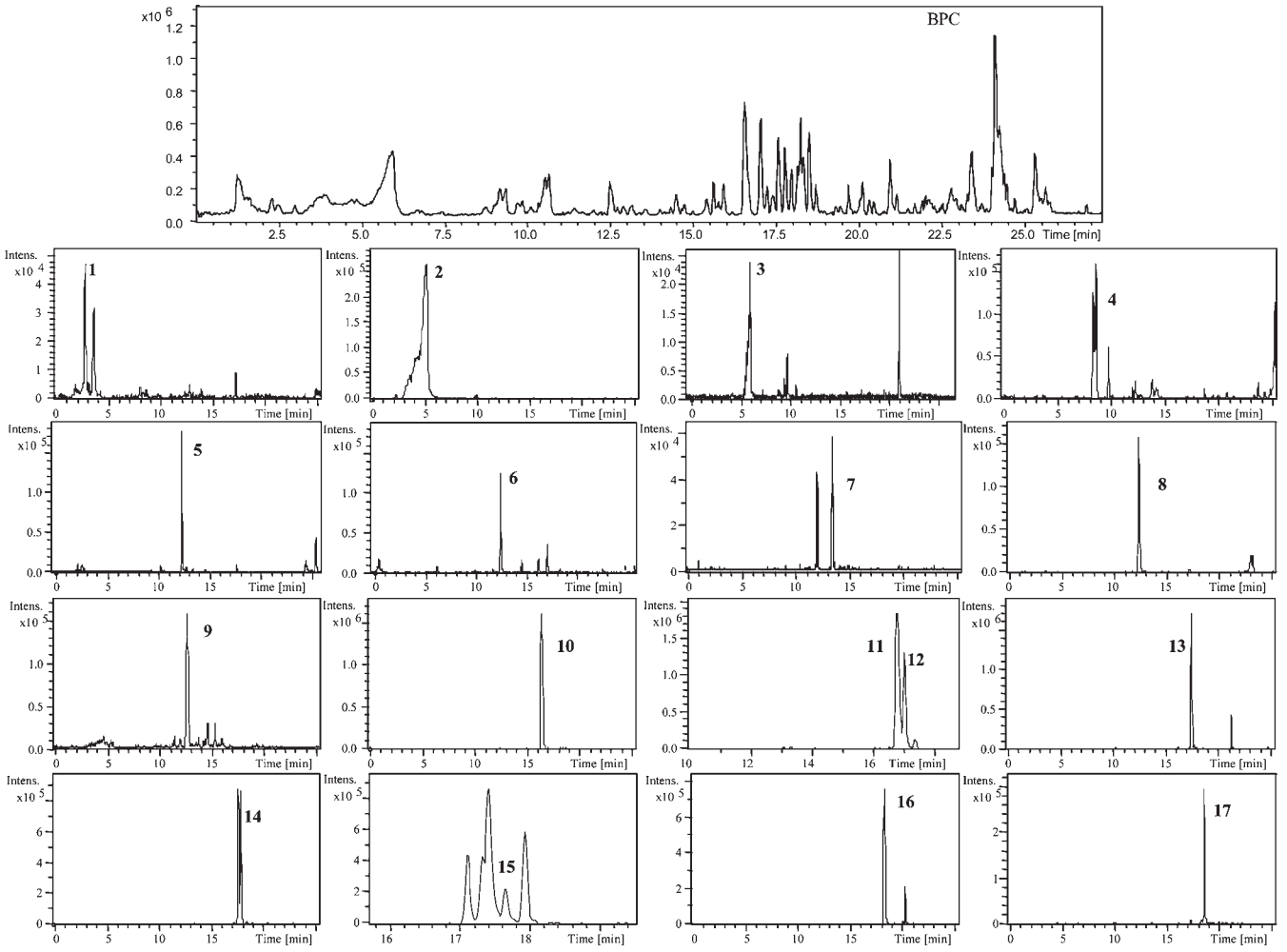

Figure 3. BPE obtained by HPLC-ESI-TOF-MS in quinoa under optimized conditions and EICs of identified free phenolic compounds. See Table 1 for identification of phenolic compounds (Kancolla variety).

Table 1. Phenolic Compounds and Saponins Tentatively Identified in Quinoa (Kancolla Variety)

			m/z						
	possible compound	retention time (min)	experimental	calculated	fragment	molecular formula	tolerance (ppm) in GMF	error (ppm)	σ
			free phenolic compounds						
1	1-*O*-galloyl-β-D-glucose	4.6	331.0697	331.0710		$C_{13}H_{16}O_{10}$	10	7.2	0.0321
2	acacetin/questin/apigenin-7-methylether	6.5	283.0664	283.0612		$C_{16}H_{12}O_5$	20	18.4	0.0156
3	protocatechuic acid 4-*O*-glucoside	7.11	315.0725	315.0722		$C_{13}H_{16}O_9$	5	1.1	0.0130
4	vanillic glucoside	9.1	329.0879	329.0878	167	$C_{14}H_{18}O_9$	5	0.2	0.0230
5	penstebioside	11.7	605.2459	605.2451		$C_{27}H_{42}O_{15}$	5	1.3	0.0183
6	canthoside A/2-hydroxybenzoate 2-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside	11.8	445.1350	445.1351	283	$C_{19}H_{26}O_{12}$	5	0.2	0.0095
7	ferulic acid 4-*O*-glucoside	12.1	355.1040	355.1035	193	$C_{16}H_{20}O_9$	5	1.4	0.0120
8	ethyl-*m*-digallate	12.3	349.0530	349.0565		$C_{16}H_{14}O_9$	15	10.2	0.0252
9	(epi)-gallocatechin	12.6	305.0643	305.0667		$C_{15}H_{13}O_7$	10	7.7	0.0301
10	quercetin 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside	15.8	755.2056	755.2040		$C_{33}H_{40}O_{20}$	5	2.0	0.0050
11	kaempferol 3-*O*-β-D-apiofuranosyl(1‴→2″-*O*-[α-L-rhamnopyranosyl (1‴→6″]-β-D-galactopyranoside	16.8	741.1934	741.1884		$C_{32}H_{38}O_{20}$	10	6.1	0.0203

Table 1. Continued

	possible compound	retention time (min)	*m/z* experimental	*m/z* calculated	fragment	molecular formula	tolerance (ppm) in GMF	error (ppm)	σ
12	kaempferol 3-*O*-β-D-apiofuranosyl(1‴→2″-*O*-[α-L-rhamnopyranosyl (1‴→6″]-β-D-galactopyranoside Isomer	17	741.1895	741.1884		$C_{32}H_{38}O_{20}$	5	1.5	0.0047
13	kaempferol 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside (mauritianin)	17.3	739.2116	739.2091	285	$C_{33}H_{40}O_{19}$	5	3.4	0.0226
14	quercetin 3-*O*-[β-D-apiofuranosyl(1‴→2″)]-β-D-galactopyranoside	17.5	595.1317	595.1305		$C_{26}H_{28}O_{16}$	5	2	0.0042
15	rutin	17.6	609.1508	609.1461		$C_{27}H_{30}O_{16}$	10	7.7	0.0150
16	quercetin-glucuronide	18.3	477.0706	477.0675	301	$C_{21}H_{18}O_{13}$	10	6.5	0.0294
17	quercetin 3-*O*-glucoside	18.5	463.0895	463.0882	301	$C_{21}H_{20}O_{12}$	5	2.9	0.0131
			saponins						
A	3-*O*-β-D-glucopyranosyl- (1→3)-α-L-arabinopyranosyl-3,23, 30-trihydroxyolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester	21.0	943.4959	943.4908	781	$C_{47}H_{76}O_{19}$	5	5.4	0.0289
B	3-*O*-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester	22.6	1001.4999	1001.4963	839	$C_{49}H_{78}O_{21}$	5	3.6	0.0269
C	3-*O*-hexose-pentose-pentose phytolaccagenic acid 28-*O*-hexose	22.8	1103.5217	1103.5279	941/779	$C_{53}H_{84}O_{24}$	10	6.2	0.0156
D	3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester	23.4	1133.5361	1133.5385	971/809	$C_{54}H_{86}O_{25}$	5	2.4	0.0130
E	3-*O*-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester	23.5	809.4343	809.4329	515	$C_{42}H_{66}O_{15}$	5	1.7	0.0131
F	3-*O*-β-D-glucopyranosyl-(1→3)-α-larabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester	23.6	971.4877	971.4857	809/515	$C_{48}H_{76}O_{20}$	5	2.0	0.0230
G	3-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranosyl serjanic acid 28-*O*-β-D-glucopyranosyl ester	24.3	969.4792	969.4701	-	$C_{48}H_{74}O_{20}$	10	9.9	0.0183
H	3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucuronopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester	24.7	941.4796	941.4752	779	$C_{47}H_{74}O_{19}$	5	4.8	0.0234
I	3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester	25.0	927.4951	927.4958	765	$C_{47}H_{76}O_{18}$	5	0.7	0.0120
J	3-*O*-β-D-glucopyranosyl-(1→3)-α-L-arabinopyranosyl serjanic acid 28-*O*-β-D-glucopyranosyl ester	25.4	955.4934	955.4908	793/499	$C_{48}H_{76}O_{19}$	5	2.8	0.0095
K	3-*O*-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester	26.1	793.4317	793.4379	-	$C_{42}H_{66}O_{14}$	10	6.2	0.0252
L	3-*O*-β-D-xylopyranosyl-(1→3)-β-D-glucuronopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester	26.2	925.4798	925.4802	631	$C_{47}H_{74}O_{18}$	5	0.5	0.0179
			bound phenolic compounds						
1	benzoic acid	12.5	121.0299	121.0295		$C_7H_6O_2$	5	2.9	0.0545
2	vanillic	12.8	167.0353	167.0350		$C_8H_8O_4$	5	2.0	0.0113
3	vanillin	14.8	151.0407	151.0401		$C_8H_8O_3$	5	4.0	0.0514
4	*p*-coumaric acid	15.7	163.0399	163.0401		$C_9H_8O_3$	5	0.9	0.0576
5	ferulic acid	17.4	193.0506	193.0506		$C_{10}H_{10}O_4$	5	0.2	0.0155

after an alkaline hydrolysis. Table 1 summarizes the information obtained by Generate Molecular Formula after carrying out mass spectra of the bound phenolic compounds of quinoa. Figure 5 illustrates the chromatogram at λ = 280 nm of the bound phenolic fraction of quinoa and the EICs of the identified compounds.

Five phenolic compounds, principally phenolic acids, have been detected in this fraction: peak 1 at 12.5 min and *m/z* 121.0299 was tentatively identified as benzoic acid. Peaks 2, 3, 4, and 5 at *m/z* 167.0353, 151.0407, 163.0399, and 193.0506, respectively, were tentatively identified according to the bibliography[19,20] as vanillic acid, vanillin, *p*-coumaric acid, and ferulic acid, respectively.

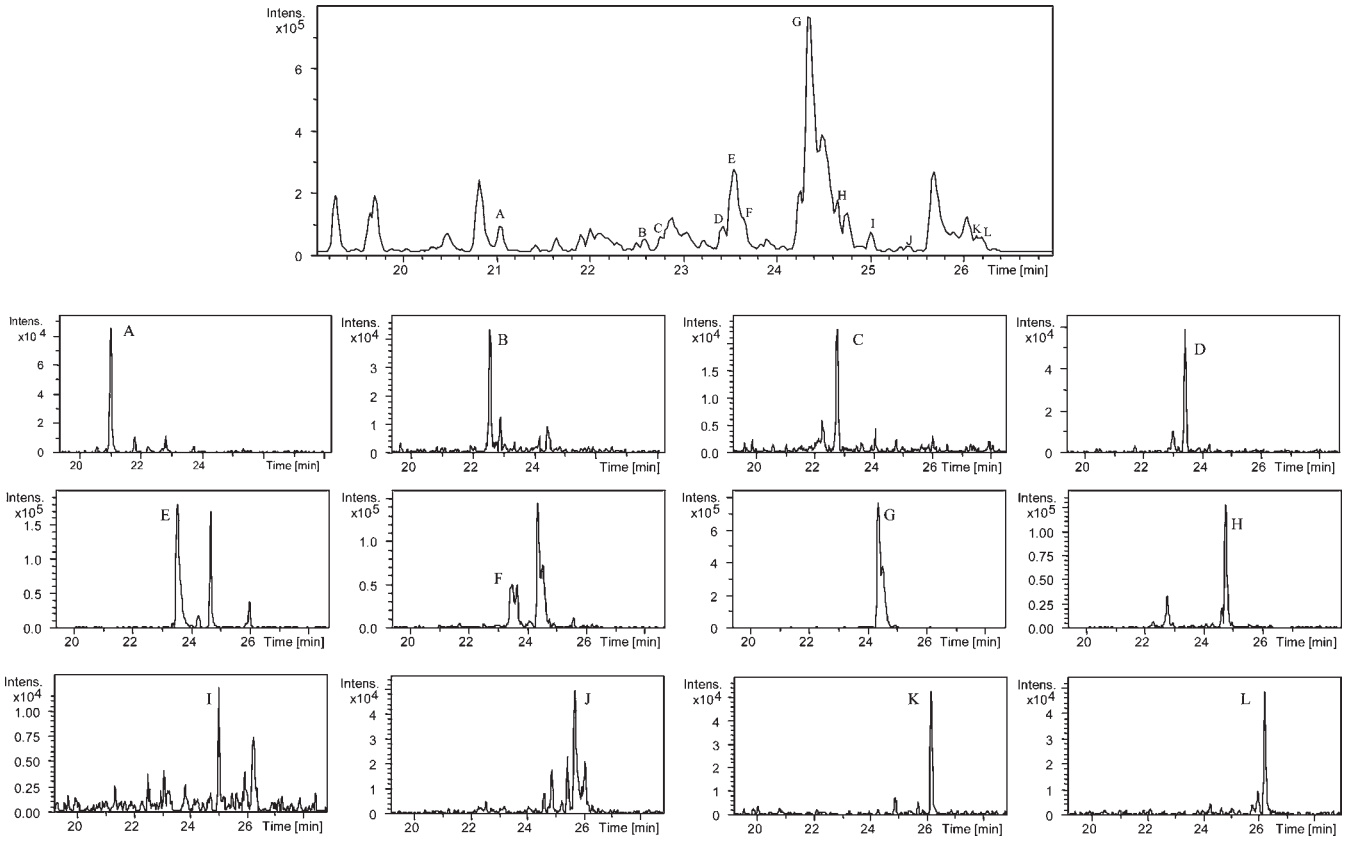

Figure 4. BPC of the saponin fraction and the EICs of the identified saponins in quinoa extracts. See Table 1 for peak identification letters (Kancolla variety).

As it can be seen, the principal component of the bound phenolic fraction of quinoa is ferulic acid. This finding is in agreement with literature where it is described that quinoa excels among dicotyledons because its high amounts of ferulic acid set out in its cell walls.[20,34]

Validation of the Method. Validation experiments were carried out to obtain the analytical parameters of the method. Three calibration curves were prepared using the following standards: rutin, ferulic acid, and oleanolic acid in the range of concentrations from the limit of quantification (LOQ) to 500, 1000, and 1000 μg/mL, respectively. Seven calibration points were used for each of them, and the analyses were replicated five times for each calibration point (n = 5). The different parameters of each one have been summarized in Table 2: sensitivity [relative standard deviations (RSDs) (%)], linearity (r^2), calibration range, correlation coefficient, and matrix effect.

All calibration curves showed good linearity between different concentrations depending on the analytes studied. The calibration plots indicate good correlation between peak areas and analyte concentrations, and regression coefficients were higher than 0.990 in all cases. The LOQ was determined as the signal-to-noise ratio of 10:1, and the limit of detection (LOD) was determined as signal-to-noise ratio of 3:1. The LOD was found to be within the range 0.053−0.243 μg/mL, while the LOQ was within 0.175−0.609 μg/mL.

Intraday and interday precisions were developed to evaluate the repeatability of HPLC-ESI-TOF-MS method. A methanol−water extract was injected (n = 6) on the same day (intraday precision) for 3 consecutive days (interday precision, n = 18). The RSDs of analysis time and peak area were determined.

The intraday repeatability (expressed as % RSDs) of the retention times was from 0.14 to 2.57%, whereas the interday repeatability was from 1.05 to 2.09%. The intraday repeatability (expressed as % RSDs) of the total peak area was 0.39%, whereas the interday repeatability was 1.24%.

The matrix effect was evaluated by comparing peak areas of all analytes in spike-after preparation samples with the corresponding peak areas in neat solution. Two different concentrations were evaluated by analyzing five samples at each level. As shown in Table 2, no obvious matrix effects were found for all of the analytes as the results ranged from 99.1 to 103.3%, which was within the acceptable limit.

Quantification of Free and Bound Polar Compounds in Quinoa. The quantification was performed by using the previous calibration curves. The calibration curve of rutin at λ = 280 nm was used to quantify flavonoid derivatives such as acacetin, penstebioside, gallocatechin, (epi)-gallocatechin, quercetin 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside, kaempferol 3-*O*-β-D-apiofuranosyl-(1‴→2″-*O*-[α-L-rhamnopyranosyl-(1‴→6″]-β-D-galactopyranoside, kaempferol 3-*O*-β-D-apiofuranosyl-(1‴→2″-*O*-[α-L-rhamnopyranosyl(1‴→6″]-β-D-galactopyranoside, kaempferol 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside (mauritianin), quercetin 3-*O*-[β-D-apiofuranosyl(1‴→2″)]-β-D-galactopyranoside, rutin, quercetin-glucuronide, and quercetin 3-*O*-glucoside.

Phenolic acid derivatives were quantified using the calibration curve of ferulic acid at λ = 280 nm (1-*O*-galloyl-β-D-glucose,

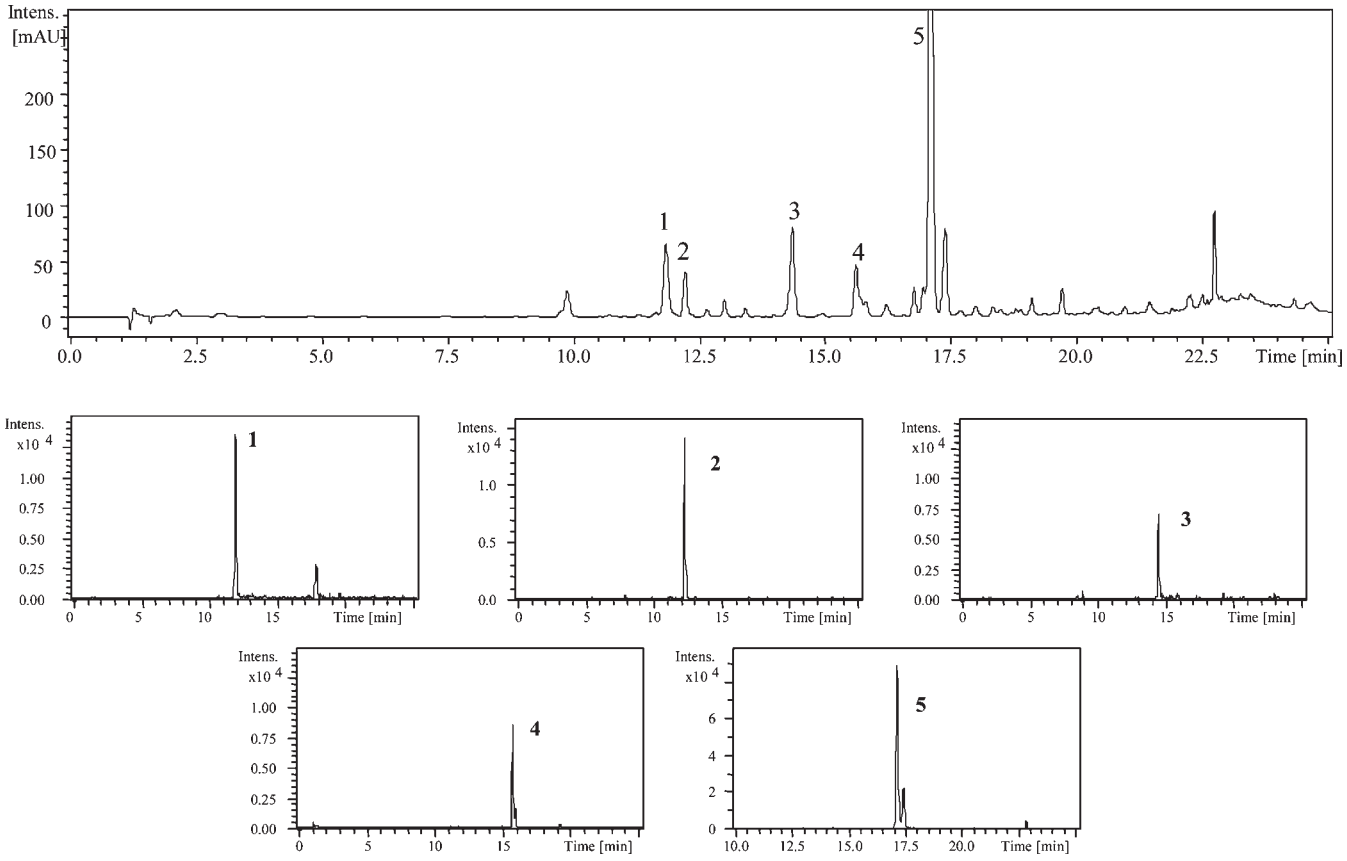

Figure 5. Chromatogram at λ = 280 nm of the bound phenolic fraction of quinoa and the EICs of the identified compounds. See Table 1 for identification of phenolic compounds (Kancolla variety).

Table 2. Analytical Parameters of the Method Proposed

		μg/mL					
analyte	RSD (%)	LOD	LOQ	calibration range (μg/mL)	calibration equations	r^2	matrix effect mean ± SD (%)
ferulic acid	0.23	0.053	0.175	LOQ−500	$y = 38476.0x - 352.9$	0.9965	99.1 ± 2.6
rutin	0.31	0.204	0.609	LOQ−1000	$y = 6402.4x - 10.4$	0.9991	103.3 ± 4.7
oleanolic acid	0.95	0.243	0.577	LOQ−1000	$y = 143.9x + 32078.0$	0.9967	101.7 ± 3.9

Table 3. Results Expressed in g Analyte/kg of Quinoa (n = 7, Value = X ± SD)

		Witulla	Kancolla
	free phenolic compounds		
1	1-*O*-galloyl-β-D-glucose[b]	0.054 ± 0.002	0.021 ± 0.001
2	acacetin/questin/apigenin-7-methylether[a]	1.064 ± 0.037	0.573 ± 0.075
3	protocatechuic acid 4-*O*-glucoside[b]	0.014 ± 0.001	0.013 ± 0.001
4	vanillic glucoside[b]	0.017 ± 0.001	0.022 ± 0.002
5	penstebioside[a]	0.011 ± 0.002	0.014 ± 0.001
6	canthoside A/2-hydroxybenzoate 2-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside[a]	0.013 ± 0.001	0.013 ± 0.001
7	ferulic acid 4-*O*-glucoside[b]	0.029 ± 0.002	0.023 ± 0.001
8	ethyl-*m*-digallate[b]	0.023 ± 0.002	0.014 ± 0.001
9	(epi)-gallocatechin[a]	0.016 ± 0.001	0.120 ± 0.001
10	quercetin 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside[a]	0.614 ± 0.031	0.489 ± 0.031
11	kaempferol 3-*O*-β-D-apiofuranosyl(1‴→2″-*O*-[α-L-rhamnopyranosyl (1‴→6″]-β-D-galactopyranoside[a]	0.341 ± 0.026	0.287 ± 0.022

Table 3. Continued

		Witulla	Kancolla
12	kaempferol 3-*O*-*β*-D-apiofuranosyl(1‴→2″-*O*-[*α*-L-rhamnopyranosyl (1‴→6″]-*β*-D-galactopyranoside isomer[a]	0.133 ± 0.008	0.199 ± 0.011
13	kaempferol 3-*O*-(2,6-di-*α*-L-rhamnopyranosyl)-*β*-D-galactopyranoside (mauritianin)[a]	0.486 ± 0.029	0.375 ± 0.036
14	quercetin 3-*O*-[*β*-D-apiofuranosyl(1‴→2")]-*β*-D-galactopyranoside[a]	0.411 ± 0.029	0.120 ± 0.008
15	rutin[a]	0.155 ± 0.007	0.085 ± 0.007
16	quercetin-glucuronide[a]	0.364 ± 0.018	0.306 ± 0.016
17	quercetin 3-*O*-glucoside[a]	0.057 ± 0.006	0.072 ± 0.003
	total	3.803 ± 0.291	2.746 ± 0.181
	saponins[c]		
A	3-*O*-*β*-D-glucopyranosyl- (1→3)-*α*-L-arabinopyranosyl-3,23,30-trihydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester	2.203 ± 0.132	1.956 ± 0.078
B	3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester	0.581 ± 0.097	0.933 ± 0.058
C	3-*O*-hexose-pentose-pentose phytolaccagenic acid 28-*O*-hexose	2.515 ± 0.125	0.945 ± 0.062
D	3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester	1.456 ± 0.099	1.382 ± 0.108
E	3-*O*-*α*-L-Arabinopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester	6.429 ± 0.006	7.101 ± 0.236
F	3-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-larabinopyranosyl phytolaccagenic acid 28-*O*-*β*-D-glucopyranosyl ester	1.789 ± 0.177	1.306 ± 0.122
G	3-*O*-*α*-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosyl serjanic acid 28-*O*-*β*-D-glucopyranosyl ester	39.621 ± 1.259	35.940 ± 0.409
H	3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucuronopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester	9.066 ± 0.018	3.276 ± 0.004
I	3-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester	2.245 ± 0.018	1.171 ± 0.012
J	3-*O*-*β*-D-glucopyranosyl-(1→3)-*α*-L-arabinopyranosyl serjanic acid 28-*O*-*β*-D-glucopyranosyl ester	1.044 ± 0.013	0.490 ± 0.003
K	3-*O*-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester	1.926 ± 0.036	1.194 ± 0.018
L	3-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucuronopyranosyl oleanolic acid 28-*O*-*β*-D-glucopyranosyl ester	6.969 ± 0.043	1.225 ± 0.051
	total	75.844 ± 0.720	56.918 ± 0.713
	bound phenolic compounds[d]		
1	benzoic acid	0.019 ± 0.001	0.021 ± 0.002
2	vanillic	0.014 ± 0.001	0.015 ± 0.001
3	vanillin	0.016 ± 0.001	0.022 ± 0.001
4	*p*-coumaric acid	0.014 ± 0.001	0.017 ± 0.001
5	ferulic acid	0.076 ± 0.006	0.089 ± 0.001
	total	0.139 ± 0.007	0.164 ± 0.003

[a] Quantified with the calibration curve of rutin. [b] Quantified with the calibration curve of ferulic acid. [c] Saponins were quantified with the calibration curve of oleanolic acid. [d] Bound phenolic compounds were quantified with the calibration curve of ferulic acid.

protocatechuic acid 4-*O*-glucoside, vanillic glucoside, ferulic acid 4-*O*-glucoside, ethyl-*m*-digallate, benzoic acid, vanillic, vanillin, *p*-coumaric acid, and ferulic acid).

Saponins were quantified using the calibration curve of oleanolic acid. The methanolic extracts of Kancolla and Witulla quinoa varieties were analyzed by the optimized HPLC-DAD-ESI-TOF-MS method, and the quantitative results are presented in Table 3.

It has to be taken into account that the response of the standards can be different from the response of the analytes present in the quinoa sample, and consequently, the quantification of these compounds is only an estimation of their actual concentrations.

Free phenolic compounds have been found to be in the range of 2.746−3.803 g/kg of quinoa, while bound phenolic compounds are present in a concentration that varies from 0.139 g/kg in Witulla variety and 0.164 g/kg in Kancolla variety. As it can be observed, most phenolic compounds in quinoa are in the free form.

The most abundant phenolic compounds are the flavonoid derivatives (peaks 10, 11, 12, 13, and 14) corresponding to 41.41% of the total free phenolic fraction for Witulla variety and 53.54% for Kancolla variety. In the bound phenolic fraction, the phenolic compound present in the highest concentration in quinoa is the ferulic acid, 0.076 g/kg in Witulla variety and 0.089 g/kg in Kancolla variety.

Saponins have been found to be in a concentration that ranges from 5.6 to 7.5% of the total composition of whole quinoa flour in the varieties of quinoa studied. Peak G, 3-*O*-α-L-arabinopyranosyl-(1→3)-β-D-glucuronopyranosyl serjanic acid 28-*O*-β-D-glucopyranosyl ester, is the saponin present in the highest concentration (39.621 g/kg in Witulla variety and 35.940 g/kg in Kancolla variety) followed by peak E, 3-*O*-α-L-arabinopyranosyl phytolaccagenic acid 28-*O*-β-D-glucopyranosyl ester, found in the concentration of 6.429 g/kg in Witulla variety and 7.101 g/kg in Kancolla variety.

Phenolic compounds and saponins have been found in concentrations slightly higher than those reported in literature. The phenolic content and also saponins vary depending on the cultivar, agronomic conditions, climatic conditions, etc. Because of that, to compare the content of these compounds, it is not easy. Moreover, this is the first time that phenolic compounds of quinoa have been identified by HPLC-DAD-ESI-TOF-MS, and also, new compounds have been tentatively identified. This fact can contribute to obtaining higher concentrations of phenolics. Besides, most papers use Folin−Ciocalteu for quantification, and it is important to highlight that results obtained with a separative technique and those obtained with a spectrometric technique can be different (Folin−Ciocalteu can suffer interferences due to the presence of proteins or other substances).

In this work, a powerful HPLC method coupled to a TOF-MS has been used to study the polar fraction of quinoa for the first time. The characterization of the extracts of quinoa has been carried out allowing the simultaneous determination of phenolic compounds and saponins in a reduce analysis time (less than 27 min). This methodology made possible the identification and quantification of 29 compounds in the free polar fraction of quinoa and five compounds in the bound polar fraction.

Thus, the hyphenation of HPLC to MS, which combines the advantages of a fused-core C18 column, represents a valuable tool and a good alternative for simultaneous and faster characterization of phenolic compounds and saponins in quinoa. It is also important to highlight that, to our knowledge, this methodology provides shorter analysis times and allows the identification of seven new compounds in quinoa.

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REFERENCES

(1) Pearsall, D. The origins of plant cultivation in south america. In *The Origins of Agriculture. An International Perspective*; Cowan, C. W., Watson, P. J., Eds.; Smithsonian Institution Press: Washington, London, 1992; pp 173−205.

(2) Jacobsen, S. E.; Jorgensen, I.; Stolen, O. Cultivation of quinoa (*Chenopodium quinoa*) under temperate climatic conditions in Denmark. *J. Agric. Sci.* **1994**, *122*, 47–52.

(3) Carmen, M. L. Acclimatization of quinoa (*Chenopodium quinoa*, Willd) and canihua (*Chenopodium pallidicaule*, Aellen) to Finland. *Ann. Agric. Fennae* **1984**, *23*, 135–144.

(4) Walker, K. C.; Entwistle, G. E.; Taylor, B. R.; Booth, E. J.; Batchelor, S. E.; Mitchell, P.; Robertson, J. S.; Walters, M. Rationalisation of new crop options in Scotland. *Aspects Appl. Biol.* **1994**, *40*, 151–157.

(5) Repo-Carrasco, R.; Espinoza, C.; Jacobsen, S.-E. Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*). *Food Rev. Int.* **2003**, *19*, 179–189.

(6) Ng, S.; Anderson, A.; Coker, J.; Ondrus, M. Characterization of lipid oxidation products in quinoa (*Chenopodium quinoa*). *Food Chem.* **2007**, *101*, 185–192.

(7) Alvarez-Jubete, L.; Arendt, E. K.; Gallagher, E. Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. *Int. J. Food Sci. Nutr.* **2009**, *60*, 240–257.

(8) Ogungbenle, H. N. Nutritional evaluation and functional properties of quinoa (*Chenopodium quinoa*) flour. *Int. J. Food Sci. Nutr.* **2003**, *54*, 153–158.

(9) Berghofer, E.; Schoenlechner, R. Grain amaranth. In *Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential*; Belton, P. S., Taylor, J. R. N., Eds.; Springer-Verlag: Berlin, Germany, 2002; pp 219−260.

(10) Taylor, J. R. N.; Parker, M. L. Quinoa. In *Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential*; Belton, P. S., Taylor, J. R. N., Eds.; Springer-Verlag: Berlin, Germany, 2002; pp 93−122.

(11) Alvarez-Jubete, L.; Wijngaard, H.; Arendt, E. K.; Gallagher, E. Polyphenol composition and in vitro antioxidant activity of amaranth, quinoa buckwheat and wheat as affected by sprouting and baking. *Food Chem.* **2010**, *119*, 770–778.

(12) Kupper, C. Dietary guidelines and implementation for celiac disease. *Gastroenterology* **2005**, *128*, S121–S127.

(13) Alvarez-Jubete, L.; Arendt, E. K.; Gallagher, E. Nutritive value of pseudocereals and their increasing use as functional glutenfree ingredients. *Trends Food Sci. Technol.* **2010**, *21*, 106–113.

(14) Scalbert, A.; Manach, C.; Morand, C.; Remesy, C.; Jimenez, L. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 287–306.

(15) Repo-Carrasco-Valencia, R.; Hellström, J. K.; Pihlava, J.-M.; Mattila, P. H. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*). *Food Chem.* **2010**, *120*, 128–133.

(16) Nsimba, R. Y.; Kikuzaki, H.; Konishi, Y. Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus spp*. seeds. *Food Chem.* **2008**, *106*, 760–766.

(17) Pasko, P.; Barton, H.; Zagrodzki, P.; Gorinstein, S.; Fołta, M.; Zachwieja, Z. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chem.* **2009**, *115*, 994–998.

(18) Gorinstein, S.; Medina Vargas, O. J.; Jaramillo, N. O.; Arnao Salas, I.; Martinez Ayala, A. L.; Arancibia-Avila, P.; Toledo, F.; Katrich, E.; Trakhtenberg, S. The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. *Eur. Food Res. Technol.* **2007**, *225*, 321–328.

(19) Paśko, P.; Sajewicz, M.; Gorinstein, S.; Zachwieja, Z. Analysis of selected phenolic acids and flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts by HPLC. *Acta Chromatogr.* **2008**, *20*, 661–672.

(20) Renard, C. M. G. C.; Wende, G.; Booth, E. J. Cell wall phenolics and polysaccharides in different tissues of quinoa (*Chenopodium quinoa* Willd). *J. Sci. Food Agric.* **1999**, *79*, 2029–2034.

(21) Zhu, N.; Sheng, S.; Li, D.; Lavoie, E. J.; Karwe, M. V.; Rosen, R. T.; Ho, C.-T. Antioxidative flavonoid glycosides from quinoa seeds (*Chenopodium quinoa* Willd). *J. Food Lipids* **2001**, *8*, 37–44.

(22) Dini, I.; Tenore, G. C.; Dini, A. Phenolic constituents of Kancolla seeds. *Food Chem.* **2004**, *84*, 163–168.

(23) Estrada, A.; Li, B.; Laarveld, B. Adjuvant action of *Chenopodium quinoa* saponins on the induction of antibody responses to intragastric and intranasal administered antigens in mice. *Comp. Immunol. Microbiol. Infect. Dis.* **1998**, *21*, 225–236.

(24) Hirose, Y.; Fujita, T.; Ishii, T.; Ueno, N. Antioxidative properties and flavonoid composition of *Chenopodium quinoa* seeds cultivated in Japan. *Food Chem.* **2010**, *119*, 1300–1306.

(25) Bonoli, M.; Marconi, E.; Caboni, M. F. Free and bound phenolic compounds in barley (*Hordeum vulgare* L.) flours. Evaluation of the extraction capability of different solvent mixtures and pressurized liquid methods by micellar electrokinetic chromatography and spectrophotometry. *J. Chromatogr. A* **2004**, *1057*, 1–12.

(26) Cavaliere, C.; Foglia, P.; Gubbiotti, R.; Sacchetti, P.; Saperi, R.; Laganá, A. Rapid-resolution liquid chromatography/mass spectrometry for determination and quantitation of polyphenols in grape berries. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3089–3099.

(27) Ferrer, I.; García-Reyes, J. F.; Mezcua, M.; Thurman, E. M.; Fernández-Alba, A. R. Multi-residue pesticide analysis in fruits and vegetables by liquid chromatographyetime- of-flight mass spectrometry. *J. Chromatogr. A* **2005**, *1082*, 81–90.

(28) Manchón, N.; D'Arrigo, M.; García-Lafuente, A.; Guillamón, E.; Villares, A.; Ramos, A.; Martínez, J. A.; Ristagno, M. A. Fast analysis of isoflavones by high-performance liquid chromatography using a column packed with fused-core particles. *Talanta* **2010**, *82*, 1986–1994.

(29) Boros, C.; Stermitz, F. Iridoids. An updated review, part II. *J. Nat. Prod.* **1991**, *54*, 1173–1246.

(30) Kanchanapooma, T.; Kasaib, R.; Yamasaki, K. Iridoid and phenolic diglycosides from *Canthium berberidifolium*. *Phytochemistry* **2002**, *61*, 461–464.

(31) Abou-Zaid, M. M.; Helson, B. V.; Nozzolillo, C.; Arnason, J. T. Ethyl m-digallate from red maple, *Acer rubrum* L., as the major resistance factor to forest tent caterpillar, Malacosoma disstria Hbn. *J. Chem. Ecol.* **2001**, *27*, 2517–2527.

(32) Madl, T.; Sterk, H.; Mittelbach, M. Tandem mass spectrometric analysis of a complex triterpene saponin mixture of *Chenopodium quinoa*. *J. Am. Soc. Mass Spectrom.* **2006**, *17*, 795–806.

(33) Kuljanabhagavad, T.; Wink, M. Biological activities and chemistry of saponins *from Chenopodium quinoa* Willd. *Phytochem. Rev.* **2009**, *8*, 473–490.

(34) Ishii, T. Structure and function of feruloylated polysaccharides. *Plant Sci.* **1997**, *127*, 111–127.