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Antioxidative properties and flavonoid composition of *Chenopodium quinoa* seeds cultivated in Japan

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ABSTRACT

To evaluate the nutritional advantages of quinoa seeds (*Chenopodium quinoa* Willd.) cultivated in Japan, antioxidative properties and flavonoid composition were determined and compared to corresponding data for conventionally-used cereals and pseudo-cereals, including quinoa seeds from South America. The antioxidant activities of these grains against DPPH radicals were strongly associated with the total phenolic content of the tested samples. The crude extracts of quinoa seeds cultivated in Japan exhibited higher antioxidative effects than those from South America and other cereals, excluding buckwheat. Four flavonol glycosides were isolated and identified from the Japanese quinoa seeds, and the chemical composition of the flavonoids – quercetin and kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnopyranosyl)-β-galactopyranosides (**1** and **4**), quercetin 3-*O*-(2″,6″-di-*O*-α-rhamnopyranosyl)-β-glucopyranoside (**2**), and quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl)-β-galactopyranoside (**3**) – was evaluated through quantitative determination. Trioside **2** was isolated for the first time from quinoa seeds. These glycosides were not detected in extracts from any of the tested grains except quinoa. The aglycone quercetin content of the Japanese quinoa seeds is higher than in the seeds from South America and buckwheat. The amounts of quercetin and kaempferol formed via acidic hydrolysis in quinoa are much higher than those of conventionally-used edible plants. The quinoa seeds cultivated in Japan are the most effective functional foodstuff – in terms of being a source of antioxidative and bioactive flavonoids – among cereals and pseudo-cereals.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a grain crop of Andean origin and the family *Chenopodiacea*; it is a pseudo-cereal used principally in the same manner as wheat and rice. Until recently, its cultivation was restricted to subsistence farming in some regions of South America (Bhargava, Shukla, & Ohri, 2006). Although a lesser-known plant, there has been increasing interest in quinoa due to its perceived superior nutritional quality compared to other grains. Quinoa seeds contain carbohydrates (77.6%), protein (12.9%), a balanced amino acid spectrum of high lysine and methionine contents, lipids (6.5%), and is rich in dietary fibre (Ando et al., 2002). Quinoa seeds are also rich in mineral nutrients (3.0%), and the K, Ca, Mg, P, and Fe contents are much higher than those of conventional cereals (Konishi, Hirano, Tsuboi, & Wada, 2004). In the last few decades, quinoa has been evaluated as a food with excellent nutritional characteristics by the National Research Council and the National Aeronautics and Space Administration (NASA) (Schlick & Bubenheim, 1993) and has been noted as a new foodstuff in the world. There has been growing interest in a number of countries, especially in Europe, in initiating introduction and research work. Nevertheless, little cultivation has occurred in Japan.

Beyond their basic nutritional function of supplying nutrients, foods also have health-promoting and/or disease-preventing properties. Polyphenols have beneficial effects on health and are ubiquitous in plant foods. Recent studies have identified flavonoid conjugates in quinoa seeds harvested in South America: kaempferol and quercetin oligomeric glycosides (Dini, Tenore, & Dini, 2004; Zhu et al., 2001). Flavonoids, one of the typical polyphenols in vegetable, fruits, and tea, can prevent degenerative diseases such as coronary heart disease (Arts & Hollman, 2005), atherosclerosis (Kurosawa et al., 2005), cancers (Rice-Evans & Packer, 1998), diabetes, and Alzheimer's disease (Youdim, Shukitt-Hale, & Joseph, 2004), through antioxidative action and/or the modulation of several protein functions. It is important to determine the amount and composition of flavonoids in edible parts of vegetables, fruits, and teas. Murota and Terao (2003) reported that flavonoid bioactivity can be attributed to aglycone structures, not sugar moieties.

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Therefore, qualitative and quantitative analyses of aglycone moieties from flavonoid glycosides provide important information on their bioavailability. No paper has explained the quantitative relationships between these compounds in quinoa seeds.

In Japan, the traditional Japanese diet has recently given way to European and American styles; this has led to an increase in lifestyle diseases such as cancer, coronary heart disease, and diabetes. In response, there has been a stronger demand for foods that are healthier and/or have added value. This means that people not only want food that contains fundamental nutrients, but also functional ingredients that are sufficiently effective at maintaining health.

In the course of our screening program for functional foodstuffs in Japan, we cultivated quinoa and isolated antioxidative ingredients from the quinoa seeds. No investigation of phenolic compounds from quinoa seeds grown in Japan has been reported prior to our own work, although there has been a recent article published on the antioxidant potency of various extracts and fractions of quinoa seeds produced in Japan (Nsimba, Kikuzaki, & Konishi, 2008).

The aim of this study is to evaluate the nutritional advantages of quinoa seeds cultivated in Japan. The usefulness of quinoa as a foodstuff was evaluated by structural and quantitative determination of flavonol glycosides, the content of aglycones formed *via* acidic hydrolysis, and radical-scavenging activity; these were compared to corresponding data for conventional cereals and pseudocereals.

2. Materials and methods

2.1. Plant materials

Quinoa (*C. quinoa* Willd.) was supplied by Yamanashi Prefectural Agritechnology Center; it was cultivated in five farms in Yamanashi, Japan over the period 2005–2008. The quinoa *ecotype* tested in this experiment was the sea-level NL-6 variety. Samples 1, 2, 4, and 6 were harvested during summer, while samples 3 and 5 were harvested during winter. After harvest, the seeds were dried in a room and slightly polished by a rice-cleaning machine. Amaranthus seeds (*Amaranthus* spp.) of the new Asteka variety were provided by the National Agriculture and Food Research Organization (NARO). The other cereal and pseudo-cereal crops were purchased at a local market in Kofu City (Yamanashi, Japan). Cereals: brown rice, proso millet, foxtail millet, Japanese barnyard millet, wholewheat flour, barley (pressed grain), oatmeal, ryeflour, Job's tears, and cornflour. Pseudo-cereals: four samples of quinoa seeds produced in South America (three in Bolivia and one in Peru), two samples of buckwheat flour, buckwheat seed, and two samples of amaranthus. These varieties were unknown.

2.2. General methods

Quercetin, morin and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma–Aldrich (St. Louis, MO); kaempferol and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Extrasynthese (Genay, France); Folin–Ciocalteu's reagent, L-ascorbic acid, and rutin trihydrate were purchased from Kanto Chemicals Co. (Tokyo, Japan). All other chemicals used were of analytical reagent grade.

UV spectra were recorded on a UV-1700 Shimadzu (Kyoto, Japan) spectrophotometer. NMR spectra were run on a Bruker AVANCE-400 spectrometer (^{1}H at 400 MHz; ^{13}C at 100 MHz) with TMS as the internal standard. The MS data were recorded on a Jeol JMS 700 mass spectrometer. Elemental analysis was performed using a Flash EA 1112 CHNS analyser. HPLC separations were performed on a JASCO 980 series pumping system with a Shimadzu SPD-10A UV–VIS detector.

2.3. Preparation of crude extract solutions from quinoa and other grains

Grain samples (100 mg) were milled to a powder, using a coffee grinder as needed, and extracted with MeOH:H_2O (2:1 v/v; 5 ml) for 60 min at 50 °C. The crude extracts were filtered through a 0.45-μm membrane filter and made up to 10 ml in a volumetric flask with MeOH:H_2O (2:1 v/v).

2.4. Total phenolic content of crude extract solutions from quinoa and other grains

The total phenolic contents of crude extract solutions were determined according to Folin–Ciocalteu's method (Singleton & Rossi, 1965). Three millilitres of water and 1 ml of crude grain extract solution were mixed with 1 ml of 5-fold diluted Folin–Ciocalteu's reagent and 1 ml of 10% sodium carbonate. The mixture was allowed to stand for 1 h at room temperature and the absorbance was measured at 760 nm against a blank. The total phenolic content in the extract is expressed as mg equivalents of gallic acid/100 g fresh weight (FW).

2.5. Scavenging ability by crude extract solutions from quinoa and other grains on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The extract solution of a grain (2 ml) was mixed with MeOH solution containing DPPH radicals (0.15 mM, 2 ml). The mixture was shaken vigorously and left to stand for 30 min in the dark before the absorbance was measured at 517 nm against a blank. The scavenging activity was calculated using the following equation: DPPH radical-scavenging ability (%) = $[(A - B)/A] \times 100$.

A and B are the control and sample absorbances at 517 nm of the reaction mixture, respectively. The scavenging ability (%) of Trolox was plotted against the concentration. The antioxidant activity of the grain is defined as the concentration (μmol) of Trolox with the antioxidant equivalent to 100 g of fresh weight (FW).

2.6. Isolation of flavonol glycosides from quinoa seeds harvested in Japan

The quinoa seeds (1.0 kg), harvested from Yamanashi in 2005, were powdered and extracted with MeOH:H_2O (10 L, 2:1 v/v). The extracts were evaporated *in vacuo* and partitioned between EtOAc and H_2O. The water layer was evaporated under reduced pressure for the removal of EtOAc and absorbed on an ODS column (20 × 250 mm). After washing with water, the column was eluted with MeOH:H_2O (35:65 v/v) and 100% MeOH to give fractions 1 (6.17 g), and 2 (11.52 g). Fraction 1 was loaded repeatedly onto a preparative ODS column (20 × 250 mm, YMC-Pack ODS-A) using 1% aq. AcOH:MeOH (7:3 and 6:4 v/v) as the mobile phase, to yield compounds **1** (556 mg), **2** (113 mg), **3** (388 mg), and **4** (302 mg).

2.7. Quercetin 3-O-(2″,6″-di-O-α-rhamnopyranosyl)-β-galactopyranoside (*1*)

Yellow amorphous powder. FAB-MS (neg.) *m/z*: 755 $[M-H]^-$, 301 $[M-H-$triose moiety$]^-$. UV (MeOH) λ_{max} nm (logε): 356 (4.27), 256 (4.36). Found: C, 47.22; H, 5.55%. Calcd. for $C_{33}H_{40}O_{20}\cdot 4.5\ H_2O$: C, 47.31; H, 5.90%.

*2.8. Quercetin 3-O-(2″,6″-di-O-α-rhamnopyranosyl)-β-glucopyranoside (**2**)*

Yellow amorphous powder. FAB-MS (neg.) *m*/*z*: 755 $[M-H]^-$, 301 $[M-H-$triose moiety$]^-$. UV (MeOH) λ_{max} nm (logε): 354 (4.25), 256 (4.35). Found: C, 47.28; H, 5.75%. Calcd. for $C_{33}H_{40}O_{20}\cdot 4.5\ H_2O$: C, 47.31; H, 5.90%.

*2.9. Quercetin 3-O-(2″-O-β-apiofuranosyl-6″-O-α-rhamnopyranosyl)-β-galactopyranoside (**3**)*

Yellow amorphous powder. FAB-MS (neg.) *m*/*z*: 741 $[M-H]^-$, 301 $[M-H-$triose moiety$]^-$. UV (MeOH) λ_{max} nm (logε): 357 (4.25), 256 (4.33). Found: C, 47.52; H, 5.34%. Calcd. for $C_{32}H_{38}O_{20}\cdot 3.5\ H_2O$: C, 47.71; H, 5.63%.

*2.10. Kaempferol 3-O-(2″,6″-di-O-α-rhamnopyranosyl)-β-galactopyranoside (**4**)*

Yellow amorphous powder. FAB-MS (neg.) *m*/*z*: 739 $[M-H]^-$, 285 $[M-H-$triose moiety$]^-$. UV (MeOH) λ_{max} nm (logε): 349 (4.21), 266 (4.29). Found: C, 48.14; H, 5.65%. Calcd. for $C_{33}H_{40}O_{19}\cdot 4.5\ H_2O$: C, 48.24; H, 6.01%.

2.11. Quantitative analysis of flavonol glycosides in quinoa and other grains by HPLC

According to Section 2.3, the tested grains were extracted with MeOH:H_2O (2:1 v/v). The crude extracts were filtered and evaporated to dryness under reduced pressure and suspended in water. The extract solution was passed through InertSep C18 (GL Science), eluted with 70% MeOH and made up to 5 ml in a volumetric flask. The flavonol glycosides in the eluate were analysed with HPLC. The HPLC conditions were as follows: column, ODS (4.6 × 250 mm, Inertsil ODS-3, GL Science); mobile phase, **A** (H_2O:MeCN:AcOH 90:8:2 v/v/v) and **B** (H_2O:MeCN:AcOH 80:18:2 v/v/v); flow rate, 1 ml/min; temperature, 40 °C; detection, UV at 370 nm. The applied elution conditions were: 0–20 min, 25% **B** isocratic; 20–40 min, linear gradient from 25% to 50% **B**; 40–60 min, linear gradient from 50% to 100% **B**.

2.12. Acid hydrolysis of glycosides in crude extract solution from quinoa and other grains

One millilitre of extract solution, according to Section 2.11, was transferred into a 25-ml test tube; 1.6 M HCl aq. soln. (2.0 ml), morin in MeOH (7.2 μg/1.0 ml) as an internal standard, and ascorbic acid in MeOH (1.6 mg/500 μl) as an antioxidant were then added and refluxed for 1 h in boiling water. 6 M NaOH aq. soln. (270 μl) was added and the resulting solution was filtered through 0.45-μm PTFE filters and adjusted with 10 ml of MeOH in a volumetric flask. The HPLC conditions for aglycone analysis were as follows: solvent, 0.01 M H_3PO_4:EtOH (62.5:37.5 v/v); column, ODS (6 × 150 mm, A-312, YMC); flow rate, 1.0 ml/min; temperature, 40 °C; detection, UV at 370 nm.

2.13. Statistics

The data was processed using Microsoft Office Excel 2002 and is presented as the mean value ± SD of three determinations.

3. Results and discussion

3.1. Antioxidative effect of quinoa seeds cultivated in Japan

Functional foods possess several important biological properties, such as antithrombotic, anti-inflammatory, anticancer, and antiviral activities. These activities may be partially related to their antioxidant activity, especially their free radical-scavenging ability. DPPH is a relatively stable free radical used extensively in evaluating the antioxidant activity of natural products; antioxidant activity is largely attributable to the amount of phenolic compounds. Fifteen samples of quinoa seeds cultivated in Japan, with differences in terms of the harvest season and farms, were extracted with MeOH:H_2O (2:1 v/v); the crude extracts were used to determine their DPPH radical-scavenging abilities. The range in scavenging ability was from 502 ± 6 to 950 ± 20 μmol equivalent of Trolox/100 g FW.

3.2. Antioxidative properties of quinoa seeds compared with other grains

To evaluate the antioxidative activity of quinoa seeds cultivated in Japan, the DPPH radical-scavenging ability and total phenolic content were compared to corresponding data for 10 conventional cereals – namely, brown rice, proso millet, foxtail millet, Japanese barnyard millet, wholewheat flour, barley (pressed grain), oatmeal, ryeflour, Job's tears, and cornflour – and three pseudo-cereals – namely, four samples of quinoa seeds produced in South America, three samples of buckwheat, and three samples of amaranthus. As demonstrated in Fig. 1, there is a good correlation between the total phenolic contents and the DPPH radical-scavenging abilities of the tested grains. The antioxidative effect of quinoa seeds from Japan was higher than those from South America and other grains excluding buckwheat. The results suggest that quinoa seeds cultivated in Japan have phenolic compounds responsible for their high antioxidant property.

3.3. Isolation and structural determination of four flavonol glycosides from quinoa seeds

Samples of the quinoa seeds cultivated in Japan were milled and extracted with several solvents such as hexane, EtOAc, EtOH, MeOH, and MeOH:H_2O (2:1 v/v). The crude extract with MeOH:H_2O (2:1 v/v) exhibited the highest antioxidant potential and phenolic content among the solvent extracts (data not shown). The MeOH:H_2O extract of whole flour (1.0 kg) from the quinoa seeds was partitioned by solvent extraction and solid phase extraction. The repeated preparative HPLC of the crude glycosidic fraction afforded four flavonol glycosides **1**–**4**. The structures of **1**–**4** were

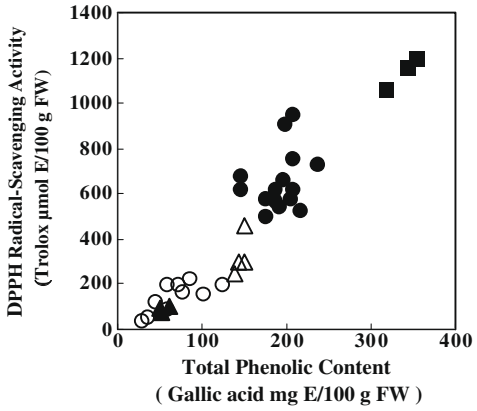


Fig. 1. Correlation between total phenolic content and DPPH radical-scavenging activity. Values are means (n = 3). ●: quinoa seeds cultivated in Japan, △: quinoa seeds produced in South America, ○: cereals (brown rice, proso millet, foxtail millet, Japanese barnyard millet, wholewheat flour, barley (pressed grain), oatmeal, ryeflour, Job's tears and cornflour), ■: buckwheat (flour and seeds), ▲: amaranthus.

determined by spectroscopic methods including UV, MS and NMR, and identified through direct comparison of their physical properties with those previously reported. The ^{1}H and ^{13}C NMR spectral data of **1**–**4** were assigned based on DEPT, HSQC, and HMBC measurements (Tables 1 and 2). The compounds **1**, **3**, and **4** had been previously reported as flavonol glycosides from quinoa seeds grown in South America and identified as quercetin 3-*O*-(2″,6″-di-*O*-α-rhamnopyranosyl)-β-galactopyranoside (**1**) (Dini et al., 2004), quercetin 3-*O*-(2″-*O*-β-apiofuranosyl-6″-*O*-α-rhamnopyranosyl)-β-galactopyranoside (**3**) (Zhu et al., 2001), and kaempferol 3-*O*-(2″,6″-di-*O*-α-rhamnopyranosyl)-β-galactopyranoside (**4**) (Dini et al., 2004), which has been named mauritianin (Nishibe et al., 1996).

The mass spectrum of **2** in negative ion mode showed an $[M-H]^-$ ion at *m*/*z* 755. A $C_{33}H_{40}O_{20}$ molecular formula was deduced from the MS and NMR data. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**, except for the small shifts in the saccharide moiety corresponding to the β-galactose of **1**. The proton signals for the saccharide moiety of **2**, except at H-6″a, were shifted 0.07–0.49 ppm upfield against those of **1**; the carbon signals, except at C-1″, were 1.0–3.3 ppm downfield. The coupling constant between H-4″ and 5″ of **2** ($J_{H4''-H5''}$ = 9.4 Hz) was larger than that of **1** (3.3 Hz). The large coupling constant ($J_{H1''-H2''}$ = 7.7 Hz) indicated a β-anomeric configuration for glucose. These facts supported the fact that the β-galactopyranose moiety in **1** was replaced with the β-glucopyranose in **2**. In the HMBC spectra of **2**, the cross-peak were observed between a carbon at C-3 (δ_C 134.5) and an anomeric proton at H-1″ (δ_H 5.59) in glucose; between C-2″ (δ_C 80.1) and H-1‴ (δ_H 5.22) in rhamnose; and between C-6″ (δ_C 68.3) and H-1⁗ (δ_H 4.50) in another rhamnose. These indicate an *O*-glycosidic substitution at C-3 and a glycosidic linkage at C-2 and C-6 of glucose with two rhamnopyranosyl units, respectively. From these results, the structure of **2** was established as quercetin 3-*O*-(2″,6″-di-*O*-α-rhamnopyranosyl)-β-glucopyranoside (**2**). Compound **2** was isolated for the first time from quinoa seeds, though it was isolated from aerial parts of *Chenopodium album*, another species within this genus (Chludil, Corbino, & Leicach, 2008). The structures of **1**–**4** are depicted in Fig. 2.

3.4. Quantitative analysis of flavonol glycosides in quinoa seeds and other grains by HPLC

The content of flavonol glycosides was determined on six samples of quinoa seeds cultivated in Japan (with differences in harvest

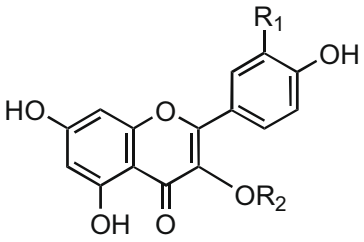
Table 1
^{1}H NMR spectral data for the flavonol glycosides (400 MHz, CD_3OD).

	1	2	3	4
Aglycone				
H-6	6.17 (*d*, 2.1)	6.18 (*d*, 2.1)	6.17 (*d*, 2.0)	6.18 (*d*, 2.0)
H-8	6.37 (*d*, 2.1)	6.37 (*d*, 2.1)	6.37 (*d*, 2.0)	6.38 (*d*, 2.0)
H-2′	7.69 (*d*, 2.0)	7.59 (*d*, 2.2)	7.72 (*d*, 2.1)	8.06 (*d*, 8.9)
H-3′				6.89 (*d*, 8.9)
H-5′	6.87 (*d*, 8.5)	6.87 (*d*, 8.3)	6.86 (*d*, 8.5)	6.89 (*d*, 8.9)
H-6′	7.55 (*dd*, 8.5, 2.0)	7.61 (*dd*, 8.3, 2.2)	7.61 (*dd*, 8.5, 2.1)	8.06 (*d*, 8.9)
3-Glucosyl				
H-1		5.59 (*d*, 7.7)		
H-2		3.64 (*dd*, 9.4, 7.7)		
H-3		3.55 (*t*, 9.4)		
H-4		3.34 (*t*, 9.4)		
H-5		3.33 (*ddd*, 9.4, 5.6, 1.5)		
H-6a		3.82 (*dd*, 11.4, 1.5)		
H-6b		3.40 (*dd*, 11.4, 5.6)		
3-Galactosyl				
H-1	5.69 (*d*, 7.8)		5.43 (*d*, 7.7)	5.61 (*d*, 7.8)
H-2	3.95 (*dd*, 9.4, 7.8)		3.95 (*dd*, 9.6, 7.7)	3.93 (*dd*, 9.5, 7.8)
H-3	3.73 (*dd*, 9.4, 3.3)		3.71 (*dd*, 9.6, 4.6)	3.70 (*dd*, 9.5, 3.7)
H-4	3.81 (*t*-like, 3.3)		3.80 (*t*-like, 4.6)	3.77 (*t*-like, 3.7)
H-5	3.67 (*m*)		3.65 (*m*)	3.64 (*m*)
H-6a	3.75 (*ddd*, 11.0, 3.3)		3.72 (*dd*, 10.0, 5.0)	3.72 (*dd*, 10.1, 5.8)
H-6b	3.47 (*dd*, 11.0, 6.4)		3.42 (*dd*, 10.0, 6.6)	3.44 (*dd*, 10.1, 6.6)
2″-Rhamnosyl				
H-1	5.21 (*d*, 1.4)	5.22 (*d*, 1.4)		5.21 (*d*, 1.4)
H-2	3.99 (*dd*, 3.3, 1.4)	4.00 (*dd*, 3.3, 1.4)		4.00 (*dd*, 3.4, 1.4)
H-3	3.78 (*dd*, 9.5, 3.3)	3.80 (*dd*, 9.7, 3.3)		3.79 (*dd*, 9.6, 3.4)
H-4	3.33 (*t*, 9.5)	3.26 (*t*, 9.7)		3.34 (*t*, 9.6)
H-5	4.04 (*dd*, 9.5, 6.2)	4.08 (*dd*, 9.7, 6.2)		4.06 (*dd*, 9.6, 6.2)
H-6 (CH_3)	0.94 (*d*, 6.2)	1.00 (*d*, 6.2)		0.97 (*d*, 6.2)
2″-Apiofuranosyl				
H-1			5.46 (*d*, 1.4)	
H-2			4.06 (*d*, 1.4)	
H-4a			4.05 (*d*, 9.6)	
H-4b			3.71 (*d*, 9.6)	
H-5a			3.75 (*d*, 11.6)	
H-5b			3.65 (*d*, 11.6)	
6″-Rhamnosyl				
H-1	4.55 (*d*, 1.7)	4.50 (*d*, 1.4)	4.53 (*d*, 1.4)	4.52 (*d*, 1.4)
H-2	3.59 (*dd*, 3.3, 1.7)	3.59 (*dd*, 3.4, 1.4)	3.58 (*dd*, 3.3, 1.4)	3.57 (*dd*, 3.3, 1.4)
H-3	3.51 (*dd*, 9.5, 3.3)	3.47 (*dd*, 9.5, 3.4)	3.51(*dd*, 9.5, 3.3)	3.50 (*dd*, 9.6, 3.3)
H-4	3.27 (*t*, 9.5)	3.23 (*t*, 9.5)	3.27 (*t*, 9.5)	3.27 (*t*, 9.6)
H-5	3.54 (*dd*, 9.5, 6.2)	3.43 (*dd*, 9.5, 6.2)	3.53 (*dd*, 9.5, 6.2)	3.52 (*dd*, 9.6, 6.1)
H-6 (CH_3)	1.18 (*d*, 6.2)	1.08 (*d*, 6.2)	1.18 (*d*, 6.2)	1.18 (*d*, 6.1)

The values in the parenthesis indicate multiplicity and coupling constants (*J* Hz).

Table 2
^{13}C NMR spectral data for the flavonol glycosides (100 MHz, CD_3OD).

	1	2	3	4
Aglycone				
C-2	158.4	159.0	158.4	158.7
C-3	134.6	134.5	135.0	134.5
C-4	179.3	179.3	179.4	179.5
C-5	163.2	163.2	163.1	163.2
C-6	99.7	99.8	99.8	99.8
C-7	165.6	165.7	165.7	165.7
C-8	94.6	94.7	94.6	94.7
C-9	158.4	158.5	158.4	158.5
C-10	105.9	105.9	105.8	105.9
C-1′	123.3	123.5	123.2	123.1
C-2′	117.4	117.4	117.4	132.3
C-3′	145.9	145.9	145.9	116.2
C-4′	149.7	149.6	149.7	161.3
C-5′	116.1	116.2	116.2	116.2
C-6′	123.0	123.6	123.2	132.3
3-Glucosyl				
C-1		100.5		
C-2		80.1		
C-3		79.0		
C-4		71.9		
C-5		77.1		
C-6		68.3		
3-Galactosyl				
C-1	101.1		101.6	100.9
C-2	77.4		76.7	77.6
C-3	75.7		75.3	75.8
C-4	70.9		70.7	70.7
C-5	75.3		75.2	75.3
C-6	66.9		67.0	67.1
2″-Rhamnosyl				
C-1	102.6	102.7		102.7
C-2	72.4	72.4		72.5
C-3	72.3	72.3		72.3
C-4	74.1	74.1		74.1
C-5	69.9	70.0		69.9
C-6	17.4	17.6		17.6
2″-Apiofuranosyl				
C-1			110.7	
C-2			78.1	
C-3			80.9	
C-4			75.5	
C-5			66.3	
6″-Rhamnosyl				
C-1	101.8	102.3	101.8	101.9
C-2	72.1	72.2	72.1	72.1
C-3	72.3	72.3	72.3	72.4
C-4	73.9	73.9	73.9	73.9
C-5	69.7	69.8	69.7	69.7
C-6	18.0	17.9	18.0	18.0



	R_1	R_2
1:	OH	2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranosyl
2:	OH	2,6-di-*O*-α-rhamnopyranosyl-β-glucopyranosyl
3:	OH	2-*O*-β-apiofuranosyl-6-*O*-α-rhamnopyranosyl-β-galactopyranosyl
4:	H	2,6-di-*O*-α-rhamnopyranosyl-β-galactopyranosyl

Fig. 2. Structures of flavonol glycosides isolated from quinoa seeds.

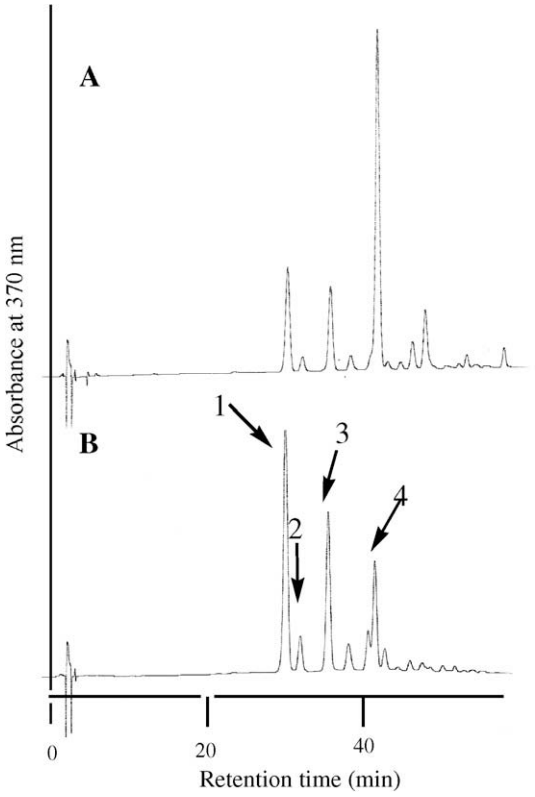


Fig. 3. HPLC profiles (370 nm) for flavonol glycosides in quinoa extracts. (**A**) Quinoa seeds produced in Bolivia and (**B**) in Japan.

season and farms) in comparison to four quinoa seeds grown in Bolivia and Peru. Along with the quinoa seeds, 10 grains of cereals, three amaranthus samples, and three buckwheat samples were prepared with MeOH:H_2O extraction followed with SPE pretreatment. The MeOH:H_2O extracts of quinoa seeds and other grains were evaluated by HPLC analysis of **1**–**4**. Typical HPLC chromatograms of quinoa seed extracts are shown in Fig. 3. The sample of A was produced in Bolivia and B in Japan.

Compounds **1**, **2**, **3**, and **4** were eluted at 31.5, 33.3, 36.8, and 42.7 min, respectively. The recovery with extraction was examined using quercetin 4′-*O*-glucopyranoside as the internal standard. Quercetin monoglucoside (7.08 μg), isolated from onions, was added to every extraction and eluted at 65.2 min under these HPLC conditions. The recovery of quercetin monoglucoside was in the range 90–99% and is considered to be sufficient for quantitative determination of the flavonols. Compounds **1**–**4** were isolated from quinoa seeds as pure chemicals to give linear calibration curves. These molecular formulas containing water crystals were determined based upon elemental analysis results; the flavonol contents are expressed as mg anhydrous/100 g of fresh weight (FW). Compounds **1**, **2**, **3**, and **4** were detected in extracts from all of the tested quinoa seeds but were not in the extracts from the other grains of cereals and pseudo-cereals. Table 3 shows the flavonol contents in the quinoa seed.

The composition of flavonol glycosides was somewhat different depending upon the place of production. In the quinoa seeds from

Table 3
Content of flavonol glycosides and their derivative aglycones by acidic hydrolysis from quinoa seeds.

Sample no.	Place of production	Harvesting season	Content of flavonol glycosides (mg/100 g FW)				Total content of **1**–**4** (mg/100 g FW)	Content of aglycones formed via acidic hydrolysis (mg/100 g FW)	
			1	**2**	**3**	**4**		Quercetin	Kaempferol
1	Japan	Summer	83.8 ± 0.8	14.0 ± 0.2	47.2 ± 0.3	41.1 ± 0.4	186.1	67.4 ± 0.9	18.4 ± 0.3
2	Japan	Summer	69.0 ± 0.5	9.8 ± 0.2	42.5 ± 1.1	34.6 ± 0.8	155.9	55.0 ± 0.3	15.9 ± 0.5
3	Japan	Winter	79.5 ± 1.5	15.0 ± 0.3	46.7 ± 0.3	48.8 ± 1.9	190.0	64.9 ± 0.6	20.3 ± 0.7
4	Japan	Summer	87.3 ± 2.5	11.2 ± 0.2	51.7 ± 1.2	42.6 ± 1.2	192.8	68.0 ± 2.1	18.0 ± 0.8
5	Japan	Winter	51.5 ± 0.4	10.4 ± 0.2	34.4 ± 0.2	33.8 ± 0.2	130.1	45.3 ± 0.9	15.0 ± 0.5
6	Japan	Summer	83.9 ± 1.9	9.9 ± 0.3	48.9 ± 1.4	39.3 ± 0.7	182.0	61.0 ± 1.8	16.7 ± 0.2
7	Bolivia	–	53.3 ± 0.8	6.9 ± 0.0	36.9 ± 0.3	78.7 ± 1.6	175.8	42.9 ± 1.2	36.6 ± 0.6
8	Bolivia	–	24.3 ± 0.7	3.3 ± 0.2	21.5 ± 0.6	113.3 ± 2.9	162.4	22.5 ± 0.7	52.1 ± 0.6
9	Bolivia	–	45.4 ± 1.3	5.8 ± 0.2	28.5 ± 0.8	113.3 ± 3.0	193.0	34.7 ± 0.9	50.1 ± 0.9
10	Peru	–	42.0 ± 0.5	6.0 ± 0.2	30.1 ± 0.2	93.0 ± 0.3	171.1	34.7 ± 1.2	41.2 ± 1.7

Values expressed as a mean of three determinations ± standard deviation. –: harvest season is unknown.

Japan, the major compound was quercetin glycoside **1**. The total content of quercetin glycosides, namely **1**, **2**, and **3**, was much higher than that of kaempferol glycoside **4**, while the content of **4** in the seeds from South America was higher than that of quercetin glycosides **1**–**3**. In order for comparisons based on mole equivalents, the flavonol glycoside contents were re-expressed as μmol/100 g of FW and are shown graphically in Fig. 4. For the samples from Japan, the total content of **1**, **2**, and **3** was 3.5-fold higher than that of **4** in samples 1, 2, 4, and 6, which were harvested in summer, whereas that of **1**, **2**, and **3** was 2.8-fold higher than **4** in samples 3 and 5, which were harvested in winter. Since this was observed in all tested quinoa seeds from Japan (data for other tested seeds is not shown), it was deduced that the sunlight is responsible for the accumulation of quercetin glycosides. According to numerous reports on flavonoid antioxidant activity, quercetin glycosides are more active than the corresponding kaempferol derivatives. In this study, the crude extracts from the quinoa seeds cultivated in Japan showed stronger antioxidant activity than those from South America; quantitative analysis supports the assumption that the antioxidant properties of quinoa seeds are correlated with the amount of quercetin glycosides. As it has been reported that some flavonoids accumulate in stressed plants and are related to chemical defence strategies (Chludil et al., 2008), the difference in glycoside concentration between the quinoa seeds from Japan and South America may be explained by differences in genetic background rather than the environmental conditions.

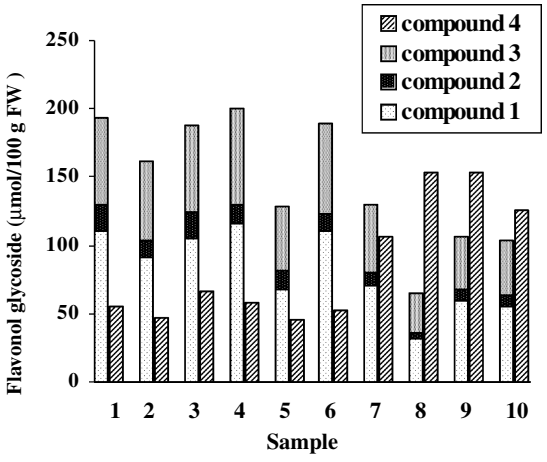


Fig. 4. Flavonol glycosides contents of quinoa seeds. **1**–**6**: quinoa cultivated in Japan; **7**–**9**: quinoa produced in Bolivia; **10**: quinoa produced in Peru. Values are means ($n = 3$).

3.5. Acidic hydrolysis of flavonol glycosides in extracts from quinoa seeds and other grains

Flavonoids are mostly present as glycosides in plants. During intestinal absorption, these glycosides are mostly hydrolysed to their aglycones, followed by conjugation to glucuronides and/or sulphates (Murota & Terao, 2003); the bioactivity is attributed to aglycone structures and not sugar moieties (Bors, Heller, Michel, & Saran, 1990). Since quercetin and kaempferol are beneficial to human health, a better understanding of the levels of the two aglycones is needed. In order to determine the content of quercetin and kaempferol, including their glycosidic form, the crude extracts of all tested grain samples were hydrolysed with hydrochloric acid and injected after neutralisation into the HPLC system. The recovery of morin as an internal standard with hydrolysis was in the range 92–102% and sufficient for quantitative determination of the two aglycones. After hydrolysis of the extracts from quinoa seeds with acid, none of the four flavonol glycosides were detected in the reactant. The aglycones quercetin and kaempferol were measured at up to 2.1 and 2.2 μg, respectively. Quercetin was detected in quinoa and buckwheat, whereas kaempferol was found in quinoa. These aglycones were not detected in tested samples of 10 cereals and amaranthus. The amount of quercetin and kaempferol formed *via* hydrolysis from quinoa seeds is shown in Table 3. The content of quercetin in buckwheat flour was approximately 20 mg/100 g FW. The amount of quercetin in quinoa seeds was much higher than in buckwheat, which was abundant in rutin.

For easy comparison of the amount of the two aglycones formed by hydrolysis with the flavonol glycosides, the amounts were re-expressed as μmol/100 g of FW and are shown graphically in Fig. 5. The amount of quercetin in samples 1–6, which were from Japan, was 150 ± 3 to 225 ± 7 μmol/100 g FW, which is about three times higher than that of kaempferol (52.3 ± 1.8 to 71.0 ± 2.5 μmol/100 g FW). The amount of quercetin in samples 7–10, which were grown in South America, was 74.6 ± 2 to 142 ± 4 μmol/100 g FW, which is lower than that of kaempferol (128 ± 2 to 182 ± 2 μmol/100 g FW). The amounts of quercetin in quinoa seeds from Japan were higher than those from South America, whereas the amounts of kaempferol in the seeds from Japan were much lower than those from South America. These results coincided with the quantitative analysis of the flavonol glycosides. On average, the total contents of **1**, **2**, and **3** reached 89.2% of the amount of aglycone quercetin and the contents of **4** reached 87.5% of the amount of aglycone kaempferol. Furthermore, Sakakibara, Honda, Nakagawa, Ashida, and Kanazawa (2003) deduced that quantification by hydrolysis may produce a loss of content due to the decomposition and polymerisation of flavonols. The results showed that all derivatives related to quercetin and kaempferol, except for the four glycosides, could

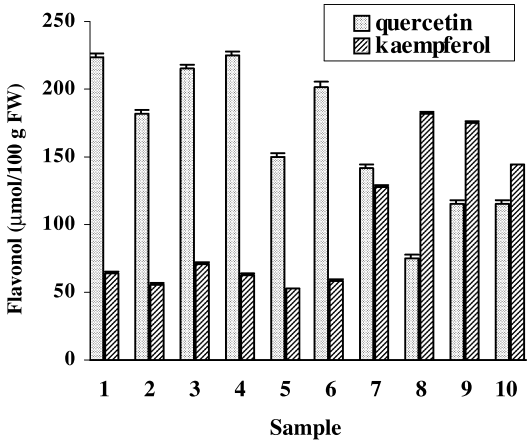


Fig. 5. Content of quercetin and kaempferol aglycones formed *via* acidic hydrolysis. **1**–**6**: quinoa cultivated in Japan; **7**–**9**: quinoa produced in Bolivia; **10**: quinoa produced in Peru. Vertical bars represent the standard deviation of each data point ($n = 3$).

not be detected in the quantitative analysis. The isolation and determination of these undetected derivatives are currently in progress.

The content of aglycone quercetin in buckwheat was found to be 50.7 ± 3.4 to 66.2 ± 2.4 μmol/100 g FW, which is much lower than in quinoa seeds. According to the present results, almost all conventionally-used cereals do not possess quercetin or kaempferol in any form, except for quinoa seeds and buckwheat. Quinoa seeds are concluded to be the most effective foodstuff as a source of flavonols among cereals and pseudo-cereals.

4. Conclusion

Dietary flavonoids are thought to have health benefits, possibly due to antioxidant and anti-inflammatory properties. Based on numerous reports on the relationship between their structure and radical-scavenging activity, quercetin is the strongest antioxidant among flavonoids; *in vitro* studies have revealed diverse biological effects, including apoptosis induction, antimutagenesis, protein kinase C inhibition, lipoxygenase inhibition, histamine-release inhibition, superoxide dismutase-like activity, modulation of cell cycle, angiogenesis inhibition, and inhibition of angiotensin-converting enzyme II (Formica & Regelson, 1995). In the present study, quinoa seeds were confirmed to possess large amounts of quercetin and kaempferol glycosides. The amount of quercetin in quinoa seeds from Japan was higher than those grown in South America and buckwheat. Since higher concentrations of flavonoid derivatives in edible plants enhance the neutraceutical value in terms of health-promoting effects, the flavonoid content of edible plants has been collected from various literature and publicised on the web site (U.S. Department of Agriculture, 2007). On the database, several examples of plants rich in quercetin are onions (21.4 mg/100 g), green tea leaves (dry, 662 mg/100 g), capers (234 mg/100 g), lovage leaves (170 mg/100 g), buckwheat (23.1 mg/100 g), etc. The quinoa seeds cultivated in Japan are the most effective functional foodstuff in terms of being a source of antioxidative and bioactive flavonoids among cereals, pseudo-cereals, and regularly-consumed plant foods. A study on the effects on bioactivity by dosing mice with quinoa seeds is currently in progress.

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