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ORIGINAL PAPER

Wild almond (*Amygdalus pedunculata* Pall.) as potential nutritional resource for the future: studies on its chemical composition and nutritional value

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Abstract
Wild almond germplasm resources have still not received enough attention in the chemical composition and application of seeds. The aim of this study was to evaluate the phytochemical composition and analyze the nutritional value of longstalk almond seeds (*Amygdalus pedunculata* Pall., a native species of China and Mongolia). In order to evaluate the nutritional value of samples, parameters including the protein, amino acids, vitamins, minerals and the oxidative stability were measured. Results indicated that the seeds were rich in crude fat (49.34 g/100 g) and crude protein (22.30 g/100 g). The average content of amygdalin was 3.55%. The amino acids in the seeds were abundant but incomplete. The content of unsaturated fatty acid in the seed oil reached 97.89%, which was mostly comprised of oleic acid and linoleic acid. Five different sugars, namely fructose, glucose, sucrose, maltose, and lactose, were detected in the seeds. With regard to mineral composition, the seeds contained ten mineral elements and high concentrations of Zn, Ca, and Se. The nuts were also an excellent source of vitamin E, vitamin B3, folate, phytosterols, and phenolic and flavonoid compounds. The oxygen radical absorbance capacity (ORAC) values (18.64 ± 0.2 μmol TE/g) indicated that the seeds were a good dietary source of antioxidants. In addition, these findings are important for the nutrition sciences, because fatty acids, lipid soluble vitamins, phytosterols, flavonoid, phenolic compounds and ORAC, in particular, seem to have considerable effect on health.

Keywords *Amygdalus pedunculata* Pall. · Chemical composition · Fatty acids · Amino acids · Minerals · Tocopherols · ORAC

Introduction

Wild almond genetic resources have received considerable attention for nut chemical compositions and uses [1–4]. Among them, the longstalk almond plant (*Amygdalus pedunculata* Pall.) is an important sand-fixation plant in northwest China that has great ornamental and medicinal value. The seeds contain oil that has been deemed acceptable for human consumption. The kernel oil development cycle (60–80 days after pollination) is only half that of the development cycles of other oil plants, such as walnut oil (140 days after pollination). As bitter almonds, longstalk almond nuts are often used as Chinese medicine materials because of rich amygdalin [5]. The seeds are edible after removing amygdalin via treatment with boiling water. The wild longstalk almond plant, which is a woody oil plant, exhibits potential for cultivation on marginal lands in warm, arid environments.

Among nut species, the cultivated almond plant is scientifically known as *Prunus dulcis* (Mill.) D.A. Webb (synonyms: *Prunus amygdalus* or *Amygdalus communis*). The longstalk almond belongs to the same *Rosaceae* family and is related to stone fruits like peaches, plums, and cherries [6]. The cultivated almond is the number one tree nut produced on a global basis, so most previous chemical composition and nutritional analyses have been conducted on cultivated almonds. Cultivated almonds are rich in nutrients and phytochemicals, such as monounsaturated fatty acids, polyunsaturated fatty acids, phytosterols, arginine

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and potassium, which are associated with improvements in heart health and obesity-related diseases [6, 7]. Meanwhile, the U.S. Food and Drug Administration (FDA) has determined that almonds are an excellent source of vitamin E and manganese.

Polyphenols are other health-promoting compounds contained in cultivated almonds [1]. Cultivated almonds contain a number of polyphenols that are responsible for a variety of bioactivities, among which antioxidant activity has been frequently studied [8, 9]. Phenolic compounds, including phenolic acids and flavonoids, are believed to play important roles in the antioxidant effectiveness of cultivated almonds [10, 11], which have been shown to provide protection against cancer and cardiovascular disease [6, 12]. The almond is one of the most important natural sources of antioxidants, which are known to possess health-promoting properties [8]. The chemical composition and the nutritional value of longstalk almonds are not clear. Thus, the present study investigates whether the phytochemicals in longstalk almonds are similar to those found in cultivated almonds.

The present work extensively investigates the chemical compositions and nutritional values of longstalk almonds. The results contribute to a better understanding of the potential applications of these particular almonds. Phytochemical composition of Amygdalus pedunculata was analyzed in detail for the first time in this study. The usefulness of long carpopodium nuts as a source of dietary protein, fats, carbohydrates, and micro and macro minerals, was evaluated with the goal of providing nutritionists with useful information regarding the nutritional potential of this food source. International development agencies working in the field of nutrition and food technologists interested in the potential industrial applications of these nuts should also benefit from the information provided by this study.

Materials and methods

Samples and sample preparation

The nuts analyzed in this study work collected from one region (North) of the Shenmu County (China) in the Mu Us desert, as this region reportedly produces the highest product yield per annum. The samples were sun-dried on location, sealed in plastic bags and transported to the laboratory.

Prior to chemical and antioxidant analyses, the nuts were shelled manually, screened to remove bad seeds, and then chopped in an appliance mill (model A327R1, Moulinex, Spain). All results are expressed on a dry weight basis.

Determination of chemical composition

Moisture, ash, and crude fiber contents were determined in accordance with the method described by Adeyeye and Ajewole [13] with some modifications. Briefly, moisture content was determined by heating 2.0 g of each sample to a constant weight in a crucible placed in an oven maintained at 105 °C. Ash was determined by incineration; 1.0-g samples were placed in a muffle furnace maintained at 550 °C for 5 h. Crude protein (% total nitrogen × 6.25) was determined by the Kjeldahl method [14] using 1.0-g samples. Crude fat was obtained by exhaustively extracting 5.0 g of each sample in a Soxhlet apparatus using petroleum ether (boiling range 40–60 °C) as the extractant [15]. Dietary fiber content was determined using the method described by Costa et al. [16]. The carbohydrate content was estimated by subtracting the values obtained for moisture, protein, lipid, ash, and total dietary fiber contents from 100. The energy value was estimated by using Atwater conversion factors of 4 kcal (proteins and carbohydrates), and 9 kcal (lipids) [17].

Fatty acid and amino acid profiles

For fatty acid analysis, crude fat was obtained from finely chopped seeds (ca. 5 g), which were extracted with light petroleum ether (boiling range 40–60 °C) in a Soxhlet apparatus; the remaining solvent was removed by vacuum distillation.

The determination of fatty acid profiles was implemented by preparing the methyl esters as described by Amaral et al. [18]. The examination of the fatty acid methyl esters was achieved by gas chromatography applying a Shimadzu GC-14B (Shimadzu Corporation, Tokyo, Japan), equipped with a flame ionization detector and integrator.

Amino acids were analyzed using the method of Sousa et al. [19]. Briefly, the samples were hydrolyzed in 6 N HCl under a nitrogen atmosphere for 24 h. The hydrolyzed solution was subjected to Eppendorf LC 3000 (Eppendorf, Germany) automatic amino acid analyzer.

The amino acid score (AAS) was estimated using the following formula: (mg of amino acid in 1 g of test protein /mg of amino acid in requirement pattern) × 100.

Sugars

Extraction and analysis of water-soluble sugars were performed according to the method of Cai and Zhang [20]. Concentrations of fructose, glucose, sucrose, maltose, and

lactose in kernels were determined by HPLC with an Agilent ZORBAX Bonus-RPUSP L60 (250 × 4.6 mm, 5 µm) and a refractive index detector (Agilent Technologies, Inc., USA). The mobile phase was 1.0 mL/min of 85% CH_3CN ($CH_3CN/H_2O = 85/15$, v/v).

Minerals

The mineral levels were assessed in crushed and homogenized samples prepared by organic wet digestion in accordance with the methodology described by Köksal et al. [21]. For organic digestion, the samples were treated with a mixture of concentrated nitric and perchloric acids at a high temperature. The macro- and microelements were solubilized, subjected to different treatments, and diluted for further quantitative evaluation. The quantification of elements was performed by spectrophotometry using a standard curve for each mineral. To determine the concentration of calcium, iron, and manganese, we used an atomic absorption spectrophotometer and acetylene. A flame photometer was used to determine potassium (768 nm), and a visible-light spectrophotometer was used to determine phosphorus (420 nm).

Determination of vitamin composition

Vitamins were detected using the method described by Köksal et al. [21]. Soluble and insoluble vitamins were determined by HPLC. For the analysis of vitamin B1, B2, B6, folic acid, biotin, ascorbic acid, and niacin, a SUPELCO Discovery C_{18} column (150 × 4.6 mm, ID 5 µm) and PDA detector at 220 nm were used. The column temperature was 35 °C. Mobile phase was K_2HPO_4/methyl alcohol (99:1) at 1 mL/min flow rate. For the determination of insoluble vitamins, such as retinol and tocopherols (α-tocopherol, γ-tocopherol and δ-tocopherol), n-hexane/isopropanol (99:1) at a flow rate 1 mL/min, was used as mobile phase. Vitamins were separated with a Lichrosorb Si60 (250 × 4 mm, ID 5 µm) column at 35 °C.

Sterol composition

Sterol composition was evaluated by GLC/FID/capillary column according to the method of Amaral et al. [18]. In brief, 250 mg of oil was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminum oxide column, on which fatty acid anions were retained and sterols passed through. The sterol fraction from the unsaponifiable matter was separated by thin-layer chromatography (TLC) on silica gel 20 × 20 cm; layer thickness of 0.25 mm, using hexane/diethyl ether (1/1 [v/v]) as the developing solvent, re-extracted from the TLC material and afterwards the composition of the sterol fraction determined by gas chromatography (GC) using betulin as an internal standard. The compounds were separated on a DB 5MS (J & W Scientific, Folsom, CA, USA) (30 m long, 0.25 mm ID, 0.25 µm film thickness). Other parameters were: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program: 250–300 °C at 2°C/min. All determinations were carried out in triplicate.

Determination of total phenols and phenolic acid contents

Total phenols were determined spectrophotometrically using Folin–Ciocalteu's reagent as described by Singleton and Rossi [22]. The results were expressed as mg of Catechol per 100 g seeds. The 11 phenolic acids (gallic acid, chlorogenic acid, syringic acid, ferulic acid, vanillic acid, sinapic acid, caffeic acid, gentisic acid, p-hydroxybenzoic acid, p-coumalic acid, protocatechuic acid) were extracted and analyzed by HPLC as described by the method of Chen et al. [23].

Total flavonoids and flavonol determination

Total flavonoids were measured according to the method of Wolfe et al. [24] with some modifications. The results were expressed as mg of Rutin per 100 g of seeds. Using optimal extraction conditions, a 10.0-g plant sample was extracted with 50 mL of 90% methanol for 60 min at 60 °C under ultrasonic irradiation. The resulting sample was centrifuged at 6000 rpm for 5 min. Supernatants were removed in a 150-mL triangle bottle, after which the extraction was repeated, and the extracts were combined and concentrated under vacuum to less than 10 mL. The solution was further filtered through a 0.45-µm nylon membrane filter. Fifty microliters of this extract were then diluted to 1 mL using distilled-deionized water. A 10-µL aliquot of this diluted solution was injected onto an HPLC column, and the 6 common flavonoids (L-epicatechin, quercetin, luteolin, vitexin, kaempferol, and hyperoside) were analyzed by HPLC [9].

Determination of antioxidant activity

The ORAC procedure used an automated plate reader (KC4, Bio Tek, USA) with 96-well plates [25]. Analyses were conducted in phosphate buffer at pH 7.4 and 37 °C. Peroxyl radicals were generated using 2, 2′-azobis (2-amidino-propane) dihydrochloride, which was prepared fresh for each run. Fluorescein was used as the substrate. Fluorescence conditions were as follows: excitation at 485 nm and emission at 520 nm. The standard curve was linear between 0 and 50 µM Trolox. The results were expressed as µM TE/g dry mass.

Table 1 Proximate chemical composition of longstalk almonds (*Amygdalus pedunculata* Pall.)

Component (g/100 g)[a]	Composition
Moisture	3.66 ± 0.11 g
Ash	2.50 ± 0.01 g
Carbohydrates	12.49
Amygdalin	3.55 ± 0.21 g
Crude protein (N × 6.25)	22.30 ± 0.20 g
Crude oil	49.34 ± 0.78
Total dietary fibers	9.71 ± 0.09
Soluble fibers	3.37 ± 0.13
Insoluble fibers	6.34 ± 0.22
Energy value (kcal/100 g)	583.22

[a]Data are means ± standard deviations of 3 replicates, excepting carbohydrates, which were calculated by subtracting the values of the other components from 100

Table 2 Fatty acid composition (in percentages) of longstalk almond tuber oil

Fatty acid[a]	%
Saturated fatty acids	
Palmitic (16:0)	1.54 ± 0.02
Stearic (18:0)	0.57 ± 0.02
Monounsaturated fatty acid	
Palmitoleic (16:1)	0.15 ± 0.00
Oleic (18:1)	68.80 ± 0.60
cis-11-Eicosenoic (20:1)	0.17 ± 0.02
Polyunsaturated fatty acid	
Linoleic (18:2)	28.69 ± 0.63
Linolenic (18:3)	0.08 ± 0.02
Saturated fatty acids	2.11
Monounsaturated fatty acid	69.11
Polyunsaturated fatty acid	28.77
Total unsaturated fatty acids	97.89

[a]Data are means ± standard deviations of three replicates

Results and discussion

Table 1 shows the proximate chemical composition of longstalk almond seeds. Fat was the predominant component (49.34 g/100 g), followed by proteins (22.30 g/100 g) and carbohydrates (12.49 g/100 g). Proteins and fats accounted for more than 71% of the almond kernel weight, confirming the high-energy value of longstalk almonds (583.22 kcal/100 g). The protein and lipid contents and the energy value determined herein for longstalk almonds were similar to the values obtained for cultivated almonds (29.65 g/100 g, 50.00 g/100 g and 570.20 kcal/100 g, respectively) [19]. Previous studies have shown that most edible nuts are rich in lipids, which range from 26.1% in coconut seeds to 75.8% in macadamia seeds. The lipids content determined herein for longstalk almonds is in agreement with a previous study of almonds, cashew nuts, and pistachios [6]. In addition, longstalk almond seeds contained a high amount of dietary fibers (9.71 g/100 g, mainly insoluble dietary fibers), in agreement with a previous study of almonds (10.44 g/100 g) [19].

The fatty acid composition of longstalk almond kernel oil was determined by gas chromatography, as shown in Table 2. A high content of unsaturated fatty acids was found in longstalk almond oil (approximately 97.89% of total fatty acids), which is the highest determination in vegetable oils [6]. Monounsaturated fatty acids (mainly oleic acid) represented the principle component (69.11%) of total fatty acids in longstalk almonds, followed by polyunsaturated fatty acids (28.77%, mainly linoleic acid) and saturated acids (2.11%, mainly palmitic acid). The fatty acid profile determined for longstalk almonds was as follows (expressed as percentage of total fatty acids): palmitic (1.54), stearic (0.57), oleic (68.80), palmitoleic (0.15), cis-11-Eicosenoic (0.17), linoleic (28.69) and linolenic (0.08). Among foods with favorable fatty acid profiles, nuts have received particular attention because epidemiological studies have indicated an association between increased consumption of nuts and protection from coronary heart disease (CHD). Like other nuts, longstalk almond nuts are low in saturated fatty acids (SFA) and high in unsaturated fatty acids, giving longstalk almond nuts the same health-benefits status as other nuts [6, 26, 27].

Table 3 presents the amino acid composition and contents of the longstalk almond. As the results show, the kernels were a rich source of acidic amino acids (Glu 25.55%+Asp 11.55%). In addition, every 100 g of longstalk almond protein contained 34.24 g of essential amino acids, 2.43 g of sulfur-containing amino acids and 8.12 g of aromatic amino acids (Table 3). The total essential amino acid content of the longstalk almond (342.4 mg/g protein) was higher than those of other fruits, including peanuts [6]. Based on the required pattern of essential amino acids [28], the protein content determined herein for longstalk almonds meets 65.4% of nutritional requirements. However, similar to other woody oil plant proteins, the proteins in longstalk almonds are incomplete. Lysine was deficient compared to the FAO- and WHO-recommended essential amino acid patterns for adults, whereas there are adequate amounts of other essential amino acids. Therefore, the limiting amino acid of the longstalk almond was lysine (first limiting amino acid). Previous studies have also shown that lysine is the limiting amino acid in other nuts, such as Brazil nuts, traditional cashew nuts, hazelnuts, pistachios, almonds, and macadamia nuts [29, 30]. Five different sugars, namely fructose, glucose, sucrose, maltose, and lactose, were identified in longstalk

Table 3 Amino acid composition and amino acid scores (AAS), according to the WHO/FAO/UNU-required pattern, of longstalk almonds

Amino acid (mg amino acid/g protein)	Required pattern[a]	Longstalk almond[b]
Indispensable (essential)		
His	16.0	23.7
Ile	31.0	31.4
Leu	61.0	72.8
Lys	48.0	**31.4**
Met + Cys	24.0	24.3
Phe + Tyr	41.0	81.2
Thr	25.0	26.8
Trp	6.6	7.9
Val	40.0	42.9
Total	292.6	342.4
AAS (%)	100.0	65.4
Dispensable (non-essential)		
Asp	–	115.5
Glu	–	255.5
Ala	–	46.0
Arg	–	98.2
Gly	–	60.9
Pro	–	40.5
Ser	–	41.1
Total	–	657.7

[a]Prior et al. [25]

[b]Data are means of two replicates. The bold value indicates the first limiting amino acid

Table 4 Content and percentages of free sugars in longstalk almonds (g /100 g)

Sugars	Content[a]	%
Fructose	0.29 ± 0.03	5.09
Glucose	0.99 ± 0.05	17.67
Sucrose	4.04 ± 0.31	72.17
Maltose	0.18 ± 0.04	3.29
Lactose	< 0.10	< 1.79
Total	5.59 ± 0.36	

[a]Values are the means of three repetitions

almonds (Table 4). The total sugar content of the longstalk almond was 5.59 g/100 g kernels. Sucrose was the predominant sugar (72.17% of total sugar content), followed by glucose (17.67% of total sugar content). Other sugars (fructose, maltose, and lactose) were present in low amounts (in percentages of total sugar content, 5.09% fructose, 3.29% maltose, and < 1.79% lactose). Sucrose has been identified as the major constituent in cultivated almonds [31], pecans, and macadamia nuts [32]. Previous studies have suggested that several factors, such as variety, environmental factors, maturity, growing seasons, storage conditions, and time, could influence the sugar content of nuts [6, 33].

Table 5 Mineral compositions of longstalk almonds

Minerals[a] (mg/100 g)	Longstalk almond
P	520.00 ± 12.20
Se (μg/100 g)	26.00 ± 3.20
Cu	5.95 ± 0.25
Zn	46.00 ± 3.00
Mg	207.47 ± 5.23
Fe	4.43 ± 0.38
Mn	0.96 ± 0.06
K	703.22 ± 36.37
Na	3.08 ± 0.13
Ca	244.02 ± 21.52

[a]Data are means ± standard deviations of three replicates

The mineral contents of longstalk almond kernels were determined by ICP-AES. Longstalk almond kernels were found to be rich in some minerals, such as calcium (Ca, 244.02 mg/100 g), potassium (K, 703.22 mg/100 g), phosphorus (P, 520.00 mg/100 g), and magnesium (Mg, 207.47 mg/100 g) (Table 5). The amounts of minerals in longstalk almonds were comparable to those in cultivated almonds (K, 728.0 mg/100 g; P, 474.0 mg/100 g, Ca, 248.0 mg/100 g; Mg, 275.0 mg/100 g) [6]. Notably, longstalk almonds showed high concentrations of zinc (Zn, 46.0 mg/100 g), much higher than zinc-rich pine nuts (6.5 mg/100 g) and cashews (5.8 mg/100 g). Considering the nutritional importance of zinc as an antioxidant and its limited availability in plant-based foods, longstalk almonds can be considered a good source of Zn. In addition, high amounts of selenium were also detected in longstalk almonds (26.0 μg/100 g); in fact, the zinc levels in longstalk almonds were found to be lower only to levels in Brazil nuts [6]. Thus, longstalk almonds are an excellent source of zinc, calcium, and selenium.

The vitamin contents of longstalk almonds are shown in Table 6. Seven different vitamins (thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, folate, and vitamin E) were detected in longstalk almond kernels. Vitamin A, vitamin C and vitamin D were not detected in longstalk almonds. The thiamine content detected was 0.35 mg/100 g, which was higher than those of other almonds (*Prunus dulcis*) [34] and walnuts, but lower than those found in cashew nuts, pecans and hazelnuts [6]. A 100-g serving provides approximately 23.33% of the recommended DV (daily value) [35] (Table 6). The riboflavin content (0.41 mg/100 g) was less than that of other almonds [34] but greater than those of cashew nuts, hazelnuts, pecans and walnuts [6]. A 100-g serving of longstalk

Table 6 Vitamin compositions of longstalk almonds

Vitamin	Composition per 100 g dry weight amount (mg)	%DV[a]
Vitamin A (μg/g)	0.00	0.00
Thaiamine (vit. B1)	0.35	23.33
Riboflavin (vit. B2)	0.41	24.12
Niacin (vit. B3)	4.57	22.85
Pantothenic acid (B5)	0.39	3.90
Vitamin B6	0.18	9.00
Folate (vit. B9) (μg/g)	71.40	17.85
Vitamin C	0.00	0.00
Vitamin D	0.00	0.00
Vitamin E	37.01	185.05

[a]Percent Daily Values (%DV) are for adults or children aged 4 or older and are based on a 2000-calorie reference diet. The daily values may be higher or lower based on individual needs Fourie and Basson [32]

almonds supplies approximately 24.12% of the DV (Table 6). The concentration of niacin (4.57 mg/100 g) was greater than that found in tree nuts [6]. A 100-g serving provides approximately 22.85% of the DV (Table 6). The pantothenic acid (B_5) content (0.39 mg/100 g) was comparable to that of other almonds [34] but less than those of cashews, hazelnuts, pecans, and walnuts [6]. A 100-g serving provides less than 3.90% of the DV (Table 6). The vitamin B_6 content (0.18 mg/100 g) was lower than those of cashew nuts, hazelnuts and walnuts but higher than that of other almonds [34] and equal to that of hazelnuts [6]. A 100-g serving provides approximately 9.0% of the DV (Table 6). The folic acid content (71.4 mg/100 g) was less than those of hazelnuts and walnuts but more than that of almonds [6, 34]. A 100 g serving provides more than 17% of the DV (Table 6).

The vitamin E content of longstalk almonds (37.01 mg/100 g) was greater than other tree nuts [6]. Vitamin-E tocopherols are a class of plant phenolics often encountered in foods and possess important antioxidant and nutritional properties [36]. The γ-tocopherol content in oil extracted from longstalk almond kernels was approximately 33.35 mg/100 g (Table 7), indicating the prevalence of γ-tocopherol in longstalk almonds; α- and δ-tocopherols were present in lower amounts (1.43 and 2.23 mg/100 g, respectively), while β-tocopherol was present in trace amounts. Previous studies have suggested that, in oils, γ-tocopherol is a more potent antioxidant than other tocopherols [37], but its poor vitamin-E activity in biological systems has also been reported [38].

Table 7 Vitamin E content (mg/100 g) in the longstalk almond

Oils	Tocopherols[a]			
	α	β	γ	δ
	1.43 ± 0.00	ND	33.35 ± 1.55	2.23 ± 0.32

[a]Values are means of three repetitions

Phytosterols are important dietary components that contribute to reducing serum cholesterol levels. Therefore, the present study also investigated the composition and content of phytosterols in longstalk almonds (Table 8). Among the phytosterols and phytostanols identified and quantified in

Table 8 Content and percentages of phytosterols in longstalk almonds (mg /100 g)

Sterols	Content[a]	%
24-methylenecholesterol	1.90 ± 0.10	0.50
Campesterol	19.31 ± 0.92	5.09
Stigmasterol	0.82 ± 0.07	0.22
Δ^7-campesterol	2.45 ± 0.16	0.65
Clerosterol	3.68 ± 0.24	0.97
β-sitosterol	281.14 ± 11.23	74.07
Sitostanol	7.28 ± 0.20	1.92
Δ^5-avenasterol	48.74 ± 1.61	12.84
$\Delta^{5,24}$-stigmastadienol	4.62 ± 0.51	1.21
Δ^7-stigmastenol	5.04 ± 0.28	1.33%
Δ^7-avenasterol	4.56 ± 0.12	1.20
Total phytosterols	379.54 ± 15.47	

[a]Values are the means of three repetitions

Table 9 Total phenolic content in whole longstalk almonds

Polyphenol class	Subclass	Content (mg/100 g)
Phenols	Total phenols (mg Catechol/100 g)	62.30 ± 1.20
	Gallic acid	0.60 ± 0.03
	Chlorogenic acid	0.56 ± 0.02
	Syringic acid	4.03 ± 0.17
	Ferulic acid	0.09 ± 0.00
	Vanillic acid	ND[a]
	Sinapic acid	ND
	Caffeic acid	ND
	Gentisic acid	ND
	p-Hydroxybenzoic acid	ND
	p-Coumalic acid	ND
	Protocatechuic Acid	ND
Flavonoids	Total flavonoids (mg Rutin/100 g)	290.80 ± 9.90
	L-Epicatechin	9.37 ± 0.11
	Quercetin	1.24 ± 0.02
	Luteolin	ND
	Vitexin	ND
	Kaempferol	ND
	Hyperoside	ND

[a]*ND* not detected; values are the means of three repetitions

longstalk almonds (24-methylenecholesterol, campesterol, stigmasterol, Δ^7-campesterol, clerosterol, β-sitosterol, sitostanol, Δ^5-avenasterol, $\Delta^{5,24}$-stigmastadienol, Δ^7-stigmastenol, Δ^7-avenasterol), β-sitosterol constituted 74.07% of the total, while Δ^5-avenasterol (12.84%) and campesterol (5.09%) ranked second and third, respectively (Table 8). The remaining phytosterols and cholesterols constituted less than 8.0% of the total. Longstalk almonds had a high total phytosterol content (379.54 mg/100 g), greater than those of almonds, cashew nuts, hazelnuts, pecans and walnuts [6]. These results suggest that longstalk almonds are a good source of phytosterols, which could interfere with cholesterol absorption and reduce serum LDL cholesterol levels [39].

The amount of total phenolics was 62.30 mg catechol/100 g dry material (Table 9). The phenolic acids isolated and identified in longstalk almonds were gallic acid, chlorogenic acid, syringic acid, and ferulic acid. The amount of total flavonoids in longstalk almond kernels was 290.80 mg Rutin /100 g. Several studies have suggested that phenolic compounds have anti-carcinogenic properties [40] and can reduce the risk of CVD by inhibiting LDL oxidation and platelet aggregation and lowering blood pressure [41]. Polyphenolics in almonds and walnuts have been shown to be effective inhibitors of LDL oxidation in animals and humans in vitro. Syringic acid, a phenolic compound present in longstalk almonds, has been shown to exert antimicrobial [42], antidiabetic and anti-endotoxic effects [43]. Contreras et al. have also suggested that L-epicatechin may prevent the alpha-induced loss of the integrity of the Caco-2 cell barrier in tumor necrosis [44].

The antioxidant activities of the aroma-active compounds in the extracted oil was determined using an oxygen radical absorbance capacity (ORAC) assay with fluorescein as the fluorescent probe. The average ORAC value was determined to be 18.64 ± 0.22 Trolox equivalents (μmol TE g^{-1}) (Fig. 1). The results indicate that longstalk almonds are a good dietary source of antioxidants.

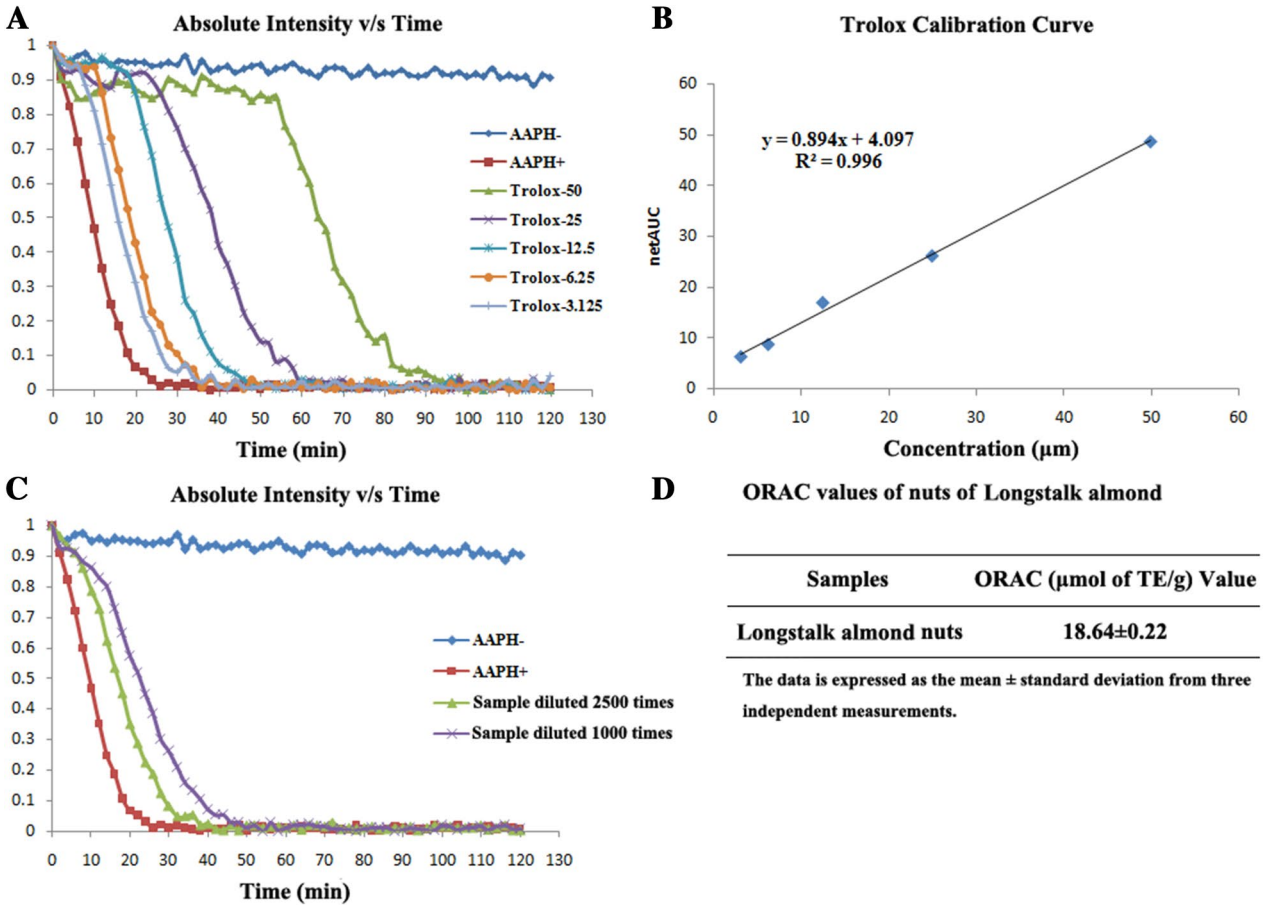

Fig. 1 The ORAC values of longstalk almonds. **a** Fluorescence decay curves of fluorescein induced by AAPH in the presence of different Trolox concentrations in phosphate buffer at pH 7.4 (results are normalized to initial fluorescence signals obtained after the addition of AAPH). **b** Calibration curve for the Trolox standard. **c** Fluorescence decay curves of different concentration samples (used in 1:2500 and 1:1000 dilutions). **d** ORAC values of longstalk almonds

Conclusions

The nutritional composition of longstalk almonds is similar to that of cultivated almonds but also has its own characteristics. The present study investigated the proximate, vitamins, minerals, and phytochemical compositions of longstalk almond seeds. The results indicate that longstalk almonds have a high nutrient density and a high content of quality proteins. As a rich source of oils and unsaturated fatty acids and its significant contribution of essential vitamins and phytosterols, longstalk almonds can be seen as a potential source of useful edible oils and cosmetics. Its high content of plant sterols, particularly β-sitosterol and campesterols, also indicates that this plant can be used to prevent or at least minimize the risk of heart disease. In addition, longstalk almonds are an excellent source of Zn (46.00 mg/100 g), Ca (244.02 mg/100 g), and Se (26.00 μg/100 g). ORAC results indicate that longstalk almond nuts are a good source of antioxidants. The presence of phenolics and flavonoids supports its antioxidant properties. In the present study, some polyphenol compounds were detected; however, many polyphenols were still left intact, such that their compositions require further analysis. Future studies are required to isolate, characterize, and elucidate the structures of bioactive compounds contained in these seeds and to determine their best use for industrial formulations.

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References

1. S.D. Colic, M.M. Fotiric Aksic, K.B. Lazarevic, G.N. Zec, U.M. Gasic, D.C. Dabic Zagorac, M.M. Natic, Fatty acid and phenolic profiles of almond grown in Serbia. Food Chem. **234**, 455–463 (2017)
2. K. Sorkheh, S. Kiani, A. Sofo, Wild almond (*Prunus scoparia* L.) as potential oilseed resource for the future: studies on the variability of its oil content and composition. Food Chem. **212**, 58–64 (2016)
3. S.M.B. Hashemi, S. Raeisi, Evaluation of antifungal and antioxidant properties of edible coating based on apricot (*Prunus armeniaca*) gum containing Satureja intermedia extract in fresh wild almond (*Amygdalus scoparia*) kernels. J. Food Meas. Charact. **12**, 362–369 (2018)
4. Z. Amirshaghaghi, K. Rezaei, M.H. Rezaei, Characterization and functional properties of protein isolates from wild almond. J. Food Meas. Charact. **11**, 1725–1733 (2017)
5. C.F. Chau, S.H. Wu, The development of regulations of Chinese herbal medicines for both medicinal and food uses. Trends Food Sci. Tech. **17**, 313–323 (2006)
6. C. Alasalvar, F. Shahidi, *Tree Nuts: Composition, Phytochemicals, and Health Effects* (CRC Press, Boca Raton, 2008), pp. 1–37
7. E. Ros, S. Rajaram, J. Sabaté, Nuts and novel biomarkers of cardiovascular disease. Am. J. Clin. Nutr. **89**, 1649S–1656S (2009)
8. S. Keser, E. Demir, O. Yilmaz, Phytochemicals and antioxidant activity of the almond kernel (*Prunus dulcis* Mill.) from Turkey. J. Chem. Soc. Pak. **36**, 534–541 (2014)
9. P.E. Milbury, C.Y. Chen, G.G.D. And, J.B. Blumberg, Determination of flavonoids and phenolics and their distribution in almonds. J. Agric. Food Chem. **54**, 5027–5033 (2006)
10. A. Moure, M. Pazos, I. Medina, H. Domínguez, J.C. Parajó, Antioxidant activity of extracts produced by solvent extraction of almond shells acid hydrolysates. Food Chem. **101**, 193–201 (2007)
11. M. Pinelo, M. Rubilar, J. Sineiro, M.J. Nunez, Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). Food Chem. **85**, 267–273 (2004)
12. S. Arranz, G. Chiva-Blanch, P. Valderas-Martínez, A. Medina-Remón, R.M. Lamuela-Raventós, R. Estruch, Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. Nutrients **4**, 759–781 (2012)
13. A. Adeyeye, K. Ajewole, Chemical composition and fatty acid profiles of cereals in *Nigeria*. Food Chem. **44**, 41–44 (1992)
14. J. Kjeldahl, Determination of protein nitrogen in food products. Encyc. Food Agric. **28**, 757–765 (1983)
15. K. Sawicka-Kapusta, Fat extraction in the Soxhlet apparatus, in *Methods for Ecological Energetics*, ed. by B.W. Grodzinski, R.Z. Klekowski, A. Duncan (Blackwell Scientific Publications, Oxford, 1975), pp. 288–293
16. G.E.D.A. Costa, K.D.S. Queiroz-Monici, S.M.P.M. Reis, A.C.D. Oliveira, Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chem. **94**, 327–330 (2006)
17. A.L. Merrill, B.K. Watt, *Energy Value of Foods* (United States Government Printing Office, Washington, 1955)
18. J.S. Amaral, S. Casal, J.A. Pereira, R.M. Seabra, B.P. Oliveira, Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. J. Agric. Food Chem. **51**, 75–79 (2003)
19. A.G.D.O. Sousa, D.C. Fernandes, A.M. Alves, J.B.D. Freitas, M.M.V. Naves, Nutritional quality and protein value of exotic almonds and nut from the Brazilian Savanna compared to peanut. Food Res. Int. **44**, 2319–2325 (2011)
20. X.X. Cai, X.Y. Zhang, Analysis of fructose, glucose, sucrose, lactose and maltose in food by high performance liquid chromatography with evaporative light scattering detector. Chin. J. Health Lab. Tech. **6**, 03 (2007)
21. A. Köksal, N. Artik, A. Şimşek, N. Güneş, Nutrient composition of hazelnut (*Corylus avellana* L.) varieties cultivated in Turkey. Food Chem. **99**, 509–515 (2006)
22. V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. **16**, 144–158 (1965)
23. J.Y. Chen, P.F. Wen, W.F. Kong, Q.H. Pan, S.B. Wan, W.D. Huang, Changes and subcellular localizations of the enzymes involved in phenylpropanoid metabolism during grape berry development. J. Plant Physiol. **163**, 115–127 (2006)
24. K. Wolfe, X. Wu, R.H. Liu, Antioxidant activity of apple peels. J. Agric. Food Chem. **51**, 609–614 (2003)
25. R.L. Prior, H. Hoang, L. Gu, X. Wu, M. Bacchiocca, L. Howard, M. Hampsch-Woodill, D. Huang, B. Ou, R. Jacob, Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. J. Agric. Food Chem. **51**, 3273–3279 (2003)

26. A.N. Yildirim, F. Akinci-Yildirim, B. Şan, Y. Sesli, Total oil content and fatty acid profile of some almond (*Amygdalus communis* L.) Cultivars. Pol. J. Food Nutr. Sci. **66**, 173–178 (2016)
27. Y. Zhu, C. Taylor, K. Sommer, K. Wilkinson, M. Wirthensohn, Influence of deficit irrigation strategies on fatty acid and tocopherol concentration of almond (*Prunus dulcis*). Food Chem. **173**, 821–826 (2015)
28. WHO, Protein and amino acid requirements in human nutrition. World Health Organ. Tech. Rep. Ser. **935**, 1–265 (2007)
29. S. Ruggeri, M. Cappelloni, L. Gambelli, S. Nicoli, E. Carnovale, Chemical composition and nutritive value of nuts grown in Italy. Ital. J. Food Sci. **10**, 243–252 (1998)
30. M. Venkatachalam, S.K. Sathe, Chemical composition of selected edible nut seeds. J. Agric. Food Chem. **54**, 4705–4714 (2006)
31. F. Sauracalixto, J. Canellas, A. Garciaraso, Gas chromatographic analysis of sugars and sugar-alcohols in the mesocarp, endocarp, and kernel of almond fruit. J. Agric. Food Chem. **32**, 1018–1020 (1984)
32. P.C. Fourie, D.S. Basson, Sugar content of almond, pecan, and macadamia nuts. J. Agric. Food Chem. **38**, 101–104 (1990)
33. K. Nomura, Y. Ogasawara, H. Uemukai, M. Yoshida, Change of sugar content in chestnut during low temperature storage. Acta Hortic. **398**, 265–276 (1995)
34. C.Y. Chen, L. Karen, B. Jeffrey, A nutrition and health perspective on almonds. J. Agr. Food Chem. **86**, 2245–2250 (2006)
35. FAO and WHO, Vitamin and Mineral Requirements in Human Nutrition. 2nd edn. (World Health Organization, Geneva, 2005), pp. 1–362
36. R. Brigeliusflohé, M.G. Traber, Vitamin E: function and metabolism. Faseb J. **13**, 1145–1155 (1999)
37. H.S. Olcott, O.H. Emerson, Antioxidants and the autoxidation of fats. IX. The antioxidant properties of the tocopherols. J. Am. Chem. Soc. **59**, 1008–1009 (1937)
38. G.W. Burton, M.G. Traber, E. Vitamin, Antioxidant activity, biokinetics, and bioavailability. Annu. Rev. Nutr. **10**, 357–382 (1990)
39. G.R. Thompson, S.M. Grundy, History and development of plant sterol and stanol esters for cholesterol-lowering purposes. Am. J. Cardiol. **96**, 3–9 (2005)
40. C.S. Yang, J.M. Landau, M.T. Huang, H.L. Newmark, Inhibition of carcinogenesis by dietary polyphenolic compounds. Annu. Rev. Nutr. **21**, 381–406 (2000)
41. B.W. Bolling, C.Y. Chen, D.L. McKay, J.B. Blumberg, Tree nut phytochemicals: composition, antioxidant capacity, bioactivity, impact factors. A systematic review of almonds, Brazils, cashews, hazelnuts, macadamias, pecans, pine nuts, pistachios and walnuts. Nutr. Res. Rev. **24**, 244–275 (2011)
42. K.P. Chong, S. Rossall, M. Atong, In vitro antimicrobial activity and fungitoxicity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against ganoderma boninense. J. Agr. Sci. **11**, 15–20 (2011)
43. J. Muthukumaran, S. Srinivasan, R.S. Venkatesan, V. Ramachandran, U. Muruganathan, Syringic acid, a novel natural phenolic acid, normalizes hyperglycemia with special reference to glycoprotein components in experimental diabetic rats. J. Acute Dis. **2**, 304–309 (2013)
44. T.C. Contreras, E. Ricciardi, E. Cremonini, P.I. Oteiza, (–)-Epicatechin in the prevention of tumor necrosis alpha-induced loss of Caco-2 cell barrier integrity. Arch. Biochem. Biophys. **573**, 84–91 (2015)