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Original Research Article

Phenolic compound composition in immature seeds of fava bean (*Vicia faba* L.) varieties cultivated in Chile

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ABSTRACT

Fava beans (*Vicia faba* L.) are a popular food in many countries. However, there is a lack of information about the phenolic composition of some important edible varieties. Polyphenols in fava beans are located in several parts of the plant (e.g. leaves, roots and seeds) but their occurrence in immature seeds is most important for human nutrition. The objective of this work was to study the phenolic composition of the major groups of polyphenols in 10 varieties of immature fava beans. Total phenolics ranged from 817 to 1337 mg gallic-acid equivalent per kilogram and condensed tannin content ranged from 309 to 958 mg (+)-catechin equivalent per kilogram. Different procyanidins, prodelphinidins, flavonols and flavones were identified using high performance liquid chromatography. Mean levels of total proanthocyanidins were 2233 mg/kg while the sum of flavonols and flavones was 252 mg/kg. The results from this study revealed clear differences in the phenolic composition among different varieties of immature *V. faba* L. seeds and demonstrates that there is ample phenotypic variability for future selections studies for traits such as nutritional value, taste, and ease of production.

1. Introduction

Polyphenols constitute a group of compounds derived from secondary plants metabolism and that are widely distributed in plants and foods of plant origin. Legumes are an important food source and have a significant role in traditional diets in many regions of the world (Amarowicz and Pegg, 2008). Legume seeds are rich in many nutrient components including protein, starch, dietary fiber, fatty acids and nutrients (vitamins, trace minerals). They are also a rich source of many bioactive non-nutrient compounds including phenolic antioxidants (Messina, 1999; Shahidi et al., 2001; Troszynska and Ciska, 2002).

The genus *Vicia* is an important protein source for humans in various parts of the world due to its nutrient composition (Köpke and Nemecek, 2010). The fava bean (*Vicia faba* L.) is an important winter crop in Mediterranean areas and is mostly a spring crop in other regions of Europe and South America and is one of the major plant food item for the Nile River populations (Duc, 1997; Salih and Mustafa, 2008). In Chile, this legume is grown for human consumption as a vegetable, both fresh and frozen, in an area of approximately 2200 ha (ODEPA, 2012).

Phenolic compounds contribute to the overall antioxidant activities of plant foods. Proanthocyanidins (condensed tannins) are a class of phenolic compounds widely distributed in the plant kingdom. Proanthocyanidins are the predominant phenolic compounds in several legume seeds such as lentils (Dueñas et al., 2003; Xu and Chang, 2010).

Various nutritional benefits of fava beans have been described (Apaydin et al., 2000; Macarulla et al., 2001; Salih and Mustafa, 2008). Fava bean seeds contain 22.4–36% protein, 57.8–61% carbohydrates, 12% fiber and 1.2–4% lipids (Hedley, 2001). Due to its chemical composition, fava bean is a suitable food for diabetics and may help prevent heart disease and reduce levels of blood glucose (Karlström et al., 1987; Rizkalla et al., 2002). Bekkara et al. (1998) compared phenolic composition patterns and antioxidant and bioactive properties in seeds from two varieties of *V. faba* (var. Blandine and Alfred), and found that the var. Alfred seed coats were dominated by catechin derivatives, condensed tannins and flavones. The phenolic patterns of the cotyledon and whole seeds were very similar and consisted mainly of phenolic acids, with (+)-catechin and flavones as minor compounds. The seed coat of var. Blandine had phenolic acids, flavones, flavonols

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and dihydroflavonols, while the phenolic profile of whole seeds and cotyledons mainly consisted of phenolic acids (Bekkara et al., 1998). Broad beans are a relatively rich source of flavan-3-ols containing more than 150 mg/kg (De Pascual-Teresa et al., 2000; Dueñas et al., 2006). In addition, pods of fava beans contain caffeoyl-L-malic acid (phaseolic acid) at up to 100 mg/kg (Winter and Herrmann, 1986).

A large amount of genetic variation in floral biology, seed size and composition has been documented in fava beans (Duc, 1997; Vilariño et al., 2009). There have been several studies of phenolic composition in dry fava bean seeds as this is the main means of consumption in the world (Bekkara et al., 1998; Anderson et al., 1999). However, but much less is known about the nutritional value of immature seeds, which is the way the beans are generally consumed in Chile.

As part of a feasibility study for genetic improvement of *V. faba* in Chile, the objective of this study was to compare the composition and contents of different phenolic compounds in the immature seeds from the most-widely consumed varieties in Chile.

2. Materials and methods

2.1. Chemical and reagents

Gallic acid, (+)-catechin and (-)-epicatechin standards were acquired from Sigma Chemical Co. (St. Louis, MO, U.S.A). The flavonols quercetin glucoside, myricetin glucoside and the flavones apigenin glucoside were purchased from Extrasynthèse (Genay, France). Polyethylene membranes of 0.22 and 0.45 μm pore size were acquired from EMD Millipore (Billerica, MA, U.S.A). Folin-Ciocalteau reagent, ammonium iron (II) sulfate, butanol, methanol, hydrochloric acid (HCl), diethyl ether, ethyl acetate, anhydrous sodium sulfate, acetic acid and acetonitrile were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade or higher.

2.2. Plant material

The immature fava bean varieties used in this study are described in Table 1. This study employed two types of growth-habit varieties. The determinate plant type is characterized by a terminal inflorescence. In contrast, the indeterminate plant type produces flowers, but continues to grow and flower for as long as conditions are favorable. In general, these varieties are taller than determinate varieties and produce flowers and fruit along the leaf axis (Robertson and Filippetti, 1991). These varieties were grown in Santiago, Metropolitan Region (33° 34′ SL; 70° 38′ WL) of Chile in 2008. The seeds were harvested for fresh consumption at the same physiological stage (between 65 and 70% humidity) in three replicates of fifty plants each. Each replicate employed the same cultural practices, such as irrigation, fertilization, weeds and disease management, as are commonly used for *V. faba* varieties in the Metropolitan Region of Chile. The fava beans were hand-harvested to eliminate those that were cracked or otherwise damaged. In all varieties, fava beans of each replicate were stored in separate plastic bags at −20 °C prior to analyses.

Table 1
Evaluated fava bean varieties.

Varieties	Distributor	Growth habit
Súper Aguadulce Agrical	Agrical, Chile	Indeterminate
Súper Aguadulce Anasac	Anasac, Chile	Indeterminate
Portuguesa INIA	INIA, Chile	Indeterminate
Luz de Otoño	Fitó, Spain	Indeterminate
Reina Mora[a]	Fitó, Spain	Indeterminate
Alargá	IFAPA, Spain	Determinate
Retaca	IFAPA, Spain	Determinate
Verde Bonita	IFAPA, Spain	Determinate
HBP/SO A/2005	Icarda, Syria	Indeterminate
HBP/SO B/2005	Icarda, Syria	Indeterminate

[a] Only this variety turns its hull purple when its dry, the rest turn whitish.

2.3. Extraction of phenolic compounds from faba beans

The extractions of phenolic compounds from entire beans in all varieties for each replicates were performed as follows: 20 g of seeds were ground with 80 mL of a solution of methanol/water (80:20, v/v) acidified with HCl (0.01%). The extracts were macerated for 2 h at room temperature (20 °C) using a mechanical stirrer model MaxQ 2000 (Barnstead International Inc., Dubuque, IA, U.S.A). The solid portion was separated from the extracts and then this process was repeated twice more with the solid portion. The solids were then discarded and the three liquid phases were combined and centrifuged for 15 min at 2200 *g* (Labofuge 400, Heraeus, Hanau, Germany). The supernatant was then filtered through 0.45 μm pore size membrane and the extract was stored refrigerated until further analysis.

2.4. Spectrophotometric analyses

Total phenolics were quantified using the Folin-Ciocalteu reagent as in Singleton et al. (1999). Gallic acid was used to obtain the standard curve (0–900 mg/L) and the results were expressed as mg gallic acid equivalent (GAE)/kg. Condensed tannins were measured with the acid butanol assay (Porter et al., 1986). The calibration curve was estimated using (+)-catechin as the standard (0–500 mg/L) and the results were expressed as mg (+)-catechin equivalents (CE)/kg. Absorbance measurements were made with a Perkin Elmer UV–Visible spectrophotometer model Lambda 25 (PerkinElmer, Waltham, MA, U.S.A.).

2.5. HPLC-DAD/ESI-MS analysis of low molecular weight phenolic compounds

A 100 mL aliquot of fava bean solution was extracted four times with 20 mL of diethyl ether and four times with 20 mL of ethyl acetate to concentrate phenolic compounds. The organic fractions were combined, dehydrated with 2.5 g of anhydrous sodium sulphate and evaporated to dryness under vacuum at 30 °C using a rotary evaporator (Rotavapor R-210, Büchi AG, Flawil, Switzerland). The solid residue was dissolved in 2 mL of methanol/water (1:1, v/v) solution and filtered through a 0.22 μm pore size membrane.

A chromatographic system used for quantification of individual phenolic compounds. This consisted of an Agilent technologies 1100 series high performance liquid chromatograph (Santa Clara, CA, U.S.A) equipped with a diode-array detector (DAD), model G1315B; a quaternary pump, model QuatPump G1311A; a degasser, model G1379A; a thermostatted column compartment, model G1316A and an autosampler, model G1329A. Aliquots (20 μL) of the final solution were subjected to reversed-phase chromatographic separation at 20 °C on a Nova Pack C_{18} column (300 × 3.9 mm i.d., 4 μm particle size; Waters Corp, Mildford, MA, U.S.A.). A diode array detector was set from 210 to 400 nm with an acquisition speed of 1 s. Two mobile phases were used as follows: A, water/acetic acid (98:2, v/v), and B, water/acetonitrile/acetic acid (78:20:2, v/v/v). The gradient profile was 0–55 min, 100–20% A; 55–70 min, 20–10% A; 70–80 min, 10–5% A; 80–90 min, 100% B, with a flow rate of 1 mL/min. For identification and confirmation through mass spectra, we used a chromatographic system

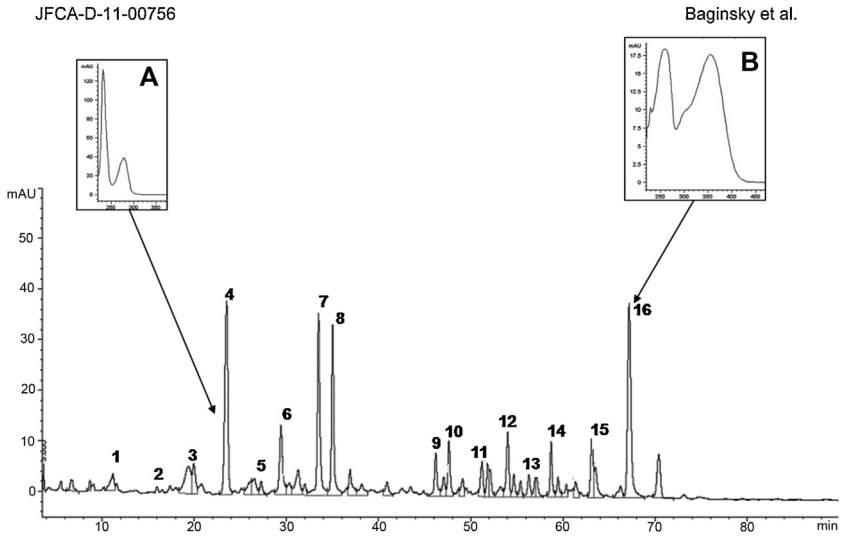

Fig. 1. HPLC-DAD chromatogram at 280 nm of the phenolic compounds of the extract from fava bean var. HBP/SO A/2005. (A) UV-spectra corresponding to procyanidins; (B) UV-spectra corresponding to flavonols. More details are encountered in Table 3.

consisting of a Hewlett-Packard Series 1100 M high performance liquid chromatograph equipped with a diode array detector and a quadrupole mass spectrometer with an electrospray interface (HPLC-DAD/ESI-MS; Palo Alto, CA, U.S.A). The solvent gradient and column used were the same as for HPLC-PAD but with a flow rate of 0.7 mL/min. ESI conditions were as follows: drying gas (nitrogen) flow, 10 L/min; temperature, 340 °C; nebulizer pressure, 40 psi; capillary voltage, 4000 V. The ESI was operated in negative mode and the mass spectra were recorded from *m*/*z* 100 to *m*/*z* 2500 using the following fragmentation program: from *m*/*z* 0 to 200 (100 V), from *m*/*z* 200 to 1000 (200 V) and from *m*/*z* 1000–2500 (250 V). Chromatographic peaks were identified by comparing retention times, UV spectra and data of UV spectral parameters (Bartolomé et al., 1993, 1996) recorded with the photodiode array detector (DAD), with results from the standards (Fig. 1). In addition, ESI-MS spectra were also used to confirm chemical structure of the separated compounds. Compounds with the same shape and wavelength maxima of the UV spectrum as the procyanidins and prodelphinidins, for which standards were not available, were identified as proanthocyanidins by HPLC-DAD. The same criterion was used for those compounds with the UV spectra corresponding to the shape and wavelength maxima of the UV spectrum of the glycosides from quercetin, myricetin or apigenin, identified as flavonols and flavones.

Quantifications were made using the external standard method, with commercial standards (Dueñas et al., 2002). The calibration curves were obtained by injection of standard solutions under the same conditions and range of concentrations as observed in the test samples. Proanthocyanidins (procyanidins and prodelphinidins) were quantified with the curve of (+)-catechin, quercetin derivatives and quercetin glycosides with the curve of quercetin, myricetin and myricetin glycosides with the curve of myricetin, and apigenin glycosides with the curve of apigenin.

2.6. Statistical analysis

Analyses were performed in triplicate and the data are presented as means. Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Statgraphics Plus Version 5.1 (Statistical Graphics Corp, Rockville, MD, U.S.A).

3. Results and discussion

The total phenolics and total condensed tannin contents of the immature fava bean seeds are summarized in Table 2. Among the different fava bean varieties analyzed, total phenolics ranged from 817.02 to 1337.82 mg GAE/kg, while total condensed tannins ranged from 309.28 to 958.77 mg CE/kg, with a mean value of 1096.02 ± 63.67 mg GAE/kg and 453.09 ± 23.48 mg CE/kg, respectively.

The Alargá variety had 38.9% more total phenolics than Luz de Otoño, the variety with the lowest concentration of these compounds. However the latter variety had the highest total

Table 2
Total phenols and condensed tannins in immature seeds of fava beans.

Varieties	Total phenols (mg GAE/kg)	Total condensed tannins (mg CE/kg)
Súper Aguadulce Anasac	1067.26 ± 55.62	402.06 ± 10.78
Súper Aguadulce Agrical	1185.97 ± 87.25	309.28 ± 34.14
Portuguesa INIA	1144.26 ± 64.37	309.28 ± 16.56
Luz de Otoño	817.02 ± 71.05	958.77 ± 26.89
Reina Mora	1326.06 ± 54.61	371.14 ± 33.91
Alargá	1337.82 ± 84.22	502.58 ± 42.11
Retaca	940.01 ± 42.84	448.46 ± 17.22
Verde Bonita	1015.93 ± 63.23	340.21 ± 25.72
HBP/SO A/2005	1103.63 ± 44.11	402.06 ± 11.28
HBP/SO B/2005	1112.18 ± 69.44	487.12 ± 16.23
Mean	1096.02 ± 63.67	453.09 ± 23.48

Data are expressed as mean ± standard deviation ($n = 3$). GAE: gallic acid equivalent; CE: (+)-catechin equivalent.

Table 3
Phenolic compounds identified by HPLC-DAD/ESI-MS in immature fava bean varieties.

Peak n°	Rt[a] (mean)	λ_{max} (nm)	$[H-M]^-$ (m/z)	Fragments (m/z)	Compounds	Faba bean variety[bc]									
						SAN	SAG	LO	PI	RM	ALA	RET	VB	HBA	HBB
1	10.6	277	593	305	Prodelphinidin dimer	–	–	–	–	*	*	–	*	*	*
2	17.4	277	593	305, 289	Prodelphinidin dimer	–	–	–	–	*	*	*	*	*	*
3	19.5	277	577	289	Prodelphinidin dimer	*	*	–	–	*	*	–	*	*	*
4	23.0	279	289		(+)-Catechin	*	*	*	*	*	*	*	*	*	*
5	27.5	280	577	289	Procyanidin dimer	*	*	–	*	*	*	*	*	*	*
6	30.2	280	865	577, 289	Procyanidin trimer	*	*	*	–	*	*	*	*	*	*
7	33.9	279	289		(-)-Epicatechin	*	*	*	*	*	*	*	*	*	*
8	35.9	280	577	289	Procyanidin dimer	*	*	*	*	–	–	*	*	*	*
9	46.0	354, 300(s)[d], 256	609	301	Quercetin 3-*O*-rutinoside	*	*	*	*	*	*	*	*	*	*
10	47.6	267, 295(s), 339	431		Apigenin 7-*O*-galactoside	*	*	*	*	*	*	*	*	*	*
11	51.2	267, 295(s), 339	431		Apigenin 7-*O*-glucoside	*	*	*	*	*	–	–	–	*	*
12	54.0	354, 300(s), 256	463	301	Quercetin 3-*O*-galactoside	*	*	*	*	*	–	*	*	*	*
13	56.1	349, 300(s), 261	479	317	Myricetin-3-*O*-glucoside	*	*	*	*	*	–	*	*	*	*
14	58.3	354, 300(s), 256		301	Quercetin derivative	*	*	*	*	*	*	–	–	*	*
15	63.3	354, 300(s), 256	447	285	Quercetin 3-*O*-glucoside	*	*	*	*	*	*	*	*	*	*
16	67.4	371, 255	463	301	Myricetin	*	*	*	*	*	*	*	*	*	*

[a]Rt: retention time referenced to HBA variety; [c]*: detected; –: not detected; [d](s): shoulder.
[b]SAN: Súper Aguadulce Anasac; SAG: Súper Aguadulce Agrical; LO: Luz de Otoño; PI: Portuguesa INIA; RM: Reina Mora; AL: Alargá; RET: Retaca; VB: Verde Bonita; HBA: HBP/SO A/2005; HBB: HBP/SO B/2005.

condensed tannins concentration of all the varieties studied, with 67.74% more than the Portuguesa INIA variety, the sample with the lowest concentration of these compounds. Total condensed tannins showed considerable variations among the samples and significant differences ($p \leq 0.05$) were found among most fava bean varieties. These results are lower than those reported by other authors for other *V. faba* varieties (Almeida et al., 2008; Chaieb et al., 2011). Some authors, when studying the phenolic content in ethanolic extracts of different plant parts, concluded that polyphenolic content depends more on the plant parts being test

Table 4
Concentration of phenolic compounds (mg/kg of fresh plant material) in immature seeds of ten varieties of fava beans.

Peak n^{o}	Compounds	S. A. Anasac	S. A. Agrical	Luz de Otoño	Portuguesa INIA	Reina Mora
1	Prodelphinidin dimer	nd	nd	nd	nd	336.36 ± 11.57
2	Prodelphinidin dimer	nd	nd	nd	nd	201.80 ± 9.90
3	Prodelphinidin dimer	199.01 ± 18.97	81.71 ± 15.56	nd	nd	501.79 ± 13.11
4	(+)-Catechin	643.90 ± 20.45	613.16 ± 10.88	151.19 ± 9.34	198.16 ± 15.21	897.66 ± 22.35
5	Procyanidin dimer	78.41 ± 7.78	71.58 ± 8.46	nd	46.53 ± 6.87	252.19 ± 9.79
6	Procyanidin trimer	146.52 ± 22.35	168.14 ± 13.73	64.60 ± 7.46	nd	470.10 ± 7.27
7	(-)-Epicatechin	653.67 ± 18.45	646.66 ± 19.54	148.22 ± 11.76	528.42 ± 22.61	703.91 ± 14.56
8	Procyanidin dimer	195.41 ± 16.37	258.64 ± 14.65	178.91 ± 7.87	527.28 ± 18.65	nd
	Total proanthocyanidins	1916.92 ± 104.37	1839.89 ± 82.82	542.92 ± 27.09	1300.39 ± 63.34	3363.81 ± 88.55
9	Quercetin 3-*O*-rutinoside	56.91 ± 9.61	23.95 ± 3.67	7.40 ± 2.11	53.97 ± 6.78	20.46 ± 2.89
10	Apigenin 7-*O*-galactoside	20.66 ± 3.45	27.93 ± 4.25	24.90 ± 3.67	14.62 ± 1.29	31.77 ± 1.89
11	Apigenin 7-*O*-galactoside	31.13 ± 1.11	26.51 ± 3.28	8.97 ± 2.21	32.44 ± 2.79	31.79 ± 2.89
12	Quercetin 3-*O*-galactoside	82.12 ± 3.97	50.30 ± 4.76	8.33 ± 3.12	57.55 ± 3.87	53.43 ± 4.78
13	Myricetin 3-*O*-glucoside	25.28 ± 2.98	37.72 ± 2.76	9.99 ± 1.89	11.94 ± 1.98	25.53 ± 1.65
14	Quercetin derivative	19.06 ± 2.76	26.34 ± 3.68	nd	12.79 ± 2.76	23.14 ± 1.98
15	Quercetin 3-*O*-glucoside	22.73 ± 4.10	14.50 ± 2.89	12.94 ± 2.45	7.53 ± 1.28	5.14 ± 1.10
16	Myricetin	119.49 ± 6.37	50.08 ± 1.99	28.99 ± 1.11	99.10 ± 4.89	28.23 ± 2.12
	Total flavonols + flavones	377.38 ± 34.35	257.33 ± 27.28	101.52 ± 16.56	289.94 ± 25.64	219.49 ± 19.30
Peak n^{o}	Compounds	Alargá	Retaca	Verde Bonita	HBP/SO A/2005	HBP/SO B/2005
1	Prodelphinidin dimer	336.32 ± 3.78	nd	206.79 ± 9.89	47.03 ± 2.87	549.18 ± 9.48
2	Prodelphinidin dimer	320.44 ± 5.45	219.47 ± 7.83	199.17 ± 3.78	292.90 ± 6.76	81.82 ± 3.29
3	Prodelphinidin dimer	422.89 ± 12.89	nd	165.56 ± 2.98	1150.95 ± 24.35	19.99 ± 1.21
4	(+)-Catechin	978.34 ± 18.32	330.64 ± 8.76	932.84 ± 13.24	84.62 ± 8.76	180.57 ± 4.65
5	Procyanidin dimer	139.58 ± 8.87	170.88 ± 4.95	113.61 ± 6.89	263.99 ± 5.65	1352.55 ± 15.87
6	Procyanidin trimer	782.01 ± 13.65	119.11 ± 6.22	522.65 ± 8.78	59.95 ± 2.34	56.01 ± 2.71
7	(-)-Epicatechin	675.44 ± 5.90	310.01 ± 4.86	558.66 ± 9.53	572.78 ± 11.65	773.94 ± 11.56
8	Procyanidin dimer	nd	200.88 ± 9.43	50.84 ± 1.38	82.00 ± 4.98	48.19 ± 1.77
	Total proanthocyanidins	3655.02 ± 68.86	1350.99 ± 42.05	2750.12 ± 56.47	2554.22 ± 67.36	3062.25 ± 50.54
9	Quercetin 3-*O*-rutinoside	19.29 ± 1.11	19.90 ± 1.98	17.90 ± 2.56	34.50 ± 2.45	43.12 ± 1.21
10	Apigenin 7-*O*-galactoside	168.08 ± 2.23	56.08 ± 2.76	42.35 ± 3.21	13.42 ± 1.27	34.84 ± 0.77
11	Apigenin 7-*O*-galactoside	nd	nd	nd	15.68 ± 0.99	47.56 ± 2.32
12	Quercetin 3-*O*-galactoside	nd	64.44 ± 4.76	66.15 ± 5.11	74.91 ± 3.32	92.41 ± 1.89
13	Myricetin 3-*O*-glucoside	nd	12.36 ± 1.02	10.40 ± 0.78	31.31 ± 1.45	41.98 ± 3.23
14	Quercetin derivative	20.56 ± 1.01	nd	nd	10.38 ± 0.98	28.88 ± 2.44
15	Quercetin 3-*O*-glucoside	23.34 ± 2.38	12.79 ± 1.29	25.91 ± 1.98	9.05 ± 1.56	17.94 ± 1.32
16	Myricetin	18.81 ± 2.11	61.58 ± 3.75	27.10 ± 2.23	71.13 ± 3.87	45.74 ± 2.67
	Total flavonols + flavones	250.08 ± 8.84	227.15 ± 15.56	189.81 ± 15.87	260.38 ± 15.89	352.47 ± 15.85

Data are expressed as mean ± standard deviation ($n = 3$); nd: not detected.

than the cultivars (Romani et al., 2003; Marinova et al., 2005). In Chile, faba beans consumption is performed with immature seeds with high moisture content. In this case, the high concentration of water in the samples could decrease the amount of phenolic compounds in the bean extracts.

To obtain more-complete data on the phenolic composition of fava beans, samples were analyzed by HPLC-DAD-MS. An HPLC-DAD chromatogram is presented in Fig. 1, with peaks that correspond to those in Table 3. Phenolic compounds were identified by UV spectra and ESI-MS spectrometric data (Table 3) and included several proanthocyanidins such as procyanidins and prodelphinidins, monomers such as (+)-catechin and (-)-epicatechin, flavonols derivatives of quercetin and myricetin, and flavones such as apigenin derivatives. A summary of the concentrations of phenolic compounds are shown in Table 4. Significant differences ($p \leq 0.05$) in the content of these compounds were found among most of the ten fava bean varieties.

Previous studies (Hussein et al., 1990; Merghem et al., 2004) demonstrated that proanthocyanidins are important phenolic compounds of *V. faba* beans. Here, the Alargá variety had the highest concentration of proanthocyanidins (3655.02 ± 68.86 mg/kg), 6.7 times greater than the Luz de Otoño variety, which had 542.92 ± 27.09 mg/kg of these compounds. Most notable were the high concentrations of (+)-catechin in the samples from Reina Mora, Alargá and Verde Bonita. In addition, there was a notable difference in the concentration of total phenols relative with condensed tannins in Luz de Otoño variety, which is possibly the results of other compounds (e.g. sugars, proteins) that can produce a skewed spectrophotometric results because of spectral interference, as was observed by Escarpa and González (2001) in legumes such as green beans. Additional studies, combined with comprehensive analyses of the chemical composition of sugars, mineral, proteins, and other parameters are essential to corroborate these findings.

Flavonol myricetin and quercetin derivatives and flavone apigenin derivatives were observed in some of the studied varieties. This is consistent with others studies that have reported that in *V. faba* beans, the flavonols are mainly myricetin, kaempferol and quercetin, both free aglycones and glycosides (Nozzolillo et al., 1989; Tomás-Barberán et al., 1991). The concentration of flavonol monoglycosides range between 1 and 20 micrograms of flavonoid/l00 g of fresh plant material - and for di- and triglycosides range between 0.08 and 22.2 miligrams of flavonoid/l00 g of fresh plant material, depending on the compound and stage of maturity of the pods of *V. faba* (Tomás-Barberán et al., 1991).

The samples corresponding to S.A. Anasac and HBP/SO B/2005 varieties had the highest content of the sum of flavonol and flavone concentrations (377.38 ± 34.35 and 352.47 ± 15.85 mg/kg, respectively), while Luz de Otoño and Verde Bonita varieties had the lowest (101.52 ± 16.56 and 189.81 ± 15.87 mg/kg, respectively).

The concentration and presence of these compounds in plants will vary depending upon many factors, including plant genetic variation, soil composition, cultural factors and climate (Haselgrove et al., 2000). In this study, all varieties were grown in the same field and only in one season, so the observed differences in the concentration of phenolic compounds among varieties are likely to primarily reflect genotypic variation. Genetic factors may also explain the differences in overall phenolic composition and low molecular weight phenols observed relative to those reported by other authors, in addition with to differences in climatic and cultural conditions. Further studies that take into account different years with different environmental conditions are needed to quantify the influence of environmental conditions on phenolic composition in this type of annual crop.

4. Conclusions

The analysis of ten samples of fresh faba bean varieties produced in Chile for their phenolic compound content, total phenolics and condensed tannins, and the individualized phenols by HPLC demonstrated considerable variation in their total phenolic and condensed tannin content. Reina Mora and Alargá had the highest content of total phenolics and Luz de Otoño the highest content of total condensed tannins. In contrast, Alargá had the highest concentration of proanthocyanidins and Super Aguadulce Anasac had the highest concentration of the sum of flavonols and flavones. The food industry may prefer fava beans with high phenolic content to promote the benefits for human health with the consumption of vegetable with a higher content of antioxidant compounds. The large amount of variation observed among the ten varieties is promising for future efforts to develop and improve strains of fava beans in Chile for maximal nutritional content, taste, and phenotypic growth characteristics.

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References

Almeida, D., Geiner, R., Furtunado, D., Trigueiro, I., Araújo, M., 2008. Content of some antinutritional factors in bean cultivars frequently consumed in Brazil. International Journal of Food Science and Technology 43, 243–249.

Amarowicz, R., Pegg, R.B., 2008. Legumes as a source of antioxidants. European Journal of Lipid Science and Technology 110, 855–878.

Anderson, J.W., Smith, B.M., Washnock, C.S., 1999. Cardiovascular and renal benefits of dry bean and soybean intake. American Journal of Clinical Nutrition 70, 464S–474S.

Apaydin, H., Ertan, S., Özekmekçi, S., 2000. Broad bean (*Vicia faba*) – A natural source of L-dopa-Prolongs on periods in patients with Parkinson's disease who have on-off fluctuations. Movement Disorders 15, 164–166.

Bartolomé, B., Bengoechea, M.L., Gálvez, M.C., Pérez-Ilzarbe, F.J., Hernández, T., Estrella, I., Gómez-Cordovés, C., 1993. Photodiode array detection for elucidation of the structure of phenolic compounds. Journal of Chromatography A 655, 119–125.

Bartolomé, B., Hernández, T., Bengoechea, M.L., Quesada, C., Gómez-Cordovés, C., Estrella, I., 1996. Determination of some structural features of procyanidins and related compounds by photodiode-array detection. Journal of Chromatography A 723, 19–26.

Bekkara, F., Jay, M., Viricel, M.R., Rome, S., 1998. Distribution of phenolic compounds within seed and seedlings of two *Vicia faba* varieties differing in their seed tannin content and study of their seed and root phenolic exudations. Plant and Soil 203, 27–36.

Chaieb, N., González, J.L., López-Mesas, M., Bouslama, M., Valiente, M., 2011. Polyphenols content and antioxidant capacity of thirteen faba bean (*Vicia faba* L.) genotypes cultivated in Tunisia. Food Research International 44, 970–977.

De Pascual-Teresa, S., Santos-Buelga, C., Rivas-Gonzalo, J.C., 2000. Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. Journal of Agricultural and Food Chemistry 48, 5331–5337.

Duc, G., 1997. Faba bean (*Vicia faba* L.). Field Crops Research 53 (1–3) 99–109.

Dueñas, M., Hernández, T., Estrella, I., 2002. Phenolic composition of the cotyledon and the seed coat of lentils (*Lens culinaris* L.). European Food Research and Technology 215, 478–483.

Dueñas, M., Hernández, T., Estrella, I., 2006. Assessment of in vitro antioxidant capacity of the seeds coat and cotyledon of legumes in relation to their phenolic contents. Food Chemistry 98, 95–103.

Dueñas, M., Sun, B.A., Hernández, T., Estrella, I., Spranger, I., 2003. Proanthocyanidins composition in the seed coat of lentils (*Lens culinaris* L.). Journal of Agricultural and Food Chemistry 51, 7999–8004.

Escarpa, A., González, M.C., 2001. Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Analytica Chimica Acta 427, 119–127.

Haselgrove, L., Botting, D., Van Heeswijck, R., Hoj, P.B., Dry, P.R., Ford, C., Iland, P.G., 2000. Canopy microclimate and berry composition: the effect of bunch exposure on the phenolic composition of *Vitis vinifera* L cv. Shiraz grape berries. Australian Journal of Grape and Wine Research 6, 141–149.

Hedley, C., 2001. Carbohydrates in Grain Legume Seeds. Improving Nutritional Quality and Agronomic Characteristics. CABI Publishing, Wallingford, UK.

Hussein, L., Fattah, M.A., Salem, E., 1990. Characterization of pure proanthocyanidins isolated from the hulls of faba beans. Journal of Agricultural and Food Chemistry 38, 95–98.
Karlström, B., Vessby, B., Asp, N.G., Boberg, M., Lithell, H., Berne, C., 1987. Effects of leguminous seeds in a mixed diet in non-insulin-dependent diabetic patients. Diabetes Research 5, 199–205.
Köpke, U., Nemecek, T., 2010. Ecological services of faba bean. Field Crops Research 115, 217–233.
Macarulla, M.T., Medina, C., De Diego, M.A., Chavarri, M., Zulet, M.A., Martinez, J.A., Noel-Suberville, C., Higueret, P., Portillo, M.P., 2001. Effects of the whole seed and a protein isolate of faba bean (*Vicia faba*) on the cholesterol metabolism of hypercholesterolaemic rats. British Journal of Nutrition 85, 607–614.
Marinova, D., Ribarova, F., Atanassova, M., 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy 40, 255–260.
Merghem, R., Jay, M., Brun, N., Voirin, B., 2004. Qualitative analysis and HPLC isolation and identification of procyanidins from *Vicia faba*. Phytochemical Analysis 15, 95–99.
Messina, M.J., 1999. Legume and soybeans: overview of their nutritional profiles and health effects. American Journal of Clinical Nutrition 70, 439S–450S.
Nozzolillo, C., Ricciardi, L., Lattanzio, V., 1989. Flavonoid constituents of seed coats of *Vicia faba* (Fabaceae) in relation to genetic control of their color. Canadian Journal of Botany 67, 1600–1604.
ODEPA (Oficina de Estudios y Políticas Agrarias). (2012). Boletín estadístico de hortalizas frescas: superfice, precios y comercio exterior. Retrieved July 11, 2012 from: h*ttp://www.odepa.gob.cl.
Porter, L.J., Hritsch, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.
Rizkalla, S.W., Bellisle, F., Slama, G., 2002. Health benefits of low glycaemic index foods, such as pulses in diabetic patients and healthy individuals. British Journal of Nutrition 88, S255–S262.
Robertson, L., Filippetti, A., 1991. Alternative plant type of faba bean. Options Méditerranéennes 10, 33–39.
Romani, A., Vignolini, P., Galardi, C., Aroldi, C., Vazzana, C., Heimler, D., 2003. Polyphenolic content in different plant parts of soy cultivars grown under natural conditions. Journal of Agricultural and Food Chemistry 51, 5301–5306.
Salih, M., Mustafa, A., 2008. A substance in broad beans (*Vicia faba*) is protective against experimentally induced convulsions in mice. Epilepsy and Behavior 12 (1) 25–29.
Shahidi, F., Chavan, U.D., Naczk, M., Amarowicz, R., 2001. Nutrient distribution and phenolic antioxidants in air-classified fractions of beach pea (*Lathyrus maritimus* L.). Journal of Agricultural and Food Chemistry 49, 926–933.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, Oxidants and antioxidants Part A 299, 152–178.
Tomás-Barberán, F.A., Garcia-Grau, M.M., Tomas-Lorente, F., 1991. Flavonoid concentration changes in maturing broad bean pods. Journal of Agricultural and Food Chemistry 39, 255–258.
Troszynska, A., Ciska, E., 2002. Phenolic compounds of seed coats of white and coloured varieties of pea (*Pisum sativum* L.) and their total antioxidant activity. Czech Journal of Food Sciences 20, 15–22.
Vilariño, M., Métayer, J.P., Crépon, K., Duc, G., 2009. Effects of varying vicine convicine and tannin contents of faba bean seeds (*Vicia faba* L.) on nutritional values for broiler chicken. Animal Feed Science and Technology 150, 114–121.
Winter, M., Herrmann, K., 1986. Esters and glucosides of hydroxycinnamic acids in vegetables. Journal of Agricultural and Food Chemistry 34, 616–620.
Xu, B.J., Chang, S.K.C., 2010. Phenolic substance characterization and chemical and cell-based antioxidant activities of 11 lentils grown in the Northern United States. Journal of Agricultural and Food Chemistry 58, 1509–1517.