Biocatalysis and Agricultural Biotechnology 8 (2016) 126–129

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Free amino acids, including canavanine, in the seeds from 32 *Vicia* species belonging to subgenus *Vicilla*

Cristina Megías, Isabel Cortés-Giraldo, Julio Giron-Calle, Manuel Alaiz, Javier Vioque *

Department of Food Phytochemistry, Instituto de la Grasa (C.S.I.C.), Campus Universidad Pablo de Olavide, Carretera de Utrera Km 1, 41013 Sevilla, Spain

ARTICLE INFO

Article history:
Received 27 January 2016
Received in revised form
17 June 2016
Accepted 1 September 2016
Available online 3 September 2016

Keywords:
Vicia
Free amino acids
Canavanine

ABSTRACT

The free amino acid composition of the seeds from 32 *Vicia* species has been determined. Free amino acids ranged from 0.25 g/100 g flour in *V. onobrychioides* to 3.94 g/100 g flour in *V. incana*. Canavanine and metabolically related amino acids were the most abundant. Canavanine by itself was the most abundant in many species, especially those belonging to sections *Cracca* and *Panduratae*. Section *Cracca* includes the species with the highest contents in canavanine, going from as low as 0.09 g/100 g flour in *V. sicula* to as much as 3.27 g/100 g flour in *V. incana*. Our results show that several *Vicia* species are very rich in canavanine and may represent a source of this amino acid for functional applications.

1. Introduction

In legumes, secondary compounds include polyphenols, alkaloids, and free amino acids. Free amino acids include the 20 amino acids found in proteins, and non-protein amino acids. The latter have been traditionally considered antinutritional compounds. There are several examples of these amino acids in *Vicia*. Thus, *β*-Cyano-L-alanine is present in subgenus *Vicia*, including *V.* sativa (Megías et al., 2014), and canavanine is found in subgenus *Vicilla*, including *V. disperma* (Megías et al., 2015).

Canavanine is an arginine analogue that is incorporated into proteins in plant predators, resulting in aberrant non-functional proteins (Rosenthal and Dahlman, 1991). In addition to defense from predators, canavanine has been related with drought tolerance (Enneking, 1995) and allelopathy (Nakajima et al., 2001).

Despite its antinutritional properties, canavanine is also considered a functional compound since inhibits cell proliferation (Bence et al., 2002) and induces apoptosis (Jang et al., 2002). Another interesting property of canavanine is the selective inhibition of inducible nitric oxide (NO) synthase. This could limit excessive NO during inflammatory or infectious diseases, without affecting constitutive NO production (Teale and Atkinson, 1994; Abd El-Gawad and Khalifa, 2001).

The presence of canavanine in *Vicia* species belonging to subgenus *Vicilla* was described more than 40 years ago using thin layer chromatography (Bell and Tirimanna, 1965). We have now carried out quantification of free amino acids, including canavanine, in 32 species belonging to subgenus *Vicilla*, by RP-HPLC. The main objective was to screen *Vicia* species belonging to subgenus *Vicilla* for new taxa that could be of interest as sources of canavanine or other free amino acids.

2. Experimental

2.1. Materials

D, L α-aminobutyric acid, amino acid standards and acetonitrile were from Sigma-Aldrich. Diethyl ethoxymethylenemanolate was from Fluka. All other chemicals were of analytical grade.

2.2. Plant material

Vicia seed samples were provided by the following seedbanks: Centro de Recursos Fitogenéticos, Spain; Margot Forde Forage Germplasm Centre, New Zealand; Gene Bank Gatersleben, Germany; Millenium Seed Bank, United Kingdom; National Plant Germplasm System, USA and Gene Bank Department Division, Czech Republic. *V. benghalensis*, *V. disperma* and *V. monardii* were collected in southern Spain and identified by the authors.

2.3. Determination of free amino acids

Vicia seeds were ground and the resulting flour was extracted by stirring in aqueous ethanol (10% (w/v) in 60% (v/v) ethanol) for 1 h. The pellets after centrifugation at 12000*g* for 20 min were extracted twice more, and the combined supernatants were used for determination of free amino acids by RP-HPLC. Free amino

* Corresponding author.
E-mail address: jvioque@cica.es (J. Vioque).

http://dx.doi.org/10.1016/j.bcab.2016.09.001
1878-8181/

Table 1
Free amino acid composition of *Vicia* species. Data are the average ± standard deviation of the number of populations analyzed (n). Duplicates of each population were carried out.

Amino acids (g/100 g amino acids)	Sec. *Cassubicae* *V. cassubica*	Sec. *Cassubicae* *V. orobus*	Sec. *Cracca* *V.benghalensis*	Sec. *Cracca* *V. cirrhosa*	Sec. *Cracca* *V. costata*	Sec. *Cracca* *V. cracca*	Sec. *Cracca* *V. dasycarpa*	Sec. *Cracca* *V. disperma*	Sec. *Cracca* *V. eriocarpa*	Sec. *Cracca* *V. glauca*
Aspartic acid	9.91	4.38	1.91	4.01	4.80 ± 0.21	5.18 ± 1.02	11.98 ± 10.61	2.29	5.84 ± 0.47	4.48
Glutamic acid	12.91	4.23	3.86	2.75	14.32 ± 0.58	3.22 ± 0.49	3.62 ± 1.02	1.86	4.67 ± 0.42	7.19
Asparagine	30.99	3.14	3.44	3.69	13.33 ± 2.77	0.98 ± 0.07	8.97 ± 6.64	1.70	4.13 ± 1.29	2.76
Serine	0.00	3.21	0.56	0.27	0.46 ± 0.37	0.19 ± 0.03	0.31 ± 0.20	0.19	0.19 ± 0.05	1.69
Glutamine	0.00	0.00	0.41	0.00	0.00 ± 0.00	0.10 ± 0.14	0.06 ± 0.10	0.01	0.54 ± 0.29	0.00
Histidine	1.62	7.17	6.70	0.11	0.84 ± 0.84	0.78 ± 0.13	0.96 ± 0.55	1.25	0.40 ± 0.24	0.73
Glycine	1.40	0.27	0.14	0.30	2.74 ± 1.01	0.16 ± 0.01	0.24 ± 0.03	0.26	0.21 ± 0.02	0.25
Threonine	6.57	0.17	9.52	0.15	1.46 ± 0.87	0.18 ± 0.13	0.13 ± 0.05	5.48	0.46 ± 0.45	6.32
Arginine	0.00	7.62	3.07	0.42	14.58 ± 6.43	1.40 ± 0.41	4.87 ± 4.18	1.71	2.45 ± 2.46	0.91
Alanine	0.94	0.73	0.51	0.27	0.79 ± 0.19	0.53 ± 0.05	0.47 ± 0.09	0.31	0.32 ± 0.10	0.73
Proline	0.00	0.00	0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00
Tyrosine	0.47	0.27	0.36	0.13	0.10 ± 0.10	0.14 ± 0.03	0.13 ± 0.05	0.11	0.11 ± 0.03	0.13
Valine	2.34	0.46	0.45	0.31	1.07 ± 0.06	0.32 ± 0.01	0.37 ± 0.14	0.13	0.51 ± 0.30	0.40
Methionine	0.00	0.00	0.43	0.00	0.00 ± 0.00	1.37 ± 0.08	1.61 ± 1.52	0.23	0.69 ± 0.15	0.48
Cysteine	0.00	0.00	0.00	0.00	0.00 ± 0.00	0.86 ± 0.30	0.59 ± 0.66	0.00	0.54 ± 0.11	2.08
Isoleucine	0.72	0.20	0.15	0.05	0.39 ± 0.16	0.18 ± 0.08	0.11 ± 0.01	0.09	0.19 ± 0.06	0.19
Tryptophan	0.00	0.00	0.30	0.00	0.00 ± 0.00	0.25 ± 0.35	0.36 ± 0.52	0.20	0.47 ± 0.19	0.18
Leucine	0.86	1.13	0.06	0.00	0.43 ± 0.04	0.23 ± 0.05	0.11 ± 0.02	0.05	0.32 ± 0.10	0.23
Phenylalanine	1.60	0.42	0.11	0.40	1.08 ± 0.33	0.39 ± 0.01	0.30 ± 0.18	0.17	0.32 ± 0.22	0.54
Lysine	0.88	0.47	0.33	0.17	0.45 ± 0.09	0.27 ± 0.03	0.18 ± 0.05	0.19	0.20 ± 0.01	0.21
Canavanine	28.86	66.10	67.67	86.94	43.14 ± 6.79	83.28 ± 2.78	64.64 ± 4.76	83.77	77.44 ± 1.63	70.49
Total free amino acids (g/100 g flour)	0.46	0.97	2.1	1.21	0.56 ± 0.16	1.87 ± 0.43	2.65 ± 0.63	2.99	1.86 ± 0.25	2.32
Canavanine (g/100 g flour)	0.13	0.63	1.42	1.05	0.24 ± 0.01	1.55 ± 0.01	1.71 ± 0.03	2.51	1.44 ± 0.00	1.64
Asp+Glu+Asn+Gln+Thr+Arg+Canav	89.2	85.6	89.9	93.7	91.6	94.3	94.3	88.0	95.5	82.2
n	1	1	1	1	3	2	3	1	2	1

Amino acids (g/100 g amino acids)	Section *Cracca*									
	V. hirsuta	*V. incana*	*V. ludoviciana*	*V. monantha*	*V. monardii*	*V. montbretii*	*V. palaestina*	*V. pseudocracca*	*V. sicula*	*V. scandens*
Aspartic acid	14.36 ± 3.63	1.76	3.98 ± 2.52	17.05 ± 4.90	4.05 ± 0.17	5.53 ± 1.10	3.10 ± 0.63	6.74	9.04	7.62
Glutamic acid	15.16 ± 1.40	5.10	3.89 ± 1.49	8.08 ± 1.26	2.94 ± 0.19	5.90 ± 1.33	3.05 ± 1.44	10.82	16.12	5.79
Asparagine	19.08 ± 2.35	1.96	7.21 ± 8.81	8.57 ± 2.03	3.85 ± 1.74	13.05 ± 4.00	3.25 ± 0.85	11.78	31.88	1.80
Serine	1.17 ± 0.09	0.18	0.23 ± 0.12	0.38 ± 0.13	0.30 ± 0.23	0.33 ± 0.01	0.17 ± 0.18	0.55	0.78	0.54
Glutamine	10.10 ± 13.66	0.21	2.93 ± 5.31	0.12 ± 0.12	0.16 ± 0.01	0.71 ± 0.68	0.35 ± 0.32	0.67	0.00	0.00
Histidine	0.74 ± 0.10	0.40	4.51 ± 8.56	2.31 ± 1.80	0.49 ± 0.07	0.52 ± 0.19	0.21 ± 0.23	1.74	0.97	0.30
Glycine	0.97 ± 0.28	0.27	0.15 ± 0.21	0.62 ± 0.50	0.10 ± 0.02	0.37 ± 0.11	0.23 ± 0.05	0.45	1.29	0.34
Threonine	1.05 ± 0.43	4.25	4.82 ± 5.34	0.65 ± 0.49	12.47 ± 0.42	5.46 ± 1.99	5.42 ± 3.39	0.10	4.40	0.65
Arginine	3.81 ± 0.47	0.43	11.18 ± 19.23	11.52 ± 8.71	2.70 ± 0.92	4.45 ± 4.08	2.66 ± 1.42	11.26	10.05	2.84
Alanine	1.01 ± 0.20	0.30	0.28 ± 0.16	0.93 ± 0.16	0.45 ± 0.19	0.61 ± 0.38	0.30 ± 0.11	1.00	1.63	4.72
Proline	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00
Tyrosine	0.58 ± 0.16	0.04	0.12 ± 0.06	0.29 ± 0.08	0.10 ± 0.00	0.07 ± 0.01	0.08 ± 0.05	0.13	0.29	0.47
Valine	4.01 ± 1.10	0.30	0.17 ± 0.13	0.98 ± 0.53	0.21 ± 0.11	0.52 ± 0.18	0.20 ± 0.19	0.94	0.00	0.95
Methionine	0.29 ± 0.21	0.47	0.00 ± 0.00	0.66 ± 0.70	0.42 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.43	0.00	0.00
Cysteine	0.29 ± 0.30	0.49	0.00 ± 0.00	1.03 ± 0.89	0.30 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.59	0.00	0.00
Isoleucine	0.94 ± 0.60	0.13	0.07 ± 0.05	0.34 ± 0.12	0.18 ± 0.10	0.12 ± 0.04	0.09 ± 0.03	0.37	0.39	0.37
Tryptophan	0.00 ± 0.00	0.10	0.00 ± 0.00	0.11 ± 0.19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.16	0.00	0.00
Leucine	0.64 ± 0.28	0.18	0.06 ± 0.04	0.24 ± 0.03	0.13 ± 0.01	0.22 ± 0.14	0.27 ± 0.19	0.25	0.65	0.10
Phenylalanine	1.17 ± 0.52	0.27	0.13 ± 0.16	0.34 ± 0.30	0.22 ± 0.07	0.26 ± 0.04	0.21 ± 0.10	0.60	1.39	0.81
Lysine	0.51 ± 0.05	0.23	0.26 ± 0.16	0.36 ± 0.14	0.16 ± 0.00	0.21 ± 0.04	0.25 ± 0.15	0.42	0.54	0.28
Canavanine	24.13 ± 4.06	82.90	60.01 ± 25.72	45.44 ± 11.41	70.78 ± 2.87	61.66 ± 8.86	80.16 ± 5.09	49.97	20.54	72.41
Total free amino acids (g/100 g flour)	0.50 ± 0.02	3.94	1.49 ± 0.63	0.95 ± 0.07	2.15 ± 0.09	1.76 ± 0.58	2.92 ± 1.02	0.89	0.44	1.92

Table 1 (*continued*)

Amino acids (g/100 g amino acids)	Section *Cracca*									
	V. hirsuta	*V. incana*	*V. ludoviciana*	*V. monantha*	*V. monardii*	*V. montbretii*	*V. palaestina*	*V. pseudocracca*	*V. sicula*	*V. scandens*
Canavanine (g/100 g flour)	0.12 ± 0.00	3.27	0.89 ± 0.02	0.43 ± 0.00	1.52 ± 0.00	1.09 ± 0.05	2.34 ± 0.05	0.45	0.09	1.39
Asp+Glu+Asn+Gln+Thr+Arg+Canav	87.8	96.6	94.0	91.5	97.0	96.7	98.0	91.3	92.0	91.1
n	2	1	4	3	2	3	5	1	1	1

Amino acids (g/100 g amino acids)	Sec. *Cracca* *V. tenuifolia*	Sec. *Cracca* *V. villosa*	Sec. *Ervilia* *V. ervilia*	Sec. *Ervoides* *V. articulata*	Sec. *Ervum* *V. parviflora*	Sec. *Ervum* *V. pubescens*	Sec. *Ervum* *V. tetrasperma*	Sec. *Panduratae* *V. cretica*
Aspartic acid	2.09 ± 1.05	10.20 ± 6.58	21.98 ± 0.19	33.48 ± 9.17	7.76	12.41	9.51 ± 0.21	2.79
Glutamic acid	2.34 ± 0.26	7.04 ± 2.37	27.52 ± 0.99	11.94 ± 0.25	10.49	9.34	5.70 ± 0.31	2.42
Asparagine	1.63 ± 0.42	5.84 ± 3.11	19.31 ± 2.75	10.44 ± 1.73	11.49	9.63	15.87 ± 0.60	3.56
Serine	0.11 ± 0.02	0.14 ± 0.15	0.95 ± 0.32	1.15 ± 0.29	0.72	0.25	0.49 ± 0.04	0.22
Glutamine	0.16 ± 0.04	0.30 ± 0.09	0.42 ± 0.09	0.34 ± 0.10	0.31	0.65	0.77 ± 0.19	0.12
Histidine	0.32 ± 0.09	2.57 ± 0.81	1.16 ± 0.07	5.99 ± 7.46	2.77	3.71	0.37 ± 0.02	0.13
Glycine	0.12 ± 0.00	0.39 ± 0.06	2.97 ± 1.08	0.85 ± 0.45	0.37	1.85	0.20 ± 0.01	0.18
Threonine	6.00 ± 1.15	0.27 ± 0.11	1.03 ± 0.12	0.73 ± 0.30	0.63	0.35	0.53 ± 0.04	5.76
Arginine	0.68 ± 0.29	3.49 ± 1.50	5.34 ± 0.92	9.65 ± 0.31	36.44	47.34	9.99 ± 1.09	0.22
Alanine	0.20 ± 0.09	0.79 ± 0.15	3.24 ± 0.86	2.06 ± 0.08	0.52	0.87	0.58 ± 0.11	0.23
Proline	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.00 ± 0.00	0.00
Tyrosine	0.30 ± 0.02	0.17 ± 0.08	0.55 ± 0.25	0.48 ± 0.46	0.23	0.65	0.10 ± 0.03	0.08
Valine	0.11 ± 0.01	0.56 ± 0.16	1.09 ± 0.20	0.58 ± 0.21	1.49	2.30	2.31 ± 0.18	0.28
Methionine	0.57 ± 0.25	0.50 ± 0.07	0.12 ± 0.17	0.79 ± 0.38	0.00	0.39	0.84 ± 0.28	0.00
Cysteine	0.23 ± 0.03	1.16 ± 0.04	0.00 ± 0.00	0.80 ± 0.06	0.00	0.00	0.00 ± 0.00	0.00
Isoleucine	0.08 ± 0.02	0.31 ± 0.00	0.57 ± 0.07	0.47 ± 0.20	0.47	0.35	0.16 ± 0.03	0.09
Tryptophan	0.04 ± 0.00	0.30 ± 0.20	0.00 ± 0.00	1.56 ± 0.19	0.53	5.10	0.96 ± 1.36	0.00
Leucine	0.07 ± 0.03	0.22 ± 0.02	3.79 ± 0.91	0.61 ± 0.45	0.42	0.40	0.15 ± 0.04	0.07
Phenylalanine	0.15 ± 0.00	0.53 ± 0.04	0.76 ± 0.11	0.15 ± 0.01	0.76	0.64	0.75 ± 0.05	0.31
Lysine	0.15 ± 0.03	0.26 ± 0.07	0.68 ± 0.11	0.58 ± 0.11	0.56	0.56	0.23 ± 0.02	0.12
Canavanine	84.66 ± 3.74	64.97 ± 15.18	8.42 ± 3.00	17.37 ± 3.18	24.04	3.22	50.48 ± 0.57	83.42
Total free amino acids (g/100 g flour)	3.15 ± 0.09	1.29 ± 0.69	0.33 ± 0.15	0.69 ± 0.00	0.61	0.67	1.09 ± 0.05	2.86
Canavanine (g/100 g flour)	2.67 ± 0.00	0.84 ± 0.11	0.03 ± 0.00	0.12 ± 0.00	0.15	0.02	0.55	2.79
Asp+Glu+Asn+Gln+Thr+Arg+Canav	97.6	92.1	84.0	84.0	91.2	82.3	92.9	98.3
n	2	2	2	2	1	1	2	1

Amino acids (g/100 g amino acids)	Sec. *Pedunculatae* *V. onobrychioides*	Sec. *Vicilla* *V. dumetorum*	Sec. *Vicilla* *V. pisiformis*	Sec. *Vicilla* *V. sylvatica*
Aspartic acid	18.10	5.02 ± 4.24	1.90	3.68 ± 1.55
Glutamic acid	29.27	9.01 ± 4.39	1.88	4.17 ± 0.95
Asparagine	20.25	38.45 ± 4.27	1.75	3.26 ± 2.14
Serine	3.40	0.11 ± 0.15	0.07	0.19 ± 0.15
Glutamine	0.69	0.48 ± 0.11	0.00	0.04 ± 0.06
Histidine	0.96	0.47 ± 0.09	78.43	0.78 ± 0.48
Glycine	1.79	0.37 ± 0.25	0.78	0.33 ± 0.25
Threonine	2.85	0.48 ± 0.16	0.00	0.26 ± 0.14
Arginine	7.25	4.63 ± 2.30	10.38	1.55 ± 0.63
Alanine	2.33	1.05 ± 0.05	0.73	0.34 ± 0.22
Proline	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00
Tyrosine	0.71	0.21 ± 0.09	0.36	0.08 ± 0.07
Valine	4.18	0.09 ± 0.13	0.00	0.22 ± 0.07
Methionine	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00
Cysteine	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00
Isoleucine	0.73	0.17 ± 0.02	0.16	0.04 ± 0.04
Tryptophan	2.00	0.00 ± 0.00	0.00	0.00 ± 0.00
Leucine	0.75	0.23 ± 0.04	0.24	0.21 ± 0.15

acids including canavanine were analyzed by RP-HPLC after derivatization using diethyl ethoxymethylenemanolate (Megías et al., 2015).

3. Results and discussion

The free amino acids composition of the seeds from 32 *Vicia* species is shown in Table 1. In section *Cassubicae*, *V. cassubica* has 0.46% free amino acids, of which 28.9% is canavanine. *V. orobus* has 0.97% free amino acids of which 66.1% is canavanine. *V. ervilia* (sect. *Ervilia*) has 0.33% free amino acids representing canavanine 0.03% dry flour. *V. articulata* (sect. *Ervoides)* has 0.69% free amino acids, representing canavanine 17.37% of these. In section *Ervum* total free amino acids ranged from 0.61% to 1.09% dry flour. Canavanine contents varied considerably within this section, ranging from 3.22% to 50.48% of free amino acids. In section *Panduratae,* analysis of seeds from *V. cretica* revealed 2.86% free amino acids and canavanine represents 83.42% of total free amino acids. In *V. onobrichioides* (sect. *Pedunculatae*) canavanine was found at only 0.01% dry flour. In section *Vicilla* content in total free amino acids ranged from 0.7% dry flour in *V. dumetorum* and *V. pisiformis*, to 1.58% in *V. sylvatica*. By last, section *Cracca* includes species with the highest contents in canavanine, going from 0.09% to 3.27% dry flour. This represents relative canavanine concentrations from 20.5% in *V. sicula* up to 86.9% of free amino acids in *V. incana*. The content in total free amino acids ranged from 0.44% dry flour in *V. sicula* to 3.94% in *V. incana*.

In addition to canavanine, *Vicia* seeds present a high content in amino acids metabolically related to canavanine including arginine, aspartic acid, asparagine, glutamic acid, glutamine, and threonine. This group of amino acids represents more than 80% total free amino acids in all species, with the only exception of *V. pisiformis* (sect. *Vicilla*) which is characterized by a very high relative content in histidine, 78.4%.

The legumes non-protein-amino-acid-accumulating clade is compoused of species, such as *Vicia*, that accumulate free amino acids in their seeds (Cardoso et al., 2013). It comprises about 9464 species and 305 genera. This clade includes many genera important in animal and human nutrition such as *Glycine*, *Phaseolus*, *Vigna*, *Canavalia*, *Astragalus*, *Cicer*, *Medicago*, *Trifolium*, *Lathyrus*, *Pisum*, *Lens* and *Vicia* among others. Canavanine contents vary considerably between the species included in this clade. For example, *Medicago sativa* (alfalfa) and *Vicia* share similar canavanine contents, varying in the first from 1.4% to 1.8% dry matter (Rosenthal and Nkomo, 2000). *Canavalia* species, such as *C. ensiformis*, where canavanine was first described, contains higher canavanine contents varying from 2.5% to 5.1% dry matter (Sridhar and Seena, 2006). However, highest canavanine contents have been observed in taxonomically distant legumes, such as *Robinia pseudoacacia* (9.75% dry matter), *Wisteria floribunda* (12.26%), and *Dioclea megacarpa* (12.71%) (Rosenthal, 1977).

Because of the potential therapeutic applications, canavanine is now starting to be considered as much more than an antinutritional or toxic component in plants. *Vicia* species in sections *Cracca* and *Panduratae,* with high canavanine contents, represent a good source of canavanine. Besides their high content in canavanine, the economic feasibility of using these plants for producing this amino acid would also be supported by their good agronomic properties, and by the availability of methods for easy purification of canavanine as recently described (Megías et al., 2016). An additional advantage of the purification of canavanine from *Vicia* using this method is that it also results in production of a canavanine-free protein concentrate that could be used for animal or human nutrition.

Acknowledgements

This work was carried out with the financial support of Junta de Andalucía (Grant number AGR-257). Thanks are due to the following seed banks for providing *Vicia* seeds: Centro de Recursos Fitogenéticos, Spain; Margot Forde Forage Germplasm Centre, New Zealand; Genebank Gatersleben of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany; Millenium Seed Bank of the Kew Royal Botanic Gardens, United Kingdom; National Plant Germplasm System of the United State Department of Agriculture, USA and Genebank Department Division of Genetics and Plant Breeding Research Institute of Crop Production, Czech Republic.

References

Abd El-Gawad, H.M., Khalifa, A.E., 2001. Quercetin, coenzyme Q(10) and L-canavanine as protective agents against lipid peroxidation and nitric oxide generation in endotoxin-induced shock in rat brain. Pharmacol. Res. 43, 257–263.
Bell, E.A., Tirimanna, A.S.L., 1965. Associations of amino acids and related compounds in the seeds of forty-seven species of Vicia: their taxonomic and nutritional significance. Biochem. J. 97, 104–111.
Bence, A.K., Worthen, D.R., Adams, V.R., Crooks, P.A., 2002. The anti proliferative and immunotoxic effects of L-canavanine and L-canaline. Anticancer Drugs 13, 313–320.
Cardoso, D., Pennington, R.T., de Queiroz, L.P., Boatwright, J.S., Van Wyk, B.-E., Wojciechowski, M.F., Lavin, M., 2013. Reconstructing the deep-branching relationships of the papilionoid legumes. S. Afr. J. Bot. 89, 58–75.
Enneking, D., 1995. Post-harvest detoxification: the key to alternative Vicia grain legumes?. In: Yusuf, H.K.M., Lambein, F. (Eds.), Lathyrus sativus and Human Lathyrism: Progress and Prospects. University of Dhaka, Dhaka, pp. 85–92.
Jang, M.H., Jun, D.Y., Rue, S.W., Han, K.H., Park, W., Kim, Y.H., 2002. Arginine antimetabolite L-canavanine induces apoptotic cell death in human Jurkat T cells via caspase-3 activation regulated by Bcl-2 or Bcl-xL. Biochem. Biophys. Res. Commun. 295, 283–288.
Megías, C., Cortés-Giraldo, I., Girón-Calle, J., Vioque, J., Alaiz, M., 2014. Determination of β-Cyano-L-alanine, γ-Glutamyl-β-cyano-L-alanine, and common free amino acids in Vicia sativa (Fabaceae) seeds by reversed-phase high-performance liquid chromatography. J. Anal. Meth. Chem. 2014, 1–5.
Megías, C., Cortés-Giraldo, I., Girón-Calle, J., Vioque, J., Alaiz, M., 2015. Determination of I-canavanine and other free amino acids in Vicia disperma (Fabaceae) seeds by precolumn derivatization using diethyl ethoxymethylenemalonate and reversed-phase high-performance liquid chromatography. Talanta 131, 95–98.
Megías, C., Cortés-Giraldo, I., Girón-Calle, J., Alaiz, M., Vioque, J., 2016. Purification of canavanine from the legume *Vicia disperma*. Biocatal. Agric. Biotechnol. 5, 150–154.
Nakajima, N., Hiradate, S., Fujii, Y., 2001. Plant growth inhibitory activity of L-canavanine and its mode of action. J. Chem. Ecol. 27, 19–31.
Rosenthal, G.A., 1977. Nitrogen allocation for L-canavnine synthesis and its relationship to chemical defense of the seed. Biochem. Syst. Ecol. 5, 219–220.
Rosenthal, G.A., Dahlman, D.L., 1991. Incorporation of L-canavanine into proteins and the expression of its antimetabolic effects. J. Agric. Food Chem. 39, 987–990.
Rosenthal, G.A., Nkomo, P., 2000. The natural abundance of L-canavanine, an active anticancer agent, in alfalfa, medicago sativa (L.). Pharm. Bull. 38, 1–6.
Sridhar, K.R., Seena, S., 2006. Nutritional and antinutritional significance of four unconventional legumes of the genus Canavalia – a comparative study. Food Chem. 99, 267–288.
Teale, D.M., Atkinson, A.M., 1994. L-canavanine restores blood-pressure in a rat model of endotoxic-shock. Eur. J. Pharmacol. 271, 87–92.