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Fatty Acid Distribution in the Seed Flour of Wild *Vicia* Species from Southern Spain

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Abstract The fatty acid distribution in the seed flour from 31 Vicia taxa distributed throughout southern Spain was analyzed by gas chromatography. Fatty acids ranged from myristic acid to araquidic acid. Linoleic acid (from 28.7 to 66.3% of the fatty acids), oleic acid (from 7.2 to 32.5% of the fatty acids) and linolenic acid (from 2.7 to 16.6% of the fatty acids) were the most abundant among unsaturated ones and palmitic acid among saturated ones. The total unsaturated to saturated fatty acids ratio ranged between 2.6 in V. hirsuta and 4.2 in V. hybrida. Polyunsaturated to monounsaturated fatty acids ratio ranged between 1.3 in V. ervilia and 9.0 in V. pyrenaica. The ω -6 to ω -3 ratio ranged between 1.7 in *V. articulata* and 17.3 in V. faba. The fatty acids distribution observed in the Vicia species studied supports the use of these plants as a source of important dietary lipids.

Keywords *Vicia* · Fatty acids · Seed flours · Legumes

Introduction

Legumes are, together with cereals, the main plant sources of proteins in human diets. However, legume intake has decreased in recent decades in many Western countries [1], despite the fact that legumes have a high content of good quality proteins [2], and also include dietary fiber,

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R. Juan · J. E. Pastor Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, 41012 Sevilla, Spain carbohydrates and a low content of saturated fats. The nutritional benefits of legume consumption have been recognized and related to components, such as fiber, proteins or some minor compounds, such as certain lipids, or polyphenols [3]. Thus, the positive effect of legume intake for the prevention of diseases like diabetes mellitus, coronary heart diseases or colon cancer have been recognized [4].

Plant oils and animal fats are an important component of the human diet, both as a source of energy and as carbon building blocks. Among the main plant oils in the human diet are soy, canola, palm, peanut and sunflower. Oil from plants such as palm and coconut are rich in short chain saturated fatty acids while those of soybean and canola are rich in the more healthy polyunsaturated fatty acids. In general, legumes have a low content of seed lipids, although with a high percentage of unsaturated fatty acids that are recommended from a nutritional and functional point of view [5]. The main fatty acids in legume seed lipids are palmitic, oleic, linoleic and linolenic acids [6].

Omega-3 unsaturated fatty acids are the object of increasing interest due to their health promoting activity related to the reduction of cardiovascular diseases associated with their ingestion [7]. Thus, the consumption of foods rich in ω -3 long chain polyunsaturated fatty acids has suggested to impart multiple positive health benefits. Thus, although present in low amounts, the fatty acids of the seed oil of legumes may have health promoting effects beyond their nutritional characteristics.

The *Vicia* genera belong to the tribe *Fabeae* [8]. These genera include around 190 species located mainly in the Mediterranean region. *Vicia* genera, as other legumes, may grow under drought stress conditions and on poor soils due their capacity to fix atmospheric nitrogen. The best known specie in *Vicia* is the faba bean (*V. faba* L.), that it is an



important pulse, fodder crop and vegetable [9]. Other *Vicia* species marginally cultivated are *V. ervilia*, *V. narbonensis*, *V. sativa*, *V. benghalensis*, *V. articulata*, *V. pannonica* or *V. villosa*. Among these, *V. sativa*, the common vetch, is the most cultivated, mainly in the Mediterranean region.

Vicia, as most legumes, have low oil seed contents. Thus, the reported seed oil contents V. faba ranged from 2.3 to 2.9% [10]. Also, V. faba is the best studied with respect to the fatty acid composition of its seed oil, where a predominance of $C_{18:2}$, $C_{18:1}$ and $C_{16:0}$ has been observed.

In the last decade, a great deal of the world phytodiversity has been lost because local varieties and species have been substituted by commercial ones with a high yield and genetic uniformity. To protect this biodiversity, a diversification of cultivated plant species is necessary, and this can be achieved by increasing the research and knowledge of local species. In this work, the seed flour fatty acid distribution of 28 species from Vicia legumes has been studied. Wild populations of these species were collected in Southern Spain. To our knowledge, this is the first time that wild populations of Vicia species have been studied from a seed flour fatty acid distribution point of view. The aim of the present research was to analyze and compare the fatty acid distribution of the collected Vicia species to determine if they show a favorable seed flour distribution from a nutritional and functional point of view.

Materials and Methods

Material

Fully matured seed samples of the *Vicia* species studies were collected from wild populations located in Andalusia (southern Spain). The seeds were collected from ten specimens in a given population and stored at -20 °C until the fatty acid distribution of the seed flour was determined. Fatty acid standards were purchased from Sigma (#1892, #1894, #1898) (Tres Cantos, Madrid, Spain). All other reagents were of analytical grade.

Determination of Fatty acid Distribution

Fatty acid distribution of seed flours was determined by gas chromatography as methyl esters according to Garcés and Mancha [11] with modifications. Seeds were ground with a domestic blender (190 W power) (Moulinex, Barcelona, Spain). One milliliter of methylation solution (methanol, 39%; sulfuric acid, 5%; dimethoxipropane, 5%; toluene, 2%) and heptane (1 mL) were added to 50 mg of seed flour and incubated at 85 °C for 50 min. The upper phase (1 mL) was dried under nitrogen and redissolved in 50 μL of heptane. A 2-μL sample of this solution was taken for

the analysis of fatty acid methyl esters by gas chromatography.

An HP 5890 series II gas chromatograph equipped with a HP Carbowax 20 M capillary column (25 m length and 0.2 mm ID) was employed. Hydrogen was used as the carrier gas for the gas chromatography analysis at a pressure of 2 kg/cm². Temperatures of the injector, detector and oven were 225, 250 and 170 °C, respectively. Fatty acid methyl esters were identified by comparison with standards.

Statistical Analysis

Results are expressed as the mean values \pm standard deviation of several samples except for species with only one population. The data were statistically analyzed by one way analysis of variance (ANOVA). Means were compared by Scheffe's test; significance was accepted at the 5% level ($P \le 0.05$). Cluster analysis of different taxa was performed using the PRIMER-pc program, employing the Bray–Curtis index of dissimilarity [12]. The dissimilarity index was transformed to the index of similarity (1 – dissimilarity index) \times 100.

Results and Discussion

The main seed flour fatty acids in the *Vicia* species studies ranged from $C_{14:0}$ to $C_{20:0}$ with a predominance of unsaturated fatty acids of the series C_{18} (Table 1). Among saturated fatty acids, $C_{16:0}$ was the most abundant with amounts ranging from 12.5% in *V. hybrida* to 21.3% in *V. incana*. $C_{16:0}$ contents in *V. hybrida* were significantly different only from the three species with the highest $C_{16:0}$ contents (*V. hirsuta*, *V. incana* and *V. pyrenaica*). The second most abundant saturated fatty acid was $C_{18:0}$. *V. pubescens* had the highest content (7.5%) of this fatty acid and was significantly different from the amounts observed in the ten species with $C_{18:0}$ lowest percentages (Table 1).

Consumption of foods rich in saturated fatty acids from 12 to 16 carbon lengths is positively associated with low-density lipoprotein and cardiovascular disorders, whereas $C_{18:0}$ is considered neutral in this respect [13]. Thus, $C_{18:0}$ is less hypercholesterolemic than $C_{16:0}$ [14]. Therefore, species with a low ratio $C_{14:0} + C_{15:0} + C_{16:0}/C_{18:0}$ would be more appropriate for human nutrition. The lowest ratio was observed in V. parviflora and the highest in V. monantha subsp. calcarata (Table 2). Most cultivated species, V. faba and V. sativa, had intermediate ratios with 5.7 and 5.4, respectively. In general, $C_{14:0} + C_{15:0} + C_{16:0}/C_{18:0}$ ratio in the Vicia studied were higher than those observed in the related genera Lathyrus, Lens and Pisum [15]. Other saturated fatty acids present in minor amounts were $C_{14:0}$, $C_{15:0}$



Table 1 Distribution of fatty acid (%) in the seed flour of the *Vicia* species studies (means \pm SD)

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Species	Clades	и	$C_{14:0}$	$C_{15:0}$	$C_{16:0}^{*}$	$C_{16:1}$	$C_{18:0}^{***}$	$C_{18:1}^{**}$	$C_{18:2}^{***}$	$C_{18:3}***$	$C_{20:0}$
V. articulata	Ą	3	2.9 ± 1.3	0.6 ± 0.2	17.4 ± 1.3^{ab}	0.6 ± 0.4	$2.4\pm0.2^{\rm a}$	29.7 ± 3.4 ^{bc}	$28.7\pm1.9^{\rm a}$	$16.6\pm2.9^{\rm d}$	1.1 ± 0.1
V. ervilia	А	ϵ	3.1 ± 0.9	0.8 ± 0.2	17.2 ± 0.8^{ab}	0.9 ± 0.1	$2.4\pm0.3^{\rm a}$	$32.5\pm0.9^{\rm c}$	36.8 ± 2.4^{ab}	5.3 ± 0.2^{ab}	1.0 ± 0.2
V. $faba$	А	_	0.2	0.2	17.5^{ab}	0.2	3.1^{ab}	27.8^{abc}	46.8^{abcd}	2.7^{abcd}	1.5
V. narbonensis	А	ϵ	1.9 ± 1.2	0.3 ± 0.3	14.3 ± 0.7^{ab}	0.5 ± 0.3	$3.1 \pm 0.8^{\mathrm{ab}}$	$30.1 \pm 4.7^{\mathrm{bc}}$	$45.5 \pm 4.6^{\mathrm{abc}}$	$3.2 \pm 0.2^{\mathrm{a}}$	1.1 ± 0.3
V. parviflora	C	2	2.9 ± 0.7	0.4 ± 0.2	15.6 ± 0.4^{ab}	0.5 ± 0.04	$5.9 \pm 0.6^{\mathrm{ab}}$	$12\pm3.5^{\rm a}$	$46.9 \pm 2.3^{\mathrm{abc}}$	$14.8\pm1.8~^{\rm cd}$	1.0 ± 0.2
V. pubescens	C	κ	3.1 ± 2.1	0.8 ± 0.2	14.6 ± 0.4^{ab}	0.6 ± 0.2	$7.5\pm0.2^{\mathrm{b}}$	13 ± 2.4^{ab}	$42.5\pm2.9^{\rm abc}$	$16.6\pm1.6^{\rm d}$	1.3 ± 0.03
V. hybrida	D	4	2.0 ± 1.2	0.6 ± 0.2	$12.5\pm0.9^{\rm a}$	0.2 ± 0.1	$3.7 \pm 0.3^{\mathrm{ab}}$	10 ± 1.1^{a}	$66.3 \pm 2.5^{\mathrm{d}}$	$4.1 \pm 0.3^{\mathrm{ab}}$	0.0 ± 0.0
V. monardi	О	ε	0.7 ± 0.3	0.2 ± 0.05	17 ± 0.9^{ab}	0.2 ± 0.2	$2.2\pm0.3^{\rm a}$	$9.1 \pm 2.9^{\mathrm{a}}$	$63.3\pm2.8~^{\rm cd}$	$6.3 \pm 1.3^{\mathrm{abc}}$	1.0 ± 0.2
V. pyrenaica	О	_	0.3	0.4	17.7 ^{ab}	0.4	3.9^{ab}	7.2 ^{abc}	61.0^{bcd}	8.2^{abcd}	6.0
V. hirsuta	ш	ε	2.7 ± 1.4	0.6 ± 0.2	$20.4\pm0.6^{\rm b}$	0.3 ± 0.1	$2.8\pm0.4^{\rm a}$	11.6 ± 1.1^{ab}	$48.9 \pm 3.3^{\mathrm{abcd}}$	$11.3 \pm 0.8^{\mathrm{abcd}}$	1.4 ± 0.2
V. lathyroides	Щ	ϵ	0.4 ± 0.1	0.5 ± 0.1	19.3 ± 0.6^{ab}	0.7 ± 0.1	$4.4 \pm 0.3^{\mathrm{ab}}$	11.9 ± 1.5^{ab}	$48.3 \pm 2.0^{\text{abcd}}$	$13.4 \pm 0.5^{\mathrm{bcd}}$	1.1 ± 0.1
V. incana	ш	2	1.0 ± 0.8	0.4 ± 0.03	$21.3 \pm 4.2^{\mathrm{b}}$	0.8 ± 0.3	5.1 ± 2.6^{ab}	$9.8 \pm 2.8^{\mathrm{ab}}$	$50.5 \pm 7.9^{\mathrm{abcd}}$	$9.9 \pm 3.8^{\mathrm{abcd}}$	1.2 ± 0.7
V. viciodes	ш	ε	0.4 ± 0.1	0.2 ± 0.1	$21.0\pm2.9^{\rm b}$	0.4 ± 0.1	3.2 ± 0.5^{ab}	$14.6 \pm 4.3^{\mathrm{abc}}$	49.6 ± 3.0^{abcd}	$9.5 \pm 2.6^{\mathrm{abcd}}$	1.1 ± 0.3
V. angustifolia	F1	S	1.9 ± 0.9	0.4 ± 0.1	17.6 ± 0.6^{ab}	0.2 ± 0.2	$3.3 \pm 0.3^{\mathrm{a}}$	$9.2\pm1.8^{\rm a}$	$55.5 \pm 2.9^{\mathrm{bcd}}$	$10.7 \pm 1.6^{\mathrm{abcd}}$	1.2 ± 0.2
V. lutea ssp. lutea var. hirta	F1	5	1.4 ± 0.2	0.6 ± 0.4	15.7 ± 0.7^{ab}	0.6 ± 0.2	$4.5\pm0.3^{\mathrm{ab}}$	$12.0\pm1.7^{\rm a}$	$55.2 \pm 1.0^{\mathrm{bcd}}$	9.2 ± 1.2^{abcd}	0.8 ± 0.1
V. sativa ssp. sativa	F1	5	3.0 ± 1.0	0.7 ± 0.4	16.5 ± 0.5^{ab}	0.8 ± 0.3	$3.7 \pm 0.3^{\mathrm{ab}}$	$13.3\pm1.5^{\rm ab}$	$53.6 \pm 2.6^{\mathrm{bcd}}$	$7.3 \pm 1.5^{\mathrm{abc}}$	1.1 ± 0.2
V. lutea ssp. cavanillesii	F1	_	1.7	0.3	18.2^{ab}	9.0	3.7^{ab}	$13.6^{ m abc}$	54.9^{abcd}	6.1^{abcd}	6.0
V. pseudocracca	F1	S	1.0 ± 1.2	0.4 ± 0.2	17.5 ± 2.5^{ab}	0.3 ± 0.3	3.7 ± 0.8^{ab}	13.1 ± 2.7^{ab}	$54.6 \pm 4.4^{\mathrm{bcd}}$	$7.9 \pm 1.5^{\mathrm{abcd}}$	1.5 ± 0.2
V. cordata	F1	κ	1.5 ± 0.1	0.2 ± 0.02	17.2 ± 0.8^{ab}	0.1 ± 0.1	$3.5\pm0.4^{\mathrm{ab}}$	12.9 ± 0.6^{ab}	$55.3 \pm 1.2^{\mathrm{bcd}}$	$8.2 \pm 0.9^{\mathrm{abcd}}$	1.1 ± 0.1
V. peregrina	F1	$_{\infty}$	1.9 ± 0.7	0.4 ± 0.03	$14.3\pm3.3^{\rm ab}$	0.2 ± 0.1	$4.1 \pm 1.9^{\mathrm{ab}}$	$17.5\pm2.1^{\rm abc}$	$55.8 \pm 2.1^{\mathrm{bcd}}$	5.1 ± 1.2^{ab}	0.7 ± 0.1
V. altissima	F1	1	0.2	0.3	19.3^{ab}	0.3	3.1^{ab}	$15.9^{ m abc}$	54.2^{abcd}	5.9^{abcd}	8.0
V. monantha ssp. calcarata	F1	$_{\infty}$	0.9 ± 0.7	0.2 ± 0.1	18.7 ± 1.1^{ab}	0.4 ± 0.1	$1.9\pm0.2^{\rm a}$	$15.5\pm2.4^{\rm abc}$	$57.7 \pm 2.0^{\text{bcd}}$	$4.1 \pm 0.5^{\mathrm{ab}}$	0.6 ± 0.05
V. lutea ssp. lutea var.lutea	F2	$_{\infty}$	1.1 ± 0.7	1.0 ± 1.2	15.1 ± 0.9^{ab}	0.5 ± 0.4	$4.0 \pm 0.7^{\mathrm{ab}}$	$21.4\pm5.0^{\rm abc}$	$47.8 \pm 3.8^{\text{abcd}}$	$8.4 \pm 0.9^{ m abcd}$	0.7 ± 0.1
V. lutea ssp. vestita	F2	7	1.8 ± 1.5	0.9 ± 0.9	14.6 ± 1.2^{ab}	0.8 ± 0.2	3.5 ± 0.1^{ab}	$22.9\pm2.1^{\rm abc}$	$50.1 \pm 0.3^{\mathrm{abcd}}$	$4.6 \pm 0.3^{\mathrm{abc}}$	0.8 ± 0.04
V. onobrychioides	F2	7	2.1 ± 1.7	0.6 ± 0.4	$14.9\pm0.3^{\rm ab}$	0.5 ± 0.3	$3.5\pm0.0^{\mathrm{ab}}$	$21.2 \pm 4.3^{\rm abc}$	$51.3 \pm 2.8^{\text{abcd}}$	$5.2\pm0.06^{\rm abc}$	0.7 ± 0.02
V. benghalensis	F2	4	0.3 ± 0.03	0.2 ± 0.02	17.6 ± 1.8^{ab}	0.1 ± 0.1	$2.7\pm0.2^{\rm a}$	$20.3 \pm 7.3^{\mathrm{abc}}$	$51.3 \pm 5.2^{\mathrm{bcd}}$	$6.0 \pm 1.0^{\mathrm{ab}}$	1.5 ± 0.1
V. disperma	F2	9	1.2 ± 0.8	0.4 ± 0.1	17.5 ± 0.7^{ab}	0.2 ± 0.3	$3.5\pm0.6^{\rm a}$	$18.9 \pm 2.2^{\mathrm{abc}}$	$52.4 \pm 1.3^{\mathrm{bcd}}$	$5.1 \pm 0.4^{\mathrm{ab}}$	0.8 ± 0.4
V. glauca ssp. giennensis	F2	_	0.3	0.3	17.4^{ab}	0.3	2.9^{ab}	$18.5^{ m abc}$	53.5^{abcd}	5.7^{abcd}	1.1
V. dasycarpa	F2	3	1.5 ± 1.1	0.4 ± 0.3	19.0 ± 1.2^{ab}	0.1 ± 0.1	$3.2 \pm 0.3^{\mathrm{ab}}$	$18.8 \pm 0.2^{\mathrm{abc}}$	$47.5 \pm 2.1^{\mathrm{abcd}}$	$8.6 \pm 0.8^{\mathrm{abcd}}$	0.9 ± 0.1
V. eriocarpa	F2	4	1.4 ± 0.4	0.3 ± 0.1	17.6 ± 1.5^{ab}	0.4 ± 0.3	$3.0\pm0.1^{\rm a}$	$18.3 \pm 4.7^{\mathrm{abc}}$	$50.1 \pm 3.1^{\mathrm{abcd}}$	$8.2 \pm 1.1^{\rm abcd}$	0.7 ± 0.5
V. tenuifolia	F2	4	3.2 ± 1.4	0.6 ± 0.4	17.5 ± 1.2^{ab}	0.3 ± 0.3	$2.6\pm0.5^{\rm a}$	$17.5\pm1.8^{\rm abc}$	$50.2 \pm 3.8^{\mathrm{abcd}}$	$7.2 \pm 1.8^{\mathrm{abc}}$	0.9 ± 0.1
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Clades assignments result from cluster analysis shown in Fig. 1

Different superscript small letters indicate significant differences between values in the same column (Scheffe's test)

n Number of populations studied

*P < 0.05, **P < 0.01, ***P < 0.001

Table 2 Different parameters based on the fatty acid distribution of the seed flour of the Vicia species studied

Species	Clades	и	C _{14:0} + C _{15:0} + C _{16:0} /C _{18:0}	TUFAs	TSFAs	TUFAs/ TSFAs	PUFAs***	MUFAs***	PUFAs/ MUFAs	C18:1/ C18:2	ω-6/ω-3
V. articulata	A	3	8.5	75.6 ± 2.2	24.4 ± 2.2	3.1	$45.3 \pm 2.1^{\text{abc}}$	30.3 ± 3.0^{ab}	1.5	1.0	1.7
V. ervilia	Ą	3	8.8	75.4 ± 1.7	24.6 ± 1.7	3.1	42.1 ± 2.6^{a}	33.3 ± 1.0^{a}	1.3	6.0	6.9
V. faba	Ą	1	5.7	77.4	22.6	3.4	49.4 abcd	28.0^{abcd}	1.8	9.0	17.3
V. narbonensis	Α	3	5.3	79.3 ± 1.0	20.7 ± 1.0	3.8	48.7 ± 4.7^{ab}	$30.6 \pm 4.8^{\mathrm{abc}}$	1.6	0.7	14.2
V. parviflora	C	5	3.2	74.3 ± 0.8	25.7 ± 0.8	2.9	$61.8 \pm 3.4^{\rm d}$	$12.5 \pm 3.4^{\mathrm{bcd}}$	4.9	0.3	3.2
V. pubescens	C	3	2.5	72.7 ± 2.5	27.3 ± 2.5	2.7	$59.1 \pm 3.5^{\mathrm{bcd}}$	$13.6 \pm 2.3^{\mathrm{abcd}}$	4.3	0.3	2.6
V. hybrida	О	4	4.1	80.7 ± 1.9	19.3 ± 1.9	4.2	70.4 ± 2.5^{d}	$10.3 \pm 1.0^{\rm d}$	8.9	0.2	16.2
V. monardi	О	3	7.9	78.9 ± 1.3	21.1 ± 1.3	3.7	69.6 ± 3.1^{d}	9.3 ± 3.0^{d}	7.5	0.1	10.0
V. pyrenaica	О	1	4.7	6.92	23.1	3.3	69.2 ^{bcd}	7.7abcd	0.6	0.1	7.4
V. hirsuta	ш	3	8.3	72.1 ± 2.5	27.9 ± 2.4	2.6	60.2 ± 3.0^{d}	11.9 ± 1.1^{abcd}	5.1	0.2	4.3
V. lathyroides	田	3	4.5	74.3 ± 1.2	25.7 ± 1.2	2.9	61.7 ± 2.1^{d}	$12.6 \pm 1.5^{\mathrm{abcd}}$	4.9	0.2	3.6
V. incana	田	2	4.4	71.0 ± 6.6	29.0 ± 6.6	2.5	$60.5 \pm 4.1^{\mathrm{d}}$	$10.5 \pm 2.5^{\mathrm{abcd}}$	5.7	0.2	5.1
V. vicioides	Э	3	6.7	74.0 ± 3.9	26.0 ± 3.9	2.9	$59.0 \pm 2.2^{\mathrm{bcd}}$	$15.0 \pm 4.1^{\mathrm{abcd}}$	3.9	0.3	5.2
V. angustifolia	F1	S	5.9	75.6 ± 1.1	24.4 ± 1.1	3.1	66.2 ± 2.0^{d}	9.4 ± 1.9^{d}	7.1	0.2	5.2
V. lutea ssp. lutea var. hirta	F1	S	3.9	77.0 ± 1.0	23.0 ± 1.0	3.4	64.4 ± 1.3^{d}	$12.6\pm1.6~^{\rm cd}$	5.1	0.2	0.9
V. sativa ssp. sativa	FI	5	5.4	75.0 ± 2.0	25.0 ± 2.0	3.0	60.9 ± 3.4^{d}	$14.1 \pm 1.5^{\mathrm{bcd}}$	4.3	0.2	7.3
V. lutea ssp. cavanillesii	F1	1	5.5	75.2	24.8	3.1	61.0^{d}	14.2^{abcd}	4.3	0.2	0.6
V. pseudocracca	F1	S	5.1	75.9 ± 2.2	24.1 ± 2.2	3.1	62.5 ± 3.1^{d}	$13.4 \pm 2.6^{\text{bcd}}$	4.7	0.2	6.9
V. cordata	FI	3	5.4	76.5 ± 0.9	23.5 ± 0.9	3.3	63.5 ± 0.5 cd	$13.0 \pm 0.6^{\mathrm{bcd}}$	4.9	0.2	6.7
V. peregrina	F1	3	4.0	78.6 ± 0.9	21.4 ± 0.9	3.7	$60.9 \pm 1.2^{\mathrm{abcd}}$	$17.7 \pm 2.1^{\text{abcd}}$	3.4	0.3	10.9
V. altissima	F1	1	9.9	76.2	23.8	3.2	60.1^{abcd}	16.1 ^{abcd}	3.7	0.3	9.2
V. monantha ssp. calcarata	F1	3	10.2	77.6 ± 2.0	22.4 ± 2.0	3.5	$61.8 \pm 2.1^{\mathrm{bcd}}$	$15.8 \pm 2.3^{\mathrm{abcd}}$	3.9	0.3	14.1
V. lutea ssp. lutea var.lutea	F2	3	4.3	78.1 ± 2.7	21.9 ± 2.7	3.6	$56.1 \pm 3.6^{\text{abcd}}$	21.9 ± 4.6^{abcd}	2.6	0.4	5.7
V. lutea ssp. vestita	F2	2	4.9	78.5 ± 1.2	21.5 ± 1.2	3.6	$54.7 \pm 0.5^{\mathrm{abcd}}$	23.8 ± 1.8^{abcd}	2.3	0.5	10.9
V. onobrychioides	F2	2	5.1	78.3 ± 1.8	21.7 ± 1.8	3.6	$56.6 \pm 2.8^{\mathrm{abcd}}$	$21.7 \pm 4.6^{\mathrm{abcd}}$	2.6	0.4	6.6
V. benghalensis	F2	4	6.5	77.7 ± 2.0	22.3 ± 1.9	3.5	$57.3 \pm 5.9^{\text{abcd}}$	$20.4 \pm 7.3^{\mathrm{abcd}}$	2.8	0.4	9.8
V. disperma	F2	9	5.5	76.6 ± 1.0	23.4 ± 1.0	3.3	$57.5 \pm 1.4^{\text{abcd}}$	$19.1 \pm 2.1^{\mathrm{abcd}}$	3.0	0.4	10.3
V. glauca ssp. giennensis	F2	1	6.2	78.1	21.9	3.6	59.2^{abcd}	18.9^{abcd}	3.1	0.3	9.4
V. dasycarpa	F2	3	6.5	74.9 ± 1.1	25.1 ± 1.1	3.0	$56.1 \pm 1.3^{\mathrm{abcd}}$	$18.8 \pm 0.2^{\mathrm{abcd}}$	3.0	0.4	5.5
V. eriocarpa	F2	4	6.4	76.9 ± 2.1	23.1 ± 2.1	3.3	$58.2 \pm 4.1^{\mathrm{abcd}}$	$18.7 \pm 4.9^{\mathrm{abcd}}$	3.1	6.4	6.1
V. tenuifolia	F2	4	8.0	75.2 ± 2.3	24.8 ± 2.3	3.0	$57.4 \pm 2.9^{\mathrm{abcd}}$	$17.8 \pm 2.1^{\text{abcd}}$	3.2	0.3	7.0
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Clades assignments result from cluster analysis shown in Fig. 1

n Number of populations studied, TUFAs total unsaturated fatty acids TSFAs total saturated fatty acids, TUFAs/TSFAs total unsaturated fatty acids, PUFAs/MUFAs polyunsaturated fatty acids/monounsaturated fatty acids, MUFAs monounsaturated fatty acids, monounsaturated fatty acid Data are expressed as the averages ± standard deviation. Different superscript small letters indicate significant differences between values in the same column (Scheffe's test)

 $^*P < 0.001$

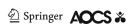


Fig. 1 Clustering based on the seed flour fatty acid distribution of studied legumes, according to the Bray–Curtis similarity index (1 – dissimilarity index) × 100. Cultivated species are marked with an asterisk



and $C_{20:0}$. However, no significant differences in their contents between the species studied was observed.

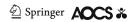
Among unsaturated fatty acids, the most abundant in decreasing order were $C_{18:2}$, $C_{18:1}$ and $C_{18:3}$ (Table 1) except for V. lathyroides, V. parviflora, V. pubescens, V. pyrenaica and V. angustifolia in which $C_{18:3}$ values were higher than $C_{18:1}$ contents and V. hirusta and V. incana where both fatty acids showed similar contents. The $C_{18:2}$ contents ranged from 28.7% in V. articulata to 66.3% in V. hybrida. The $C_{18:2}$ content in the latter species was significantly different ($P \le 0.001$) from those observed in V. articulata, V. ervilia, V. narbonenis, V. parviflora and V. pubescens.

The highest percentages of $C_{18:1}$ were observed in V. *ervilia* with 32.5%. Twelve of the studied species had $C_{18:1}$ contents significantly lower ($P \le 0.001$) than those reported for V. *ervilia*. In most of the studied species, the

quantity of $C_{18:1}$ was lower than $C_{18:2}$, and therefore the $C_{18:1}/C_{18:2}$ ratio was below one (Table 2), as with other legumes [16]. A negative correlation ($r^2 = 0.48$) between these two fatty acids was observed, which supports previous findings on *Lathyrus* [15, 17], probably because $C_{18:1}$ is the substrate for the biosynthesis of $C_{18:2}$.

The $C_{18:3}$ contents ranged from 2.7% in V. faba to 16.6% in V. articulata. $C_{18:3}$ content in V. articulata was significantly different ($P \le 0.001$) with respect to the 12 *Vicia* species with lowest contents of this fatty acid.

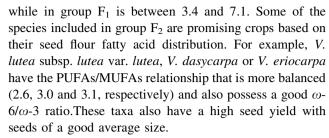
All the species studied had higher contents of total unsaturated fatty acids (TUFAs) than saturated ones (TSFAs) (Table 2). These results are in accordance with those obtained by other authors in other legume species [16, 18] and [19]. TSFAs contents ranged between 19.3% in *V. hybrida* and 29.0% in *V. incana* (Table 2) without significant differences between the *Vicia* species studied.



TUFAs contents ranged between 71.0% in *V. incana* and 80.7% in *V. hybrida* without significative differences between the *Vicia* species studied.

In addition, the total amount of unsaturated fatty acids it is also important to establish the relative proportion of each fatty class. For example, in order to be recommend, a diet rich in polyunsaturated fatty acids (PUFA) should have a balanced proportion between ω -6 and ω -3 fatty acids [20-22]. $C_{18:2}$ and $C_{18:3}$ are the precursors of many eicosanoids with opposite effects in the human body. Eicosanoids derived from C_{18:2}, such as arachidonic acid, increase the risk of cardiovascular diseases [23], while eicosapentaenoic and docosahexaenoic acids derived from C_{18:3} have opposite effects. Thus, the antithrombotic, hypolipidemic, and antiinflammatory effects of ω -3 fatty acids have been reported [24]. Thus, research suggests that a high ω -6/ ω -3 ratio may contribute to an increase in cardiovascular diseases [25]. According to the FAO [26], this ratio should be between 5/1 and 10/1, although some authors suggest that a 4/1 proportion is better, since an increase in $C_{18:3}$ improves the health of people suffering from asthma or arthritis [22]. The optimal proportion may also vary as a function of the severity of the genetic predisposition and illness [22]. Among the species studied, the ω -6/ ω -3 ratio ranged from 1.7% in V. articulata to 17.3 in V. faba (Table 2). The ω -6/ ω -3 ratios between 5 and 10 were observed in 19 of the 31 taxa studied, and therefore indicating a more balanced distribution of polyunsaturated fatty acids.

The analysis of similarity shows that all species have an affinity close to 80% (Fig. 1). A similar affinity have been observed for the Lathyrus, Lens and Pisum genera, that belong together with Vicia to the tribe Fabeae, when their seed oil fatty acid composition was analyzed [15]. Studied Vicia taxa are divided into two well-defined groups characterized by their C_{18:1} contents. Group A includes the four Vicia species with the highest amounts of this fatty acid, and therefore are the species with the lowest PUFAs/ MUFAs relationship (below 1.8). All species included in this group A (V. articulata, V. ervilia, V. faba and V. narbonensis) are or have been cultivated. In group B are found the remaining species with higher PUFAs/MUFAs relationship due to the lower C_{18:1} contents. In group B, another four well-defined groups can be recognized. The first is group C with the lowest affinity, compounded by V. parviflora and V. pubescens. These are two annual species characterized by having the highest C_{18:0} contents. Group D includes V. hibrida, V. monardi and V. pyrenaica with a 91.4% affinity and having in common the highest $C_{18\cdot 2}$ contents. Group E is formed by the species, V. hirsuta, V. lathyroides, V. incana and V. vicioides, with highest C_{16:0} contents. Group F includes the rest of the species studied. Two subgroups can be observed, F₁ and F₂. In group F₂ PUFAs/MUFAs relation is between 2.3 and 3.2



The fat composition of the diet rather than the amount of fat intake is more important in determining the risk factors for heart disease since the dietary fat is a major factor in determining the long-term fatty acid composition of adipose tissue [25]. Hence the good balance in the TSFA/TUFA and ω -6/ ω -3 fatty acids ratios of the *Vicia* species studied may help us correct this current balance in the western diet. It has been observed that the processing and cooking of legumes do not affect the fatty acid composition, while antinutritional compounds present in legumes are removed or degraded [27–29], thus making cooked legumes a possible source of important fatty acids.

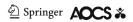
Conclusion

Most of the *Vicia* species studied had a good fatty acid distribution according to the FAO requirements, i.e., having a high content of unsaturated acids and a good ω -6/ ω -3 ratio. Thus, the good fatty acid composition, together with the high nutritional quality of other components of these pulses, like proteins, may be useful for the revalorization of these plants and the extension of their cultivars resulting in better human nutrition and conservation of phytodiversity.

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