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EFFECT OF PROCESSING METHODS ON THE CONTENT OF PHENOLIC COMPOUNDS IN *Vicia faba* L. TISSUES GROWN IN FIELD AND GREENHOUSE

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ABSTRACT

Environmental conditions during the growth of *Vicia faba* plants and post-harvest processing methods influence its contents of secondary metabolites. In this study, total phenolic compounds (TP) and total flavonoids (TF) were quantified in broad bean plants at 10, 15 and 20 days after emergence (DAE), in floral buds and open flowers developed in the field or in a greenhouse with soil (GH-S) or tezontle (GH-T) as substrate. The effects of post-harvest processing, namely oven-drying and freeze-drying, were also evaluated. The analysis of variance showed, in all growth conditions, that the content of TP and TF varied significantly ($P \leq 0.05$ or $P \leq 0.01$) according to the age of the plant or the flowering stage, the processing methods and the interaction between those factors. Field-grown plants at 10, 15 and 20 DAE exhibited a higher mean value of TP (113.55 $mg \cdot g^{-1}$ of gallic acid equivalent in DM) and TF (126.60 $mg \cdot g^{-1}$ of quercetin equivalent in DM) with oven-dried samples, compared with those plants harvested in GH-S and GH-T conditions. Drying in the oven was most efficient in conserving phenolic compounds in field plants while freeze-drying preserved the levels of metabolites in greenhouse plants more effectively. In order to obtain the maximum content of phenolic compounds in minimal time, it is suggested to grow broad beans in the field, harvest plants at 10 DAE, and process them by oven-drying.

Additional keywords: Bioactive compound, broad bean, drying method, field experiment, freeze-drying

RESUMEN

Efecto de métodos de procesamiento sobre el contenido de compuestos fenólicos en tejidos de *Vicia faba* L. cultivada en campo e invernadero

Durante el cultivo de plantas de *Vicia faba* las condiciones ambientales y los métodos de procesamiento post-cosecha influyen en sus contenidos de metabolitos secundarios. En este estudio, compuestos fenólicos totales (CFT) y flavonoides totales (FT) fueron cuantificados en plantas de haba a los 10, 15 y 20 días después de emergencia (DDE), en botones florales y flores abiertas cultivadas en campo, o en invernadero con suelo (INV-S) o tezontle (INV-T) como sustrato. También se evaluó el efecto del secado en estufa o liofilización. El análisis de varianza mostró en todas las condiciones de crecimiento, que los contenidos de CFT y FT variaron significativamente ($P \leq 0,05$ o $P \leq 0,01$) de acuerdo a la edad de la planta o la etapa de floración, los métodos de procesamiento y la interacción entre los factores. Las plantas cultivadas en campo a los 10, 15 y 20 DDE mostraron un valor medio más alto de CFT (113,55 $mg \cdot g^{-1}$ equivalente de ácido gálico en materia seca) y FT (126,60 $mg \cdot g^{-1}$ equivalente de quercetina en materia seca) con muestras secadas en estufa, comparadas con las plantas cosechadas bajo condiciones de INV-S e INV-T. El secado en estufa fue más eficiente en conservar compuestos fenólicos en plantas de campo mientras que liofilizado preservó de manera más efectiva los niveles de metabolitos en plantas de invernadero. Para obtener el máximo contenido de compuestos fenólicos en un tiempo mínimo, se sugiere cultivar habas en campo, cosechar las plantas a 10 DDE y procesarlas mediante secado en estufa.

Palabras clave adicionales: Compuestos bioactivos, experimento en campo, haba, liofilizado, método de secado

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INTRODUCTION

Broad bean (*Vicia faba* L.) is a cool-season legume that was originally domesticated in the Near East (Cubero, 1974), with a second center of diversity generated later in Mexico (Molina and Córdova, 2006). The species has a high nutritional value (Calixto et al., 2020), as well as medicinal and agricultural properties (as the ability to fix nitrogen to the soil, increasing its fertility, and it is used as a cover crop to prevent soil erosion) (Prabhu and Rajeswari, 2018). The plant has been proposed as a strategic food crop to serve the growing world population. In addition to high levels of protein and fiber, the species contains bioactive compounds (BCs) with pharmacological properties that can contribute to the improvement of human health (Multari et al., 2015). Recently it has been documented that the plants belonging to the genus *Vicia* are of great interest as a source of many bioactive compounds with potential health beneficial properties, such as antioxidant and anti-inflammatory activities (Salehi et al., 2020).

Among the BCs identified in *V. faba*, those belonging to the phenolic class of phytochemicals are particularly important since they can serve as antioxidants by capturing free radicals and/or chelating metal ions (Creus, 2004). Phenolic compounds (mainly flavonoids) found in immature and mature seeds and in the pod, seed coat and cotyledon of *V. faba* (Chaieb et al., 2011; Boukhanouf et al., 2016) that possess anti-hypertensive, anti-diabetic and chemo-preventive activities can protect against disease development (Turco et al., 2016).

The presence of phenolic compounds is not limited to the seeds, but extends to other plant organs/tissues, which also contain high levels of such compounds that are not used for human consumption. This suggests that *V. faba* tissues could provide important bioactive compounds for pharmacological uses. For example, flavonols such as kaempferol and quercetin glycosides have been detected in the leaves of plants (Neugart et al., 2015) while isoflavones with estrogenic function, such as daidzein and genistein, have been found in stems (Fuentes et al., 2020) and seedlings (Kirakosyan et al., 2004). In addition, *V. faba* has been shown to be rich in levo-3,4-dihydroxyphenylalanine (L-Dopa), a non-protein amino acid with the structure of a phenolic acid that acts to restore dopamine to the brain (Siqueira et al., 2013). This finding is important because of the known association of Parkinson's disease with the progressive degeneration and death of dopaminergic neurons and the decline in brain dopamine. Restoration of dopamine improves the functioning of the central nervous system (Florán and Rangel, 2005).

Although it would be possible to exploit *V. faba* for its phenolic compounds, a number of different factors can influence the quantity of BCs present in plant tissues. For example, Etemadi et al. (2018) have shown that the biosynthesis/accumulation of L-Dopa in broad bean varies according to growing conditions (irrigation, N-fertilization) and post-harvest processing methods (freeze-, oven- or air-drying). Moreover, it is known that cooking methods involving boiling or steaming generally decrease the content of phenolic compounds and their antioxidant activity (Boukhanouf et al., 2016). Since there are no comparable reports concerning the production of secondary compounds by *V. faba*, we hypothesized that, for this species, the accumulation of phenolic compounds will vary according to age and processing methods. Therefore, we have determined the amounts of total phenolic compounds (TP) and total flavonoids (TF) in bean plants 10, 15 and 20 days after emergence (DAE) and in floral buds and open flowers of plants cultivated in the field or in a greenhouse with soil or red tezontle (volcanic rock) as substrate. In addition, we have evaluated the effects of processing methods (oven-drying and freeze-drying) on the total contents of phenolics and flavonoids.

MATERIALS AND METHODS

Reagents and standards. All reagents and solvents employed in the study were of analytical grade, and solutions were prepared in distilled or deionized water, as appropriate. Folin-Ciocalteu's phenol reagent (# 47641), aluminium chloride and reference standards of gallic acid and quercetin were from Sigma-Aldrich. We also used methyl alcohol, sodium carbonate, sodium nitrite and sodium hydroxide.

Plant material. Seeds of *V. faba* cultivar Calvario were obtained from San Pedro-Cholula, Puebla, Mexico. Field-grown plants were cultivated

according to traditional farming practices (land preparation using plow and harrow, with a distance of 90 cm between rows and 70 cm between plants, planting two to three seeds per point, fertilization a month and a half after the plant was emerged and in the flowering stage, and irrigation as needed), in San Agustín Calvario (19°03' N, 98°20' W) from seeds sown in September 2017.

Greenhouse-grown plants were established at the same location very close to sowing in field in October 2017. A low-tech greenhouse with a plastic cover 800 gauge (200 microns thick) was used providing 15 % shade and blocking 76 % of UV radiation, under natural sunlight conditions. In each sowing, five seeds were placed at depths of 2 cm in plastic bags (8 L capacity) containing 7 kg of either soil substrate (50 % composted cow manure and 50 % field soil) or red-tezontle (rock of volcanic origin) based substrate. Soil-grown greenhouse plants (GH-S) were irrigated with water, while those grown in tezontle (GH-T) were irrigated additionally with Steiner universal nutrient solution (Steiner, 1984) diluted 1:3 at 7 DAE and with 100 % nutrient solution from 10 DAE onwards. In both regimes, weeding was performed when necessary and plants were thinned leaving two plants per bag at 20 DAE. The temperature inside the greenhouse was recorded every hour during plant growth using an automatic Hobo H08-004-02 data logger, while for field experiments the maximum and minimum monthly temperatures were obtained from CONAGUA (2019), weather station 21065 located at 19°03'00'' N, 98°10'00'' W (Table 1).

Table 1. Monthly maximum and minimum temperatures during planting and development of *Vicia faba* in the field and greenhouse

		Field		Greenhouse	
		Maximum (°C)	Minimum (°C)	Maximum (°C)	Minimum (°C)
2017	September	24.6	12.7	–	–
	October	24.3	10.6	33.1	9.4
	November	25.1	6.3	38.1	6.2
	December	23.7	4.1	33.4	5.1
2018	January	–	–	31.1	4.2

Temperatures in the field were obtained from CONAGUA weather station 21065, while greenhouse temperatures were recorded using an Hobo automatic data logger

Plants grown at field and greenhouse were monitored every second day between 7:00 and 9:00. Emergence was established when the epicotyl (stem) was exposed above soil level and, at this point, the plant was marked with a unique colored tag so that its identity and age could be established at follow-up analysis. The sampling of all tissues was carried out at the same season (fall) and time of day around 10:00. At 10, 15 and 20 DAE (± 1 day), 20 plants cultivated under each of the three growing conditions (FG, GH-S and GH-T) were harvested without roots. For each batch of 20 plants, 10 plants were oven-dried and 10 plants were freeze-dried (each representing a sample). During the flowering stage, 300 floral buds were collected at approximately 60 DAE and 300 open flowers were harvested at around 70 DAE. Of these samples, half were oven-dried and half were freeze-dried.

Post-harvesting processing. Plant materials were processed on the day of harvesting. Samples to be oven-dried were placed in paper bags and maintained on metal trays in a forced-air oven at 38 °C for 12 to 24 h (depending on the size of the seedlings) until the remaining moisture level was in the region of 7 to 10 %. Samples destined for freeze-drying were cut into sections of approximately 0.5 cm^2 immediately after harvesting and stored in a freezer at -20 °C for 48 h. Tissues were subsequently freeze-dried in a cascade benchtop freeze dryer (Labconco) at -80 °C and constant vacuum (11 Pa) over two cycles of 4 h each until a moisture content of 7 to 10 % was attained. Dried samples from either process were ground in a Krups GX4100 domestic mill, screened at 420 μm and the resulting powder stored in amber jars at room temperature (22±2 °C).

Extraction and analysis of phenolic compounds. Powdered plants or flowers (10 mg) were mixed with methanol (10 mL) and placed in a Tianjin Yihuan automatic instrument technology AS5150B, ultrasonic bath, for 30 min (180 w power, 40KHz ultrasonic frequency and 22 ± 2 °C of temperature). Samples were then centrifuged at 4,600 *g* for 2 min in a refrigerated (4 °C) Hermle Z326K bench top centrifuge, and the supernatants stored at -20 °C.

Plant samples (n = 18) comprised one freeze-dried sample and one oven-dried sample each of FG, GH-S and GH-T plants collected at 10, 15 and 20 DAE. Flower samples comprised one freeze-dried and one oven-dried sample each of floral buds from FG, GH-S and GH-T plants (n = 6), and one freeze-dried and one oven-dried sample each of open flowers from FG, GH-S and GH-T plants (n = 6). All samples were extracted in triplicate.

The TP and TF contents of each extract were determined in triplicate using the Folin-Ciocalteu and the $AlCl_3$ methods, respectively, as described by Herald et al. (2012) but with minor modifications. For TP, 25 µL aliquots of samples or the reference standard were mixed with the test reagents in the wells of a microplate and maintained for 1 h in the dark at room temperature (22±2 °C) inside a Varioskan Flash (Thermo Fisher Scientific) multimode spectral microplate reader. After this time, the assay solutions were agitated for 30 s and their absorbances recorded at 725 nm. A calibration curve was constructed using methanolic solutions of gallic acid with concentrations in the range 0 to 200 $\mu g \cdot mL^{-1}$. Values of TP were determined from the calibration curve $y = 0.0065x + 0.1026$; $R^2 = 0.9969$ and expressed as $mg \cdot g^{-1}$ of gallic acid equivalents (GAE) in dry matter basis (DM). For TF, 25 µL aliquots of samples or the reference standard were mixed with deionized water (100 µL) and $NaNO_2$ (5 % w/v in distilled water; 10 µL) in the wells of a microplate and the assay solutions left to stand for 5 min at room temperature (22 ± 2 °C). After this time, 15 µL aliquots of $AlCl_3$ (10 % w/v in methanol) were added to the assay solutions, the mixtures were agitated and left to stand for 6 min before being mixed with NaOH (50 µL; 1 M) and deionized water (50 µL). The resulting assay solutions were thoroughly mixed, maintained for 30 min in the dark at room temperature inside the microplate reader, mixed again for 1 min and their absorbances measured at 400 nm. A calibration curve was constructed using methanolic solutions of quercetin with concentrations in the range 0 to 550 $\mu g\ mL^{-1}$. Values of TF were determined from the calibration curve $y = 0.0017x + 0.1$; $R^2 = 0.9986$ and expressed as $mg \cdot g^{-1}$ of quercetin equivalent (QE) in DM.

Statistical analyses. Mean TP and TF values of plants and flowers cultivated under field and greenhouse conditions (GH-S and GH-T) were analyzed according to a completely randomized design (factorial arrangement of treatments). TP and TF values of plants, floral buds and open flowers harvested from field-grown, GH-S and GH-T were subjected to analysis of variance (Proc ANOVA procedure) followed by the Tukey test ($P \leq 0.05$) using the statistical analysis system program 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Analysis of variance of the content of total phenolic compounds and total flavonoids. The analysis of variance of the plants for the different growth conditions (Table 2), presented highly significant differences ($P \leq 0.01$) for age, processing methods and the interaction between both factors (DAE x PM) for the TP and TF variables. With the exception of the processing method factor that only presented significant difference ($P \leq 0.05$) and the DAE x PM interaction did not present significance with the TP variable in the field condition. Also, the greenhouse-soil condition with the processing method factor did not present significance with the TF variable.

The analysis of variance of the flower data for the different growth conditions (Table 3), showed that there were significant differences ($P \leq 0.05$ or $P \leq 0.01$) among flowering stage, processing methods and the interaction between both factors (FS x PM) for the TP and TF variables. Except some factors did not present statistical difference such as flowering stage factor with TP and processing method with TF in field conditions. In the same way, for TF under greenhouse-soil conditions there were no significant differences in the FSxPM interaction, and under greenhouse-tezontle conditions, the factor of processing

methods and the interaction did not present differences either.

Table 2. Analysis of variance of total phenolic compounds and total flavonoids in plants of *Vicia faba* grown in different conditions

Variable	Mean	CV	Mean square			
			Age (DAE)	PM	DAE x PM	Error
			Field			
TP (mg·g^{-1} GAE, DM)	109.63	9.51	6107.49 **	736.39 *	272.43 ns	108.76
TF (mg·g^{-1} QE, DM)	110.07	9.08	1956.62 **	13109.08 **	721.09 **	100.04
			Greenhouse-soil (GH-S)			
TP (mg·g^{-1} GAE, DM)	78.49	6.71	7876.62 **	1981.27 **	2201.94 **	27.81
TF (mg·g^{-1} QE, DM)	57.29	12.66	6073.79 **	0.42 ns	2806.23 **	52.69
			Greenhouse-tezontle (GH-T)			
TP (mg·g^{-1} GAE, DM)	81.88	8.94	14649.71 **	21770.34 **	302.67 **	53.71
TF (mg·g^{-1} QE, DM)	83.31	13.20	9835.91 **	14687.43 **	1586.71 **	121.07

TP: total phenolic compounds; TF: total flavonoids; CV: coefficient of variation; DAE: days after emergence; PM: processing method; GAE: gallic acid equivalent; QE: quercetin equivalent; DM: dry matter basis; ns: no significant; * and **: significant ($P \leq 0.05$) and highly significant differences ($P \leq 0.01$), respectively

Table 3. Analysis of variance of total phenolic compounds and total flovonoids in flowers of *Vicia faba* grown in different conditions.

Variable	Mean	CV	Mean square			
			FS	PM	FS x PM	Error
			Field			
TP (mg·g^{-1} GAE, DM)	157.84	1.79	19.42 ns	173.85**	83.02 **	8.01
TF (mg·g^{-1} QE, DM)	157.16	10.56	7325.34 **	1029.62 ns	10025.49 **	275.89
			Greenhouse-soil (GH-S)			
TP (mg·g^{-1} GAE, DM)	152.90	0.47	6.35 **	15.27 **	164.65 **	0.52
TF (mg·g^{-1} QE, DM)	103.23	10.43	11009.49 **	3120.34 **	12.44 ns	115.95
			Greenhouse-tezontle (GH-T)			
TP (mg·g^{-1} GAE, DM)	155.09	0.54	173.95 **	104.65 **	3.74 *	0.71
TF (mg·g^{-1} QE, DM)	118.00	9.33	4865.91 **	213.52 ns	94.94 ns	121.33

TP: total phenolic compounds; TF: total flavonoids; CV: coefficient of variation; DAE: days after emergence; PM: processing method; GAE: gallic acid equivalent; QE: quercetin equivalent; DM: dry matter basis; ns: no significant; * and **: significant ($P \leq 0.05$) and highly significant differences ($P \leq 0.01$), respectively

Total phenolic compounds and total flavonoids during the development of *V. faba* plants and flower stage. The concentrations of TP and TF varied significantly according to the age of the plant, irrespective of the growing conditions. Plants cultivated in the field or under GH-S or GH-T conditions and harvested at 10 DAE presented amounts of TP that were significantly higher ($P \leq 0.05$) than those of older plants regardless of the processing methods (Table 4). On the other hand, the highest concentrations of TF were not always present in 10 DAE plants under all growth conditions, as exemplified by freeze-dried samples of plants harvested from GH-S and GH-T at 15 DAE (62.36 and 113.61 mg·g^{-1} QE, DM, respectively), which contained levels of TF that were not statistically different ($P > 0.05$) than those of 10 DAE plants. In the general, TP and TF contents decreased with the age of the plant. According to the results shown in Table 4, the highest concentrations of TP (134.92 mg·g^{-1} GAE, DM) and TF (135.64 mg·g^{-1} QE, DM) were obtained from 10 DAE plants cultivated in the field and subsequently oven-dried.

The concentrations of TP and TF in floral buds and open flowers were statistically different in most of the treatments (Table 5). The levels of TP in floral buds were generally significantly higher than those of open flowers, irrespective of the growing conditions or processing methods

applied. However, floral buds harvested from field-grown plants and subsequently freeze-dried presented a TP value that was lower (but not significantly so) than that of the corresponding open flowers, while buds obtained from GH-S plants and oven-dried contained significantly ($P \leq 0.05$) less TP (151.77 mg·g^{-1} GAE, DM) than the corresponding open flowers (155.41 mg·g^{-1} GAE, DM). In floral buds, the concentrations of TP ranged between 151.77 and 159.58 (mg·g^{-1} GAE, DM), with the highest levels found in oven-dried buds harvested from GH-T plants. In open flowers, the contents of TP ranged from 149.50 to 162.56 mg·g^{-1} GAE, DM, with the highest levels found in freeze-dried open flowers harvested from field-grown plants.

Table 4. Total contents of phenolic compounds and flavonoids in *Vicia faba* plants at 10, 15 and 20 days after emergence according to the growing conditions and processing method (n=9)

	Age (DAE)	Field		Greenhouse-soil (GH-S)		Greenhouse-tezontle (GH-T)	
		FD	OD	FD	OD	FD	OD
Total phenolic compounds -TP (mg·g^{-1} GAE, DM)	10	124.47 ± 7.35 aB	134.92 ± 10.16 aA	95.60 ± 3.83 aB	109.80 ± 3.54 aA	130.23 ± 7.70 aA	96.96 ± 5.72 aB
	15	91.37 ± 5.12 cA	89.96 ± 16.39 cA	86.14 ± 3.46 bA	61.13 ± 7.68 bB	103.98 ± 13.80 bA	53.48 ± 3.06 bB
	20	101.31 ± 7.18 bB	115.77 ± 12.18 bA	73.00 ± 6.58 cA	45.26 ± 5.02 cB	75.32 ± 4.26 cA	31.33 ± 3.45 cB
Mean		105.72 ± 15.51	113.55 ± 22.63	84.91 ± 10.53	72.07 ± 28.57	103.18 ± 24.62	60.59 ± 28.14
Total flavonoids - TF (mg·g^{-1} QE, DM)	10	108.57 ± 9.52 aB	135.64 ± 11.44 aA	61.32 ± 11.20 aB	90.09 ± 8.70 aA	109.84 ± 12.12 aA	97.85 ± 8.93 aB
	15	83.54 ± 8.99 bB	131.97 ± 10.42 aA	62.36 ± 5.41 aA	56.21 ± 4.22 bB	113.61 ± 17.32 aA	67.05 ± 5.63 bB
	20	88.53 ± 11.08 bB	112.18 ± 7.03 bA	48.48 ± 6.72 bA	25.30 ± 4.73 cB	78.97 ± 11.77 bA	32.57 ± 5.38 cB
Mean		93.55 ± 14.72	126.60 ± 14.09	57.39 ± 10.12	57.20 ± 27.67	100.81 ± 20.72	65.82 ± 28.01

DAE: days after emergence; FD: freeze-drying; OD: oven-drying; GAE: gallic acid equivalent; QE: quercetin equivalent; DM: dry matter basis. For each growth condition, distinct small letters mean difference within ages, and distinct capital letters mean differences within processing methods, according to the Tukey test ($P \leq 0.05$)

Table 5. Total contents of phenolic compounds and flavonoids in *V. faba* floral buds and open flowers according to the growing conditions and processing method

	Flowering stage	Field		Greenhouse-soil (GH-S)		Greenhouse-tezontle (GH-T)	
		FD	OD	FD	OD	FD	OD
Total phenolic compounds -TP (mg·g^{-1} GAE, DM)	Floral buds (60 DAE)	157.78 ± 5.49 aA	156.34 ± 0.62 aA	154.92 ± 0.36 aA	151.77 ± 0.75 bB	155.27 ± 0.96 aB	159.58 ± 0.92 aA
	Open flowers (70 DAE)	162.56 ± 0.96 aA	154.67 ± 0.72 bB	149.50 ± 1.10 bB	155.41 ± 0.43 aA	151.30 ± 0.50 bB	154.23 ± 0.89 bA
Mean		160.17 ± 4.54	155.51 ± 1.07	152.21 ± 2.91	153.59 ± 1.97	115.42 ± 17.27	120.58 ± 16.09
Total flavonoids - TF (mg·g^{-1} QE, DM)	Floral buds (60 DAE)	148.91 ± 12.63 bB	195.66 ± 23.68 aA	111.28 ± 11.58 aB	132.28 ± 13.49 aA	126.02 ± 16.98 aA	134.64 ± 5.10 aA
	Open flowers (70 DAE)	154.06 ± 9.59 aA	130.00 ± 17.05 bB	75.43 ± 9.94 bB	93.94 ± 6.97 bA	104.81 ± 9.66 bA	106.53 ± 8.78 bA
Mean		151.49 ± 11.15	162.83 ± 39.33	93.36 ± 21.24	113.11 ± 22.35	153.28 ± 2.18	156.90 ± 2.89

DAE: days after emergence; FD: freeze-drying; OD: oven-drying; GAE: gallic acid equivalent; QE: quercetin equivalent; DM: dry matter basis. For each growth condition, distinct small letters mean difference within ages, and distinct capital letters mean differences within processing methods, according to the Tukey test ($P \leq 0.05$)

The contents of TF in floral buds were also significantly higher than those of open flowers, harvested from field-grown plants and freeze-dried, irrespective of growing conditions or processing methods, with the single exception of buds (Table 5). The values of TF in floral buds

varied between 111.28 and 195.66 mg·g^{-1} QE DM, with the highest levels found in oven-dried buds harvested from field-grown plants. The range of TF concentrations found in open flowers (75.43 to 154.06 mg·g^{-1} QE, DM) was slightly less wide than that established for floral buds.

Effects of processing method in the content of total phenolic compounds and total flavonoids in *V. faba* of different ages and stages of flowering. The processing methods affected the contents of both TP and TF, although the profiles differed according to growing conditions, plant age and flower development. With oven-drying, the highest concentrations of TP were detected mainly in plants grown in the field and harvested at 10 or 20 DAE (134.92 and 115.77 mg·g^{-1} GAE, DM, respectively) (Table 4). However, oven-dried plants at 15 DAE contained an amount of TP (89.96 mg·g^{-1} GAE, DM) that was not significantly different from that (91.37 mg·g^{-1} GAE, DM) of the corresponding freeze-dried plants. Oven-drying also favored the conservation of TF in field-grown plants since concentrations in samples collected at 10, 15 and 20 DAE were significantly higher than those determined in their freeze-dried counterparts. In contrast, GH-T plants harvested at 10, 15 and 20 DAE, as well as GH-S plants harvested at 15 or 20 DAE, exhibited significantly higher concentrations of TP when processed by freeze-drying.

A similar trend was detected for TF in which freeze-dried samples generally showed a higher content than their oven-dried counterparts, especially for GH-T plants harvested at 15 and 20 DAE. These results verify that oven-dried samples of almost all of the plants harvested in the field at different ages exhibited higher contents of TP and TF than those that had been freeze-dried. In contrast, the concentrations of TP and TF of greenhouse-grown plants of almost all ages were higher in the freeze-dried samples. Although processing exerted a significant effect on the concentrations of TP in floral buds and open flowers, no marked trends were observed. For example, the content of TP in buds harvested in the field was not affected by the processing method, but freeze-dried samples of open flowers exhibited significantly higher levels of TP (162.56 (mg·g^{-1} GAE, DM) than oven-dried samples (154.67 mg·g^{-1} GAE, DM) (Table 5). In contrast, floral buds and open flowers harvested from the greenhouse were affected differently by growing conditions and stage of flower development. Thus, for GH-S plants, oven-dried open flowers exhibited a higher content of TP (155.41 mg·g^{-1} GAE, DM) while for floral buds the content of TP was higher with freeze-drying (154.92 mg·g^{-1} GAE, DM). For GH-T plants, both floral buds and open flowers presented higher quantities of TP when processed by oven-drying. In contrast, the content of TF was notably different, since oven-drying generally conserved the constituents better than freeze-drying except in the case of open flowers harvested in the field, which showed higher concentrations of TF with freeze-drying.

DISCUSSION

The amounts of TP and TF in vegetative tissue of *V. faba* were found to be widely different from those in flowers, an outcome likely related to changes in the synthesis of metabolites during plant development. Such a situation has been described previously for *Camellia sinensis* in which the contents of phenolic compounds in root, stem, shoot and 1st, 2nd, 3rd and 4th leaf tissues varied considerably (Jiang et al., 2013). In broad bean, the mean concentrations of TP and TF in the different growing conditions were markedly higher in flowers (Table 5) possibly because secondary metabolites accumulate mostly at the flowering stage of plant development, as is the case for *Rumex crispus* and *R. obtusifolius* (Feduraev et al., 2019). These authors suggested that the enhanced content of phenolic compounds in *Rumex* spp. is due to the increase in activity of phenylalanine ammonium lyase (PAL) when flowering begins.

In the present study, the average TP values from plants harvested at 10 DAE ranged between 95.60 and 134.92 mg·g^{-1} GAE, DM, regardless of the growing condition of plants or drying method employed. Taking into consideration the water content (~ 80 %) of fresh plant tissue (Duan et al., 2021), these values are comparable with those reported by Randhir and Shetty (2003) for broad bean seedlings harvested at 12, 16 and 20 days after germination, in which the highest concentration of TP (21 mg·g^{-1} fresh matter) was

exhibited by 16-day-old seedlings. However, it should be noted that, in contrast to the present study, these researchers used natural elicitors to stimulate synthesis of the phenolic metabolites. According to Boukhanouf et al. (2016), the TP content of immature broad bean seeds (testa + cotyledons) from northern Algeria was 36.4 $mg \cdot g^{-1}$ GAE, DM DM while the TF content was 0.623 $mg \cdot g^{-1}$ QE, DM, although these values decrease by 67 and 73 %, respectively, when the seeds mature. Although we analyzed a different cultivar of *V. faba*, our results suggest that the average TP content of plants (90 $mg \cdot g^{-1}$ GAE, DM) is likely to be 2-fold and 7-fold higher than those of immature (green) and mature (dry) seeds, respectively, regardless of the growing conditions or processing method. Furthermore, the average TF content of broad bean plants (83.55 $mg \cdot g^{-1}$ QE, DM) may be 100-fold higher than that of immature seeds and around 500-fold higher than mature seeds.

The concentrations of TP in flowers of wild medicinal species such as *Crataegus monogyna*, *Cytisus multiflorus*, *Malva sylvestris* and *Sambucus nigra* vary between 5 and 55 mg/g DM (Barros et al., 2012). However, the edible flowers of some plants contain TP levels that are markedly higher, such as *Rosa hybrida* (129.0-198.4 $mg \cdot g^{-1}$ GAE, DM) and *Camellia japonica* (56.7-107.6 $mg \cdot g^{-1}$ GAE, DM), and these species have been considered as potential sources of phenolic compounds that benefit health (Trinh et al., 2018). The floral buds and open flowers of *V. faba* can also be proposed as valuable edible sources of BCs because of their high concentrations of TP (149.50-162.56 $mg \cdot g^{-1}$ GAE, DM) and TF (75.43-195.66 $mg \cdot g^{-1}$ QE, DM) (Table 5).

A number of studies have shown that growing conditions (field or greenhouse) exert significant effects on the content of primary and secondary metabolites in soybean seeds (John et al., 2016, 2017). In our study, the amounts of TP and TF were higher in field plants mostly with oven-dried samples than in those grown in the greenhouse, a finding that differs from that of John et al. (2016) in which different varieties of soybean grown in a greenhouse (22.2 °C; photoperiod of up to 16 h) produced higher yields of isoflavones (daidzin, genistin, glycitein, daidzein and genistein) than plants grown in the field (21.7 °C; natural photoperiod). Moreover, when soybeans were cultivated in the field and the greenhouse under similar conditions of temperature and photoperiod, the accumulation of proteins and isoflavones remained higher in the greenhouse plants (John et al., 2017). The discrepancy between these results and those presented herein may be explained by differences in species and tissues analyzed and/or by differences in growing conditions in the field and in the greenhouse that prevailed in our study. In addition, the higher accumulation of phenolic compounds in field-grown plants could be attributed to their direct exposure to solar radiation. The plastic covering of the greenhouse employed in our study provided 15 % shade and blocks 76 % of UV radiation, giving rise to a reduction in the incidence of radiation on the crop and likely affecting the synthesis of phenolic compounds. It is known that, during plant growth, the accumulation of phenolic compounds is affected by various environmental factors, including UV radiation, temperature and fertilization, among others. For example, Julkunen et al. (2015) reported that flavonoid synthesis responds to UV radiation since the accumulation of these compounds depends on the season and even the hour of the day.

In agreement with our findings, Andreotti et al. (2006) reported that the leaves of pear (*Pyrus communis* L.) plants grown under field conditions contained higher levels of phenolic compounds than those grown under greenhouse conditions. The authors attributed the difference to environmental conditions, especially to UV radiation, since natural light in the orchard promoted higher concentrations of hydroxy-cinnamic acids and flavonoids (flavone-3-ols and flavonol glucosides) and these metabolites are members of groups of phenolic compounds that absorb UV rays along with natural radiation.

According to Løvdal et al. (2010), accumulation of some types of flavonoids by tomato (*Solanum lycopersicum*, cv. Suzanne) leaves is favored by synergic effects of temperature, light conditions and nutrition. In particular, these authors noted that anthocyanins increased when the growth temperature decreased from 24 to 18 or 12 °C, while flavonols increased when the light intensity increased from 100 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Similar trends were observed in our study in which flavonoid accumulation in field-grown plants and flowers was favored by higher

luminosity and lower (23-25 °C) growing temperatures in comparison with greenhouse conditions (Table 1). Apparently, the temperature under which a plant develops exerts a significant effect on the content of secondary metabolites, and this is particularly so when plants are cultivated at low temperatures. According to Olennikov et al. (2017), the levels of TP, TF, total flavonoid glucosides, total flavonoid aglycones and total flavanone glucosides (102.31, 98.19, 82.36, 15.8 and 20.7 mg·g^{-1} DM, respectively) in *Dracocephalum palmatum* seedlings grown in the greenhouse at low temperature (1 °C) were markedly higher than those (16.7, 14.1, 12.1, 2 and 3 mg·g^{-1} DM, respectively) of plants cultivated in the greenhouse at 20 °C.

Sinkovič et al. (2017) reported that cultivation in hydroponics increased the concentration of BCs in chicory (*Cichoruim intybus* L.) plants, particularly those that had been fertilized with nutritive solutions enriched in K and, to a lesser extent, N and P. These findings are in accord with our results in that *V. faba* planted and cultivated in hydroponics (GH-T) presented a higher abundance of TP and FT, mainly with freeze-dried samples than those grown in the greenhouse in soil (GH-S) (Table 4).

There is evidence that the drying method affects the contents of BCs in plants. For example, the TP content of freeze-dried (-40 to -50 °C at 0.7 Pa) and oven-dried (forced air oven at 55 °C for 6-7 h) leaves of *Eruca sativa* Miller were not significantly different (8.7 *vs* 8.5), while freeze-drying preserved a higher content of TF (3.3 g·100 g^{-1} QE, DM) than oven-drying (2.4 g·100 g^{-1} QE, DM) (Alruwaih and Yaylayan, 2017). In addition, Çoklar and Akbulut (2017) analyzed the TP content of grapes (*Vitis vinifera* L.) that had been oven-dried (60 °C for 17 h), freeze-dried (-110 °C for 48 h) or sun-dried (for 7 d), and found that freeze-drying was more efficient than the other two methods in that it conserved 98 % of the TP (20.2 mg·g^{-1}) present in fresh fruit. These findings suggest that freeze-drying is better for the preservation of TP and TF. Nevertheless, the results of our study reveal that freeze-dried samples of *Vicia faba* did not conserve the contents of phenolic compounds in plants grown under all of the conditions studied. Thus, oven-dried plants that had been cultivated in the field exhibited higher contents of TP and TF than their freeze-dried counterparts, while the opposite was the case for greenhouse-grown plants (Table 4). However, in our study oven-drying was carried out at a lower temperature (38 °C) than those (55 and 60 °C) employed in the investigations cited above, and it is known that higher temperatures can cause a loss of some phenolic compounds, such as flavonoids, that differ in their thermostability (Alruwaih and Yaylayan, 2017). Moreover, our results are in agreement with those reported by Ortiz et al. (2019) in which oven-dried (38 °C) samples of field-grown broad bean plants exhibited higher TP contents than samples that had been freeze-dried at -80°C. These authors suggest that the higher contents obtained with oven-drying could be attributed to the drying temperature, which was set lower than that conventionally employed to dry plant tissues (>60 °C) in order to avoid degradation of the thermolabile compounds.

In the present study, we processed field-grown and greenhouse-grown plants using the same drying method and the results suggest that the differences in TP and TF contents obtained are likely due to the type of phenolic compounds synthesized under the particular growing condition, with higher levels of UV radiation promoting flavone or flavonol synthesis and lower temperatures promoting anthocyanin synthesis. It is evident, therefore, that the growing conditions influenced the types of phytochemicals that accumulated, each of which would show discrete levels of resistance/sensitivity to the low (freeze-drying) or high (oven-drying) temperatures employed in processing. Following a study of the dehydration of blueberries (*Vaccinium corymbosum* L.), Mejía et al. (2008) reported that levels of quercetin were particularly affected by oven processing, while ellagic acid and kaempferol were affected more by freeze-drying. Interestingly, anthocyanin aglycones were better conserved by freeze-drying than by oven-drying (291.8 *vs* 67.9 mg·100 g^{-1} pelargonidin-3-glucoside) while aglycones of a group of polyphenolic compounds showed higher retention with oven-drying than with freeze-drying (505.4 *vs* 474 g·100 g^{-1} GAE).

Studies that evaluate the effects of stages of development and processing methods on the content of BCs in plant species are, in general, somewhat limited. The results of the present

investigation provide evidence of changes in the accumulation of total phenolic compounds and total flavonoids in broad bean plants and flowers at different stages of development. In addition, we monitored the influence of the processing method employed (oven-drying and freeze-drying) on the contents of BCs in the plants sown under different growing conditions (field, GH-S and GH-T). This information is particularly important since sensitivity to oven- or freeze-drying will vary significantly between individual metabolites. However, since process times and temperatures employed in the different drying methods vary considerably, the elucidation of ideal drying conditions for metabolites of interest is problematic. In the case of broad bean plants, the problem is exacerbated by the scarcity of information concerning the exact nature of the phenolic compounds synthesized by field- and greenhouse-grown plants. Evaluation of these factors is relevant in the selection of growing and drying conditions that would provide the highest levels of BCs in broad bean plants. It is inferred that *Vicia faba* plants containing high levels of TP and TF will provide enhanced beneficial effects on health because of the antioxidant activities that can decrease or prevent disease. In addition, *V. faba* contain L-DOPA, which is used to treat Parkinson's disease (Patil et al., 2013), and isoflavones that have a chemopreventive function against some types of cancer (Křížová et al., 2019).

The plants and flowers of *V. faba* may be ideal to obtain extracts for use in functional products, nutraceuticals or drug development. Although the flowers contain higher levels of TP and TF than vegetative tissues, we recommend 10 DAE plants as a natural source of BCs since the biomass required for processing can be obtained more rapidly.

CONCLUSIONS

The conditions under which broad bean plants develop and the method employed in processing the harvested tissues exerts significant effects on the concentrations of total phenolic compounds (TP) and total flavonoids (TF). The contents of both TP and TF showed a decreasing trend as plants aged from 10 days after emergence (DAE) to 20 DAE, regardless of the growing conditions or processing method. Among the tissues evaluated the flowers present the most content of bioactive compounds (BCs) unlike plants, mainly when the flower was in the flora button. Oven-dried tissues of plants grown in the field conserved more TP and TF, while freeze-drying better conserved tissues obtained in the greenhouse. In the tissues of *Vicia faba* high concentrations of TP and TF were detected, findings that provide guidelines for further research on the type of metabolites with biological activity that may be present in the tissues of the species. In addition, a drying method as sophisticated and expensive as freeze drying is not necessary to preserve TP and TF in *Vicia faba* plants. It is valuable to note that the tissues of broad bean could be used for the extraction of BCs. Based on these results; we recommend field cultivation of broad bean plants with harvesting at 10 DAE and subsequent oven-drying in order to obtain the highest yields of TP and TF.

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