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EFFECT OF ROASTING AND BOILING ON THE CONTENT OF VICINE, CONVICINE AND L-3,4-DIHYDROXYPHENYLALANINE IN *VICIA FABA* L.

ANABERTA CARDADOR-MARTÍNEZ[2], KARINA MAYA-OCAÑA[1], ALICIA ORTIZ-MORENO[1], BRAULIO E. HERRERA-CABRERA[3], G. DÁVILA-ORTIZ[1], MERCEDES MÚZQUIZ[4], MERCEDES MARTÍN-PEDROSA[4], CARMEN BURBANO[4], CARMEN CUADRADO[4] and CRISTIAN JIMÉNEZ-MARTÍNEZ[1,5]

[1]Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México D.F. C.P. 11340
[2]Instituto Tecnológico de Estudios Superiores de Monterrey, Campus Querétaro, Querétaro, Mexico
[3]Colegio de Posgraduados Campus Puebla, Puebla, Mexico
[4]Dpto. Tecnología de Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Madrid, Spain

[5]Corresponding author.
TEL: 52-5557296000 ext. 62462;
FAX: 52-5557296000 ext. 62463;
EMAIL: crisjm_99@yahoo.com

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ABSTRACT

The pyrimidine glycosides, vicine and convicine, are compounds of the genus *Vicia*, whereas their aglycone derivatives, divicine and isouramil, are responsible for favism occurrence, and L-3,4-dihydroxyphenylalanine (L-DOPA) is a nonprotein amino acid precursor of dopamine in healthy neurons. The present work evaluated the effect of thermal treatment, which consisted of roasting and boiling, on the content of vicine, convicine and L-DOPA in mature cotyledons of 10 varieties of Mexican *Vicia faba* L. and determined the concentration of glycosides and L-DOPA by high-performance liquid chromatography. The raw cotyledons showed 2.88–6.10, 0.63–1.68 and 0.28–0.44 vicine, convicine and L-DOPA, respectively. The 12 and 40% and 30 and 61% decrease in vicine and convicine content, respectively, were observed to be the effect of roasting and boiling the samples. L-DOPA was totally removed by both thermal processes. In all analyzed samples, vicine was the main compound; however, there were significant differences between varieties and thermal treatments.

PRACTICAL APPLICATIONS

Vicine and convicine glycosides are strongly implicated in favism, a hemolytic anemia in humans, and in poor performance of laying hens. There are reports of decreased egg weight, increase in the fragility of the yolk and a number of blood stains, and decreased fertility and hatchability of eggs. The characterization of new varieties of faba bean in their content of potentially toxic glycosides allows selection of those with the lowest possible content of such compounds. Also, the application of heat treatments that reduce glycosides will allow the use of seeds or flour to develop harmless food and feed ingredients.

INTRODUCTION

The seed of broad bean (*Vicia faba* L.) is a legume seed used as a source of proteins in different regions around the world for human and animal consumption (Waldroup and Smith 1989; Olvera *et al.* 2001) because its protein content oscillates to around 24–32% (Waldroup and Smith 1989). This legume seed has other important nutritional compounds, such as fiber, minerals and vitamin B complex (Agustin and Klein 1989). Nonetheless, the broad beans have compounds that are not considered nutritionally important, which modifies the bioavailability of nutrient, by influence on their assimilation, use and digestibility. Among these nonnutritional compounds are the pyrimidine glycosides vicine (2,6-diamino-4,5-dihydroxypyrimidine-5-[β-D-glucopyranoside]) and convicine (2,4,5-trihydroxy-6-aminopyrimidine-5-[β-D-glucopyranoside]). These pyrimidine glycosides vicine and convicine are hydrolyzed by β-glycosidase of the anaerobic microflora in the intestinal tract; by effect of these hydrolytic enzymes, their aglycone derivatives, divicine and isouramil, respectively, are released. The latter compounds are responsible for the hereditary disease known as favism, which leads to acute hemolytic anemia (Mckay 1992). Favism occurs after consumption of broad beans, in 10–20% of patients with glucose-6-P-dehydrogenase deficiency in their red blood cells (Cappellini and Fiorelli 2008; Schuurman *et al.* 2009). The first symptoms of favism appear about 6–24 h after ingestion of broad beans and comprise tiredness, headache, dizziness, nausea, vomiting, shiver, paleness, abdominal and lumbar pain, and fever (Luzzatto *et al.* 2001). Then, hemolysis of variable intensity and severity, which, in the worst cases, is accompanied by hemoglobinuria, occurs around 5–30 h next to broad bean consumption. A few hours later, jaundice is observed, and in some cases, acute renal infection, which might lead to death, developed (Mager *et al.* 1980). Even though vicine and convicine have been associated with favism and with low productivity of laying hens (Jamalian 1978; Schuurman *et al.* 2009; Vilariño *et al.* 2009), other works have proven the benefits of these compounds in preventing cardiac arrhythmia, and under certain conditions, they are capable of inhibiting the growth of the malaria parasite (Golenser *et al.* 1983; Roth *et al.* 1983). Divicine has also an antitumor property because in the presence of O_2, it generates H_2O_2, which in concomitancy with the ion Fe^{2+} releases OH^-; the latter ion causes damage to the cell membrane and triggers the tumor cell lysis (Cowden *et al.* 1987). From these works, the effects of pyrimidine glycosides could be beneficial or not for human health.

On the other hand, L-3,4-dihydroxyphenylalanine (L-DOPA) is a nonprotein amino acid that has been identified as useful for controlling Parkinson's disease, hypertension, renal insufficiency (renal failure) and hepatic cirrhosis (Rabey *et al.* 1992). This amino acid is the immediate precursor of dopamine in healthy neurons that are still present in patients with Parkinson's disease, and its administration incompletely relieves the dopamine deficiency (Horst *et al.* 1973; Randhir and Shetty 2004; Nagatsu and Sawada 2009). It is well known that the content of these compounds in broad bean seeds is generally affected by genetic and environmental factors, such as cultivar type, year, location and temperature.

Recent works indicate that ingestion of broad beans increases the duration of the on-periods in patients with Parkinson's disease with on and off cycles. This extended on-period has not been observed after administration of synthetic L-DOPA at high doses (Apaydin *et al.* 2000). Thereby, the availability of this compound in natural sources, such as broad bean seeds, which have important levels of L-DOPA, denotes a promising alternative (Liu *et al.* 2000).

The broad bean for human consumption can be used as tender or mature. In Mexico, it is commonly consumed after thermal processes such as roasting, frying and cooking through a boiling treatment. The nutritional importance of this seed and its content of nonnutritional factors depend on the type of processing, the seed variety, genetic, climatic and edaphic conditions (Duffus and Slaugther 1992).

Given the above, the objective of the present work was to evaluate the effect of the wet and dry thermal treatments, boiling and roasting, respectively, on the content of vicine, convicine and L-DOPA in cotyledons of 10 varieties of mature Mexican broad beans.

MATERIALS AND METHODS

Biological Material

Ten different varieties of broad bean (*V. faba* L.), which were cultivated in 2007 at the town of Tlachichuca, in the Mexican state of Puebla (the major state in broad bean production in Mexico), were used for this work. These varieties belong to the collection of broad beans from the central region of Mexico held by the Germplasm Bank of the Mexican College of Postgraduates Campus Puebla. The seeds showed great extent of variability with respect to characteristics such as seed yield, length of the plant life cycle and color of the seed hull. Some characteristics of each studied seed are described in Table 1. Because the seeds were cultivated under the same climatic and edaphic conditions, and by following the same production system during the same time period, the effect of external factors on the samples and on the content of vicine, convicine and L-DOPA might be discarded.

Each of the 10 broad bean seed varieties was split into three groups: in the first group, the seed was separated into cotyledon and hull, and then the cotyledon was milled up to obtain flour which was subsequently analyzed; this flour was named "cotyledon without treatment" (CWT). In the second group, the whole seeds were roasted at 120C for 10 min; afterward, the cotyledon and the hull were separated from each other; the cotyledon was ground until obtaining flour which was analyzed later ("roasted cotyledon" or RC). In the third group, the cotyledon (without hull) was placed in Erlenmeyer flasks of 500 mL capacity, and water was added up to a 1:10 ratio; the cooking time was 20 min at 121C; after that, the sample along with cooking water was freeze-dried and milled up to flour. Cooking temperatures and times were selected after a sensory acceptance analysis was conducted in consumers.

TABLE 1. CHARACTERISTICS OF THE *VICIA FABA* VARIETIES

Seed variety	Size and color of the seed or hull	Seed height (cm)	Life cycle duration (days)	Harvest
Col-25	Small–yellow	91.0	66	Early
Col-89	Tarragona*–yellow	105.0	78	Late
Col-93	Tarragona–yellow	96.5	71	Mid
Col-146	Small–purple	124.7	65	Early
Col-160	Medium–white	101.7	72	Mid
Col-181	Tarragona–parraleña†	104.5	71	Mid
Col-281	Tarragona–yellow	109.7	78	Late
Col-288	Tarragona–yellow	108.7	78	Late
Inglesa (ING)	Small–white	60.0	46	Early
Zacatecas (ZAC)	Cochinera‡–green	52.5	56	Early

* Big.
† Purple-yellow.
‡ Intermediate to small and medium sizes.

Determination of Vicine, Convicine and L-DOPA

The content of the pyrimidine glycosides, vicine and convicine, and the nonprotein amino acid L-DOPA was determined by the method of Burbano *et al.* (1993), which is based on that of Marquardt and Frohlich (1981). These compounds were extracted from 0.05 g of flour with 5 mL of 0.83 M perchloric acid using an Ultraturrax homogenizer (T25 basic, IKA Labortechnik, Staufen, Germany) at 4C. Then, the extract was centrifuged at 15,000 g for 15 min and filtered through 0.45 μm nylon membranes (Millipore, Tulla Green, Carringtwohill, Ireland). The resultant solution was injected (20 μL) into a high-performance liquid chromatograph (Beckman System Gold, Beckman, CA) with an UV-visible detector fixed at 280 nm and using a C_{18} column (Spherisorb ODS, 250 × 4.6 mm, 5 μm, Waters, Dublin, Ireland); a buffer solution of 0.05 M ammonium phosphate, pH = 2.0, was used as mobile phase under isocratic flow conditions at 1.0 mL/min. Each sample was analyzed in duplicate.

The quantification of the compounds was performed by means of standards of vicine and convicine obtained from Dr. Marquardt (University of Manitoba, Canada) and a commercial standard of L-DOPA (Serva, Heidelberg, Germany). Calibration curves were drawn for the three components. There was linearity in the range: 0.009–0.310 mg/mL for vicine; 0.004–0.134 mg/mL for convicine and 0.047–0.567 mg/mL for L-DOPA. The retention times for vicine, convicine and L-DOPA were 6.0, 7.5 and 12.8 min, respectively. The total glycosides were obtained from vicine + convicine.

Statistical Analysis

Multifactorial analysis of variance was used to identify the influence of each studied factor (life cycle, seed variety and thermal treatment). Pairwise comparisons of means were performed by the Tukey's method for each significant factor. The principal component analysis (PCA) was also used to examine the relationships between the quantitative variables, discarding *a priori* given structures of variables or individuals. PCA is a simple, nonparametric method of extracting relevant information from confusing data sets. With minimal additional effort, PCA provides a roadmap on how to reduce a complex data set to a lower dimension to reveal the sometimes hidden, simplified structure that often underlies it. The statistical software STATISTICA 8.0 (StatSoft Inc., Tulsa, OK) was used for this purpose.

RESULTS AND DISCUSSION

Pyrimidine Glycosides

The contents of vicine and convicine for raw, roasted and boiled cotyledons (BCs) are shown in Tables 2 and 3 for each studied variety of *V. faba* L. The content of pyrimidine glycosides in raw cotyledons of the 10 different varieties ranged from 2.88 (Col-181) to 6.10 Zacatecas (ZAC) mg/g of vicine and from 0.60 (Col-93) to 1.68 Inglesa (ING) mg/g of convicine. Vicine was always the main pyrimidine glycoside.

Regarding RCS, the content of vicine was in the interval 2.75–5.40 mg/g of cotyledon; these values were statistically lower ($P < 0.05$) than that for raw cotyledons. Col-160 did not show a decrease in the content of vicine by effect of roasting, whereas this parameter was diminished by 12% for Col-89. The highest vicine content was for the ZAC variety; however, this parameter was 11.5% lower for the RC than that of raw cotyledon.

In relation to boiled cotyledons, the values for vicine content ranged from 2.54 (Col-181) to 6.04 (ZAC) mg/g of sample. The ZAC variety, with the highest vicine content in CTW, showed the lowest decrease (0.9%) when boiling,

TABLE 2. VICINE CONTENT IN RAW, ROASTED AND BOILED COTYLEDONS OF THE 10 VARIETIES OF *VICIA FABA* L. (MG/G OF SAMPLE)

Variety	CWT	RC	% decrease	BC	% decrease
Col-25	4.77 ± 0.09[c]	4.72 ± 0.03[b,c]	1.15	3.61 ± 0.20[c]	24.37
Col-89	4.39 ± 0.08[d,e]	3.87 ± 0.09[d]	12.01	4.12 ± 0.06[b]	6.15
Col-93	3.40 ± 0.06[f]	3.21 ± 0.04[e]	5.45	2.77 ± 0.08[f]	18.56
Col-146	4.46 ± 0.07[d]	3.99 ± 0.16[d]	10.60	4.08 ± 0.12[b]	8.41
Col-160	4.66 ± 0.01[c,d]	4.68 ± 0.03[b,c]	0.00	3.19 ± 0.20[d]	31.49
Col-181	2.88 ± 0.15[g]	2.75 ± 0.02[f]	4.77	2.54 ± 0.16[f]	11.97
Col-281	4.15 ± 0.10[e]	3.89 ± 0.03[d]	6.33	3.12 ± 0.07[d,e]	24.89
Col-288	4.64 ± 0.15[c,d]	4.53 ± 0.19[c]	2.43	2.80 ± 0.21[e,f]	39.68
ING	5.33 ± 0.13[b]	4.96 ± 0.25[a,b]	6.81	4.14 ± 0.17[b]	22.21
ZAC	6.10 ± 0.06[a]	5.40 ± 0.21[a]	11.52	6.04 ± 0.33[a]	0.90

The results were obtained by averaging two replicates from two independent measurements. Different letters in the same column make reference to statistically significant differences (Tukey's test $\alpha = 0.05$). The content values are expressed in mg of vicine per g of cotyledon, dry basis.
BC, boiled cotyledon; CWT, cotyledon without treatment; ING, Inglesa; RC, roasted cotyledon; ZAC, Zacatecas.

whereas the varieties with average vicine content reported marked reductions (even 39%), as shown for Col-288 whose vicine content shifted from 4.64 to 2.80 mg/g, by effect of boiling (Table 2).

The content of pyrimidine glycosides significantly differed for seed varieties when roasting or boiling treatment was applied to them. The general trend was a 6.06% average decrease in the content of vicine by effect of roasting, whereas the average decrease (19%) by effect of boiling was higher in 7 of the 10 varieties tested, which means at least 10% more reduction. These results show that boiling is a more effective treatment to reduce glycosides than roasting, this could be due to the slight water solubility of glycosides in water (Abd Allah *et al.* 1988), making them more available to heat treatment.

Convicine content for raw samples of *V. faba* L. and the effect of the thermal treatments on it are shown in Table 3.

In the varieties studied, the convicine content was in the interval 0.60 (Col-93)–1.68 (ING) mg/g of the sample. The ING and ZAC varieties, which reported the highest value of vicine content, were also the varieties with the highest convicine content. Their convicine content was affected by the thermal treatments; after roasting, the amount convicine content diminished from 1.53 to 0.48 mg/g of sample; while it was decreased from 1.52 to 0.23 mg/g of sample, by effect of boiling. In the Col-160 and Col-181 varieties, no reduction was observed in convicine content by effect of roasting, whereas for the other varieties, there was a decrease in this parameter by about 3.2–30.0%. The boiling process produced about 12.8–60% reduction in the content of convicine. As suggested by these results, the thermal processing may decrease the convicine content in the varieties examined.

The average decrease in the content of vicine and convicine was higher by boiling (18.86 and 22.53%, respectively) than by roasting (6.06 and 22.53%, respectively); moreover, the change in the content of convicine was higher than that for vicine. Similar results were obtained by Jamalian (1999)

TABLE 3. CONVICINE CONTENT (MG/G OF SAMPLE) IN RAW, ROASTED AND BOILED COTYLEDONS OF THE 10 VARIETIES OF *VICIA FABA* L.

Variety	CWT	RC	% decrease	BC	% decrease
Col-25	0.95 ± 0.05[c,d]	0.92 ± 0.03[c]	3.16	0.83 ± 0.03[c]	12.89
Col-89	0.72 ± 0.04[e,f]	0.64 ± 0.06[e,f]	11.15	0.82 ± 0.02[c]	–14.63
Col-93	0.60 ± 0.05[f]	0.48 ± 0.02[g]	20.00	0.23 ± 0.05[e]	61.25
Col-146	1.10 ± 0.06[c]	0.77 ± 0.05[d,e]	30.00	0.81 ± 0.04[c]	26.42
Col-160	0.65 ± 0.03[e,f]	0.78 ± 0.02[d]	–20.00	0.54 ± 0.03[d]	18.01
Col-181	0.63 ± 0.03[e,f]	0.63 ± 0.03[f]	0.00	0.52 ± 0.04[d]	17.06
Col-281	0.80 ± 0.02[d,e]	0.74 ± 0.04[d,e]	7.5	0.59 ± 0.08[d]	25.47
Col-288	1.12 ± 0.10[c]	1.01 ± 0.09[c]	9.80	0.45 ± 0.10[d]	59.96
ING	1.68 ± 0.15[a]	1.53 ± 0.13[a]	8.92	1.30 ± 0.06[b]	22.62
ZAC	1.47 ± 0.05[b]	1.34 ± 0.11[b]	8.84	1.52 ± 0.02[a]	–3.75

The results were obtained by averaging two replicates from two independent measurements. Different letters in the same column make reference to statistically significant differences (Tukey's test $\alpha = 0.05$). The content values are expressed in mg of convicine per g of cotyledon, dry basis.
BC, boiled cotyledon; CWT, cotyledon without treatment; ING, Inglesa; RC, roasted cotyledon; ZAC, Zacatecas.

through different cotyledons and flour obtained from broad beans; in that work, the elimination of convicine was almost complete, finding, in some cases, only traces of this compound. Khalil and Mansour (1995) found a decrease of 35 and 37% in the content of vicine and convicine, respectively, by effect of cooking and autoclave processing of broad beans.

The results of pyrimidinic glycoside content for raw, roasted and boiled cotyledons of the 10 different varieties under study indicated that vicine is the main compound. The ratio vicine/convicine for raw broad beans was between 3 and 7, for boiled broad beans it was in the interval 3–12, while it was 3–7 for roasted cotyledons. The ING variety was always at the bottom of those intervals, whereas the variety Col-93 was typically at the top. Similar works have reported ratios equal to 2:1 for two Spanish varieties of broad beans (Muzquiz *et al.* 2004). In that work, the results of vicine content (4.74 and 4.16 mg/g) were similar to that obtained for the raw cotyledons analyzed here; however, the content of convicine for the two Spanish varieties was higher than for the 10 Mexican varieties of *V. faba* L. (2.32 and 2.03 mg/g) (Goyoaga *et al.* 2008).

L-DOPA

L-DOPA was detected in raw cotyledons of nine varieties; the Col-181 variety was the only one lacking of this compound. After thermal treatment, L-DOPA was found only in the boiled cotyledon of the ZAC variety. All the results are summarized in Table 4. The content of L-DOPA was within the interval of 0.28–0.44 mg/g. The ZAC and Col-146 varieties showed the highest content of L-DOPA, which was comparable to the value (03 mg/g) determined by Shetty *et al.* (2001) for commercial cotyledons and markedly lower than 10 mg/g as reported for raw broad beans by Randhir and Shetty (2004).

TABLE 4. CONTENT OF L-DOPA (MG/G OF SAMPLE) IN RAW, ROASTED AND BOILED COTYLEDONS OF THE 10 VARIETIES OF *VICIA FABA* L

Variety	CWT	RC	BC
Col-25	0.37 ± 0.01[a,b,c]	nd	nd
Col-89	0.33 ± 0.00[c,d]	nd	nd
Col-93	0.29 ± 0.03[c,d]	nd	nd
Col-146	0.42 ± 0.06[a,b]	nd	nd
Col-160	0.30 ± 0.01[c,d]	nd	nd
Col-181	nd	nd	nd
Col-281	0.29 ± 0.08[c,d]	nd	nd
Col-288	0.34 ± 0.04[b,c,d]	nd	nd
ING	0.28 ± 0.02[d]	nd	nd
ZAC	0.44 ± 0.01[a]	nd	0.36 ± 0.02

The results were obtained by averaging two replicates from two independent measurements. Different letters in the same column make reference to statistically significant differences (Tukey's test $\alpha = 0.05$). The content values are expressed in mg of L-DOPA per g of cotyledon, dry basis.

BC, boiled cotyledon; CWT, cotyledon without treatment; ING, Inglesa; L-DOPA, L-3,4-dihydroxyphenylalanine; nd, no detected; RC, roasted cotyledon; ZAC, Zacatecas.

According to the results, the thermal treatments (roasting and boiling) performed under specified conditions completely eliminated the nonprotein amino acid L-DOPA, excluding the variety ZAC, which only showed a 16% decrease. Other treatments such as microwaves and germination (Shetty *et al.* 2001; Randhir and Shetty 2004; Goyoaga *et al.* 2008) increase the content of L-DOPA in broad beans.

Nowadays (Mwatseteza and Torto 2007), different works have reported other natural sources of L-DOPA such as seeds of *Mucuna pruriens*, they stated that after cooking and processing, "black" and "white" varieties showed a decrease in the L-DOPA content from 4.97 and 6.84% up to 0.26 and 0.67%, respectively.

The highest content of L-DOPA has been found in the embryonic axis; if the seed undergoes germination, this compound increases with time until the ninth day of the germination process (Goyoaga *et al.* 2008).

Unlike pyrimidine glycosides, L-DOPA is accumulated in pods, stalks and leaves, whereas the mature seeds nearly lack this compound. In very young seeds, small amounts of this phenolic amino acid might be detected and was observed to decrease as its metabolism occurs through different biosynthetic reactions (Longo *et al.* 1974; Lattanzio *et al.* 1982; Burbano *et al.* 1995; Kirakosyan *et al.* 2004). Because L-DOPA is a phenolic compound, it has antioxidant properties that are used by the seed for protection against oxidative damage during germination (Shetty *et al.* 2001).

Statistical Analysis

The multivariate analysis was based on the factorial design with the following factors: length of life cycle (early, mid, late), seed variety and thermal treatment (CWT, RC and BC); the response variables were content of vicine (mg/g), convicine (mg/g), total content of pyrimidine glycosides (vicine + convicine), percentage of vicine and convicine with respect to the total content and percentage of decrease in the content of vicine and convicine. Because the content of L-DOPA was completely reduced in almost all the seed varieties, this parameter was not considered as a response variable.

The factors with significant effect on the response variables are shown in Table 5. The effect of seed variety was statistically significant for all the response variables ($P < 0.0001$), whereas the thermal treatment was not significant for the percentage of vicine. The interaction (seed

TABLE 5. ANOVA RESULTS FOR THE MAIN FACTORS "SEED VARIETY," "THERMAL TREATMENT" AND "HARVEST" AND THEIR INTERACTION

Factor and level	Vicine	Convicine	Total glucoside	% vicine	% convicine	% decrease vicine	% decrease convicine
Harvest	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P = 0.4603$)	($P = 0.4603$)
Early	4.80^{a}	1.28^{a}	6.22^{a}	79.65^{b}	20.35^{a}	6.84^{a}	10.88^{a}
Mid	3.34^{c}	0.56^{c}	3.90^{b}	85.52^{a}	14.48^{b}	8.26^{a}	13.19^{a}
Late	3.94^{b}	0.76^{b}	4.71^{c}	$83.96^{a,b}$	$16.04^{a,b}$	10.27^{a}	12.58^{a}
Treatment	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P < 0.3145$)	($P < 0.0032$)	($P < 0.0000$)	($P < 0.0000$)
CWT	4.48^{a}	0.97^{a}	5.45^{a}	82.55^{a}	17.45^{a}	–	–
RC	4.20^{b}	0.88^{c}	5.08^{b}	82.99^{a}	$17.01^{a,b}$	6.53^{b}	10.20^{b}
BC	3.64^{c}	0.76^{c}	4.40^{c}	83.53^{a}	16.47^{b}	18.97^{a}	24.45^{a}
Variety	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)	($P < 0.0000$)
Col25	4.36^{c}	$0.90^{c,}$	5.26^{c}	$82.80^{b,c}$	$17.20^{c,d}$	$8.91^{c,d}$	$5.53^{c,d}$
Col89	$4.13^{d,e}$	0.73^{d}	4.85^{d}	$85.06^{a,b}$	$14.94^{d,e}$	$6.05^{d,e}$	$3.72^{d,e}$
Col93	3.12^{f}	0.44^{f}	3.56^{f}	88.11^{a}	11.89	8.00^{d}	27.36^{a}
Col-146	$4.18^{c,d}$	0.89^{c}	$5.07^{c,d}$	$82.53^{b,c}$	17.47^{c}	$6.34^{d,e}$	18.83^{b}
Col-160	$4.18^{c,d}$	$0.66^{d,e}$	4.83^{d}	$86.37^{a,b}$	13.63^{e}	10.91^{b}	$6.00^{c,d}$
Col-181	2.72^{g}	0.60^{e}	3.32^{f}	82.09	17.91^{c}	5.86^{e}	$6.22^{c,d}$
Col-281	3.72^{f}	0.71^{d}	4.42^{e}	83.99^{b}	16.01^{d}	$10.41^{b,c}$	10.90^{c}
Col-288	$3.99^{d,e}$	0.86^{c}	4.85^{d}	$82.84^{b,c}$	17.16^{c}	14.36^{a}	$23.14^{a,b}$
ING	4.81^{b}	1.50^{a}	6.31^{b}	76.20^{d}	23.80^{a}	9.67^{c}	10.57^{c}
ZAC	5.85^{a}	1.44^{b}	7.29^{a}	80.23^{c}	19.77^{b}	$4.51^{e,f}$	3.24^{e}

Values within parentheses indicate the significance level of each factor.
Different letters in the same column make reference to statistically significant differences.
ANOVA, analysis of variance; ING, Inglesa; ZAC, Zacatecas.

variety) × (thermal treatment) had the same influence than that found for the factor (thermal treatment).

The length of the plant life cycle had significant effect on the content of vicine and convicine as well as on the total content of pyrimidine glycosides; this effect being greater for early harvest than for mid-harvest broad beans, the lowest change was observed for the late-harvest broad beans. However, the magnitude of this effect had different behaviors for different compounds; the late-harvest seeds reported the highest content of vicine, the early harvest seeds reported the highest content of convicine. No difference was observed in relation to the decrease in the content of vicine and convicine by effect of life cycle length.

The pyrimidine glycosides vicine and convicine are synthesized by the seed during the first stages of seed germination; therefore, the content of this compound in unripe, fresh seeds is relatively high; in turn, these compounds are not present in pods and leaves. According to the work of Brown and Roberts (1972) on *V. faba* L., these compounds can be found in the germinating seed and no translocation from other tissues is produced. Ramsay and Griffiths (1996) found that the hull is the biosynthetic structure of these compounds, from which they are exported to the cotyledon and embryonic axis. However, as stated by Ramsay and Griffiths (1996), another synthetic place might be in the mother plant; thus, the metabolites could be transferred to the seed with the purpose of providing metabolizable nitrogen (Mager *et al.* 1980; Ramsay and Griffiths 1996). The results obtained in the present work indicated that the longer the life cycle, the lower the glycoside content; this suggests a similar behavior to that found by other authors: the content of pyrimidine glycosides decreases at the end of the seed germination, i.e., when seed ripening and dehydration occur (Jamalian 1978; Lattanzio *et al.* 1982).

The content of vicine and convicine and the total content of pyrimidine glycosides showed the same behavior independent of the thermal treatment applied to the seeds. As expected, the CWT reported the highest value for those parameters, followed by the RCS and finally by the BCS. Regarding the vicine content, no difference was observed between thermal treatments. These results indicate efficient reduction in the content of pyrimidine glycosides by effect of boiling, probably due to more solubilization of the compounds in comparison to the roasting treatment.

The analysis of the effect of the seed variety reported the maximum vicine content in the ZAC variety (6.1 mg/g) followed by the ING variety, whereas the variety with the minimum vicine content was Col-181 (2.88 mg/g). Regarding the content of convicine, ING (1.68 mg/g) showed the highest content and Col-93 (0.60 mg/g) the lowest one. By averaging the total content of glycosides, ZAC variety showed the highest value (7.29 mg/g) and Col-181 reported the lowest content (2.72 mg/g). The ratio vicine/total pyrimidine glycosides ranged between 76.20 (ING) and 88.11% (Col-93), the latter value was greater than any other found for the factor "thermal treatment." The interval for the percentage of convicine with respect to total content of glycosides, by effect of the variety analyzed, was 11.89–23.80%

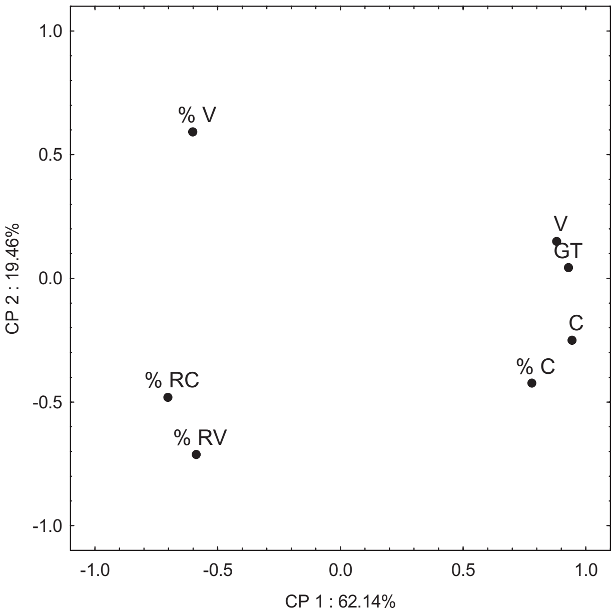

FIG. 1. PRINCIPAL COMPONENT ANALYSIS FOR THE RESPONSE VARIABLES
V = content of vicine (mg/g), C = content of convicine (mg/g), GT = total content of pyrimidine glucosides, %RV = percentage of reduction in the content of vicine by effect of the thermal treatment, % RC = percentage of reduction in the content of convicine by effect of the thermal treatment, %V = percentage of vicine with respect to the total content of pyrimidine glucosides, %C = percentage of convicine with respect to the total content of pyrimidine glucosides.

(for Col-93 and ING, respectively). The total decrease in vicine content was the highest for the Col-288 variety (14.36%); almost three times greater than that observed for ZAC variety (4.51%). The variety that showed the greatest decrease in convicine content was ING, whereas the Col-93 variety showed the lowest reduction in the same parameter. As well, the decrease in convicine content was twice the decrease in vicine content, within seed varieties and independent of the thermal treatment (Table 5).

PCA

PCA is a method that reduces data dimensionality by performing a covariance analysis between factors. In this study, the PCA was used to describe the correlations between the content of pyrimidine glycosides and the decrease in the same parameter by effect of the thermal treatments applied to the 10 seed varieties under study. Only the two first principal components (PC1 and PC2) were analyzed, as together they are responsible for 81.78% of the total decrease; 62.14 and 19.46% being the contributions from PC1 and PC2, respectively (Fig. 1). PC1 is strongly and directly correlated with the content of vicine and convicine and with the total content of glycosides (respective correlation coefficients: 0.88, 094 and 0.93), and, in lower grade, with the percentage of convicine (0.78). As well, this component is negatively correlated with the percentage of vicine, and the percentage of decrease in the content of vicine and convicine (all these correlation coefficients with a value equal to 0.60) (Fig. 1). With respect to PC2, this is inversely correlated with the percentage of convicine and with the percentage of decrease in the content of vicine and convicine (0.427, 0.716 and 0.483, respectively). The relationship of PC2 with the content of vicine and convicine and the total content of glycosides was minimal (Fig. 1). Because grouping is observed in the positive side of the variables content of vicine, content of convicine and total content of glycosides, PC1 could be redefined as the content of glycosides in the broad bean cotyledons, whereas PC2 could be redefined as percentage of glycosides, making reference to the total glycoside content or to the decrease by effect of the thermal treatment, because the variables that lie closer to the value 1 of this component are the percentage of decrease in the content of vicine and convicine, and the percentage of vicine.

Figure 2 shows the grouping of variables in the first plane of the principal components PC1 and PC2. In Fig. 2A, it is shown that early harvest samples are grouped in the positive side of PC1, with values close to 1.0 for this component, indicating that these varieties have high content of pyrimidine glycosides. The mid-harvest varieties tend to be grouped around intermediate values of the content of glycosides. In Fig. 2B, the distribution of the responses with respect to the thermal treatment is presented. The CWT and roasted are grouped in the positive side of PC1, which denotes higher content than for the BCs (grouped in the negative side of PC1 and PC2).

CONCLUSIONS

The content of vicine and convicine in the broad bean seeds was partially reduced after treatment by roasting and boiling, whereas a total decrease was found for L-DOPA, except for ZAC variety. The cotyledons of the ING and ZAC varieties had the highest content of pyrimidine glycosides, while the Col-181 and Col-93 varieties showed the lowest content of vicine and convicine. ZAC variety stood out for its content of L-DOPA even after boiling.

The vicine was the main compound in the seed varieties of *V. faba* L. under study; however, convicine was the most sensible to the thermal treatments; therefore, these compounds are partially thermostable, whereas the L-DOPA is thermolabile.

By the information acquired, the use of simple methods to reduce the content of pyrimidine glycosides and L-DOPA in broad bean has been proven; the production of faba bean

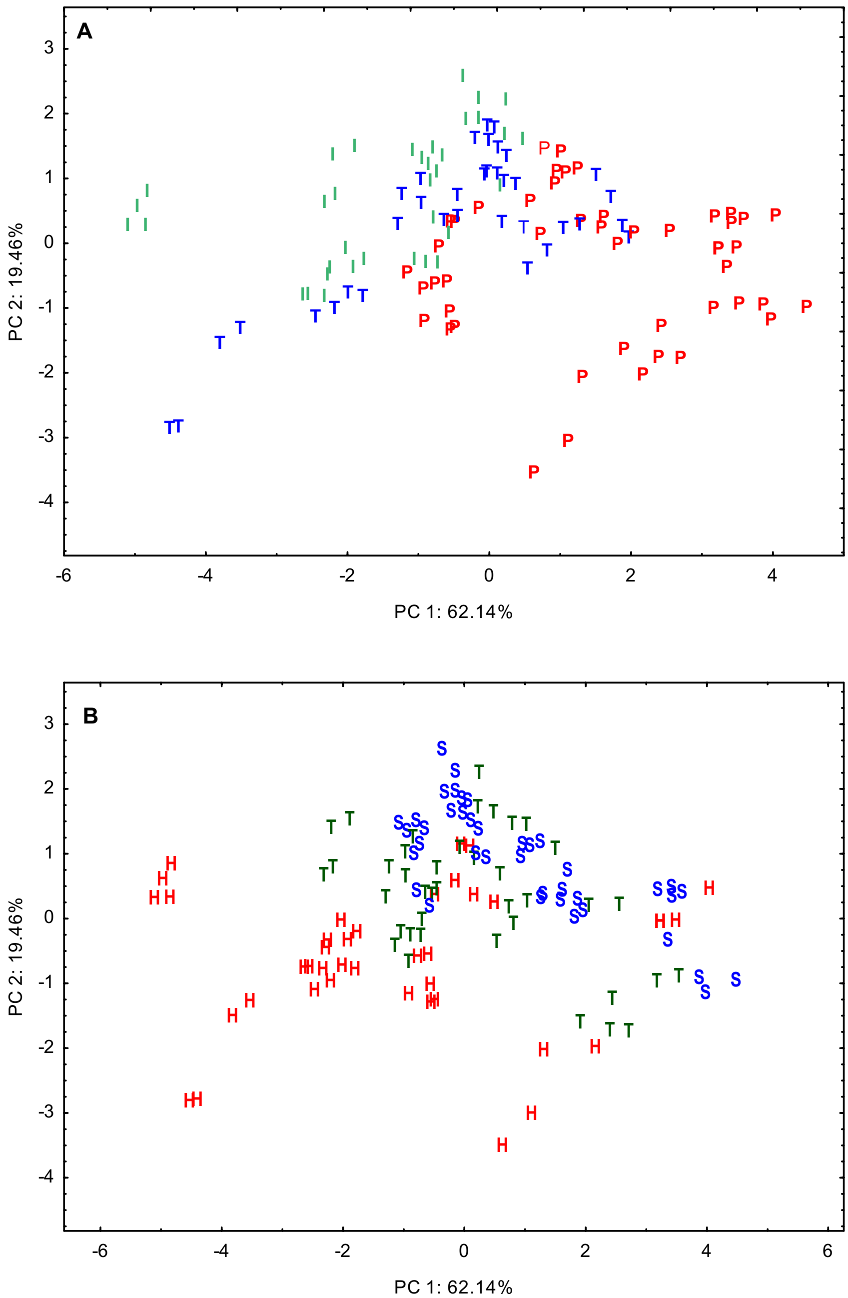

FIG. 2. PRINCIPAL COMPONENT ANALYSIS
(A) For type of harvest. I = mid, P = early, T = late. (B) For thermal treatment; S = cotyledon without treatment, H = boiled cotyledon, T = roasted cotyledon.

seeds with vicine + convicine reduced represents a real advantage in nutritional performance in food safety for humans and poultry diets. Furthermore, these compounds may be isolated and purified to take advantage for pharmaceutical applications by integrating the knowledge on phytochemistry and technology applied to broad bean, including the possibility of developing genetic modifications to reduce, eliminate or increase (especially L-DOPA) the content of these compounds in the studied varieties of *V. faba* L.

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