



Phenolic compounds profile and antioxidant activity of pea (*Pisum sativum* L.) and black bean (*Phaseolus vulgaris* L.) sprouts

Eduardo BORGES-MARTÍNEZ¹, Tzayhri GALLARDO-VELÁZQUEZ¹, Anaberta CARDADOR-MARTÍNEZ², Deyanira MOGUEL-CONCHA¹, Guillermo OSORIO-REVILLA¹, Jorge Carlos RUIZ-RUIZ³, Cristian Jiménez MARTÍNEZ^{1*} 

Abstract

Germination increases total phenolic compounds (TF) concentration and antioxidant activity (AOx). The characterization and quantification of TPC, total flavonoids content (TPC), and AOx of beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*) sprouts, germinated for ten days was performed. Results showed that the highest concentration of TPC for both sprouts was in days 6 and 7 (685.21 and 910.69 mg GAE/100 g dry matter) and TF varied only for beans. AOx of the pea sprouts was 512.64 and 6083.55 mg ET/100 g dry matter to DPPH and ORAC method, respectively, is higher than for bean. Regarding FRAP, bean sprouts showed better values (421.07 mg ET/100 g dry matter) compared to pea; in the CUPRAC analysis, sprouted bean showed better activity than a pea (85.76 and 44.05% inhibition). Germination induced variations in gallic and syringic acids in pea sprouts and of catechin and quercetin in the bean. Germination time and legume type are important factors the biological activity of sprouts.

Keywords: legumes; germination; phenolic compounds; antioxidant activity; sprouts.

Practical Application: Legume sprouts represent a good alternative for as ingredients in functional foods.

1 Introduction

Legumes have been used as food by mankind since ancient times; its consumption is common worldwide, standing out for their high content of protein and complex carbohydrates (Oghbaei & Prakash, 2016). These seeds contain different bioactive compounds, among them total phenolic compounds (Fernandez-Orozco et al., 2006) that are related with antioxidant, anti-inflammatory, and antihypertensive effects, among others (Dueñas et al., 2009; López-Amorós et al., 2006; Rawson et al., 2014; Juárez-Chairez et al., 2020). These bioactive compounds are produced as plants second metabolites, and are derived mainly from phenylalanine and, to a lesser extent, from tyrosine. They act as a defence against pathogens, parasites, and predators, and contribute to the taste, smell, color of plants (Ma et al., 2020). They include a large amount of heterogeneous substances characterized by possessing at least one aromatic ring and one hydroxyl group with a lateral functional chain (Saleh et al., 2019).

There are several techniques for preparation of legumes before their consumption; the most common is boiling, however, during this process some bioactive compounds are lost (Margier et al., 2018). Germination is a low cost alternative that improves the organoleptic properties and increases the bioactive compounds present in the seed, such as the phenolic compounds (Fernandez-Orozco et al., 2006) that are characterized for their antioxidant activity (Xu et al., 2018). Changes in TPC during germination depend on various factors as the type of legume and germination conditions (time, amount of light, and temperature) (Domínguez-Arispuro et al., 2018). Most TPC are present in the cotyledons and seed coats of legumes (López-Amorós et al., 2006).

The effect of germination on TPC is still not known accurately but, defence responses are promoted through the biosynthesis of TPC to protect themselves from biotic and abiotic stress, like photo-oxidation, reactive oxygen species (ROS), injuries, and ultraviolet light, among others (Dueñas et al., 2006). During germination, procyanidins and catechins become condensed and give origin to other molecules with a higher degree of polymerization by means of polyphenol oxidases, in consequence, diminishing their concentration (Cevallos-Casals & Cisneros-Zevallos, 2010; López-Amorós et al., 2006).

Hydrolases and polyphenol oxidases, enzymes related to TPC, increase during germination, and allow polymerization and modification of TPC, although this increase, varies with the type of legume (Nkhata et al., 2018). Likewise, during germination, there is an increase in the free phenolic compounds by hydrolysis of cell wall polymers, increasing thereby their availability. In the present work, the effect of germination on the concentration and modification of TPC with antioxidant activity in two legumes, black bean (*P. vulgaris*) and pea (*P. sativum*) was evaluated.

2 Materials and methods

2.1 Raw material

Pea seeds (*Pisum sativum* L., var Rogger) were obtained from Hidalgo state, Mexico, and bean seeds (*Phaseolus vulgaris* L. var Jamapa) from Puebla state, Mexico. Harvest 2018.

Received 08 Sept., 2020

Accepted 05 Oct., 2020

¹Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México City, Mexico

²Tecnológico de Monterrey Departamento de Bioingenierías, Santiago de Querétaro, Mexico

³Universidad Anáhuac del Mayab, Mérida, Yucatán, Mexico

*Corresponding author: crisjm_99@yahoo.com, cjimenezh@ipn.mx

2.2 Germination process

For the germination process, two-hundred seeds of each legume, previously selected, were placed on stainless steel trays and covered with moistened filter paper. Germination was performed during 10 days in darkness at 25 ± 2 °C and 70% relative humidity. After each germination day, a sample of the seeds was dried through lyophilization (JOUAN LP3, France) (-50 °C; 0.3-0.4 mPa) for 24 h. Dried sprouts were milled and sieved (180 μ m mesh) to obtain a flour of homogeneous particle size

2.3 Extraction of phenolic compounds

For the extraction of TPC, the methods proposed by Dueñas et al. (2015) was followed. 2 g of flour were mixed with 10 mL of 80% methanol acidified with HCl (pH 2.8). This mixture was stirred for 16 h in darkness at 4 °C using a Mini Rocker, Biorad (Hercules, Ca., EE.UU.) at 20 rpm, then it was centrifuged at 4000 g for 20 min in a Hermle LaborTechnik GmbH - Z 323 K (Waltham, M. EE.UU.). Three sequential extractions were performed. The obtained supernatants were combined, and the solvent evaporated in a Rotavapor® R-300, Büchi (Flawil, Suiza) at 30 °C until complete drying; lastly, the resulting powder was resuspended in 10 mL of distilled water.

2.4 Quantification and identification of TPC

Four mL of TPC extract was filtered through a C18 SEP-PAK cartridge, previously activated with methanol and water (Dueñas et al., 2015), identification of TPC was performed with the method of Khang et al. (2016) with modifications. A HPLC Agilent Technologies 1200 Series system was used with a C-18 (4 μ m \times 4.6 mm \times 250 mm, Agilent Technologies) column at 30 °C. A biphasic elution was applied (a: methanol, b: acetic acid 0.5%), the flow was of 1 mL/min and the elution time was 65 min. Each compound was identified comparing it with the retention times of the reference standards (gallic acid, ferulic acid, caffeic acid, *p*-coumaric acid, vanillin, *p*-hydroxybenzoic acid, syringic acid, catechin, quercetin, *trans*-3-hydroxycinnamic acid), and the identified compounds were quantified through calibration curves of each compound (0.005-0.08 mg/mL) at 280 nm.

2.5 Spectrophotometric quantification of total phenols

Quantification of total phenols was performed following the method developed by Singleton & Rossi (1965), with modifications for use in a multiwell plate (Varioskan Lux, Thermo Fisher, USA). Results were expressed in milligram equivalents of gallic acid per 100 g dry matter.

2.6 Determination of TF

Total Flavonoid (TF) determination was performed according to Zhishen et al. (1999), which consists in the formation of an aluminum-flavonoid complex in a basic medium (pink salmon coloration) that absorbs at 490 nm. and TPC were quantified through a calibration curve, and results were expressed as mg catechin equivalents/100 g dry matter.

2.7 Assessment of antioxidant activity

The antioxidant activity (AOx) of the phenolic extracts of sprouted beans and peas, were evaluated by means of four assays: scavenging of free DPPH radical, according to the methodology proposed by Brand-Williams et al. (1995), oxygen radical absorbance capacity (ORAC), by the method proposed by Álvarez et al. (2012); ferric ion reducing antioxidant power (FRAP), determined according to Canabady-Rochelle et al. (2015); and Cu⁺² chelating activity (CUPRAC), determined according to Dinis et al. (1994), all adapted to the multiwell plate (Varioskan Lux, Thermo Fisher, USA). All results were expressed in mg of Trolox equivalent per 100 mg dry matter.

2.8 Statistical analysis

All results are reported as means \pm standard deviation. A two-way variance analysis of replicates and comparison of means was performed with the Sidak method to establish significant differences ($p \leq 0.05$). All analyses were performed with the software packages Sigma Plot 12.0 and IBM SPSS 23.

3 Results and discussion

3.1 Quantification of TPC and TF

The content of total TPC and TF of both legumes is shown in Table 1. The total TPC shown for the bean and pea flours without germination was of 402.96 and 584.32 mg GAE/100 g dry matter., respectively; these values are within the interval of 310-1300 mg GAE/100 g dry matter reported for bean seeds and of 228-600 mg GAE/100 g dry matter reported for peas (Dueñas et al., 2006).

During germination, the total TPC concentration for both legumes increased as compared to non-sprouted seeds, obtaining a maximal concentration for bean sprouts on day 6, which represented 70.04% more than in the non-sprouted seed. For the pea, the maximal concentration of TPC was obtained on day 7, representing an increase of 55.85% with respect to non-sprouted seeds.

The results obtained for total TPC coincide with those of Cardador-Martínez et al. (2020), who reported a 1.5-times increase in black beans germinated for 7 days. (Oloyo, 2004) reported for germinated pea seeds similar values to those obtained in the present work. Tan et al. (2017) reported a 45.39% increase during 5 days of germination, these values are higher than those obtained in this work.

During germination, TPC are synthesized through the shikimic acid, pentose phosphate pathways and the phenylpropanoid pathway (López-Amorós et al., 2006). The reserve molecules like proteins and carbohydrates inside the storage tissues of the seed become mobilized fostering the plant's growth. Cevallos-Casals & Cisneros-Zevallos (2010) explain that, at the time when the seed breaks its latency state, protecting responses emerge through the synthesis of phenolic compounds. Ying et al. (2013) informed an increase in the TPC concentration in the peanut seed during germination, this increase was attributed to the plant getting prepared to avoid damage by different pathogens

Table 1. Concentration of Total Phenolic (mg GAE/100 g dry matter) and Flavonoids content (mg CE/100 g dry matter) of bean and pea sprouts.

Germination day	Total Phenolic mg GAE/100 g d.m		Flavonoids mg CE/100 g d.m.	
	Bean	Pea	Bean	Pea
0	402.96 ± 0.69 ^k	584.32 ± 0.27 ^k	5.10 ± 0.04 ^{kl}	4.53 ± 0.16 ^{kl}
1	412.81 ± 0.90 ^l	609.16 ± 0.39 ^l	6.41 ± 0.57 ^{jl}	5.40 ± 0.57 ^{dl}
2	433.30 ± 0.47 ^{il}	628.10 ± 0.20 ⁱ	7.24 ± 0.88 ^{il}	5.27 ± 0.51 ^{fl}
3	434.27 ± 0.93 ^{hli}	664.85 ± 0.25 ^h	7.91 ± 0.13 ^{hli}	5.47 ± 0.03 ^{cl}
4	487.80 ± 0.92 ^g	697.83 ± 0.50 ^g	13.49 ± 0.58 ^{gm}	5.05 ± 0.47 ^{il}
5	530.84 ± 0.46 ^f	776.56 ± 0.47 ^f	13.65 ± 0.15 ^{fm}	4.94 ± 0.75 ^{jl}
6	685.21 ± 0.46 ^a	815.72 ± 0.38 ^e	22.05 ± 0.56 ^{dn}	5.25 ± 0.02 ^{gl}
7	664.15 ± 0.11 ^b	910.69 ± 0.04 ^a	21.07 ± 0.03 ^{en}	6.02 ± 0.11 ^{al}
8	626.41 ± 0.60 ^{em}	836.08 ± 0.11 ^d	22.21 ± 0.37 ^{cn}	5.90 ± 0.01 ^{bl}
9	629.56 ± 0.03 ^{dmm}	844.01 ± 0.13 ^c	23.30 ± 0.36 ^{bn}	5.05 ± 0.51 ^{hl}
10	634.07 ± 0.12 ^{cn}	850.48 ± 0.03 ^b	23.89 ± 0.08 ^{an}	5.34 ± 1.22 ^{el}

Data represent the mean of three independent experiments ± SD. Different letter in a column indicate statistical difference ($p \leq 0.05$) through Sidak test. GAE: Gallic Acid Equivalents; CE: Catechin Equivalents; d.m: Dry matter.

or environmental factors. As can be seen in Table 1, After day 6 and 7 days, in which maximal TF concentration was reached in both legumes, these concentrations diminish.

Regarding the TPC content of non-sprouted beans and peas, this was of 5.10 and 4.53 mg CE/100 g dry matter, respectively. During the 10 germination days, the bean showed an increase in TF (468.43%), in contrast, the pea did not show any variation during the whole germination process. Like the TPC, changes in TPC during germination are influenced by diverse aspects, such as the type of legume and environmental factors (López-Amorós et al., 2006).

Dueñas et al. (2015) reported for *Phaseolus vulgaris* L. var. pinto, a TF content increase from 10.87 to 70.06 mg CE/100 g dry matter, these values are higher than those reported herein, which could be due to the bean variety and its phenolic composition. Cardador-Martínez et al. (2020) reported that the TF content of the black bean seeds increased from 16.2 to 35.5 mg CE/100 g dry matter during germination, whereas Ohanenye et al. (2020) reported a reduction of TF of 28 and 64% in soy and peanut seeds respectively, after 5 days of germination. Similar results were obtained during germination of peas in this work (Table 1); this effect is due to the activity of the polyphenol oxidase that causes enzymatic hydrolysis of TPC and polymerization of TF during germination, giving rise to a significant reduction in TPC. Results obtained with pea sprouts also agree with those of Ma et al. (2018), who showed a reduction in TPC content in pinto beans during germination.

The amount of TPC presented by beans and pea and their sprouts is greater than that presented by fruits such as pineapple, banana, lychee, papaya and their by-products (Santos-Silva et al.2020), which shows their capacity as a source of bioactive compounds

3.2 Assessment of antioxidant capacity in black beans and peas

Table 2 depicts the results obtained from the antioxidant capacity (DPPH) assessment in the black bean and pea sprouts, revealing that the antioxidant capacity was of 118.46 and 205.32 mg TE/100 g dry matter., respectively. For pea sprouts

the highest value was obtained at germination day 10. It was also observed that the AOX of both seeds increased as germination days advanced, with a maximal value at day 6 for bean sprouts (410.48 mg TE/100 g dry matter.), which represents an increase of 246.10% respect to the non-sprouted seed, and at day 7 for the pea sprouts (512.64 mg TE/100 g dry matter.), increasing 149.67%. The pea sprouts presented a higher concentration of TPC as compared to the bean sprouts. This could be a hint of why the pea extracts present a higher concentration of simple phenols like gallic or syringic acids, which have a greater capacity to scavenge free radical like the DPPH.

Results from the AOX determination through DPPH of the methanolic extracts of peas and beans sprouts, are within those reported by Dueñas et al. (2009) for *Lupinus*, with an increment from 170.3 to 690.2 mg ET/100 g dry matter on day 9 of germination, which agrees with the maximal concentration obtained for TPC in this work. Values found for the pea sprouts are within those reported by Xu et al. (2019) of 110 to 800 mg ET/100 g dry matter obtained on day 6 on the same seed, and although present difference in their constituent compounds, the values are close to those found for fruits such as blueberry and byproducts (Casas-Forero et al., 2020)

Results show an increase in AOX determined through DPPH for both seeds during germination, finding better results on the days that presented the highest TPC concentration; variation in results can be due to the influence of factors like the amount and composition of TPC in the germination days. Beninger & Hosfield (2003) attributed the DPPH free radical scavenging capacity to the presence of low- weight TPC like gallic and syringic acids.

Results obtained in this work, lie within the values reported by Dueñas et al. (2015) for sprouts of pinto beans (2000 to 8000 mg ET/100 g dry matter.) and by Wu et al. (2012), who reported an increase in AOX and of TPC during 4 germination days in black bean (1400-2200 mg ET/100 g dry matter.), and peanut seed (1339-2084 mg ET/100 g dry matter.). Xu et al. (2019) reported a 200.85 to 5000.82 mg TE/100 g of sample

Table 2. Assessment of the antioxidant and chelating activities in sprouted beans and peas (mg ET/100 g of dry matter).

Germination (days)	DPPH		ORAC		FRAP		CUPRAC (% inhibition)	
	Bean	Pea	Bean	Pea	Bean	Pea	Bean	Pea
0	118.46 ± 0.24 ^k	205.32 ± 0.16 ^{kl}	2813.70 ± 0.542 ^{klm}	3362.71 ± 3.98 ^{kl}	120.67 ± 0.23 ^{klm}	112.14 ± 0.20 ^{clqyz}	30.74 ± 0.03 ^{kl}	27.60 ± 0.19 ^{emnp}
1	134.15 ± 2.42 ^l	207.72 ± 0.27 ^{jl}	2833.28 ± 2.13 ^{ilm}	3476.50 ± 2.53 ^{jl}	121.63 ± 0.51 ^{ilm}	115.615 ± 0.35 ^{almnt}	31.39 ± 0.01 ^{jl}	22.11 ± 0.30 ^{gorty}
2	181.13 ± 1.34 ⁱ	254.58 ± 0.93 ^{im}	2849.64 ± 4.64 ^{imn}	3716.04 ± 5.60 ^{im}	123.446 ± 0.44 ^{imo}	110.75 ± 0.34 ^{dmuxy}	38.31 ± 0.42 ⁱ	19.72 ± 0.14 ^{koqvx}
3	212.29 ± 2.12 ^h	257.39 ± 1.41 ^{hm}	3203.77 ± 0.31 ^h	3716.04 ± 2.05 ^{hm}	137.518 ± 2.01 ^h	112.16 ± 0.33 ^{bpqtx}	54.69 ± 0.27 ^{hmn}	20.39 ± 0.43 ^{iruzx}
4	270.33 ± 0.32 ^{dl}	305.44 ± 0.72 ^g	3537.63 ± 1.67 ^g	4105.49 ± 3.42 ^g	185.97 ± 4.2 ^g	108.35 ± 4.59 ^{empuz}	54.91 ± 0.26 ^{gmo}	25.55 ± 0.15 ^{flmp}
5	272.67 ± 5.06 ^{dl}	334.30 ± 4.23 ^f	3806.91 ± 1.73 ^f	5209.99 ± 1.08 ^{fn}	278.58 ± 0.43 ^f	99.74 ± 0.62 ^{frw}	56.44 ± 0.47 ^{fno}	30.18 ± 0.70 ^{dn}
6	410.48 ± 0.44 ^{amnn}	390.10 ± 0.08 ^e	4353.42 ± 9.18 ^a	5238.84 ± 2.72 ^{en}	327.62 ± 0.91 ^e	95.96 ± 0.20 ^{hrv}	64.32 ± 0.36 ^{ep}	42.45 ± 0.34 ^{bs}
7	402.97 ± 3.77 ^{cmo}	512.64 ± 0.28 ^a	4066.53 ± 2.92 ^{coqr}	6083.54 4.07 ^a	341.72 ± 0.15 ^d	98.07 ± 1.32 ^{gww}	64.54 ± 0.41 ^{dp}	44.05 ± 0.39 ^{ps}
8	385.06 ± 3.85 ^{ep}	414.59 ± 3.65 ^{cmo}	4095.66 ± 6.84 ^{cops}	5447.20 ± 1.65 ^{do}	406.61 ± 0.35 ^c	83.65 ± 0.48 ^{ios}	75.06 ± 0.37 ^c	35.62 ± 0.31 ^c
9	391.11 ± 1.45 ^{dp}	414.77 ± 6.10 ^{bnp}	4073.69 ± 5.47 ^{dqqt}		415.41 ± 0.36 ^b	79.11 ± 0.21 ^{koñ}	81.39 ± 0.25 ^b	20.81 ± 0.37 ^{ivwz}
10	406.25 ± 10.06 ^{bno}	412.74 ± 10.12 ^{dop}	4141.62 ± 7.35 ^{brst}		421.07 ± 3.35 ^a	80.11 ± 0.38 ^{sn}	85.76 ± 0.14 ^a	21.52 ± 0.52 ^{hiquwy}

TE: Trolox equivalent. Data represent means ± standard deviation. Different letters in a column indicate statistical difference ($p \leq 0.05$) through a SIDAK test.

dry matter. increase in the antioxidant capacity (ORAC) of peas germinated for 6 days.

The antioxidant activity increase during germination, measured through DPPH, was seed up to 300%, whereas, through ORAC, the value increased in 200%. This difference could be due to the fact than in ORAC, the AOx of lipophilic and hydrophilic compounds is measured, whereas in DPPH only that of lipophilic compounds is measured, which is a limitation for the interpretation of AOx (Dávalos et al., 2004; Xu et al., 2019). Relevance of the ORAC analysis is that demonstrate the antioxidant capacity to neutralize the peroxy radical, which are the most abundant in the biological systems.

Table 2 depicts the results of AOx assessment through FRAP in black bean and pea seeds and sprouts. Results show the AOx of non-sprouted beans and peas was of 120.67 and 112.14 mg TE/100 g dry matter, respectively. Likewise, during the germination process, beans presented and increase in FRAP values up to 248.94% on day 10 (421.07 mg TE/100 g dry matter.) with respect to the non-sprouted seeds. In contrast, peas presented a reduction of AOx during germination (28.56%). The statistical analysis shows a non-significant difference during the first 4 days. FRAP results, indicate that AOx depends not only on the amount of TPC present in the sample, but also on the composition of phenols during the germination process, since apparently, the TPC present in peas are not able to reduce the Fe⁺³ to a state of lesser reactivity.

Shohag et al. (2012) described an increase in FRAP in soy seeds germinated for 10 days (500-790 mg TE/100 g dry matter.), and reported a 180% increase in FRAP for black beans germinated for 10 days and correlate it with an increase in TPC during germination.

3.3 Assessment of the chelating activity of Cu⁺² in the black bean and pea sprouts

Results of the Cu⁺² chelating activity (CUPRAC) of the bean and pea samples are presented on Table 2. The values obtained for non-sprouted beans and peas were 31 and 28% inhibition, however, during germination, the bean increased its chelating activity up to 86%, increasing with the germination time. In contrast, pea diminished 22% inhibition at day 10 of germination. As observed in Table 2, for pea sprouts on days 6 and 7 the highest inhibition was attained, but it never reaches a 50% reduction, whereas for bean sprouts, the highest inhibition reached was 85.76 ± 0.14 at day 10. These values demonstrate, as in the other assays, that the type of compound has a greater influence in the antioxidant capacity than its concentration. The chelating capacity of the TPC is greatly related with the synergic effect of the functional groups –OH and –COOH and with the presence of TPC, as these, aside from scavenging free radical, have the capacity the chelate metals which is higher in ortho-dihydroxy-substituted polyphenols like catechin or quercetin (Kumar & Pandey, 2013).

3.4 Characterization and quantification of phenolic compounds in the methanolic extracts of the sprouted beans and peas by HPLC

Table 3 depicts the results obtained for TPC content present in black bean (*Phaseolus vulgaris L.*) and pea (*Pisum sativum L.*) during the 10-day germination process. Identification and quantification results of TPC through HPLC (Table 3) show a variation in the composition of extracts during germination. In the sprouted beans extract, seven different phenolic compounds were identified, among them, two TPC: catechin and quercetin, and five simple phenolic acids, as well as a significant increase in catechin (449.37%), this agrees with the reports of Dueñas et al. (2015), who found an increase in catechin concentration in *P. vulgaris* germinated for 8 days.

Table 3. Concentration of phenolic compounds ($\mu\text{g/g}$ dry matter) present in bean and pea sprouts.

Germination (Days)	Gallic acid	Catechin	Vanillin	Syringic acid Bean	Ferulic acid	T-3- hydroxy cinnamic acid	Quercetin
0	6.89 \pm 0.60 ^{kl}	38.50 \pm 0.21 ^k	ND	12.73 \pm 0.58 ^{kl}	6.14 \pm 0.16 ^{kl}	ND	12.51 \pm 0.02 ^{gl}
1	7.35 \pm 1.55 ^{il}	51.73 \pm 0.08 ^j	ND	12.84 \pm 0.21 ^{jl}	9.12 \pm 0.06 ^{hm}	ND	12.37 \pm 0.24 ^{hl}
2	7.38 \pm 0.58 ^{hl}	60.11 \pm 1.09 ⁱ	ND	12.85 \pm 0.07 ^{hl}	9.06 \pm 0.54 ^{im}	ND	12.34 \pm 0.04 ^{il}
3	7.30 \pm 1.6 ^{jl}	66.83 \pm 0.26 ^h	ND	12.84 \pm 1.53 ^{il}	9.46 \pm 0.40 ^{gm}	ND	12.33 \pm 0.06 ^{kl}
4	10.84 \pm 0.57 ^{gm}	122.58 \pm 0.63 ^{el}	ND	12.98 \pm 0.18 ^{gl}	9.72 \pm 1.03 ^{fm}	ND	12.35 \pm 0.23 ^{il}
5	12.07 \pm 1.18 ^{em}	123.86 \pm 0.55 ^{dl}	23.42 \pm 0.11 ^{fm}	13.17 \pm 0.55 ^{fl}	8.96 \pm 0.08 ^{im}	6.47 \pm 0.16 th	12.71 \pm 0.65 ^{fl}
6	18.51 \pm 0.62 ^{co}	201.93 \pm 0.15 ^b	24.68 \pm 0.04 ^{em}	13.38 \pm 0.71 ^{bl}	10.98 \pm 1.58 ^{dm}	9.14 \pm 0.54 ^c	18.64 \pm 1.22 ^{em}
7	18.53 \pm 0.05 ^{bo}	154.51 \pm 0.59 ^c	4.55 \pm 0.07 ^{bo}	13.37 \pm 0.73 ^{cl}	10.72 \pm 0.08 ^{em}	8.47 \pm 0.64 ^{egh}	20.19 \pm 0.47 ^{dmm}
8	12.45 \pm 0.54 ^{dm}	71.49 \pm 0.54 ^g	3.03 \pm 0.58 ^{co}	13.25 \pm 0.22 ^{el}	14.65 \pm 0.68 ^{cn}	8.87 \pm 1.02 ^{dgh}	20.73 \pm 0.24 ^{em}
9	11.93 \pm 0.05 ^{fm}	118.58 \pm 0.24 ^f	0.88 \pm 0.27 ^{dl}	13.93 \pm 0.17 ^{al}	15.80 \pm 1.52 ^{bn}	9.90 \pm 0.55 ^{bg}	20.90 \pm 0.5 ^{bmm}
10	19.57 \pm 1.05 ^{ao}	211.51 \pm 0.53 ^a	38.85 \pm 0.33 ^a	13.29 \pm 0.55 ^{dl}	18.80 \pm 0.51 ^a	10.72 \pm 1.08 ^{ag}	21.56 \pm 0.39 ^{an}
Pea							
0	35.46 \pm 0.57 ^{jl}	32.45 \pm 0.58 ^{kl}	ND	13.35 \pm 0.03 ^{lm}	ND	ND	12.92 \pm 0.26 ^{hlm}
1	35.24 \pm 0.38 ^{kl}	41.55 \pm 0.61 ^{bm}	ND	13.65 \pm 0.53 ^{ilm}	ND	ND	12.43 \pm 0.81 ^{him}
2	35.75 \pm 0.08 ^{il}	41.04 \pm 0.54 ^{cm}	ND	12.75 \pm 0.16 ^{kl}	ND	ND	11.64 \pm 0.65 ^{kl}
3	36.38 \pm 0.61 ^{hl}	42.48 \pm 1.21 ^{am}	ND	14.78 \pm 0.72 ^{gl}	ND	ND	12.27 \pm 1.05 ^{ilm}
4	36.41 \pm 0.55 ^{gl}	37.01 \pm 0.51 ^{go}	ND	14.87 \pm 0.59 ^{fl}	ND	ND	13.58 \pm 0.22 ^{flmnp}
5	43.36 \pm 1.16 ^{em}	35.90 \pm 0.05 ^{hlo}	ND	14.52 \pm 0.57 ^{hl}	10.17 \pm 0.18 ^{fg}	ND	13.48 \pm 0.70 ^{glmnp}
6	42.42 \pm 1.02 ^{fm}	35.69 \pm 0.16 ^{ilo}	8.02 \pm 0.69 ^{ef}	15.27 \pm 0.22 ^{em}	10.50 \pm 1.10 ^{eg}	ND	16.84 \pm 0.63 ^{cnp}
7	50.75 \pm 0.58 ^{an}	37.65 \pm 0.47 ^{fo}	10.84 \pm 1.16 ^{bfg}	18.95 \pm 0.69 ^{an}	10.78 \pm 0.65 ^{dg}	ND	22.56 \pm 0.57 ^{ao}
8	44.43 \pm 0.02 ^{cm}	38.08 \pm 0.57 ^{eo}	10.19 \pm 0.58 ^{dfg}	18.79 \pm 1.06 ^{cn}	11.19 \pm 0.13 ^{eg}	ND	20.63 \pm 1.23 ^{bo}
9	43.88 \pm 0.30 ^{dm}	34.87 \pm 0.70 ^{jl}	10.43 \pm 0.75 ^{cfg}	18.70 \pm 1.04 ^{dn}	11.19 \pm 0.70 ^{bg}	ND	15.65 \pm 1.04 ^{dp}
10	47.26 \pm 1.76 ^{bn}	38.54 \pm 0.35 ^{do}	11.95 \pm 1.73 ^{ug}	18.81 \pm 2.05 ^{bn}	13.21 \pm 0.93 ^{ag}	ND	14.91 \pm 1.02 ^{emnp}

Data represent the mean of three independent experiments \pm SD. Different letter in a column indicate statistical difference ($p \leq 0.05$) through Sidak test. ND: Not detected.

Quercetin increased significantly until day 6, from there on it remained constant, reaching a maximal value on day 10 (21.56 $\mu\text{g/g}$ of dry sample). The increase of these compounds during germination could be due to induction of their synthesis in response to stress, as a protective way of the plant against pathogens, nitrogen deficiency, or the formation of structural components of the cell wall (Xu et al., 2018). On the other side, vanillin and A-T-3-hydroxycinnamic acid appear on day 6, increasing their concentration from then on.

The presence and increase of TPC in the methanolic extract of bean sprouts is an indicator of their capacity to act as antioxidants by scavenging free radical and chelating metals. It has been pointed out that the *ortho*-dihydroxy-substituted polyphenols like catechin and quercetin show a greater metal chelating activity than the simple phenols; in turn, this effect is strengthened by the presence of a double bond between carbons 2 and 3 and carbonyl groups in position 4 (Kumar & Pandey, 2013). The latter agrees with results obtained previously in which the phenolic extract of beans showed a higher metal chelating activity than the pea extracts, and this increased with increasing germination days, which could be due to the catechin and quercetin increase in the extracts. López-Amorós et al. (2006) reported an increase in the concentration of quercetin and kaempferol in black bean

seeds starting on day 6 of germination, similarly to the results obtained in this work (Table 3).

Regarding the phenolic extract of peas during the germination process (Table 3), the concentration of gallic acid increased significantly, obtaining a 43.11% increase, with a higher concentration on days 7 and 10 (50.75 and 47.26 $\mu\text{g/g}$ dry matter, respectively).

Oloyo (2004) reported that, in the 5-day germinated pea, there was a 4-times increase in the content of simple phenolic compounds. Likewise, the pea extracts presented syringic acid during all germination days, with a higher concentration on day 7. In contrast to the black bean, the pea did not present T-3-hydroxycinnamic acid. It also increases the content of ferulic acid in beans three times (10 d) and allows its synthesis in pea. Ferulic acid exerted a neuroprotective effect and enhancing behavioral function following cerebral ischemia (Lee et al., 2020).

The presence and increase in the concentration of these simple phenolic compounds in the pea extract indicated its capacity to act as an antioxidant by scavenging free radical. The anti-free radical activity of the compounds is based on the redox properties of their hydroxyl groups and the relation with different parts of their chemical structure; thus, the capacity to

eliminate the radical is related with the pattern of free hydroxyl groups in their structure (Kumar & Pandey, 2013). Likewise, the presence of these compounds agrees with results obtained previously in which the phenolic pea extract showed a better antioxidant activity in the free radical scavenging assays (DPPH and ORAC) than the phenolic bean extracts.

Table 3 also shows that, during germination of the pea seeds, the identified TPC (catechin and quercetin) did not show a significant increase as occurred in the bean seeds. This behavior agrees with the report by Ohanenye et al. (2020), who found a reduction in TPC of 28 and 64% in soy and peanut seeds, respectively, after 5 days of germination; this effect is attributed to the activity of the polyphenol oxidase enzyme, which causes enzymatic hydrolysis of TPC during germination. The effect of TPC in the pea extract could explain the reduction in the metal chelating activity of the extracts of this seed. Cevallos-Casals & Cisneros-Zevallos (2010) reported a reduction in the TPC of sprouted soy and lentil seed from day 7 of germination, probably causing oxidation of these compounds, which would be used as precursors of lignin or would become part of the structure of lignans.

The phenolic extracts of beans and peas presented vanillin on days 5 and 6 of germination; this result is similar to that reported by Khang et al. (2016), who identified this compound in sprouted black beans on day 5 and that by López-Amorós et al. (2006), who reported this result in pea sprouts on day 6. They concluded that the appearance of vanillin depends on the enzymatic degradation of lignin present in the seed.

To analyze the relation existing between total and individual phenolic compounds with the antioxidant capacity respect to AOX, the Pearson correlation index (r) was used, and it was found that in the pea, the DPPH and ORAC methods have a positive and strong correlation ($0.867 > r < 0.984$) with the total phenolic compounds and quercetin, gallic, syringic, ferulic acids, and vanillin as individual compounds. The FRAP and CUPRAC methods had mild or moderate correlations ($r \leq 0.7$) or negative correlations with the TPC. Whereas in the bean, the correlations were stronger and positive ($r > 0.9$) and were present in DPPH and ORAC, in contrast to the pea, in which correlation existed with FRAP and CUPRAC methods.

However, each compound contributes differently to the antioxidant activity. Low correlation coefficients could be because the antioxidant activity is given by the total contribution of bioactive compounds and not only by their individual fractions.

4 Conclusions

Germination is a simple and low-cost technological process to increase the consumption of legumes. This process aside of improving the taste of the products improves their biological properties, which in this case were assessed by antioxidant and chelating activities. The beneficial effect exerted by legume seeds is strengthened by germination, because during this process there are changes in the content and type of phenolic compounds, which is reflected in an increase in the antioxidant capacity. This, together with their already proven nutritional value, make legumes an important raw material for the elaboration of

functional foods that, in the future, could become an alternative for the prevention and, perhaps, control of chronic-degenerative diseases, an effect that would be important to be assessed.

Acknowledgements

Authors thank the *Consejo Nacional de Ciencia y Tecnología (CONACyT)* for the support granted through a fellowship and the IPN for the financial support provided through project SIP-202002.

References

- Álvarez, R., Carvalho, C. P., Sierra, J., Lara, O., Cardona, D., & Londoño-Londoño, J. (2012). Citrus juice extraction systems: effect on chemical composition and antioxidant activity of clementine juice. *Journal of Agricultural and Food Chemistry*, 60(3), 774-781. <http://dx.doi.org/10.1021/jf203353h>. PMID:22225414.
- Beninger, C. W., & Hosfield, G. L. (2003). Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *Journal of Agricultural and Food Chemistry*, 51(27), 7879-7883. <http://dx.doi.org/10.1021/jf0304324>. PMID:14690368.
- Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft + Technologie*, 28(1), 25-30. [http://dx.doi.org/10.1016/S0023-6438\(95\)80008-5](http://dx.doi.org/10.1016/S0023-6438(95)80008-5).
- Canabady-Rochelle, L. L. S., Harscoat-Schiavo, C., Kessler, V., Aymes, A., Fournier, F., & Girardet, J.-M. (2015). Determination of reducing power and metal chelating ability of antioxidant peptides: revisited methods. *Food Chemistry*, 183, 129-135. <http://dx.doi.org/10.1016/j.foodchem.2015.02.147>. PMID:25863620.
- Cardador-Martínez, A., Martínez-Tequitlalpan, Y., Gallardo-Velazquez, T., Sánchez-Chino, X. M., Martínez-Herrera, J., Corzo-Ríos, L. J., & Jiménez-Martínez, C. (2020). Effect of instant controlled pressure-drop on the non-nutritional compounds of seeds and sprouts of common black bean (*Phaseolus vulgaris* L.). *Molecules*, 25(6), 1464. <http://dx.doi.org/10.3390/molecules25061464>. PMID:32213962.
- Casas-Forero, N., Orellana-Palma, P., & Petzold, G. (2020). Influence of block freeze concentration and evaporation on physicochemical properties, bioactive compounds, and antioxidant activity in blueberry juice. *Food Science and Technology*. In press. <http://dx.doi.org/10.1590/fst.29819>.
- Cevallos-Casals, B. A., & Cisneros-Zevallos, L. (2010). Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. *Food Chemistry*, 119(4), 1485-1490. <http://dx.doi.org/10.1016/j.foodchem.2009.09.030>.
- Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) assay. *Journal of Agricultural and Food Chemistry*, 52(1), 48-54. <http://dx.doi.org/10.1021/jf0305231>. PMID:14709012.
- Dinis, T. C. P., Maderia, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (Acetaminophen, Salicylate, and 5-Aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Archives of Biochemistry and Biophysics*, 315(1), 161-169. <http://dx.doi.org/10.1006/abbi.1994.1485>. PMID:7979394.
- Domínguez-Arispuro, D. M., Cuevas-Rodríguez, E. O., Milán-Carrillo, J., León-López, L., Gutiérrez-Dorado, R., & Reyes-Moreno, C. (2018). Optimal germination condition impacts on the antioxidant activity and phenolic acids profile in pigmented desi chickpea (*Cicer arietinum* L.) seeds. *Journal of Food Science and Technology*, 55(2), 638-647. <http://dx.doi.org/10.1007/s13197-017-2973-1>. PMID:29391628.
- Dueñas, M., Hernández, T., & Estrella, I. (2006). Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chemistry*, 98(1), 95-103. <http://dx.doi.org/10.1016/j.foodchem.2005.05.052>.
- Dueñas, M., Hernández, T., Estrella, I., & Fernández, D. (2009). Germination as a process to increase the polyphenol content and antioxidant

- activity of lupin seeds (*Lupinus angustifolius* L.). *Food Chemistry*, 117(4), 599-607. <http://dx.doi.org/10.1016/j.foodchem.2009.04.051>.
- Dueñas, M., Muñoz-González, I., Cueva, C., Jiménez-Girón, A., Sánchez-Patán, F., Santos-Buelga, C., Moreno-Arribas, M. V., & Bartolomé, B. (2015). A survey of modulation of gut microbiota by dietary polyphenols. *BioMed Research International*, 2015, 850902. <http://dx.doi.org/10.1155/2015/850902>. PMID:25793210.
- Fernandez-Orozco, R., Piskula, M. K., Zielinski, H., Kozłowska, H., Frias, J., & Vidal-Valverde, C. (2006). Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. Zapaton. *European Food Research and Technology*, 223(4), 495-502. <http://dx.doi.org/10.1007/s00217-005-0229-1>.
- Juárez-Chairez, M. F., Cid-gallegos, M.S., Meza-Marquez, O.G., & Jimenez-Martínez, C. (2020). Biological activities of chickpea in human health (*Cicer arietinum* L.): a review. *Plant Foods for Human Nutrition*, 75(2), 142-153. <http://dx.doi.org/10.1007/s11130-020-00814-2>. PMID:32239331.
- Khang, D. T., Dung, T. N., Elzaawely, A. A., & Xuan, T. D. (2016). Phenolic profiles and antioxidant activity of germinated legumes. *Foods*, 5(2), 1. <http://dx.doi.org/10.3390/foods5020027>. PMID:28231122.
- Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, 2013, 162750. <http://dx.doi.org/10.1155/2013/162750>. PMID:24470791.
- Lee, S. H., Yeo, D., & Hong, J. H. (2020). Effect of dihydroferulic acid obtained from fermented rice bran extract on neuroprotection and behavioral recovery in an ischemic rat model. *Food Science and Technology*. In press. <http://dx.doi.org/10.1590/fst.33719>.
- López-Amorós, M. L., Hernández, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, 19(4), 277-283. <http://dx.doi.org/10.1016/j.jfca.2004.06.012>.
- Ma, M., Zhang, H., Xie, Y., Yang, M., Tang, J., Wang, P., Yang, R., & Gu, Z. (2020). Response of nutritional and functional composition, anti-nutritional factors and antioxidant activity in germinated soybean under UV-B radiation. *Lebensmittel-Wissenschaft + Technologie*, 118, 108709. <http://dx.doi.org/10.1016/j.lwt.2019.108709>.
- Ma, Z., Boye, J. I., & Hu, X. (2018). Nutritional quality and techno-functional changes in raw, germinated and fermented yellow field pea (*Pisum sativum* L.) upon pasteurization. *Lebensmittel-Wissenschaft + Technologie*, 92, 147-154. <http://dx.doi.org/10.1016/j.lwt.2018.02.018>.
- Margier, M., Georgé, S., Hafnaoui, N., Remond, D., Nowicki, M., Du Chaffaut, L., Amiot, M. J., & Reboul, E. (2018). Nutritional composition and bioactive content of legumes: characterization of pulses frequently consumed in France and effect of the cooking method. *Nutrients*, 10(11), E1668. <http://dx.doi.org/10.3390/nu10111668>. PMID:30400385.
- Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition*, 6(8), 2446-2458. <http://dx.doi.org/10.1002/fsn3.846>. PMID:30510746.
- Oghbaei, M., & Prakash, J. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: a comprehensive review. *Cogent Food & Agriculture*, 2(1), 1136015. <http://dx.doi.org/10.1080/23311932.2015.1136015>.
- Ohanenye, I. C., Tsopmo, A., Ejike, C. E. C. C., & Udenigwe, C. C. (2020). Germination as a bioprocess for enhancing the quality and nutritional prospects of legume proteins. *Trends in Food Science & Technology*, 101, 213-222. <http://dx.doi.org/10.1016/j.tifs.2020.05.003>.
- Oloyo, R. A. (2004). Chemical and nutritional quality changes in germinating seeds of *Cajanus cajan* L. *Food Chemistry*, 85(4), 497-502. [http://dx.doi.org/10.1016/S0308-8146\(02\)00454-5](http://dx.doi.org/10.1016/S0308-8146(02)00454-5).
- Rawson, A., Patras, A., Oomah, B. D., Campos-Vega, R., & Hossain, M. B. (2014). Antioxidant activity of phytochemicals and their method of analysis. In P. M. Visakh, L. B. Iturriaga & P. D. Ribotta (Eds.), *Advances in food science and nutrition* (pp. 153-256). Hoboken: John Wiley & Sons.
- Saleh, H. M., Hassan, A. A., Mansour, E. H., Fahmy, H. A., & El-Bedawey, A. E. (2019). Melatonin, phenolics content and antioxidant activity of germinated selected legumes and their fractions. *Journal of the Saudi Society of Agricultural Sciences*, 18(3), 294-301. <http://dx.doi.org/10.1016/j.jssas.2017.09.001>.
- Santos-Silva, J., Ortiz-Weyrich, D., García, L. G. C., Ramírez-Asquieri, E., Salamoni-Becker, F., & Damian, C. (2020). Effect of drying on nutritional composition, antioxidant capacity and bioactive compounds of fruits co-products. *Food Science and Technology*. In press. <http://dx.doi.org/10.1590/fst.21419>.
- Shohag, M. J. I., Wei, Y., & Yang, X. (2012). Changes of folate and other potential health-promoting phytochemicals in legume seeds as affected by germination. *Journal of Agricultural and Food Chemistry*, 60(36), 9137-9143. <http://dx.doi.org/10.1021/jf302403t>. PMID:22906127.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.
- Tan, Y., Chang, S. K. C., & Zhang, Y. (2017). Comparison of α -amylase, α -glucosidase, and lipase inhibitory activity of the phenolic substances in two black legumes of different genera. *Food Chemistry*, 214, 259-268. <http://dx.doi.org/10.1016/j.foodchem.2016.06.100>. PMID:27507474.
- Wu, Z., Song, L., Feng, S., Liu, Y., He, G., Yioe, Y., Liu, S. Q., & Huang, D. (2012). Germination dramatically increases isoflavonoid content and diversity in chickpea (*Cicer arietinum* L.) seeds. *Journal of Agricultural and Food Chemistry*, 60(35), 8606-8615. <http://dx.doi.org/10.1021/jf3021514>. PMID:22816801.
- Xu, M., Jin, Z., Ohm, J.-B., Schwarz, P., Rao, J., & Chen, B. (2018). Improvement of the antioxidative activity of soluble phenolic compounds in chickpea by germination. *Journal of Agricultural and Food Chemistry*, 66(24), 6179-6187. <http://dx.doi.org/10.1021/acs.jafc.8b02208>. PMID:29860843.
- Xu, M., Jin, Z., Simsek, S., Hall, C., Rao, J., & Chen, B. (2019). Effect of germination on the chemical composition, thermal, pasting, and moisture sorption properties of flours from chickpea, lentil, and yellow pea. *Food Chemistry*, 295, 579-587. <http://dx.doi.org/10.1016/j.foodchem.2019.05.167>. PMID:31174798.
- Ying, C. M., Azlan, A., Al-Sheraji, S. H., Hassan, F. A., & Prasad, K. N. (2013). Antioxidant activities and total phenolic content in germinated and non-germinated legume extracts following alkaline-acid hydrolysis. *Pakistan Journal of Nutrition*, 12(12), 1036-1041. <http://dx.doi.org/10.3923/pjn.2013.1036.1041>.
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555-559. [http://dx.doi.org/10.1016/S0308-8146\(98\)00102-2](http://dx.doi.org/10.1016/S0308-8146(98)00102-2).