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Influence of germination on the bioactive compounds, antioxidant capacity and thermic characteristics of *Phaseolus vulgaris* L. and *Cajanus cajan* seeds

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Summary

Aim: To evaluate the effect of germination on total phenols, antioxidant capacity, trace elements, and thermal behavior of the seeds of Pigeon peas (*Cajanus cajan* L.) and Chacha and Recline varieties of the common bean (*Phaseolus vulgaris* L.). **Methodology:** The evaluation of the total phenols was done using Folin Ciocalteu's spectrophotometric method; the antioxidant capacity through the DPPH y $ABTS^{\circ+}$ radicals; the macro and microelements using the spectrophotometric optical emission coupled inductively with plasma method ICP-OES; and the thermic behavior through differential scanning calorimetry (DSC). **Results:** The germination process influenced the total polyphenol content, antioxidant capacity, and macro and microelements in each specie that was studied. For example, for the Recline variety seedlings, the phenol content increased by 57.75% in comparison to the seed. The antioxidant capacity against DPPH and $ABTS^{\circ+}$ was greater in the seedlings in the following order: Chaucha variety>Pigeon pea>Recline variety. For the seeds, the macro and micro-element content had the following order: K>Mg>Ca>Na y Fe>Zn>Cu>Cr; for the seedlings, the greatest decrease was in K, Cr and the greatest increase was in Ca and Mn; the Na:K relationship was less than one. Finally, the thermic behavior, in reference to the gelatinization enthalpy (ΔH) was greater for the seed than the seedlings.

Keywords: Trace elements, *Cajanus cajan, Phaseolus vulgaris,* Differential scanning calorimetry, Sprout.

Resumen

Influencia de la germinación sobre los compuestos bioactivos, capacidad antioxidante y características térmicas de semillas de *Phaseolus vulgaris L.* y *Cajanus cajan*

Objetivo: evaluar el efecto de la germinación sobre los fenoles totales, capacidad antioxidante, oligoelementos y comportamiento térmico de las semillas de guandú (*Cajanus cajan* L.) y frijol común (*Phaseolus vulgaris* L.) de las variedades Chaucha y Recline. **Metodología:** la evaluación de los fenoles totales se realizó mediante el método espectrofotométrico de Folin Ciocalteu; la capacidad antioxidante a través de los radicales DPPH y $ABTS^{o+}$; los macro y microelementos utilizando la emisión óptica espectrofotométrica acoplada inductivamente con plasma método ICP-OES; y el comportamiento térmico mediante calorimetría diferencial de barrido (DSC). **Resultados:** el proceso de germinación influyó en el contenido de polifenoles totales, capacidad antioxidante y macro y microelementos en cada especie estudiada. Por ejemplo, para las plántulas de la variedad reclinada, el contenido de fenoles aumentó en un 57,75 % en comparación con la semilla. La capacidad antioxidante frente a DPPH y ABTS $^{o+}$ fue mayor en las plántulas en el siguiente orden: variedad Chaucha > guandú > variedad Recline. Para las semillas, el contenido de macro y micro elementos tuvo el siguiente orden: K>Mg>Ca>Na y Fe>Zn>Cu>Cr; para las plántulas, la mayor disminución fue en K y Cr, y el mayor incremento fue en Ca y Mn; la relación Na:K fue menor que uno. Finalmente, el comportamiento térmico, en referencia a la entalpía de gelatinización (ΔH) fue mayor para la semilla que para las plántulas.

Palabras clave: Elementos traza, *Cajanus cajan*, *Phaseolus vulgaris*, calorimetría diferencial de barrido, brote.

Resumo

Influência da germinação nos compostos bioativos, capacidade antioxidante e características térmicas de sementes de *Phaseolus vulgaris L.* e *Cajanus cajan*

Objetivo: avaliar o efeito da germinação nos fenóis totais, capacidade antioxidante, oligoelementos e comportamento térmico das sementes do feijão boer (*Cajanus cajan* L.) e das variedades Chacha e Recline do feijão comum (Phaseolus vulgaris L.). **Metodologia:** a avaliação dos fenóis totais foi feita pelo método espectrofotométrico de Folin Ciocalteu; a capacidade antioxidante através dos radicais DPPH y ABTS°+; os macro e microelementos utilizando a emissão óptica espectrofotométrica acoplada indutivamente com o método de plasma ICP-OES; e o comportamento térmico por calorimetria exploratória diferencial (DSC). **Resultados:** o processo de germinação influenciou o teor de polifenóis totais, capacidade antioxidante, macro e microelementos em cada espécie estudada. Por exemplo, para as mudas da variedade reclinada, o teor de fenóis aumentou 57,75% em relação à semente. A capacidade antioxidante contra DPPH e ABTS °+ foi maior nas mudas na seguinte ordem: variedade Chaucha>feijão boer>variedade reclinada. Para as sementes, os teores de macro e microelementos tiveram a seguinte ordem: K>Mg>-Ca>Na y Fe>Zn>Cu>Cr; para as mudas, a maior diminuição foi em K, Cr e o maior aumento foi em Ca e Mn; a relação Na:K foi menor que um. Por fim, o comportamento térmico, referente à entalpia de gelatinização (ΔH) foi maior para a semente do que para a plântula.

Palavras-chave: Oligoelementos, *Cajanus cajan*, *Phaseolus vulgaris*, calorimetria exploratória diferencial, rebentos.

Introduction

Legumes (Fabaceae/Leguminosae) are considered the second most important crop in human nutrition, after cereals (Gramineae). Leguminosae seeds constitute an essential part of the human diet, they are an excellent source of protein, bioactive compounds, minerals, and vitamins, in comparison to cereals which are more widely represented; this is the reason that it's known as "poor man's meat" [1]. The *Phaseolus* L. genre is made up of 76 species throughout the world, with those most widely represented being the: tepary bean (*P. acutifolius* A. Gary), runner bean (*P. coccineous* L.), lima bean (*P. lunalus* L.), year bean (*P. palyanthus* Greenman) and the common bean

(*P. vulgaris* L.) [2]. The last of which has a high nutritional and nutraceutical value; thus, it is considered to be a valuable nutrient source. Beans contain carbohydrates, vitamins, soluble fiber, minerals, phenolic compounds, phenolic acid, flavonoids and proanthocyanidins; all with antioxidant properties [3]. They also contain non-nutritional components and toxic compounds such as saponins, lecithin, condensed tannins, trypsin inhibitors and phytic acid, all of which interfere with the digestion of proteins and minerals [4]. At the same time, beans present phytochemical compounds, which provide additional health benefits, given that they possess preventative effects against cancer, due to having antimutagenic and antiproliferative properties [5, 6]. In addition, they are an important source of polyphenols, with effects that have been proven to protect the oxidation of low-density lipoproteins (LDL) [7]. The Pigeon pea (*Cajanus cajan)* is a species of high nutritional value and occupies a very important place in the diets of many countries around the world [8]. This Leguminosae is eaten as a vegetable (green unripe seeds) or as a legume (dry seeds) [9].

In the seedlings, the nutraceutical properties are improved, which modify the content of different metabolites; in particular the peptides and amino acids [8]. This process can cause changes in the phytochemical compounds and their functional properties due to cellular respiration and biochemical metabolism [7]. For example, the nutritional values of the seeds can be improved due to the degradation of the principal macronutrients such as carbohydrates, proteins, and organic acids; favoring the increase of simple sugars and amino acids. At the same time, the non-nutritional factors such as protease and lecithin are reduced [10]. The research that has been done up to now to determine the antioxidant potential and the polyphenol and mineral contents, specifically for the common bean and Pigeon pea, shows scarce information. Based on this framework, the objective that was proposed was to evaluate the total phenols, antioxidant capacity, macro and micro-elements, and the thermic behavior of the seeds and seedlings of Pigeon peas (*C. cajan)* and common beans (*P. vulgaris* L.) of the Chaucha and Recline varieties.

Materials and Methods

Raw Material

Chaucha and Recline varieties of the common bean (*P. vulgaris* L.) and Pigeon peas (*C. cajan)* were acquired from the seeds laboratory of the Faculty of Agronomy at Universidad Nacional Agraria de la Selva (Peru).

Germination process

The methodology recommended by Pakaj *et al.* (2014) [11] and Cardador-Martinez *et al.* (2020) [12] was followed, where the seeds were disinfected by immersion in ethanol at 70% for one minute, then soaked in distilled water at a proportion of 1:10 (m/v) for twelve hours. The water they were soaked in was eliminated and the seeds were spread out on sterile trays and stored in the dark at room temperature for five days; to avoid microbial growth, they were washed with sterile distilled water every twenty-four hours. The sprouts were dried at 50°C until they reached the constant weight, then they were ground, placed into triple laminated bags, stored in the dark, and refrigerated until they were later analyzed.

Preparation of the extract

The method started with a mix of 1 g of the sample with methanol at 80%, at a total volume of 20 mL, with an agitation rate of 250 rpm for 24 hours at room temperature. The suspension was centrifuged at 10 000 rpm, 10 minutes at 4 °C (Hettich, Mikro R22, Germany) and then the supernatant was filtered using a PVDF 0.45 μm filter and refrigerated at 4 °C until its analysis [13].

Total Phenols

These were determined by using the Folin-Ciocalteu's spectrophotometer [14] with minimal modification; gallic acid was used as the standard (10-100 $\mu g \cdot mL^{-1}$). In brief, 100 uL of the extract was mixed with 500 μL of Folin-Ciocalteu reactor (0.2 N), in a 1.5 mL polystyrene tray and it was left to react in a room temperature environment for two minutes. A saturated sodium carbonate solution (400 μL; 7.5%) was added and left to rest for two hours at room temperature and the absorbency reading was done at 740 nm. The total phenols was expressed as milligrams of equivalent gallic acid per gram of dry sample ($mg\ EAG \cdot g^{-1}$).

Antioxidant Capacity

Capacity for capturing DPPH radicals

50 μL of the extract was mixed with 950 μL of the DPPH methanolic solution (100 μM) in a 1.5 mL polystyrene tray and they were incubated in the dark at room temperature for fifteen minutes. Its absorbance at 517 nm was measured using a UV-Vis spectrophotometer (Thermo Scientific, Genesys 10, U.S.A.). The activity for the elimination of DPPH radicals was expressed as Trolox millimole equivalents per gram of the dry sample ($mmol\ ET \cdot g^{-1}$) [15].

Capacity for capturing $ABTS^{°+}$ radicals

The activity for the elimination of $ABTS^{°+}$ radicals was measured according to the method described by Re *et al.* (1999) [16], with some modifications. ABTS was dissolved in water at a concentration of 7 mM, the radical was formed by making the ABTS solution react with potassium persulfate at 2.45 mM and the prepared mix was left to rest in the dark at room temperature for sixteen hours. The solution of $ABTS^{°+}$ radicals was diluted with ethanol until an absorbency of 0.70 was reached at 734 nm (Thermo Scientific spectrophotometer, Genesys 10, U.S.A.). The mix was 10 μL of extract with 990 μL of $ABTS^{°+}$ radical and the reaction time was a fifteen minutes period, where at the end an absorbency reading was done. The results were expressed in millimole trolox equivalents per gram of the dry sample (mmol $ET·g^{-1}$).

Analysis of the micro and macro elements

Preparation of the digestion

A sample of 0.5 g in powder was weighed in a flask and 10 mL of nitric acid solution (65%) with perchloric acid (98%) was added at a relationship of 4:1(v/v). The mix was led to digest through heating, starting at 50 °C and reaching 280 to 300 °C. The complete digestion was reached at 3 h when white-colored vapor appeared. The digested sample was cooled to room temperature, filtered using Whatman N° 20 paper, and flushed with distilled water in a 25 mL flask [17, 18].

Instruments

The digested samples were analyzed with an inductively coupled plasma optical emission spectrometry (ICP OES) (Horiba brand, Ultima Expert model). Argon gas 5.0 was used (99.99 % purity, Praxair, Peru). The operational conditions were: argon gas-plasm flow of 12 L/min, nebulizer flow velocity of 2 L/min, pump velocity of 30 rpm, stabilization time of 15 s, three trials for each measurement and observation of the radial plasma in view of the total plasma. The results were expressed in $mg·100\ g^{-1}$ of the dry sample [19].

Differential scanning calorimetry (DSC) analysis

The thermic behavior of the flour was found using a calorimeter (SETARAM, Labsys Evo Robot, France). The method recommended by Alzate *et al.* (2013) [20], was followed, with slight modifications. The sample was sifted with a 60 mesh (250 μm/ 0.250 mm) and was hydrated with distilled water until a suspension of 80% humidity was reached. Forty milligrams of the flour-water suspension were weighed and loaded into an aluminum crucible (75 μL); the crucibles were hermetically sealed and left to rest for one hour at room temperature. For the differential scanning calorimetry (DSC) analysis, the equipment was calibrated with indium and an empty aluminum crucible was considered to be the reference. The velocity for heating the samples was 2 °C/min at an interval of 25-120 °C, with a nitrogen atmosphere. The onset temperature (To), the peak temperature (Tp), the completion temperature (Tc) and the gelatinization enthalpy (ΔHgel) were automatically calculated with CALISTO software.

Results and Discussion

Total phenol content in the seeds and sprouts

According to the scientific evidence, the phenolic compounds have beneficial effects at a physiological, antioxidant and therapeutic level; the total phenol content of the seeds and sprouts (Table 1) shows a significant statistical difference ($p \leq 0.05$) between the samples. For the seeds, the phenol content varied between 0.466 (Chaucha variety) and 0.136 mg $EAG \cdot g^{-1}$ (Recline variety); the results were inferior to those reported by James *et al.* (2020) [9] in red beans, 221.05 mg $EAG \cdot 100\ g^{-1}$ and *Cajanus cajan,* 196.33 mg $EAG \cdot 100\ g^{-1}$. However, they were similar to those reported by Lee *et al.* (2018) [21] in 209 varieties of the common bean from Korea, where the average was 1.3 mg $EAG \cdot g^{-1}$ (0.55 to 2.23 mg $GAE \cdot g^{-1}$). However, Gan *et al.* (2017) [10] concluded that for common beans, the phenols can vary between not detectable (ND) and 4871 mg $EAG \cdot 100g^{-1}$, with the red bean having the greatest value. It is worth pointing out that the variation in the total phenol content depends on the species of the bean, extraction method [22], climatological conditions, crop management and storage conditions, which affect the nutritional quality of the seeds [4].

Concerning sprouts, the total phenol content increased for the Chaucha by 57.7% and the Pigeon pea by 39.4%, but for the Recline, it decreased by 13.3%. With respect to the black bean sprouts, the phenols increased by 99% and there was no difference in the drying method [12]. Meanwhile, Yang *et al*. (2018) [3] reported 4.00 $mgEAG \cdot g^{-1}$ for the black bean seed and 6.74 $mgEAG \cdot g^{-1}$ for the sprout, with an increase of 40.6%; and according to Torres *et al*. (2018) [8], for the Pigeon pea, the number of total polyphenols increased by 110%. This is related to the reactivation of the polyphenol oxidase during germination, which intervenes in the synthesis of the polyphenol compounds. According to James *et al.* (2020) [9], the increase in the total phenols could be a result of the solubility of the condensed tannins when the seeds are soaking and the migration of the phenol compounds to the external layer, as a result of the germination observed during the darkening of the germinated seeds.

Table 1. Results of the total phenols and antioxidant capacity in the seeds and sprouts.

	Sample	Total phenols (mg $EAG \cdot g^{-1}$)	DPPH (mmol $ET \cdot g^{-1}$)	$ABTS^{0+}$ (mmol $ET \cdot g^{-1}$)
Seed	Chaucha Variety	0.466±0.003 ^{b}	2.766±0.008 ^{b}	1.996±0.031 ^{b}
	Recline Variety	0.136±0.003 ^{d}	0.290±0.000 ^{e}	0.066±0.003 ^{d}
	Pigeon Pea	0.270±0.000 ^{c}	0.623±0.006 ^{d}	0.593±0.267 ^{cd}
Sprout	Chaucha Variety	1.103±0.008 ^{a} (+57.7)	3.993±0.014 ^{a} (+30.7)	3.766±0.081 ^{a} (+47.7)
	Recline Variety	0.120±0.000 ^{d} (-13.3)	0.263±0.008 ^{e} (-10.2)	0.090±0.005 ^{d} (+26.6)
	Pigeon Pea	0.446±0.003 ^{b} (+39.4)	1.450±0.040 ^{c} (+57.0)	1.086±0.031 ^{c} (+45.3)

Values represent: average ± SEM; repetitions: n=3; values from the same column with different superindices are significant ($p \leq 0.05$); (+/-) = % increase /decrease. (*) mgEAG·g^{-1} of sample; (**) mmol ET·g^{-1} of sample.

Antioxidant capacities of the seeds and sprouts

Inhibition capacity for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The antioxidant capacity of the legumes in study varied between 0.290 and 2.766 mmol ET·g^{-1} sample of the Chaucha and Recline varieties, respectively. Regarding 209 common beans in Korea, an activity of trapping the DPPH IC_{50} radical between 62.3 and 643.9 was reported, with an average of 105.0 [21] and a DPPH antioxidant activity of 11.6 to 19.2 µmol ETrolox·g^{-1} of dry weight [4]. The reported variation can be justified by the fact that the phenol-compounds and the antioxidant activity of the seeds, which have different colors (red, black, brown, white beans, etc.) are influenced by the cultivators [6].

The results for the sprouts revealed a significant statistical difference. When comparing the seed and the sprout of the Chaucha variety, there was an increase of 30.7% and for the Pigeon pea, it was 57.0%. This behavior was also demonstrated by James *et al.* (2020) [9] evaluating the antioxidant capacity against the DPPH free radical for the red bean seed. It was 28.35% and by the third day of germination, 60.12%, with an increase of 52.84%, as well as, in the Pigeon pea seed which was 32.95% and germinated was 57.84%, with an increase of 43.03%. The DPPH of the mung bean seed was 0.11±0.00 mg Trolox·g^{-1}, whereas the germinated was 1.41±0.11 mg Trolox·g^{-1}, with an increase of 92.19% [11]. Germination, as a domestically developed process in many countries, improves the nutritional and functional deficiencies of the Leguminosae, increasing the antioxidant activity [22]. In the case of Pigeon pea, the activity of trapping the radicals after germination increased by 64%, possibly due to the changes that occur in the distribution of the secondary metabolites; the mobilization of the reserve proteins which are stored in the protein bodies of the cotyledons [8]. For Pigeon pea sprouts, the antioxidant capacity for DPPH increased by 63.52% [23]. The germination process modifies the antioxidant activity, measured by the capacity for capturing the DPPH free radicals, provoking an increase [24].

Capacity for inhibiting the 2,2–azinobis (3–ethylbenzothiazoline–6- sulfonic - acid) (ABTS°+)

Concerning seeds, the antioxidant capacity for the ABTS °+ varied between 0.066 and 1.996 mmol ET·g^{-1} for the Recline and Chaucha varieties, respectively. For the ABTS°+ radical, the degree of discoloration correlated to the antioxidant activity, which was revealed with the white beans and varied from 27 μmol to 43 μmol Trolox·g^{-1}; this content was very low in comparison to the red bean (0.19-0.40 mmol Trolox·g^{-1}) [6].

The effect of the germination, regarding the Chaucha variety seed, had an increase of 47.7%, the Recline variety had 26.6% and the Pigeon pea had 45.3%. Pankaj *et al.* (2014) [11] reported an increase of 92.40% for the mung bean seeds. Germination is a technique of natural bioprocessing which improves the bioactive components, the antioxidant activity and the functional properties of the food grains [24]. The antioxidant capacity of the common beans can be affected by the method of thermic processing, fermentation and germination [3]. During germination, the quantity of phenolic compounds in the Leguminosae increase due to the presence of more hydroxyl groups, which can act in reducing free radicals, which results in an improved antioxidant activity [24].

Macro and microelement content of seeds and sprouts

The content of macro-elements for the bean seeds (Table 2), the Ca was within the range of 69.95 to 121.36 mg·100 g^{-1}; sodium (Na), it was between 50.01 and 53.16 mg·100 g^{-1}; potassium (K), it was between 891.2 and 1175.4 mg·100 g^{-1} and for magnesium (Mg), it was between 157.7 and 160.83 mg·100 g^{-1}. For the Chaucha and Recline varieties, the values that were found concur with those reported by Grela *et al.* (2017) [25] for common beans (cv. Mela) with a Ca of 0.98 g·kg^{-1}, K of 8.47 g·kg^{-1} and Mg of 1.47 g·kg^{-1}. Morales-Morales *et al.* (2016) [26] reported a Ca of 1.2 and K of 19.7 g·kg^{-1} for black-eyed peas; Espinoza-Garcia *et al.* (2016) [27] reported Ca values from 148.9 to 100.1, Na from 63.9 to 85.1, K from 846.1 to 946.6, and Mg 113.7 a 125.9 mg·100 g^{-1}, for sixty-seven native bean populations in Mexico. The greatest content of macroelements for the beans was potassium. This mineral is the principal cation in intracellular liquid and participates in the function of regulating the acid-base equilibrium, muscle contraction, correct cellular membrane function and the maintenance of volume of fluids. For the Pigeon pea seeds, the greatest content of macro-elements was K at 1140.1 and the least was Na with 52.13 mg·100 g^{-1}. The results for Ca, K, Na and Mg were superior to those reported by Asouzu and Umerah (2020) [28], where the macro element values for the Pegeon pea seeds were Mg 68.24, K 2.10, Na 6.10 and Ca 7.28 mg·100 g^{-1}. The same authors indicated that Na and K are necessary for maintaining the osmotic equilibrium of body fluids, the pH of the body, regulating the irritability of muscles and nerves, controlling the absorption of glucose and improving the normal retention of proteins during growth.

For the Chaucha and Recline variety sprouts, it was shown that there was an increase in the Ca of 47.4% and 51.5%, for the Na it was 8.1% and 3.5%, but for Mg, the Chaucha variety registered an increase of 4.8%, while the Recline variety, a decrease of 0.08%. The K decreased for the Chaucha variety by 16.2% and the Recline variety by 9.8%; this behavior was cited by Devi *et al.* (2015) [29] for black-eyed pea sprouts where the Ca content increased from 5.9 % to 9.9 %, which can be attributed to the salts from Ca which are present in the water that is used during the germination. Santos *et al.* (2020) [30], in the germination of twelve varieties of lentils, obtained an increase of Zn, Ca and K; moreover, the germination of Leguminosae is associated with the reduction of phytates, and these are tied to minerals that form insoluble and unavailable compounds. For the Pigeon pea, the values that were found for Ca, Na, K and Mg were inferior to those reported by Torres *et al.* (2018) [8] for Pegeon pea sprouts, which had a Ca of 186.9±7.9 mg·100 g^{-1}, K of 956.6±26.4 mg·100 g^{-1} and Mg of 137.9±5.6 mg·100 g^{-1}. On the other hand, it is worth clarifying that the germination also provoked a greater increase, with respect to the seeds of Ca at 31.1% and a decrease of K at 10.4%. The Na: K relationship for the seeds and sprouts varied between 0.04 and 0.33. The results found were the opposite of those reported by Audu and Aremu (2011) [31] in their work with pinto beans, where the Na: K relationship was found to be greater than one; this indicates that the consumption of the bean can generate arterial hypertension problems. At the same time, it is known that a diet low in sodium (Na) and high in potassium (K), is highly recommended as a strategy for lowering arterial pressure and reducing the risk of cardiovascular disease [32].

The micro-elements (Table 2), for the bean seeds, the results of the Zn and Fe contents were within the range, Mn was slightly superior and Cu slightly inferior to those reported by Morales-Morales *et al.* (2016) [26] for black-eyed peas which had a Zn of 83.2 mg·kg^{-1} and a Fe of 36.3 mg·kg^{-1}. Moreover, Fe is an essential component of diverse heme enzymes, non-heme enzymes and transporters; Zn is an essential component of diverse dehydrogenase, protease and peptidase and, Fe is an important constituent of hemoglobin and participates in numerous biochemical roles within the body. Celmeli *et al.* (2018) [2] found that for common and modern varieties of beans the Zn ranged from 17.81 to 37.90 mg·kg^{-1} and the greatest Fe content was 133.64 mg·kg^{-1}, for local varieties. The microelements of bean sprouts, an increase of 30.2% occurred in Zn for the Chaucha variety. For the Fe and Mn, the Recline variety showed a decrease of 1.6% and 13.7%; the Cu increased for the Chaucha variety, as well as the Recline variety; and concerning the Cr, there was a decrease of 75% and 77.7%, respectively. According to Devi *et al.* (2015) [29], an increase from 0.4 to 1.4% was reported for the Fe, the value of which was lower when compared to this study. According to Santos *et al.* (2020) [30], in the germination of twelve varieties of lentils, a decrease in the Fe and Mn were found. Just as it is known that germination is an individual biological process for all seeds, it is multi-enzymatic with the preparation to bring about all of the metabolic activity that is required to generate a new plant. One of the most important enzymes in the process is phytase, which is activated during soaking and continues during the germination; it is the enzyme in charge of hydrolyzing the phytic acid, which keeps the nutrients (mineral ions, proteins, and carbohydrates) to one side for when the seed needs them [33]. For the Pigeon pea, the Fe, Mn and Cu contents reported were slightly superior to those cited by Torres *et al*. (2018) [8].

The Zn that is necessary for the correct growth and maintenance of the human body is found in various systems and biological reactions, and it is necessary for immunological, coagulation, and thyroid function, among others [28].

The Pb content in the seeds varied from 0.16 to 0.07 mg·100 g^{-1}, the Cd from 0.01 to 0.03 mg·100 g^{-1}, and the Pb and Cd decreased for the sprouts; only for Pigeon peas sprouts did it slightly increase. The Pb, it did not surpass the limits reported by Glavač *et al*. (2017) [34] in plants. The European Commission considers the limits of Pb and Cd in plants to be 1.0 mg·kg^{-1} and 5.0 mg·kg^{-1}, respectively. The presence of Pb in food is a problem for health and food security, thus norms have been established with maximum allowable limits according to Casteblanco (2018) [35]. Cd is a trace metal with no essential biological functions and it is toxic to plants, animals, and humans at low concentrations [36].

Table 2. Macro and microelement contents in Leguminoseae seeds and sprouts.

		Seeds			Sprouts		
		Chaucha variety	Recline variety	Pigeon pea	Chaucha variety	Recline variety	Pigeon pea
Macro- -elements (mg·100 g^{-1})	Ca	69.95±0.64^{e}	121.36±0.35^{d}	168.63±2.95^{b}	133.13±0.71^{c} (+47.4)	250.64±2.67^{a} (+51.5)	244.96±1.43^{a} (+31.1)
	Na	53.16±0.41^{b}	50.01±0.64^{c}	52.13±0.17^{bc}	57.85±0.52^{a} (+8.1)	51.86±0.85^{bc} (+3.5)	55.95±0.53^{a} (+6.8)
	K	1175.4±14^{a}	891.2±42^{d}	1140.1±1.2^{ab}	1010.3±24^{c} (-16.2)	811.3±27^{d} (-9.8)	1032.2±1.7^{bc} (-10.4)
	Mg	157.70±0.63^{bc}	160.83±0.49^{ab}	155.00±1.60^{c}	165.76±1. 81^{a} (+4.8)	160.7±1.23^{abc} (-0.08)	164.20±0. 83^{a} (+5.6)
	Na:K	0.04	0.05	0.33	0.05	0.06	0.05
Micro-ele- ments (mg·100 g^{-1})	Zn	2.03±0.01^{e}	2.71±0.03^{c}	2.23±0.01^{d}	2.91±0.01^{ab} (+30.2)	2.84±0.04^{b} (+4.5)	2.96±0.01^{a} (+24.6)
	Fe	13.51±0.11^{c}	14.16±0.06^{c}	13.73±0.04^{c}	15.03±0.14^{b} (+10.1)	13.93±0.08^{c} (-1.6)	15.86±0. 31^{a} (13.4)
	Mn	2.35±0.04^{d}	2.65±0.03^{c}	2.56±0.09^{cd}	8.85±0.05^{a} (+73.4)	2.33±0.03^{d} (-13.7)	4.22±0.01^{b} (+39.3)
	Cu	0.66±0.01^{e}	0.90±0.00^{b}	0.73±0.00^{c}	0.69±0.01^{d} (+4.3)	0.97±0.01^{a} (+7.2)	0.91±0.00^{b} (+19.7)
	Cr	0.21±0.01^{a}	0.16±0.00^{b}	0.11±0.00^{cd}	0.12±0.01^{c} (-75)	0.09±0.00^{d} (-77.7)	0.08±0.00^{d} (-37.5)
Heavy me- tals (**)	Pb	0.16±0.00^{a}	0.08±0.00^{b}	0.07±0.00^{bc}	0.14±0.01^{a}	0.06±0.00^{c}	0.09±0.00^{b}
	Cd	0.03±0.00^{a}	0.01±0.00^{c}	0.02±0.00^{b}	0.01±0.002^{c}	0.01±0.00^{d}	0.01±0.00^{c}

*The values represent: average ± SEM; repetitions: (n=3); values within the same row with different superindices are significative ($p \leq 0.05$); (+/-) = % increase /decrease. (**) (mg·100 g^{-1}).

Thermic behavior of the seeds and sprouts

The gelatinization of starch measured by differential scanning calorimetry (DSC) represents every one of the endothermic events, which take place during the process of the transition phase. These thermal properties of the flours in grains depend on the humidity and the endothermic transition of the thermogram DSC, which reflects the gelatinization of the flours when excess water is added. In the study, the gelatinization temperature and the enthalpic changes (ΔH) of the flour from the seeds as well as from the sprouts were examined (Table 3). For the flour from the seeds, the gelatinization from thermal transition starts with a slight endothermic peak above the baseline of the thermogram. The lowest process is initiated at 62.29 °C (Chaucha variety) and the highest at 77.48°C (Pigeon pea), which is known as the onset temperature (T_0). The peak temperature (T_p) is the temperature where the highest values of heat absorption are recorded for the seeds, which was between 68.13 °C (recline variety) and 81.28 °C (Pigeon Pea). The completion temperature (T_c) for the gelatinization of the seeds was in the range of 72.38 °C (Recline variety) and 86.93 °C (Pigeon pea), and it represents the temperature at which the process is finalized. The behavior found was similar to that reported by Maninder *et al.* (2007) [37] between the flour from *P. sativum* (FPF), which was low (T_0: 59.45 °C; T_p: 65.5 °C; Tc: 74.1 °C), and Pigeon pea (PPF) (T_o: 75.6 °C; T_p: 82.0 °C; T_c: 87.2 °C). It indicates that the difference in the gelatinization temperatures can be attributed to the differences in size, shape and distribution of the starch granules in the flours, as well as the internal disposition of the fraction within the starch granule. According to Xu *et al.* (2019) [38], the temperatures of thermic transition to the flour, from grains, are influenced by the amylose and protein content, and the distribution of amylopectin ramified chains.

The energy necessary to complete the process that is known as gelatinization enthalpy, which is calculated as the area under the curve of the gelatinization peak (ΔHgel), varied for the seeds: Chaucha variety 1.019, Recline variety 0.643 and Pigeon pea 0.714 $J \cdot g^{-1}$. Moreover, it allows for the structural and chemical changes, which influence the quality of the products derived from this material to be analyzed. The results were superior to those reported by Sanchez-Arteaga *et al.* (2015) [39]; the ΔH in six defatted beans varied from 0.34 ± 0.03 (black-eyed) to 0.71 ± 0.06 $J \cdot g^{-1}$ (pinto). The variation in the thermic profile of the bean also depends on the amylose content, the distribution of the amylopectin ramified chain, the complex lipid's amylose chain, and the proteins present in them [40].

Table 3. Temperature and Gelatinization Enthalpy of the Seeds and Sprouts.

	Sample	(To) °C	(Tp) °C	(Tc) °C	ΔHgel (J/g)
Seed	Chaucha variety	62.29±0.41[b]	77.09±0.49[b]	81.09±1.87[ab]	1.019±0.06[a]
	Recline variety	65.67±0.20[b]	68.13±0.06[d]	72.38±0.01[b]	0.643±0.014[bc]
	Pigeon pea	77.48±1.37[a]	81.28±0.24[a]	86.93±0.29[a]	0.714±0.07[b]
Sprout	Chaucha variety	63.05±0.40[b]	74.20±0.17[c]	80.22±3.47[ab]	0.442±0.06[bcd]
	Recline variety	62.48±0.09[b]	68.37±0.20[d]	74.46±0.05[b]	0.308±0.00[d]
	Pigeon pea	79.64±0.44[a]	82.48±0.36[a]	86.52±0.02[a]	0.355±0.00[cd]

Data represent (mean ± SEM). Different lower-case letters indicate statistically significant differences intraspecies ($p < 0.05$). T_o, T_p, T_c, and ΔH is the onset temperature, peak temperature, completion temperature, and enthalpy, respectively.

All of the flour from the seeds and sprouts (Fig. 1) showed only one endothermic transition from 50 to 100 °C, which reflects the gelatinization. For the Chaucha variety sprouts, the T_o was 63.05 °C, the T_p was 74.20 °C and the T_c was 80.22 °C; for the Recline variety T_o was 62.48 °C, T_p was 68.37 °C and T_c was 74.46 °C; and for Pigeon pea T_o was 79.64 °C, T_p was 82.48 °C and T_c was 86.52 °C. As can be seen, the three samples had different values of T_p; and T_p is considered the temperature at which 50% of the molecules have suffered a thermic transition. The differences observed in the thermic profiles are possibly associated with the variation in the chemical composition, thermal properties, and quality of the protein fraction [39].

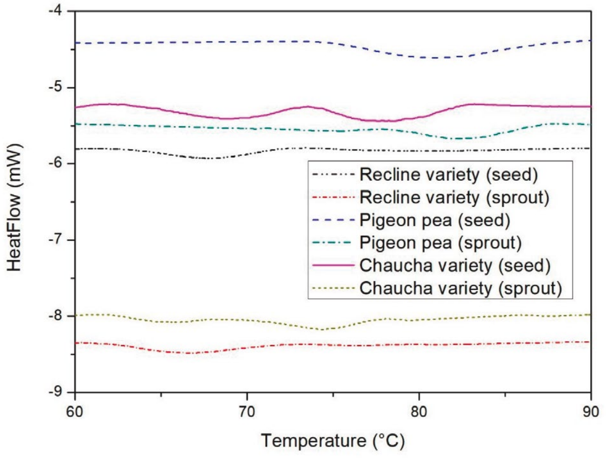

Figure 1. Thermal transitions of *C. cajan* and *P. vulgaris L*. seed and sprout through DSC.

For the sprouts, the ΔH diminished, in comparison to the seed. Jimenez *et al*. (2019) [41] reported similar effects in quinoa sprouts, probably due to the hydrolysis of the starch; with a greater amylose/amylopectin relationship, due to greater degradation of the amylopectin during germination. Xu *et al*. (2019) [38] indicated that the ΔH can be utilized to predict the energy necessary for the decomposition of the intermolecular hydrogen bond in the starch granules. In garbanzo beans and peas this diminishes by the third day of germination, which indicates that there is a reduction in necessary energy to convert the chemical composition of the flour from an orderly to a disorderly form. During germination, partial hydrolysis of the starch occurs due to the activation of enzymes that affect the hydrogen bonds of the starch, making the detachment easier when heated.

Conclusion

The sprouts are considered functional food due to the increase in the content of phenols, antioxidants, and minerals, thus, it is necessary to study the technological properties associated with functionality of these Legumes.

Conflict of interest

All authors report that they do not have any conflicts of interest.

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