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# Common bean (*Phaseolus vulgaris* L.) seed germination improves the essential amino acid profile, flavonoid content and expansion index

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**Abstract:** Common bean (*Phaseolus vulgaris* L.) is one of the most important grain legume foods for the human diet. Common bean seed is gaining attention due to its content of secondary metabolites with positive effects on human health. The present work analysed the effect of common bean germination on the essential amino acid profile, active compound content, and expansion index after extrusion processing. The content of tryptophan (Trp) in raw common bean (RCB) and in germinated common bean (GCB) seeds was 6.1 and 7.9 $mg \cdot g^{-1}$ of protein, respectively. The limiting amino acids in RCB were sulphur amino acids and Trp, and in GCB only the sulphur amino acids were the limiting amino acids. The germination process in beans increases the levels of quercetin and kaempferol and allows the synthesis of daidzein and genistein, and significantly increases the expansion index after extrusion processing. The germination of common bean could be used as a strategy to improve nutritional, nutraceutical, and processing properties.

**Keywords:** black bean; daidzein; extrusion; legume; tryptophan

For centuries, grain legumes have been a constant element in human diets, with an important role in agricultural sustainability and human health (Semba et al. 2021). The significant advantages in the agricultural production of legumes are related to their symbiotic nitrogen fixation capacity. However, compared with cereals, the contribution of grain legumes to worldwide human consumed calories is low (Foyer et al. 2016). A diet low in legumes is considered a risk factor contributing to the current global cardiovascular disease and diabetes burden (GBD 2017 Diet Collaborators 2019).

Common bean (*Phaseolus vulgaris* L.) is a major legume staple crop widely cultivated worldwide for human consumption of vegetable or dry grain, providing essential amino acids, fibre, complex carbohydrates, and phytochemicals (Weller et al. 2019). Storage proteins of common bean seeds are high in lysine (Lys) content and deficient in sulphur amino acids and tryptophan (Trp) (Vaz-Pato 2016). A diet based on bean

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and maise is marginally deficient in Trp, depending on the nicotinamide intake. A diet low in niacin demands Trp for nicotinamide and niacin synthesis (Fukuwatari and Shibata 2013). Currently, the common bean seed is gaining attention due to its content of oligosaccharides, phenolic acids, and flavonoids, active compounds associated with reducing the risk of metabolic disease development (Yang et al. 2018).

Common bean, like other legume seeds, contains antinutritional factors, and a proper processing method is necessary to make them edible for humans (Díaz-Batalla et al. 2006; Weller et al. 2019). Food extrusion is a versatile, short time and high-temperature food processing technology which transforms raw food materials into stable cooked products with enhanced organoleptic and nutritional properties. The extrusion of common bean seed eliminates antinutritional factors and improves mineral bioavailability and protein digestibility (Batista et al. 2010). During the germination of common bean seeds, storage proteins, lectins, protease inhibitors, oligosaccharides, and phytic acid are degraded, improving the common bean's nutritional value (Savelkoul et al. 1994). The contents of flavonoids and phenolic acids increase during the germination of common bean seeds, improving the nutraceutical properties (Díaz-Batalla et al. 2006; Dueñas et al. 2015; Yang et al. 2018). Extrusion of germinated common bean (GCB) seeds improves their functional properties, due to enhanced molecular interaction between proteins and starch, in a synergistic way, compared with extruded raw common bean (RCB) (De la Rosa-Millán et al. 2019). Given the important role of legumes and common bean seeds in the current alimentary systems, where the substitution of legume protein for animal protein is recommended, their potential to ameliorate the non-communicable disease burden and the necessity to find new ways to diversify their industrial uses, in the present work, the effect of common bean germination on the amino acid profile, active compound content, and expansion index was analysed.

## MATERIAL AND METHODS

**Chemicals.** 2-2-Diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, daidzein, genistein, myricetin, quercetin, and kaempferol were from Sigma (St. Louis, MO, USA). Buffers, ninhydrin, andamino acid standards were from Sykam GmbH (Eresing, Germany).

**Black common bean seeds.** Black common bean seeds (8 025 varieties) were purchased in the local market. Five hundred grams of mature black common bean seeds were ground to pass through a 0.29mm screen. Additionally, two batches of 500 g of black common bean seeds were sanitised for 20 min by immersion in a 200 ppm sodium hypochlorite solution, rinsed with distilled water and hydrated (1 mL of water·$g^{-1}$ of seeds). Hydrated seeds were germinated in the dark at 27 °C for 144 h in sealed, impermeable and sterile plastic bags, dried at 50 °C overnight, ground and sieved in a 0.29 mm screen.

**Protein and essential amino acids.** Protein was determined using the procedures described by the Association of Official Analytical Chemists [AOAC (2005): Official Method of Analysis, $18^{th}$ ed. Association of Officiating Analytical Chemists, Washington DC]. The amino acid content in raw and germinated black common bean flours was analysed, after flour hydrolysis with 6 N HCl, using an LCA K-06/Na (4.6−150 mm), ion exchange separation column, with postcolumn ninhydrin derivatisation using a Sykam amino acids analyser (S 433; Sykam GmbH, Germany). The same method was used for sulphur amino acid quantification, adding an oxidation process of samples with performic acid before acid hydrolysis (Díaz-Batalla et al. 2018). Enzymatic hydrolysis of samples with papain and reaction with *p*-dimethylaminobenzaldehyde was used to quantify Trp at 620 nm (Díaz-Batalla et al. 2018).

**Extrusion and expansion index.** Raw and germinated black common bean flours were moisturised with deionised water to obtain a 16% moisture content. Flours were processed in a single screw extruder (19/25DN; Brabender, Duisburg, Germany), using a screw of 19 mm in diameter with a compression ratio of 3 : 1, working at 170 rpm. Temperatures in the barrel were 80, 100, 120, and 150 °C for zones 1 to 4, respectively, with a 3 mm exit die, and the feeder was used at 30 rpm. The expansion index (EI) was assessed in the obtained extrudates (Díaz-Batalla et al. 2018). The extrudates were dried and ground (0.28 mm mesh) for analysis.

**Total phenolic compounds, DPPH radical scavenging capacity and flavonoids.** Raw and germinated black common bean flours were extracted with aqueous ethanol for total phenolic compound content and DPPH radical scavenging capacity (Díaz-Batalla et al. 2018). The total phenolic compound content was estimated by a spectrophotometric method using the Folin-Ciocalteu reagent with absorbance measurement at 760 nm (Díaz-Batalla et al. 2018). The free radical scavenging capacity was determined using a spectrophotometric method, the DPPH synthetic stable radical, with absorbance measurement at 515 nm (Díaz-Batalla et al. 2018). For flavonoid analysis, the ethanolic extracts of samples were hydrolysed

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with 2N HCl, and ethyl acetate extraction was used for aglycone recovery. High-performance liquid chromatography (HPLC) was used for flavonoids separation in a Dionex UltiMate 3000-DAD system (Thermo Fisher Scientific, Waltham, MA, USA) supplied with an Acclaim C-18 column (120 C-18, 4.6–100 mm). The flavonoids were detected using UV-visible spectra, and for quantification, the absorbance at 254 nm was used (Díaz-Batalla et al. 2018).

**Statistical analysis.** Chemical assays of samples were performed in triplicate. All values were presented as means ± standard deviation. The data were submitted to the ANOVA test using Statsoft Statistica 8.0, and means were compared by Tukey's test ($P \leq 0.05$).

## RESULTS AND DISCUSSION

**The protein content and essential amino acid profile.** The protein content and essential amino acid profile in RCB and GCB are shown in Table 1. Germination of common bean seeds does not change the protein content significantly. Major changes in amino acid content were evidenced in Trp and sulphur amino acids (methionine + cysteine). The contents of Trp in RCB and GCB were 6.1 and 7.9 $mg \cdot g^{-1}$ of protein, respectively. The content of sulphur amino acids in RCB and GCB were 11.3 and 13.1 $mg \cdot g^{-1}$ of protein, respectively, indicating a significant increase in the content of these essential amino acids due to the germination process. Considering the amino acid scoring patterns of the Food and Agriculture Organization (FAO 2013) recommended for humans older than 3 years, in the present work, Trp and sulphur amino acids were the limiting amino acids in RCB. However, in GCB, only sulphur amino acids were the limiting amino acids. In the present work, the protein content in RCB was similar to previously reported values for common bean (Vaz-Patto 2016). In previous works, Trp and sulphur amino acids were found as the limiting amino acids in RCB (Vaz-Patto 2016). During the germination of common bean seeds, storage proteins are degraded (Savelkoul et al. 1994), and new metabolic pathways, such as the shikimate pathway, are activated. The shikimate pathway allows for a *de novo* synthesis of aromatic amino acids (Maeda and Dudareva 2012). The higher content of Trp in GCB could be related to this metabolic activation. The germination of black common bean seed increases the Trp content, improving the essential amino acid profile and nutritional value.

**Total phenolic compounds, DPPH radical scavenging capacity and flavonoids.** The content of total phenolic compounds, DPPH radical scavenging capacity, and flavonoid content in RCB and GCB are shown in Table 2. The total phenolic compound content in RCB was similar and lower than previously reported values (Yang et al. 2018; Chávez-Mendoza et al. 2019). During the germination of RCB, the content of total phenolic compounds maintains the same values, while the values of DPPH radical scavenging capacity decreased significantly (40%). The germination of legume seeds can increase the content of soluble phenolic compounds compared with raw seeds. However, several studies have also reported a reduction in phenolic compound content and antioxidant activity during ger-

Table 1. Protein and essential amino acids content in raw common bean (RCB) and germinated common bean (GCB) (mean ± *SD*; $n = 3$)

| Amino acid | RCB | GCB | FAO* |
|---|---|---|---|
| Protein ($g \cdot 100\ g^{-1}$ DW) | 21.39 ± 0.6$^{a}$ | 22.16 ± 0.8$^{a}$ | – |
| Trp ($mg \cdot g^{-1}$ of protein) | 6.1 ± 0.3$^{b}$ | 7.9 ± 0.2$^{a}$ | 6.6 |
| Val ($mg \cdot g^{-1}$ of protein) | 41.6 ± 0.7$^{a}$ | 40.4 ± 0.8$^{a}$ | 40 |
| Ile ($mg \cdot g^{-1}$ of protein) | 38.5 ± 0.3$^{a}$ | 37.1 ± 0.5$^{a}$ | 30 |
| Thr ($mg \cdot g^{-1}$ of protein) | 48.3 ± 0.7$^{a}$ | 42.2 ± 0.3$^{b}$ | 25 |
| Leu ($mg \cdot g^{-1}$ of protein) | 81.2 ± 1.3$^{a}$ | 76.6 ± 0.9$^{b}$ | 61 |
| Lys ($mg \cdot g^{-1}$ of protein) | 96.6 ± 0.9$^{a}$ | 88.7 ± 0.6$^{b}$ | 48 |
| His ($mg \cdot g^{-1}$ of protein) | 27.2 ± 0.4$^{a}$ | 27.4 ± 0.3$^{a}$ | 16 |
| Met + Cys ($mg \cdot g^{-1}$ of protein) | 11.3 ± 0.3$^{b}$ | 13.1 ± 0.5$^{a}$ | 23 |
| Phe + Tyr ($mg \cdot g^{-1}$ of protein) | 78.6 ± 0.5$^{a}$ | 76.6 ± 0.3$^{b}$ | 41 |

*Food and Agriculture Organization recommended amino-acids scoring patterns for humans older than 3 years (FAO 2013); $^{a,b}$means with the same letter in the same line – no significant difference between samples ($P < 0.05$); RCB – raw common bean; GCB – germinated common bean; DW – dry weight; Trp – tryptophan; Val – valine; Ile – isoleucine; Thr – threonine; Leu – leucine; Lys – lysine; His – histidine; Met – methionine; Cys – cysteine; Phe – phenylalanine; Tyr – tyrosine

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Table 2. Total phenolic compounds, DPPH scavenging capacity, flavonoids content, and expansion index of raw common bean (RCB) and germinated common bean (GCB) (mean ± *SD*; *n* = 3)

| Component | RCB | GCB |
| --- | --- | --- |
| Total phenolics (mg GAE·$100^{-1}$ g DW) | 148.2 ± 6.5[a] | 149.2 ± 7.5[a] |
| DPPH (mg AAE·$100^{-1}$ g) | 161.3 ± 5.4[a] | 97.6 ± 3.7[b] |
| Quercetin (mg·$kg^{-1}$ DW) | 38.5 ± 1.4[b] | 86.8 ± 0.9[a] |
| Kaempferol (mg·$kg^{-1}$ DW) | 27.2 ± 1.7[b] | 49.4 ± 0.7[a] |
| Daidzein (mg·$kg^{-1}$ DW) | – | 85.1 ± 0.8 |
| Genistein (mg·$kg^{-1}$ DW) | – | 54.3 ± 1.2 |
| Expansion index | 1.51 ± 0.12[b] | 1.93 ± 0.11[a] |

[a,b] means with the same letter in the same line – no significant difference between samples ($P < 0.05$) ;RCB – raw common bean; GCB – germinated common bean; GAE – gallic acid equivalent; DW – dry weight; AAE – ascorbic acid equivalent

mination (Dueñas et al. 2015). Germination activates seed metabolism, including respiration and reactive oxygen species (ROS) production. Production of hydrogen peroxide in the early imbibition period of legume seeds plays important roles in cell signalling, germination, seed dormancy, and defence against pathogens. ROS levels must be regulated by enzymatic and non-enzymatic mechanisms, where secondary metabolites are important (Bailly et al. 2008). These roles of ROS and antioxidant demand during germination could explain the inconsistency in previous reports and the decreasing effect of germination on the radical scavenging capacity observed in the present study.

The levels of flavonoids in RCB were similar to previously reported values for common bean seeds (Diaz-Batalla et al. 2006; Chávez-Mendoza et al. 2019). Quercetin and kaempferol were found in RCB, but daidzein and genistein were absent. The germination process increases the levels of quercetin and kaempferol and allows the synthesis of daidzein and genistein. Germination activates seed primary and secondary metabolism, activating the shikimate and the phenylpropanoid pathways. Phenolic acids and flavonoids synthesised by these metabolic pathways in legumes have metabolic functions related to ROS modulation, pathogen control, and signalling activities, including nitrogen-fixing capacity (Vogt 2010). Increases in flavonoid content in GCB resulted from metabolic activation during germination. On the other hand, flavonoids are important molecules in human health due to their antioxidant, anti-inflammatory, and antihypertensive effects, modulation of detoxifying enzymes, hormone modulation, and anti-obesogenic activities (Chávez-Mendoza et al. 2019). The germination of black common bean seed improves flavonoid content and nutraceutical value.

**Extrusion and expansion index.** The expansion index of RCB and GCB extrudates are shown in Table 2. The extrusion of RCB produced an extrudate with an expansion index of 1.51, and the extrusion of GCB produced an extrudate with an expansion index of 1.91. Extraction processing did not affect the content of active compounds, and radical scavenging capacity was not affected by extrusion processing (results not shown). High temperature and mechanical shear during extrusion break hydrogen and covalent bonds generating an intense structural disruption in starch, fibres and proteins and changing the functional properties of foods (Patil et al. 2007). During common bean seed germination, starch and proteins are depolymerised. When this matrix is extruded, new and strong interactions between fibres, starch and proteins are developed, increasing the number and distribution of water bubbles and the expansion index (De la Rosa-Millán et al. 2019). The germination of black common bean improves expansion index and processing properties.

## CONCLUSION

The germination of common bean seeds triggers a metabolic activation that drives important changes in storage proteins, starch, anti-nutritional factors, and secondary metabolites. In the present work, the germination of the black common bean for 6 days increases the levels of Trp, quercetin, kaempferol, daidzein, and genistein and improves the expansion index when it was processed by extrusion. Further studies must be developed to establish the molecular mechanism involved in these changes. Common bean seeds' germination could improve their nutritional and nutraceutical value and diversify their industrial use through extrusion processing.

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