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Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans

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Abstract

Comparative effects of extrusion cooking and conventional processing methods on protein content and reduction of antinutritional factor (phytic acid, condensed tannins, polyphenols, trypsin, chymotrypsin, α-amylase inhibitors and haemagglutinating activity) levels in *Vicia faba* and *Phaseolus vulgaris* seeds were studied. In vitro protein and starch digestibilities were assessed. *P. vulgaris* seeds showed highest levels of condensed tannins, chymotrypsin and α-amylase inhibitory activities and haemagglutinating activity. Dehulling significantly increased protein content and greatly reduced condensed tannin and polyphenol levels in both legumes. Extrusion was the best method to abolish trypsin, chymotrypsin, α-amylase inhibitors and haemagglutinating activity without modifying protein content. Furthermore, this thermal treatment was most effective in improving protein and starch digestibilities when compared with dehulling, soaking and germination.

1. Introduction

Food legumes are characterised by a relatively large content of proteins and carbohydrates. In general, pulses also contain significant amounts of crude fibre, lipids, minerals and vitamins.

In recent years, many compounds from food legumes have been shown to cause physiological and biochemical effects, such as pancreas enlargement and growth inhibition in various species of animal (Grant, 1989; Guen & Birk, 1993; Liener, 1989). These compounds include phytic acid, condensed tannins, polyphenols, protease inhibitors (trypsin and chymotrypsin), α-amylase inhibitors and lectins. Moreover, the digestibility of legume protein and legume starch is limited by the presence of antinutrients (Lajolo, Filho & Menezes, 1991; Nielsen, 1991; Yadav & Khetarpaul, 1994) and the utilisation of pulses in both human and animal nutrition is restricted by the presence of the aforementioned factors.

A wide range of processing techniques could improve the protein and starch digestibilities of legumes and therefore their utilisation (Alonso, Orŕve & Marzo, 1998; Conan & Carré, 1989; Frias, Diaz-Pollan, Hedley & Vidal-Valverde, 1995; Gujska, & Khan, 1991; Van der Poel, 1990; Wang, Lewis, Brennan & Westby, 1997). However, it is known that certain treatments, such as heat processing, could produce, in some conditions, physicochemical changes in proteins, starch and in the other components of legume seeds affecting their final nutritional properties (Della Valle, Quillien & Gueguen, 1994; Jeunink & Cheftel, 1979).

The aim of this work was to study the efficiency of processing methods on reduction or elimination of *Vicia faba* and *Phaseolus vulgaris* antinutritional factors in order to improve nutritional value of these sources. In addition, the relation of each treatment application to in vitro protein digestibility and, in vitro starch digestibility has been assessed. A special emphasis has been placed on extrusion-cooking as a versatile, quick and efficient method to reduce antinutrients when compared with other traditional processing methods.

2. Materials and methods

2.1. Materials

V. faba L. (var. Equina) and *P. vulgaris* L. (var. Athropurpurea) seeds cultivated in Navarra (Spain), were employed for all determinations. After processing, the seeds were ground and sieved to 0.5 mm diameter particle

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size, using a SKI 100 Restch granulator mill and then stored at 4°C until analysis. All chemicals and reagents were purchased from Aldrich Chemical Co Inc. (Milwaukee, WI) and Sigma Chemical Co (St. Louis, MO).

2.2. Processing

2.2.1. Dehulling

Kidney bean and faba bean hulls were removed manually.

2.2.2. Soaking

Seeds were soaked in the dark in double-deionized water (1:5 w/v) for 12 h at 30°C in an incubator (Heraeus Vötsch HPS 500). The soaked solution was drained off, then seeds were dried at 50°C in an assisted air circulation oven (Memmert ULM 400).

2.2.3. Germination

V. faba and *P. vulgaris* seeds were pre-treated with 10% mercuric chloride solution to remove surface contamination, then three times rinsed with autoclaved bideionized water. Grains were transferred to a Petri dish lined with wet filter paper and germinated in an aired and dark incubator (Heraeus Vötsch HPS 500) either for 24, 48 or 72 h at 25°C. Sodium azide at a concentration of 0.01% was added to autoclaved bideionized water to prevent microbial growth. During germination, bideionized water (containing sodium azide) was sprinkled on seeds every 12 h. Germinated seeds (the degree of germination was 95% ± 1 and 97% ± 1 for faba beans and kidney beans, respectively) were dried in an air oven (Memmert ULM 400) at 50°C overnight.

2.2.4. Extrusion

Extrusion of faba beans and finely ground (0.5 mm) kidney beans was performed in a Clextral X-5 model BC 45 twin-screw extruder (F-42100 Firminy, France). The extruder was operated at 100 rpm and the feeder was set to deliver 383 and 385 g/min, respectively. Moisture content in the extruder barrel was constant at 25%. Extrusion temperatures, by iron-constant thermocouple at the outlet die, were 152 and 156°C, respectively. The extrudates were allowed to cool to room temperature and then were ground to pass a 0.5 mm sieve.

2.3. Analytical methods

2.3.1. Moisture content

Moisture contents of samples were determined by AOAC method 925.10 (AOAC, 1990).

2.3.2. Crude protein

Nitrogen content of flours was estimated by Kjeldahl method (AOAC, 1990) using a nitrogen autoanalyzer (Tecator Kjeltec Auto Analyzer Model 1030) and crude protein content was calculated (% N×6.25).

2.3.3. Phytic acid

Phytic acid was extracted in seed samples with 2.4% HCl (1:20 w/v). The obtained creamy mixture was centrifuged at 17,300 *g* (Sorvall® RC-5B) for 30 min at 15°C and the supernatants collected. Glass barrel Econo-columns 0.7×15 cm (Bio Rad Laboratories) were filled with 0.5 g of AG 1×4 anion-exchange resin to effect phytate purification. To assess total phytic acid content, the simple procedure based on the reaction between ferric chloride and sulfosalicylic acid was followed (Frühbeck, Alonso, Marzo & Santidrián, 1995).

2.3.4. Condensed tannins

Condensed tannins were extracted with HCl:methanol (1:100 v/v) for 7 h in faba beans and 1 h 30 min in kidney beans with mechanical shaking at room temperature and centrifuged at 5000 *g* at 15°C for 15 min. Aliquots were immediately analysed for tannin using the 0.5% vanillin assay (Broadhurst & Jones, 1978).

2.3.5. Polyphenols

The method of the Association of Official Agricultural Chemists with some modifications (Christensen, 1974) was used. Total phenols were extracted in a sample of 1 g flour with 75 ml of water. An internal standard curve was prepared by adding 10 ml of 0–0.01% tannic acid to the flasks. The contents were heated for 30 min at 70°C with constant shaking. Clear supernatants were collected after centrifuging the contents at 2500 *g* for 15 min and filtering them. Polyphenols were determined using the Folin-Denis reagent.

2.3.6. Trypsin inhibitors

Trypsin inhibitor was determined as described by Kakade, Rackis, McGhee and Puski (1974), using α-*N*-benzoyl-DL-arginine-*p*-nitroanilidehydrochloride (BAPNA) as the substrate for trypsin, with some modifications (Valdebouze, Bergeron, Gaborit & Delort-Laval, 1980). One gram of finely ground sample was extracted with 10 ml of 0.15 M phosphate buffer pH 8.1 at 4°C overnight. Extracts (200 μl) were incubated with 250 μl of trypsin solution (0.004% trypsin in 0.025 M glycine HCI buffer) and diluted to 1 ml with pH 8.1 buffer phosphate. 2.5 ml of 0.001 M BAPNA solution in pH 8.1 buffer phosphate, previously warmed to 37°C, was added. Trypsin inhibitor activity (TIA), expressed as trypsin inhibitor units/mg sample, was calculated from the absorbance read at 410 nm against a reagent blank. One trypsin unit was defined as the increase by 0.01 absorbance unit at 410 nm of the reaction mixture.

2.3.7. Chymotrypsin inhibitors

The enzyme inhibitory activity (CIA) was determined in extracts as described by Sathe and Salunkhe (1981). Seed meal is extracted by stirring in Tris-HCl buffer pH 7.6 overnight at 4°C [sample:buffer, 1:10 (w:v)]. Sample

extracts (50 μl) were incubated with 100 μl of chymotrypsin solution (0.005% chymotrypsin in Tris-HCI buffer pH 7.6) and diluted to 1 ml with pH 7.8 Tris-HCl buffer. A 2.5 ml portion was of 0.001 M benzoyl-L-tyrosine ethyl ester (BTEE), previously warmed to 30°C was added and mixed. Changes in absorbance at 256 nm were recorded immediately after substrate addition. One chymotrypsin unit was defined as the increase by 0.01 absorbance unit at 256 nm of the reaction mixture.

2.3.8. α-Amylase inhibitors

α-Amylase inhibitor activity (AIA) was evaluated according to the method of Deshpande, Sathe, Salunkhe and Cornforth (1982). A 1 g sample was extracted with 10 ml of deionized water for 12 h at 4°C and the supernatants were tested for α-amylase inhibitory activity. A 0.25 ml sample solution containing the inhibitor was incubated with 0.25 ml of α-amylase enzyme solution (0.003% in 0.2 M sodium phosphate buffer, pH 7.0, and containing 0.006 M NaCI) for 15 min at 37°C. To this mixture was added 0.5 ml of 1% starch solution (preincubated at 37°C). At the end of 3 min, the reaction was stopped by the addition of 2 ml of dinitrosalicylic acid reagent and heating in a boiling water bath for 10 min. The absorbance was recorded at 540 nm. One unit of enzyme activity was defined as that which liberates, from soluble starch, one micromole of reducing groups (calculated as maltose) per min at 37°C and pH 7.0 under the specified conditions. One unit of α-amylase activity inhibited was defined as one α-amylase inhibitory unit.

2.3.9. Haemagglutinating activity

Haemagglutination assays, using trypsin-treated rabbit erythrocytes, were carried out by a serial dilution method as described by Grant, More, McKenzie, Stewart and Pusztai (1983). One unit of haemagglutinating activity (HU) was defined as that contained in the amount of sample in the last dilution which caused 50% agglutination of the blood cells.

2.3.10. In vitro protein digestibility

A multienzyme system consisting of 1.6 mg trypsin (14,600 U/mg), 3.1 mg α-chymotrypsin (48 U/mg) and 1.3 mg peptidase (102 U/g) per ml was used for in vitro protein digestibility assay (Hsu, Vavak, Satterlee & Miller, 1977). Five millilitres of the multienzyme solution maintained in ice were added to 50 ml of a legume flour suspension (1 mg N/ml), adjusted to pH 8.0 and incubated at 37°C. The mix was stirred at 37°C and the pH change after ten minutes period ($pH_{10\,min}$) was recorded. The percent in vitro protein digestibility (IVPD) was calculated using the following equation.

$$\text{In vitro protein digestibility (\%)} = 210.46 - 18.10\ pH_{10\,min}$$

2.3.11. In vitro starch digestibility

The in vitro starch digestibility (IVSD) was determined in flours (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20°C for 2 h according to the method of Singh, Kherdekar and Jambuathan (1982). At the end of the incubation period, 2 ml of 3,5-dinitrosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard. IVSD was expressed as mg of maltose released per gram of sample on a dry weight basis. Values were corrected for the background.

2.4. Statistical analysis

The results are given as means plus or minus SD. Where appropriate, statistical analysis of variance (ANOVA) was done to determine the significant differences among means followed by Fisher's least significant difference (LSD) when the *F*-test demonstrated significance (Miller & Miller, 1993). The statistically significant difference was defined as $P < 0.05$.

3. Results and discussion

3.1. Protein content

Table 1 shows results for crude protein content of *V. faba* and *P. vulgaris* as a function of processing. Comparing both legumes, raw seeds of faba beans had significantly ($P < 0.0001$) more protein than those of kidney bean. Previous findings indicated that faba bean and kidney bean protein content varies from 229 to 385 and 211 to 394 g kg^{-1}, respectively (Kadam, Deshpande & Jambhale, 1989).

Table 1
Protein content (g kg^{-1} DM) in raw, dehulled, soaked, germinated and extruded seeds of *Vicia faba* L. and *Phaseolus vulgaris* L.[a]

	Protein content	
Treatment	*Vicia faba*	*Phaseolus vulgaris*
Raw seeds	270 ± 2.2 a,b	238 ± 3.4 a
Dehulling	313 ± 7.5 c (15.8)	253 ± 6.6 b (6.39)
Soaking	269 ± 1.8 b (0.37)	239 ± 1.8 a,c (0.63)
Germination 24 h	272 ± 4.0 a (0.70)	241 ± 3.9 a,c (1.47)
Germination 48 h	273 ± 3.5 a (1.19)	243 ± 3.8 a,c (2.06)
Germination 72 h	276 ± 3.0 a (2.11)	245 ± 4.5 c (2.90)
Extrusion	271 ± 1.9 a (0.22)	240 ± 2.9 a,c (1.18)

[a] Results are the means of 10 determinations ± SD. Means within the same column followed by same letter are not statistically significant using one-way ANOVA test ($P < 0.05$). figures in parentheses indicate the percent increase/decrease over the values of the corresponding raw seed.

Dehulling significantly ($P < 0.0001$) increased the levels of the protein of *V. faba* and *P. vulgaris* seeds. Because of their greater weight, cotyledons contribute the major amount of protein to the whole seed. The hull of *V. faba* seed has significantly more weight than that of *P. vulgaris* in proportion to the whole grain. Therefore dehulling more significantly increased protein content in *Vicia* (15.8%) than in *Phaseolus* (5.90%) seeds.

Also a gradual increase was observed from 24 to 72 h of germination. This increase, attributed to the utilisation of carbohydrates as source of energy during germination, was only significant in *P. vulgaris* at 72 h ($P = 0.0256$). The results agree with the notable increase in IVSD found during germination, presumably caused by starch digestion through amylolytic enzymes.

3.2. Phytic acid, condensed tannins and polyphenols

Data on phytic acid, condensed tannins and polyphenol contents of raw and processed seeds are summarised in Table 2. Different processing methods, such as soaking, germination (24–72 h) and extrusion cooking significantly ($P < 0.05$) reduced the levels of phytic acid, condensed tannins and polyphenols. The data agree with those found by Chau and Cheung (1997) and Alonso et al. (1998) in other legume seeds.

Compared to the raw seeds, a significant ($P < 0.005$) reduction in the levels of all three antinutrients in both legume seeds was observed after 12 h soaking, as well as after 24, 48 and 72 h of germination. In general, longer germination resulted in lower levels of phytic acid, condensed tannins and polyphenols. Thus, germination for 72 h was found the most effective treatment for phytic acid reduction. The decrease in phytic acid content has been reported by different workers to be a result of a leaching-out effect during hydration (Beleia, Thu Thao & Ida, 1993) and as a consequence of increase in phytase activity during germination (Bau, Villaume, Nicolas & Méjean, 1997).

Extrusion cooking caused a significant reduction in phytic acid content compared to raw seeds in both faba beans and kidney beans ($P < 0.0001$). Analysis performed by a high performance liquid chromatography (HPLC) method (Sandberg, Andersson, Carlsson & Sandström, 1987) revealed that during extrusion, some molecules of inositol hexaphosphate were hydrolysed to penta-, tetra- and triphosphates (data not shown). Condensed tannins and polyphenols were also significantly reduced by this thermal processing method. Thermal degradation of these molecules, as well as changes in their chemical reactivity or the formation of insoluble complexes, could explain the significant ($P < 0.0001$) reduction of the antinutrients by thermal processing (Barroga, Laurena & Mendoza, 1985; Kataria, Chauhan & Punia, 1989).

Phytic acid is accumulated in beans in the globoids, which is one of the inclusions of the protein body (Lott & Buttrose, 1978) and major amounts of bean tannins are located in the seed coat or testa, with low or negligible amounts located in the cotyledons (Bressani & Elias, 1980). Since phytates are mainly located in the cotyledons and tannins in the testa, the physical removal of testa by dehulling increased the phytic acid content and significantly decreased ($P < 0.0001$) the condensed tannin concentration. Because the different proportions of the hull in relation to the whole seed in *Vicia* and *Phaseolus*, phytates were increased significantly by dehulling only in faba bean seeds ($P < 0.0001$ and $P = 0.4838$, respectively).

Table 2
Phytic acid (g kg^{-1} DM), condensed tannins (g eq cat kt^{-1}DM) and polyphenols (g kg^{-1}DM) in raw, dehulled, soaked, germinated and extruded seeds of *Vicia faba* L. and *Phaseolus vulgaris* L.[a]

Treatment	Phytic acid	Condensed tannins	Polyphenols
Vicia faba			
Raw seeds	21.7 ± 0.2a	1.95 ± 0.05a	3.92 ± 0.39a
Dehulling	23.8 ± 0.3b (9.68)	0.15 ± 0.01b (92.3)	0.72 ± 0.16b (81.6)
Soaking	14.6 ± 0.3c (32.7)	1.02 ± 0.02c (47.7)	3.73 ± 0.13a (4.85)
Germination 24 h	10.1 ± 0.2d (53.5)	0.86 ± 0.02b,d (55.9)	3.58 ± 0.02a,c (8.67)
Germination 48 h	8.9 ± 0.1e (59.0)	0.82 ± 0.03b,d (58.0)	3.56 ± 0.11a,c (9.18)
Germination 72 h	8.5 ± 0.1e (60.8)	0.78 ± 0.02b (60.0)	3.10 ± 0.22c,d (20.9)
Extrusion	15.9 ± 0.7f (26.7)	0.89 ± 0.01d (54.4)	2.80 ± 0.04d (28.6)
Phaseolus vulgaris			
Raw seeds	15.9 ± 0.3a	3.59 ± 0.05a	2.07 ± 0.12a
Dehulling	16.2 ± 0.3a (1.89)	0.24 ± 0.00b (93.3)	0.19 ± 0.02b (90.8)
Soaking	15.0 ± 0.1b (5.66)	2.72 ± 0.04c (24.2)	1.64 ± 0.08c (20.7)
Germination 24 h	14.5 ± 0.4b (8.81)	2.03 ± 0.06d (43.5)	1.40 ± 0.06c,d (32.4)
Germination 48 h	12.9 ± 0.3c (18.9)	1.30 ± 0.01c (63.8)	1.23 ± 0.07d,e (40.6)
Germination 72 h	11.1 ± 0.2d (30.2)	1.02 ± 0.02f (71.6)	0.97 ± 0.13e (53.1)
Extrusion	12.6 ± 0.2c (21.4)	0.58 ± 0.05g (83.8)	1.12 ± 0.09d,e (45.9)

[a] Results are the means of 10 determinations ± SD. Means within the same column followed by same letter are not statistically significant using one-way ANOVA test ($P < 0.05$) Figures in parentheses indicate the percent increase/decrease over the values of the corresponding raw seed.

3.3. Trypsin, chymotrypsin, α-amylase inhibitors and haemagglutinating activity

Table 3 shows that trypsin, chymotrypsin, and α-amylase inhibitor activities of the two legume seeds were decreased significantly by processing methods such as soaking, germination (24–72 h) and extrusion. These results are in agreement with previous findings (Alonso et al., 1998; Griffiths, 1984). The relatively high concentration of AIA in kidney bean was consistent with the findings of Jaffé, Moreno and Wallis (1973). No differences were found in haemagglutinating activity when seeds were soaked, dehulled or germinated. The estimation of lectin content by haemagglutination of red cells is not a very precise method although the procedure used is a safe method for checking the efficiency of treatments with respect to seed toxicity (Grant, More, McKenzie & Pusztai, 1982).

Extrusion was the most effective method to reduce TIA, CIA and AIA when compared with three other treatments. Although there was no detectable variation in the haemagglutinating activity of dehulled, soaked and germinated seeds, this activity was very sensitive to hydrothermal treatment. Heat treatments have been shown to be very effective in destroying protease inhibiting activity and lectin (haemagglutinating) activity (Armour, Perara, Buchan & Grant, 1998).

The non-competitive inhibition of the digestive enzymes by tannins due to their affinity with protein to form complexes has been reported (Griffiths, 1981; Griffiths & Moseley, 1980). This could explain the lower than expected increase of TIA, CIA and AIA after dehulling.

3.4. Protein and starch digestibilities

Tables 4 and 5 show that both IVPD and IVSD of *V. faba* and *P. vulgaris* were increased significantly ($P < 0.005$) by dehulling, soaking, germination and extrusion. The results agree with those of Bishnoi and Khetarpaul (1993) and Chau and Cheung (1997) in legume seeds. Compared with control, extrusion cooking produced a more significant improvement of IVPD and IVSD in both legumes. The increases of digestibility produced by the different treatments were higher in starch than in protein.

Improvement of protein digestibility after processing could be attributable to the reduction or elimination of different antinutrients. Phytic acid, as well as condensed

Table 4
In vitro protein digestibility (%) in raw, dehulled, soaked, germinated and extruded seeds of *Vicia faba* L. and *Phaseolus vulgaris* L.[a]

Treatment	*Vicia faba*	*Phaseolus vulgaris*
Raw seeds	70.8 ± 0.2a	68.1 ± 0.4a
Dehulling	72.5 ± 0.3a (2.40)	71.6 ± 0.3b (5.14)
Soaking	71.3 ± 0.1c (0.71)	71.4 ± 0.5b (4.85)
Germination 24 h	73.0 ± 0.1d (3.11)	73.4 ± 0.2c (7.78)
Germination 48 h	75.2 ± 0.3e (6.21)	75.7 ± 0.4d (11.2)
Germination 72 h	78.1 ± 0.2f (10.3)	78.0 ± 0.3e (14.5)
Extrusion	87.4 ± 0.2g (23.5)	83.0 ± 0.3f (21.9)

[a] In vitro protein digestibility of casein was 93.2 ± 0.2. Results are the means of three determinations ± SD. Means within the same column followed by same letter are not statistically significant using one-way ANOVA test ($p < 0.05$). Figures in parentheses indicate the percent increase/decrease over the values of the corresponding raw seed.

Table 3
Trypsin (IU/mg DM), chymotrypsin (IU/mg DM), and α-amylase (IU/g DM) inhibitor contents and haemagglutinating activity (HU/mg DM) in raw, dehulled, soaked, germinated and extrucded seeds of *Vicia faba* L. and *Phaseolus vulgaris* L.[a]

Treatment	Trypsin inhibitors	Chymotrypsin inhibitor	α-Amylase inhibitors	Haemagglutinating activity
Vicia faba				
Raw seeds	4.47 ± 0.21a,b	3.56 ± 0.16a,b	18.9 ± 1.84a,b	49.3 ± 0.0a
Dehulling	4.99 ± 0.30a (11.6)	3.71 ± 0.13a (4.21)	20.7 ± 0.90a (9.25)	49.3 ± 0.0a (0.00)
Soaking	4.27 ± 0.17b (4.47)	3.41 ± 0.11a–c (8.43)	16.1 ± 0.59b,c (14.9)	49.3 ± 0.0a (0.00)
Germination 24 h	4.20 ± 0.04b,c (6.04)	3.25 ± 0.15b,c (8.71)	13.9 ± 0.92c (26.5)	49.3 ± 0.0a (0.00)
Germination 48 h	3.76 ± 0.08c,d (15.9)	3.18 ± 0.10b,c (10.7)	12.6 ± 0.65c (33.4)	49.3 ± 0.0a (0.00)
Germination 72 h	3.34 ± 0.19d (25.3)	3.13 ± 0.11c (12.1)	11.9 ± 1.27c (37.2)	49.3 ± 0.0a (0.00)
Extrusion	0.05 ± 0.01e (98.9)	1.68 ± 0.19d (52.8)	0.00 ± 0.00d (100)	0.2 ± 0.0b (99.6)
Phaseolus vulgaris				
Raw seeds	3.10 ± 0.24a	3.97 ± 0.16a	248 ± 4.25a	74.5 ± 0.0a
Dehulling	3.43 ± 0.18a (9.62)	4.56 ± 0.09b (14.9)	254 ± 4.09a (2.29)	74.5 ± 0.0a (0.00)
Soaking	2.93 ± 0.18a,b (5.48)	3.37 ± 0.17c (15.1)	220 ± 4.56b (11.2)	74.5 ± 0.0a (0.00)
Germination 24 h	2.87 ± 0.26a,b (7.42)	3.28 ± 0.09c (17.4)	189 ± 1.56c (23.9)	74.5 ± 0.0a (0.00)
Germination 48 h	2.43 ± 0.18b,c (21.6)	3.18 ± 0.10c (19.9)	170 ± 5.83d (31.4)	74.5 ± 0.0a (0.00)
Germination 72 h	2.20 ± 0.21c (29.0)	3.06 ± 0.11c (22.9)	164 ± 4.46d (33.8)	74.5 ± 0.0a (0.00)
Extrusion	0.43 ± 0.11d (86.1)	0.00 ± 0.00d (100)	0.00 ± 0.00e (100)	0.2 ± 0.0b (99.7)

[a] Results are the mens of 10 determinations ± SD. Means within the same column followed by same letter are not statistically significant using one-way ANOVA test ($P < 0.05$). Figures in parentheses indicate the percent increase/decrease over the values of the corresponding raw seed.

Table 5
In vitro startch digestibility (mg of maltose g^{-1}) in raw, dehulled, soaked, germinated and extruded seeds of *Vicia faba* L. and *Phaseolus vulgaris* L.[a]

Treatment	*Vicia faba*	*Phaseolus vulgaris*
Raw seeds	159 ± 3a	134 ± 2a
Dehulling	174 ± 5b (9.43)	151 ± 4b (12.7)
Soaking	167 ± 5a,b (5.03)	141 ± 4a (5.22)
Germination 24 h	187 ± 4c (17.6)	161 ± 3c (20.1)
Germination 48 h	208 ± 4d (30.8)	178 ± 4d (32.8)
Germination 72 h	222 ± 3e (39.6)	191 ± 3e (42.5)
Extrusion	290 ± 5f (82.4)	306 ± 7f (128)

[a] In vitro protein digestibility of casein was 93.2 ± 0.2. Results are the means of three determinations ± SD. Means within the same column followed by same letter are not statistically significant using one-way ANOVA test ($p < 0.05$). Figures in parentheses indicate the percent increase/decrease over the values of the corresponding raw seed.

tannins and polyphenols, are known to interact with protein to form complexes. This interaction increases the degree of cross-linking, decreasing the solubility of proteins and making protein complexes less susceptible to proteolytic attack than the same protein alone (Cheryan, 1980; Reddy, Sathe & Salunkhe, 1985). Extrusion produced a higher increase in IVPD than the other processing methods. This can be related to the higher efficiency of this thermal treatment in reduction of trypsin and chymotrypsin inhibitory activities in comparison with soaking and germination.

Enhancement of starch digestibility during dehulling, soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility (Deshpande & Cheryan, 1984). The degree of starch gelatinisation of thermally treated samples is higher than in untreated ones (Bishnoi & Khetarpaul, 1993) and it is thus more readily hydrolysed. The rupture of starch granules of legumes, making the substrate more accessible, facilitated the amylolysis. On the other hand reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility. Despite the increase in phytic acid, trypsin, chymotrypsin and α-amylase inhibitor levels produced by dehulling, both IVPD and IVSD were significantly increased by this processing method. This reveals the important role that condensed tannins play in digestive enzyme inhibition.

In this work soaking, germination and extrusion procedures were compared as to their efficiency in reducing antinutrient levels and improving IVPD and IVSD. Dehulling was the most effective in reducing the levels of condensed tannins and polyphenols. Extrusion caused the biggest reduction of protein antinutritional factors and appeared to be the most effective, improving both IVPD and IVSD.

Further biological trials must be carried out in order to evaluate the nature of modifications to nutrients and antinutrients caused by extrusion cooking and to correlate these with the subsequent nutritive values of faba bean and kidney bean. Protein and starch digestibilities of raw and processed *V. faba* and *P. vulgaris* seeds must be assessed in vivo to correlate with corresponding in vitro studies.

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