Assessment of the nutritional quality of raw and extruded *Pisum sativum* L. var. *laguna* seeds

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ABSTRACT

Pea (*Pisum sativum* L. var. *Laguna*) seeds were submitted to extrusion process at 129, 135 and 142 ºC and modifications on the proximate composition and nutritional parameters were evaluated. Peas were a good source of protein (24 g/100g), amino acids (sulphur amino acids were the limiting ones), dietary fibre (18 g/100g), carbohydrates (53 g/100g), energy (330 Kcal/100g), riboflavin and thiamine (0.1-0.2 mg/100g). Pea seeds also contained non-nutritive compounds such as α-galactosides (4 g/100g), phytic acid (0.4 g/100g) and trypsin inhibitor activity (2 TIU/mg). Extrusion cooking caused a slight increase of protein and fat content, whilst it reduced dietary fibre, thiamine and α-galactosides, and led to negligible trypsin inhibitor activity (TIA) levels. The protein quality of pea measured by biological indexes (net protein utilization, net protein ratio, relative net protein ratio, true protein digestibility and biological value) was not affected by extrusion treatments. Protein quality measured by chemical indexes (chemical score and protein digestibility corrected amino acid score) decreased in processed peas. Among extruded peas, those processed at 135 ºC presented the highest chemical indexes. Therefore, the aforementioned condition could be considered adequate for the manufacture of novel pea-derived products with high nutritive value.

Keywords: Peas, extrusion, protein quality, chemical score, PDCAAS.

1. Introduction

Legumes provide economical sources of proteins, carbohydrates, fibre, vitamins and energy for a large population all over the world and the inclusion of legumes in a diet could have beneficial physiological effects in controlling and preventing various metabolic diseases (Tharanathan & Mahadevamma, 2003). Nevertheless, nutritive utilization of legumes can be negatively affected by their content in non-nutritive factors, such as α-galactosides, trypsin inhibitors, or phytic acid, which cause flatulence, interfere with the ingestion and digestive utilization of proteins and minerals by monogastric animals (Champ, 2002).

Treatments to overcome such limiting factors involve thermal processing directed to decrease trypsin inhibitors, enhancing protein digestibility (Vidal-Valverde, Frias, Diaz-Pollan, Fernandez, Lopez-Jurado, & Urbano, 1997) and modify the conten α-galactosides and phytic acid (Martínez-Villaluenga, Frias, & Vidal-Valverde, 2008; Urbano, Lopez-Jurado, Aranda, Vidal-Valverde, Tenorio, & Porres, 2000). The extrusion cooking process is a high-temperature, short-time processing technology that leads starch gelatinization, protein denaturation and inactivation of enzymes as well as heat sensitive non-nutritive factors, and changes seem to be highly dependent on the extruder operating conditions (Bhattacharya and Prakash, 1994).

Peas (*Pisum sativum* L.) are relatively inexpensive and highly nutritious crop and processed seeds can be utilized in specific food formulations for pre-school children to improve their protein intake. In addition, they are rich in lysine and can complement cereals complying with the FAO reference pattern (FAO, 2007).

Although there is information on the literature relative to the effect of extrusion on the nutritional properties of peas (Alonso, Orue, & Marzo, 1998; Alonso, Aguirre, & Marzo, 2000; Burel, Tulli, & Kaushik, 2000), the influence of extrusion processes at different temperatures on the nutritional quality of peas habitually grown in Argentina has not been previously studied. The investigation of this topic is of great importance due to the high social and economic relevance of Peas in South America.

Therefore, the aim of this work was to assess the nutritional quality of peas (*Pisum sativum* L. var. *laguna*) extruded at different temperatures through their chemical and nutritional characterization. Proximate composition as well as the content of dietary fibre, amino acids, vitamins B_1 and B_2, and non-nutritive compounds (trypsin inhibitors, α-galactosides and phytic acid) were analysed. To assess the nutritional value of extruded pea flours biological studies were also carried out.

2. Materials and methods

2.1. Samples. Pea seeds (*Pisum sativum* L. var. *laguna*) were provided and processed by Cereal and Oilseeds Centre from National Institute of Industrial Technology (INTI, Argentina). Raw and extruded pea seed samples were studied for analytical and biological purposes.

2.2. Extrusion process. Pea seeds were grounded to a particle size smaller than 4 mm in an industrial hammer mill (Berandebi S.A., Argentina) with a 1000 Kg/h capacity and an engine of 20 HP. Following this, they were processed at a semi-industrial scale in a single screw extruder with the following specifications: barrel length, 200 mm; barrel diameter, 105 mm; 4 zone barrel; die diameter,

3.2 mm and at a screw speed at 60 rpm. The pea flour was fed at a speed of 500 Kg/h and had a residence time of 3 seconds. Three temperatures were assayed: 129, 135 and 142ºC, and the established temperature were reached in the last second. 40, 35 and 25 liters/hour of water were added during the process to each established temperature, respectively and, afterwards, the samples were allowed to reach room temperature inside the extruder. The final size of extruded products was smaller than 7 mm, according to mesh extruder specifications. Three batches were carried out for each extrusion process.

Chemical analysis

2.3.1. Protein content: Nitrogen content was determined according to the Kjeldahl method (AOAC 984.13) and nitrogen value was multiplied by 6.25 as conversion factor (AOAC, 1990).

2.3.2. Moisture content was determined by drying the samples to a constant weight at 105 ºC according to AOAC 925.09 (AOAC, 2000).

2.3.3. Ash content was measured by calcinations at 550 ºC to a constant weight, according to AOAC 923.03 (AOAC, 2000).

2.3.4. Fat content was determined gravimetrically after hexane extraction, according to AOAC 922.06 (AOAC, 2000).

2.3.5. Dietary fibre was determined with the enzymatic-gravimetric method following the AOAC 985.29 method (AOAC, 2000).

2.3.6. Available carbohydrates were estimated by difference as:

100 – (%proteins + %fat + %water + %fibre + %ash) (FAO, 2002).

2.3.7. Energy content was calculated by the Atwater general factors system (FAO, 2002).

116 *2.3.8. Protein amino acids* were determined by HPLC according to Frias et al.
117 (2009) by acid hydrolysis and alkaline hydrolysis (Trp), derivatization and HPLC
118 quantification.
119 *2.3.9. Vitamin B_1 and B_2* were determined in a single extraction procedure and
120 quantified separately by HPLC with a fluorescence detector, according to
121 Martínez-Villaluenga, Frias, & Vidal-Valverde, (2006).
122 *2.3.10. α-Galactosides* were extracted with 80% ethanol and quantified by
123 HPLC with a refractometric detector, according to Martínez-Villaluenga et al.
124 (2006).
125 *2.3.11. Inositol phosphates*: IP_6 (phytic acid or hexainositol phosphate), IP_5
126 (pentainositol phosphate), IP_4 (tetrainositol phosphate) and IP_3 (tri-inositol
127 phosphate) were extracted with 0.5M HCl and quantified by HPLC using a
128 refractometric detector, according to Frias, Doblado, Antezana, & Vidal-
129 Valverde, (2003).
130 *2.3.12. Trypsin inhibitor activity* (TIA) was spectrophotometrically determined at
131 410 nm, as in Vidal-Valverde et al., (2003).
132
133 *2.4. Protein quality evaluation*
134 *2.4.1. Protein quality evaluation by biological assays*
135 Protein quality assessment was established by net protein utilization (NPU) and
136 true protein digestibility (TD), according to the Miller and Bender method (1955).
137 Net protein ratio (NPR), relative net protein ratio (RNPR) and Biological Value
138 (BV) were also determined (Pellet and Young, 1980).
139 *Diets*: Four experimental diets have been studied: raw peas and extruded peas
140 at 129 ºC, 135 ºC and 142 ºC. At the same time, two additional diets were used

as controls: one free protein diet and the other one constituted with casein supplemented with 2% methionine. With the exception of the free protein diet, all the diets contained 10% protein (Pellet and Young, 1980) and the provided diets were the only source of protein. The rest of diet components were formulated following the recommendations given by Reeves, Nielsen, & Fahey, (1993).

Experimental design. The influence of different diets on metabolic utilization of nitrogen was studied in rats fed for 10 days. A total of 72 rats were divided into 6 groups of 12 animals for each diet. Food intake, body weight, change in body weight, nitrogen intake, and faecal nitrogen excretion were determined in all rats.

Animals: The animals were 4 weeks old (recently weaned) Wistar albino rats with an initial body weight of 55±5g. For every studied diet, 12 animals were used, which were housed from day 0 of the experiment in individual galvanized iron cages with grid floor to prevent coprophagy and to facilitate collection of faeces, according to the National Research Council (1996). The cages were located in a well-ventilated, thermostatically controlled room (20±2 ºC), humidity (50–60 %), with 12 h light/dark periods. Throughout the experimental period, all animals had free access to water and diets were consumed *ad libitum*.

Food intake: Total amount consumed daily by each rat was determined by weighing the amount of diet given, refused, and spilled. The body weight was recorded every two days during the experimental period. Faeces were collected in the last 6 days. Each group of 12 rats was divided into two for faeces collection. At the end of experimental period, animals were anaesthetised and

sacrificed with CO_2 (Close et al, 1996), and body water weight was determined by drying the carcass at 105 ºC for 72 h.

Biological indexes

True protein digestibility (TD), the proportion of food protein that is absorbed, is defined from measurements of nitrogen content of foods and faeces as follows:

$$TD = I - (F - F_k) / I$$

F: Faecal nitrogen of the animals fed the experimental diets

F_k: Faecal nitrogen of the animals fed protein free diet

Nitrogen content of the body was calculated from the water content using the equation that represents the ratio of nitrogen and body water content according to the animal's age (Miller and Bender, 1955) adapted to our experimental conditions using the following formulas (Sambucetti and Sanahuja, 1970):

$$y = N/water \times 100$$

$$y = 2.76 + 0.0293\ x$$

x: animal age (days) at the end of the experiment.

From the obtained values, NPU was determined as the porcentual ratio between the retained nitrogen and intaken nitrogen applying the following formula:

$$NPU = \frac{B - (B_k - I_k)}{I} \text{ ; where}$$

B: Body nitrogen content of animals fed the experimental diets

B_k : Body nitrogen content of the animals fed the protein free diet

I_k: nitrogen intake of animals fed free protein diet

I: nitrogen intake of animals fed experimental diets

Biological value (BV) is the proportion of absorbed nitrogen that is retained for manteinance and/or growth, and expressed by the following formula:

$$BV = (NPU / TD) \times 100$$

NPR= (Weight gain of test animal, g + weight loss of nonprotein group, g)/ protein consumed by test animal, g.

Relative NPR = (NPR of protein source /average NPR of reference protein)x100

Reference protein: casein supplemented with 2% methionine

2.4.2. Protein quality evaluation by chemical indexes

2.4.2.1. Chemical score. Chemical score (CS) is achieved by a comparison of the content of the main limiting amino acid in the raw or extruded peas with its content in the requirement pattern (Pellet and Young, 1980). In this work, CS was calculated as the average of the ratio of each essential amino acid in the food protein (raw and extruded peas) to their respective content in the recommended protein reference for 3-10 year old children (FAO, 2007).

$$CS = \frac{\text{Limiting amino acid of test protein}}{\text{The same amino acid of reference protein}}$$

The amino acid with the lowest percentage is called the limiting amino acid and this percentage is the chemical score.

2.4.2.2. Protein digestibility corrected amino acid score (PDCAAS). The protein digestibility corrected amino acid score (PDCAAS) is a method of evaluating the protein quality based on both the amino acid requirements of humans and their ability to digest it. This method uses the amino acid score corrected by true protein digestibility (TD) (FAO/WHO, 1991) according with the following formula:

$$PDCAAS = CS \times TD$$

2.5. Statistical analysis

Analytical data were expressed as the mean ± standard deviation (SD) of three independent determinations. Data were subjected to multifactor analysis of variance (ANOVA) using the least-squared difference test with the Statgraphic 5.0 Program (Statistical Graphic, Rockville, MD, USA). Biological data were expressed as the mean ± SD of 12 independent determinations. ANOVA and multiple comparison Student-Newman-Keuls tests were used.

3. Results and discussion

Table 1 summarizes the proximate composition of raw peas (*P. sativum* var. *laguna*) and the effect of extrusion processes at 129, 135 and 142 ºC. Raw pea showed contents of proteins, fat and ash of 23.6, 2.6 and 2.7 g/100 g dw, respectively. Furthermore, the amount of total dietary fibre and carbohydrates in raw pea were 18.3 and 52.8 g/100 g dw, respectively, while the total energy was 329 kcal/100 g dw. These results agree with data found in the bibliography for different cultivars of *Pisum sativum* (Souci, Fachmann, & Fraut, 2008; Paul, & Sauthgate, 1988). The extrusion process led to a slight but significant ($P \leq 0.05$) increase of protein content. Fat underwent a slight rise at 129 ºC whilst at 135 and 142 ºC caused larger increments (22%). However, the content of total dietary fibre of extruded peas experienced a significant ($P \leq 0.05$) reduction. The content of carbohydrates, ash and total energy were not modified. Proximate composition of extruded peas is consistent with previous findings (Burel et al., 2000; Stein and Bohlke, 2007).

The amino acid content of raw and extruded pea flours, expressed as g/100g d.w. is collected in Table 2. Among the non-essential amino acids, Glu,

242 Asp and Arg were present in higher amounts while Lys, Leu, Val and Phe were
243 the predominant essential amino acids, and Met+Cys were the limitans, results
244 which are within the range found in the literature (Souci, 2008; Al-Marzooqi and
245 Wiseman, 2009). When the extrusion was carried out at 129, 135 and 142ºC,
246 Pro underwent the largest reductions (28-38%), followed by Gly (10-15%) while
247 Arg decreased only when the peas were extruded at 129ºC (11%) and Glu at
248 129ºC and 142 ºC (8 and 6%, respectively). Among the essential amino acids,
249 Val, Phe and Lys showed significant reductions (10-22%), while Ile only when
250 extrusion was carried out at 129 and 142 ºC ($P \leq 0.05$) and Trp when the process
251 was performed at 142ºC (Table 2). Changes in Lys content are taken as an
252 quality indicator of the severity of thermal treatments through the formation of
253 cross-links or Maillard condensation products which reduce carbohydrates.
254 These results agree with those shown by Al-Marzooqi and Wiseman, (2009)
255 who found a slight decrease in almost all the essential amino acids of pea
256 seeds extruded at 140ºC.

257 The effect of extrusion on the content of vitamin B_1 and B_2 is shown in
258 Table 3. The content of those vitamins were 0.2 and 0.1 mg/100g, respectively.
259 Thiamin decreased ~50% after the extrusion, irrespective of the temperature
260 assayed. Riboflavin, however, was kept almost constant after the extrusion of
261 pea. Several studies have assessed the effect of extrusion in the vitamin B
262 group, mainly referred to cereals (Athar, Hardacre, Taylor, Clark, Harding, &
263 McLaughlin, 2006), and there is scarce information on extruded legumes but, in
264 general, thiamin retention is low since the heat-lability of this vitamin.

265 Table 4 shows the levels of α-galactosides in raw and extruded peas.
266 The content in unprocessed seeds was 4g/100g dw, of which stachyose

accounted for a large amount (57.6%), followed by raffinose (27.8%) and verbascose (14.6%), results that are in accordance with previously published (Vidal-Valverde et al., 2003). Extrusion induced significant (P≤0.05) reductions in α-galactosides and larger losses were achieved at 135 and 142ºC than at 129 ºC (27%, 28% and 12%, respectively). These results are in line with those reported by De Berrios, Morales, Cámara, & Sanchez-Mata, (2010) in extruded peas at 160 ºC, while Alonso et al. (2000) found reductions only in stachyose after extrusion of peas at 145ºC. The reduction in the oligosaccharides could be due to the breakage of the (2-1) furanosidic bonds during extrusion cooking (Chiang and Johnson, 1977), and the reducing sugars formed may interact with charged protein groups (Maillard reactions). This hypothesis confirms the behaviour found in sucrose content that decreased from 1.45 g/100g dw in unprocessed seeds to 1.3 or 0.8 g/100g dw, respectively, in 129ºC or 135 ºC/145 ºC extruded peas, respectively (unpublished results).

Changes in inositol phosphates content during the pea extrusion are also shown in Table 4. Phytic acid (IP_6) represented 69% of total inositol phosphates in raw pea seeds, followed by IP_5 (22%), whilst IP_4 and IP_3 were present in lower amounts (8 and 2%, respectively). The content of phytic acid agrees with values for different raw peas previously reported (Urbano et al., 2003) although it is rather lower than those shown by Alonso et al. (2000). Extrusion brought about slight decreases in phytic acid that were accompanied with tiny increments of lower inositol phosphates. Alonso et al. (2000) also found losses in phytic acid after extrusion.

Table 4 shows the levels of TIA in raw and extruded peas. Seeds initially presented a low TIA level (1.8 TIU/mg) that fell within the range found in the

292 literature for peas (Vidal-Valverde et al., 2003). Extrusion lead to a great TIA
293 reduction at 129 ºC (90%), and to its total inactivation at 135 and 142 ºC, results
294 that agree with those presented by Al-Marzooqi and Wiseman (2009) and
295 Alonso et al. (1998). Inactivation of TIA could be due to reactions involving
296 deamidation splitting of covalent bonds and interchange or destruction of
297 disulfide bonds during thermal processing (Adam, 1991).

298 The protein quality evaluation of raw and extruded peas diets measured
299 by biological indexes is shown in Table 5. Extrusion process did not significantly
300 modify ($P \leq 0.0001$) the values of NPU, TD, BV, NPR and RNPR and it is
301 highlighted the high values of TD (83-85%), and the acceptable value of BV for
302 raw and extruded pea flours (50-58%).

303 Table 6 shows the content of essential amino acids, expressed as g/100g
304 protein, and the evaluation of protein quality by chemical indexes. Met + Cys
305 were the limiting amino acids, as shown in Table 2. The percentages of other
306 essential amino acids outweigh the values of the reference protein (FAO, 2007).
307 The values obtained for protein quality assessed by CS and PDCAAS were
308 higher than those obtained by NPU. This could be explained because biological
309 studies carried out with rats underestimate the nutritional quality when sulphur
310 amino acids are limiting, possibly due to the elevated requirements of recently
311 weaned rats during growth, as Sarwar, Peace, & Botting (1989) pointed out.
312 Nevertheless, despite the differences found between both methods, the results
313 show that the protein quality of peas was affected by the extrusion treatments
314 assayed and, among them, when extrusion process was carried out at 135ºC
315 higher CS and PDCAAS were obtained (Table 6).

316

4. Conclusions

The extrusion processes increased slightly the content of protein and fat, whilst reduced the dietary fibre and thiamine content in pea flours. Furthermore, extruded pea flours provided lower amount of α-galactosides and negligible TIA than raw pea. The protein quality of pea flours measured by biological indexes was not affected by the extrusion treatments, but the chemical indexes decreased after extrusion and, among them, extruded pea obtained at 135 ºC presented the highest CS and PDCAAS. Hence, extrusion carried out in these conditions could be considered the most adequate for the manufacture of novel pea-derived products with high nutritive value.

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Table 1. Proximate composition and energy in peas of *Pisum sativum* var*. laguna* seeds*

	Raw pea	Extruded 129º C Peas	Extruded 135º C Peas	Extruded 142º C Peas
Moisture	12.62±0.04^{c}	10.55±0.09^{b}	8.55±0.03^{a}	8.24±0.03^{a}
Proteins (g/100 g dw)	23.57±0.17^{a}	24.07±0.13^{b}	24.06±0.11^{b}	24.09±0.15^{b}
Fat (g/100 g dw)	2.63±0.05^{a}	2.98±0.06^{b}	3.21±0.04^{c}	3.21±0.03^{c}
Ash (g/100 g dw)	2.74±0.04^{a}	2.82±0.03^{a}	2.81±0.03^{a}	2.72±0.05^{a}
Total Dietary Fibre (g/100 g dw)	18.28±0.13^{d}	15.27±0.11^{a}	16.93±0.16^{c}	16.02±0.15^{b}
Carbohydrates (g/ 100 g dw)	52.78	54.86	52.99	53.96
Energy (Kcal/100 g dw)	329	343	337	341

*) Values are the mean of 3 determinations ± SD. Different superscripts in the same row indicate significant difference (P≤0.05).

Table 2. Amino acid content of raw and extruded *Pisum sativum* var. *laguna* seeds (g/100g dry weight

Amino acids	Raw Peas (g/100g d.w.)	Extruded 129º C Peas (g/100g d.w.)	Extruded 135º C Peas (g/100g d.w.)	Extruded 142ºC Peas (g/100g d.w.)
Non essential amino acids (g/100g d.w.)				
Asp	2.82[a]	2.91[a]	2.86[a]	2.87[a]
Glu	4.56[b]	4.27[a]	4.56[b]	4.38[a]
Ser	1.03[a]	0.99[a]	1.06[a]	0.94[a]
Gly	1.01[b]	0.86[a]	0.87[a]	0.91[a]
Arg	1.49[b]	1.33[a]	1.46[b]	1.47[b]
Ala	0.83[b]	0.76[a]	0.73[a]	0.74[a]
Pro	0.85[b]	0.57[a]	0.61[a]	0.53[a]
Essential amino acids (g/100g d.w.)				
His	0.52[a]	0.52[a]	0.54[a]	0.55[a]
Val	1.23[b]	1.01[a]	1.08[a]	1.10[a]
Met+Cys	0.45[a]	0.38[a]	0.42[a]	0.40[a]
Ile	1.09[b]	0.96[a]	1.04[ab]	1.03[ab]
Leu	1.85[b]	1.63[a]	1.70[a]	1.67[a]
Phe	1.29[c]	1.15[b]	1.00[a]	1.04[a]
Tyr	0.59[a]	0.56[a]	0.53[a]	0.54[a]
Lys	2.18[b]	1.82[a]	1.81[a]	1.73[a]
Thr	0.84[a]	0.86[a]	0.84[a]	0.78[a]
Trp	0.52[b]	0.50[b]	0.48[ab]	0.46[a]

*) Values are the mean of 3 determinations $\pm$ SD. Different superscripts in the same row indicate significant difference (P≤0.05).

Table 3. Thiamin and riboflavin content (mg/100g d.w.) in raw and Extruded *Pisum sativum* var. *laguna* seeds*

	Raw pea	Extruded 129º C Peas	Extruded 135º C Peas	Extruded 142º C Peas
Thiamin	0.196 ± 0.012^{c}	0.104 ± 0.009^{b}	0.100 ± 0.008^{ab}	0.089 ± 0.005^{a}
Riboflavin	0.102 ± 0.004^{c}	0.096 ± 0.006^{bc}	0.087 ± 0.005^{a}	0.089 ± 0.004^{ab}

*) Values are the mean of 3 determinations ± SD. Different superscript in the same row indicate significant difference ($P \leq 0.05$).

Table 4. Non-nutritive compounds in raw and extruded *Pisum sativum* var. *laguna* seeds

	Raw pea	Extruded 129º C Peas	Extruded 135º C Peas	Extruded 142º C Peas
Raffinose (g/100g dw)	1.14±0.04^{c}	1.02±0.05^{b}	0.74±0.04^{a}	0.76±0.04^{a}
Stachyose (g/100g dw)	2.36±0.07^{c}	2.04±0.06^{b}	1.75±0.06^{a}	1.73±0.03^{a}
Verbascose (g/100g dw)	0.60±0.03^{c}	0.53±0.02^{b}	0.48±0.03^{a}	0.47±0.02^{a}
Total α-galactosides (g/100g dw)	4.10±0.11^{c}	3.60±0.05^{b}	2.98±0.10^{a}	2.95±0.05^{a}
IP6 (g/100g dw)	0.35±0.01^{a}	0.28±0.01^{b}	0.29±0.01^{b}	0.34±0.03^{a}
IP5 (g/100g dw)	0.11±0.01^{a}	0.13±0.01^{b}	0.13±0.01^{b}	0.15±0.02^{c}
IP4 (g/100g dw)	0.04±0.01^{a}	0.06±0.01^{c}	0.05±0.01^{b}	0.05±0.00^{b}
IP3 (g/100g dw)	0.01±0.00^{a}	0.02±0.00^{b}	0.02±0.00^{b}	0.01±0.00^{a}
Total IP (g/100g dw)	0.51	0.49	0.49	0.55
TIA (U TI/mg dw)	1.84±0.15^{c}	0.18±0.02^{b}	ND^{a}	ND^{a}

*) Values are the mean of 3 determinations $\pm$ SD. Different superscripts in the same row indicate significant difference (P≤0.05).

Table 5.- Evaluation *in vivo* of protein of raw and extruded *Pisum sativum* var. *laguna* seeds*

	Casein	Raw pea	Extruded129ºC pea	Extruded 135ºC pea	Extruded 142ºC pea
NPU	75.3±7.7[b]	48.08±8.6[a]	42.4±6.6[a]	42.5±5.7[a]	44.4±7.0[a]
TD	97.8±0.1[b]	82.6±0.4[a]	83.7±1.3[a]	83.6±1.4[a]	85.6±2.5[a]
B.V	77.0±7.9[b]	58.1±10.4[a]	50.6±7.9[a]	50.8 ± 6.8[a]	51.9±8.2[a]
NPR	4.9 ± 0.4[a]	2.9 ± 0.4[b]	2.6 ± 0.5[b]	2.5 ± 0.4[b]	2.5 ± 0.2[b]
RNPR	100[a]	59.6 ± 8.3[b]	54.2 ± 9.9[b]	50.6 ± 8.9[b]	51.6 ± 4.7[b]

Different superscripts in the same row indicate significant difference (P≤0.05).

NPU: Net Protein Utilization; TD: Protein digestibility; BV: Biological value; NPR: Net Protein Ratio; RNPR: Relative Net Protein Ratio.

Table 6. Protein evaluation of raw and extruded *Pisum sativum* var. *laguna* seeds by chemical indexes.

Essential amino acids	Requirements children 3-10 years old*	Raw** peas	% amino acid raw pea/ requirements	Extruded 129º C Peas**	% amino acid extruded 124 ºC pea/ requirements	Extruded 135º C Peas**	% amino acid extruded 135 ºC pea/ requeriments	Extruded 142º C Peas**	% amino acid extruded 142 ºC pea/ requirements
His	1.6	2.21	138	2.16	135	2.24	140	2.28	142
Val	4.0	5.22	130	4.20	105	4.49	112	4.57	114
Met+Cys	2.4	1.91	80	1.58	66	1.75	73	1.66	69
Ile	3.1	4.62	149	3.99	129	4.32	139	4.28	138
Leu	6.1	7.85	129	6.77	111	7.07	116	6.93	114
Phe+Tyr	4.1	7.97	194	7.11	173	6.36	155	6.56	160
Lys	4.8	9.25	193	7.56	157	7.52	157	7.18	150
Thr	2.5	3.56	142	2.33	93	3.49	140	3.24	130
Trp	0.66	2.21	335	2.08	305	2.00	303	1.91	289
Chemical score			80		66		73		69
Limiting amino acid			Met+Cys		Met+Cys		Met+Cys		Met+Cys
PDCAAS			66		55		61		59

* FAO (2007). **g/100g protein. PDCAAS = protein digestibility corrected amino acid score.