Nutritional Assessment in Vitro and in Vivo of Raw and Extruded Peas (*Pisum sativum* L.)

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The effects of extrusion cooking on the nutritional properties of *Pisum sativum* L. have been evaluated in vitro and in vivo. The treatment greatly elevated protein and starch digestibility in vitro. Also, the amounts of intact starch diminished while total free sugars increased. In addition, the levels of antinutritional factors, such as protease inhibitors and lectins, were greatly decreased. Concentrations of methionine and cystine were low in raw peas and were further reduced by extrusion treatment. The nutritional performance of rats fed extruded pea diets for 15 days was no better than that of rats given raw pea diet. This was due to the overriding effects of amino acid deficiencies in the diets. Weight gains by rats fed extruded pea diets supplemented with amino acids were, however, much higher than those achieved by rats fed supplemented raw pea diets. Food transformation index and protein efficiency ratio values were also greatly improved. Extrusion treatment did therefore significantly improve the nutritional quality of peas.

Keywords: *Extrusion-cooking; Pisum sativum; antinutritional factors; food transformation index; protein efficiency ratio*

INTRODUCTION

Peas are grown extensively in many regions of the world and make a major contribution to the total output of food legumes. Only soybean, peanut, and dry bean production exceeds that of peas. Although generally used as mature dry seeds, peas can also be consumed as green immature seeds.

The nutritive value of pea proteins is however low in comparison to animal proteins. This has been attributed to poor digestibility, a deficiency of sulfur amino acids and the presence of antinutritional factors, such as phytates, polyphenols, enzyme inhibitors (trypsin, chymotrypsin, and α-amylase) and hemagglutinins (Gwiazda et al., 1980; Evans and Boulter, 1980). Several industrial- or home-scale processes, including soaking, germination, dehulling, milling, cooking, roasting or fermentation, have been used to improve the nutritional properties of legumes (Melcion and van der Poel, 1993; Campbell and van der Poel, 1998). However, the efficacy of these treatments has been found to be variable.

In recent years, extrusion-cooking has become increasingly used in the production of foods and food ingredients such as breakfast cereals, baby foods, snacks, meat and cheese analogues, modified starches, etc. It is unique among heat-treatments in that the material is also subjected to intense mechanical shear: moistened starchy or proteinaceous foods are worked into a viscous, plastic-like dough and cooked before being forced through a die (Martín-Cabrejas et al., 1999). Some results of this process are the gelatinization of starch, denaturation of protein, and inactivation of antinutritional factors (Melcion and van der Poel, 1993). However, processing may also impair the quality and availability of some nutrients depending on the technology and conditions involved (Melcion and van der Poel, 1993).

The objectives of this study were to evaluate in vitro and in vivo the nutritional quality of a pea cultivar habitually grown in Navarra (Spain) and assess the effects of extrusion-cooking on levels of antinutritive factors and availability of pea proteins, amino acids, carbohydrates, and minerals.

MATERIALS AND METHODS

Samples. Mature pea seeds (*Pisum sativum* L. cv. Ballet) cultivated in Navarra (Spain) were used in all studies. All chemicals and reagents were purchased from Sigma Aldrich Co. (St. Louis, MO).

Extrusion. Extrusion-cooking of finely ground (0.5 mm) pea seeds was performed in a Clextral X-5 model BC 45 twin-screw extruder (F-42100 Firminy, France). The extrusion temperature at the outlet die was 145 °C, and the moisture content in the extruder barrel was constant at 25%. The extruder was operated at 100 rpm, and the feeder was set to deliver 21.5 kg h^{1}. The extrudates were allowed to cool to room temperature, reground to pass through a 0.5 mm sieve, and stored at 4 °C.

Chemical Analysis. Moisture (925.10), crude fiber (962.09), ash (942.05), ether extract (920.85), and N content (976.05) of raw and extruded pea samples were determined according to AOAC methods (1992). Crude protein was calculated using the 6.25 conversion factor for Kjeldahl N.

The amino acid composition of acid-digested samples were analyzed by the procedure of Llames and Fontaine (1994) using a Waters HPLC system in combination with the Pico-Tag method. Cysteine and methionine were analyzed after performic acid oxidation and determined as cysteic acid and methionine sulfone, respectively. Tryptophan was hydrolyzed with methanosulfonic acid (Cohen et al., 1987).

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Carbohydrate analyses were conducted by the procedures of Englyst and Cummings (1984). Amounts of free sugars were estimated by HPLC after extraction with boiling 80% ethanol solution. Starch was determined as glucose after enzyme digestion (Aman and Hesselman, 1984).

Mineral levels in samples, digested in nitric acid, perchloric acid, and hydrochloric acid, were evaluated by atomic absorption spectrometry. Phosphorus was determined by means of the ammonium molybdate metavanadate reaction (Roach, 1965).

Phytic acid was extracted in 2.4% HCl (1:20 w/v). The mixture was centrifuged at 17300*g* for 30 min at 15 °C, and the supernatants were collected and purified by anion-exchange chromatography (Dowex 1X4). Phytates were determined spectrophotometrically after reaction with the modified Wade reagent (Frühbeck et al., 1995).

Condensed tannins were extracted with HCl/methanol (1:100 w/v) for 20 min and centrifuged at 5000*g* at 15 °C for 15 min. Tannins were measured using 0.5% acidified vanillin and expressed as milliequivalents of catechin (Broadhurst and Jones, 1978).

Polyphenols were extracted in a sample of 1 g of 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. After centrifugation at 2500*g* for 15 min, polyphenols were determined using the Folin−Denis reagent (Christensen, 1974).

Trypsin inhibitors were determined by the procedure of Armour et al. (1998) using α-*N*-benzoyl-DL-arginine-*p*-nitroanilidehydrochloride (BAPNA) as substrate. One gram of finely ground sample was extracted with 10 mL of 0.15 M phosphate buffer pH 8.1 at 4 °C for 12 h. Extracts were incubated with trypsin solution (0.004% trypsin in 0.25 M glycine HCl buffer) and diluted with pH 8.1 buffer phosphate.

Chymotrypsin inhibitors were analyzed by the method of Sathe and Salunkhe (1981) using benzoyl-L-tyrosine ethyl ester (BTEE) as substrate. Seed meal was extracted (1:10 w/v) in Tris HCl buffer pH 7.6 at 4 °C for 12 h. Sample extracts were incubated with chymotrypsin solution (0.005% chymotrypsin in Tris HCl buffer pH 7.6) and diluted with Tris HCl buffer pH 7.8.

α-Amylase inhibitor activity was assessed by the method of Grant et al. (1994). The meal was extracted (1:5 w/v) with 0.02 M sodium phosphate buffer pH 6.9 containing NaCl (9 g l^{-1}) at 4 °C for 16 h.

Hemagglutination assays were carried out by a serial dilution method using trypsin-treated rabbit erythrocytes (Armour et al., 1998). One unit of hemagglutinating activity (HU) was defined as that contained in the amount of sample in the last dilution which caused 50% agglutination of the blood cells.

In Vitro Protein and Starch Digestibilities. In vitro protein digestibility was determined in pea flour suspensions (1 mg N mL^{-1}) using a multienzyme system consisting of 1.6 mg of trypsin (14 600 U mg^{-1}), 3.1 mg of α-chymotrypsin (48 U mg^{-1}), and 1.3 mg of peptidase (102 U g^{-1}) (Hsu et al., 1977). The in vitro starch digestibility was determined in flour suspensions (50 mg mL^{-1} of 0.2 M phosphate buffer, pH 6.9) using 0.5 mL of pancreatic amylase (1260 U mg^{-1}) suspension (0.4 mg mL^{-1} of 0.2 M phosphate buffer, pH 6.9) according to the method of Singh et al.. (1982).

Studies in Vivo. *Diets.* Isoproteic (110 g kg^{-1}) and isoenergetic (16.7 MJ kg^{-1}) diets in which raw (30.9 g N kg^{-1} DM) or extruded (31.4 g N kg^{-1} DM) peas were the only protein source were formulated as before (Armour et al., 1998). Casein was used as a control protein source. Some diets, including casein diet, were supplemented with individual amino acids to bring the levels up to the target requirements for rats.

Animals. Male Wistar rats (Charles River Laboratories, Rouen, France) were weaned at 19 days of age and given a stock diet (Harlan Teklad, Madison, WI) for 7 days. After this, rats were fed control diets for 4 days to ensure their adaptation to the experimental diets. Animals were housed in metabolic cages fitted with glass separators for urine and feces collection and fed for 15 d with test or control diets. The rats were housed under controlled conditions (22 ± 1 °C, 50 ± 5% RH and 12 h light/dark period), and food intake and body weight were monitored daily. Water was freely available at all times.

Experimental Design. In the first experiment, 40 rats were divided into four groups of 10 animals. Two groups were given free access to raw or extruded pea diet. The remaining groups were given casein (control) and were pair-fed to the daily intake of rats given either raw or extruded pea diet. In the second experiment, thirty rats were divided into three groups. Two groups had free access to raw or extruded pea diets that had been supplemented with amino acids to the target requirements for rats, and the control group was fed casein (control) diet.

Statistical Analysis. The Student's *t*-test was performed on chemical analyses and in vitro studies. One-way ANOVA, followed by Fisher's least significant difference (LSD) was used on in vivo studies to determine the differences among treatments. Differences were considered significant with a *p* value at the 5% level. Computations were performed with the StatView/Apple Macintosh version 4.01 non-FPU (Abacus Concepts, 1992−1993) statistical package.

Table 1. Composition of Raw and Extruded Pea (*Pisum sativum* L. cv. Ballet) Seeds[a]

nutrient (g kg^{-1})	raw peas	extruded peas
crude protein (N × 6.25)	193.1 ± 2.1[a]	196.4 ± 3.0[a]
moisture	106.2 ± 1.4[a]	83.4 ± 1.7[b]
crude fiber	33.1 ± 3.2[a]	36.4 ± 3.0[a]
ash	27.0 ± 1.0[a]	28.1 ± 1.1[a]
ether extract	8.4 ± 0.5[a]	8.5 ± 0.5[a]
carbohydrates[b]	632.2 ± 9.7[a]	647.2 ± 9.3[a]

[a] Results are the mean of five determinations ± SD. Values in the same row with distinct superscripts differ statistically $p < 0.05$. [b] Calculated by difference.

Table 2. Amino Acid Composition of Raw and Extruded Pea (*Pisum sativum* L. cv. Ballet) Seeds[a]

AA (g kg^{-1} protein)	raw peas	extruded peas
arginine	85.6 ± 0.9[a]	87.4 ± 1.2[a]
histidine	22.1 ± 0.2[a]	19.6 ± 0.2[b]
isoleucine	31.3 ± 1.7[a]	34.8 ± 1.8[a]
leucine	83.3 ± 1.2[a]	82.7 ± 0.2[a]
lysine	82.3 ± 1.4[a]	83.4 ± 1.0[a]
methionine	10.2 ± 0.3[a]	6.7 ± 0.4[b]
phenylalanine	52.6 ± 1.2[a]	54.9 ± 1.4[a]
threonine	38.9 ± 1.1[a]	40.8 ± 1.1[a]
tryptophan	9.4 ± 0.5[a]	8.0 ± 0.3[b]
valine	54.7 ± 1.0[a]	53.3 ± 1.2[a]
alanine	49.3 ± 1.4[a]	47.5 ± 1.4[a]
aspartic acid	48.5 ± 2.4[a]	48.8 ± 3.1[a]
cystine	11.4 ± 0.4[a]	4.4 ± 0.5[b]
glutamic acid	122.9 ± 3.1[a]	118.5 ± 2.6[a]
glycine	48.0 ± 1.2[a]	46.8 ± 1.3[a]
proline	49.4 ± 1.3[a]	47.6 ± 1.6[a]
serine	48.7 ± 1.8[a]	51.1 ± 1.6[a]
tyrosine	39.3 ± 0.5[a]	38.3 ± 0.8[a]

[a] Results are the mean of 5 determinations ± SD. Values in the same row with distinct superscripts differ statistically $p < 0.05$.

RESULTS AND DISCUSSION

Chemical Analysis. Extrusion-cooking of peas did not cause any significant change in their nitrogen, crude fiber, ash, ether extract, and carbohydrate contents (Table 1). The water content was, however, lower than in raw flour (Table 1). This was probably due to evaporation of water at the extruder outlet, a result of the high temperatures inside the screw channel.

Most amino acids in pea flour were unaffected by extrusion treatment (Table 2). However, methionine and cystine levels were reduced (34.3%, $p < 0.0001$ and 61.4%, $p < 0.0001$, respectively) (Table 2). Similar

Table 3. Carbohydrate Composition of Raw and Extruded Pea (*Pisum sativum* L. cv. Ballet) Seeds[a]

carbohydrate (g kg^{-1} DM)	raw peas	extruded peas
starch	493.1 ± 7.2[a]	473.3 ± 6.0[b]
total nonstarch polysaccharides	148.9 ± 2.2[a]	143.9 ± 1.3[b]
rhamnose	2.5 ± 0.2[a]	2.2 ± 0.1[a]
fucose	0.6 ± 0.1[a]	0.6 ± 0.1[a]
arabinose	34.5 ± 0.5[a]	32.0 ± 0.7[b]
xylose	12.6 ± 0.5[a]	12.6 ± 0.5[a]
mannose	1.1 ± 0.3[a]	1.2 ± 0.2[a]
galactose	17.7 ± 0.3[a]	17.2 ± 0.3[a]
glucose	59.7 ± 2.1[a]	57.1 ± 1.6[a]
uronic acids	20.5 ± 0.4	21.2 ± 0.4
total free sugars (as hexose)	82.8 ± 2.2[a]	89.5 ± 2.0[b]
glucose	ND[b]	ND
sucrose	24.8 ± 1.6[a]	27.0 ± 1.0[a]
raffinose	9.2 ± 0.3[a]	9.6 ± 0.4[a]
stachyose	23.1 ± 0.7[a]	18.6 ± 1.0[b]
verbascose	14.0 ± 0.7[a]	18.7 ± 0.5[b]

[a] Results are the mean of five determinations. Values in the same row with distinct superscripts differ statistically $p < 0.05$. [b] Not detected.

effects were observed in field beans and soybeans treated by extrusion (Jeunink and Cheftel, 1979; Cheftel, 1986; Prudêncio-Ferreira and Arêas, 1993).

Some amino acids can become unavailable after thermal treatment (Asp, 1990). This is due to the formation of cross-links or to Maillard condensation with reducing carbohydrates. Changes in lysine content are taken as an important indicator of the severity of these processes during thermal treatment (Cheftel, 1986). In the present study, unlike other reports (Cheftel, 1986), no loss of lysine content was evident in extrusion-treated peas (Table 2). This suggests that, under the conditions used, extrusion was likely to have had little adverse effect on availability of amino acids.

Extrusion cooking affected the amounts of sugar extractable from peas (Table 3). The starch and nonstarch polysaccharide contents of the extruded meal were reduced ($p < 0.01$ and $p < 0.05$, respectively). In contrast, total free sugar levels were significantly increased. Sucrose and verbascose levels were higher, but stachyose was lowered. These changes may be the result of a number of processes. The molecular weight of amylopectin is reduced after extrusion at 130 or 180 °C (Colonna et al., 1984). The mechanism involved is unknown but shear may be a major factor (Colonna et al., 1984). Hydrolysis reactions occurring during extrusion can lead to increased levels of total free sugars, as noted in the present study. However, some of the reducing sugars formed may interact with charged protein groups (Maillard reaction) resulting in apparently lowered extractability of these sugars (Asp, 1990). The lowered levels of stachyose in extruded meal may be due to this effect.

Extrusion did not appear to generally affect the mineral composition of peas (Table 4). The exception was the iron content, which was increased. This may be due to contamination of the sample as a result of wear and tear on the extruder.

The levels of most antinutritional factors in peas were reduced following extrusion treatment (Table 5). Trypsin inhibitory and lectin activities were almost completely abolished. Chymotrypsin inhibitoring activity was also lowered but not so extensively as trypsin inhibiting activity. Heat treatments were effective in inactivating lectins and protease inhibitors in soyabean (Armour et al., 1998). They would therefore have been expected to

Table 4. Mineral Composition of Raw and Extruded Pea (*Pisum sativum* L. cv. Ballet) Seeds[a]

mineral (mg kg^{-1}DM)	raw peas	extruded peas
Na	640 ± 25[a]	640 ± 21[a]
Ca	800 ± 53[a]	800 ± 67[a]
Mg	1150 ± 74[a]	1010 ± 81[a]
Zn	26.9 ± 3.2[a]	29.1 ± 2.4[a]
Fe	38.2 ± 3.5[a]	52.5 ± 4.0[b]
Cu	10.5 ± 1.1[a]	9.9 ± 0.6[a]
Mn	9.3 ± 0.3[a]	9.1 ± 0.3[a]
P	4620 ± 110[a]	4450 ± 81[a]

[a] Results are the mean of five determinations. Values in the same row with distinct superscripts differ statistically $p < 0.05$.

Table 5. Antinutritional Factors Content of Raw and Extruded Pea (*Pisum sativum* L. cv. Ballet) Seeds[a]

antinutritional factor	raw peas	extruded peas	% reduction
phytic acid (g kg^{-1} DM)	11.93 ± 0.11[a]	11.23 ± 0.15[b]	5.9
condensed tannins (g equiv cat. kg^{-1} DM)	0.24 ± 0.01[a]	0.02 ± 0.00[b]	91.7
total phenols (g kg^{-1} DM)	0.50 ± 0.01[a]	0.23 ± 0.00[b]	54.0
trypsin inhibitors (UI kg^{-1} DM)	6320 ± 230[a]	340 ± 20[b]	94.6
chymotrypsin inhibitors (UI kg^{-1} DM)	4850 ± 90[a]	1680 ± 70[b]	65.4
α-amylase inhibitors (UI kg^{-1} DM)	ND[b]	ND	
hemagglutinating activity (UH kg^{-1} DM)	6000 ± 0[a]	100 ± 0[b]	98.3

[a] Results are the mean of 10 determinations ± SD. [b] Not detected.

fully inactivate pea inhibitors since they are homologous to soyabean Bowman-Birk [trypsin/chymotrypsin] inhibitors (Domoney et al., 1993; Ferrasson et al., 1997). However, six different forms of pea inhibitor have been isolated, each of which has distinctive reaction characteristics (Domoney et al., 1993; Ferrasson et al., 1995; Welham et al., 1998). Some of these forms may be more resistant to thermal treatment than others. Alternatively, the residual chymotrypsin inhibiting activity may be due to nonproteinaceous components of the seeds, such as polyphenols.

Polyphenols in peas were lowered but not eliminated by extrusion cooking (Table 5). In particular, the condensed tannins were greatly reduced. The high temperature of extrusion may alter their molecular structure and either reduce their chemical reactivity or decrease their extractability due to a certain degree of polymerization (Melcion and van der Poel, 1993).

Extrusion cooking caused a minor reduction in phytic acid content of peas (Table 5). Analysis performed using high-performance liquid chromatography (HPLC) showed that extrusion treatment caused some inositol hexaphosphate to break to penta- and tetraphosphates (data not shown).

In Vitro Studies. The digestibility in vitro of pea proteins was significantly elevated ($p < 0.0001$) from 839 ± 1 in RP to 874 ± 1 g kg^{-1} protein in EP. This could be attributable to the reduction in antinutritional factors, particularly protease inhibitors that interfere with the action of proteolytic enzymes and tannins or phytate that complex with proteins and increase their resistance to proteolytic degradation (Trugo and Baer, 1998). However, despite this absence of significant amounts of antinutritional factors, pea proteins were

Table 6. Growth and Nutritional Performance of Rats Fed for 15 Days with Diets Containing Raw or Extruded Peas

	experiment 1[a]				experiment 2		
diet[b]	C	RP	C	EP	C	SRP	SEP
total food intake (g)	158.6 ± 15.1[a]	158.9 ± 1.8[a]	168.4 ± 19.6[a]	170.3 ± 0.8[a]	270.3 ± 17.6[b]	261.5 ± 30.3[b]	262.8 ± 18.8[b]
initial body weight (g)	96.0 ± 5.6[a]	92.6 ± 6.5[a]	95.7 ± 1.6[a]	93.7 ± 3.8[a]	91.1 ± 7.7[a]	87.0 ± 3.5[a]	88.0 ± 4.3[a]
final body weight (g)	147.0 ± 5.0[a]	129.1 ± 7.1[b]	153.6 ± 4.3[a]	131.8 ± 7.4[b]	202.9 ± 13.9[c]	172.2 ± 13.7[d]	187.7 ± 12.2[e]
FTI[c] (g/g)	3.1 ± 0.3[a]	4.5 ± 0.4[b]	3.0 ± 0.2[a]	4.5 ± 0.4[b]	2.4 ± 0.1[c]	3.0 ± 0.3[a]	2.7 ± 0.3[d]
PER[d] (g/g)	2.9 ± 0.2[a]	2.1 ± 0.3[b]	3.1 ± 0.2[a]	2.1 ± 0.2[b]	3.8 ± 0.1[c]	3.0 ± 0.3[a]	3.4 ± 0.3[d]

[a] For details, see Materials and Methods section. Values are means ± SD for 10 animals per group. Values in the same row with distinct superscripts differ statistically $p < 0.05$. [b] C, control (casein supplemented with Cys); RP, raw peas; RP, raw peas; EP, extruded peas; SRP, raw peas supplemented with Met, Ile, His, Trp and Thr; SEP, extruded peas supplemented with Met, Ile, His, Trp and Val. [c] FTI, food transformation index = food intake/body weight gain. [d] PER, protein efficiency ratio = body weight gain/protein intake.

still far less digestible (874 ± 1 g kg^{-1} protein) than a high quality protein such as casein (932 ± 2 g kg^{-1} protein). This may suggest that pea reserve proteins are inherently poorly digestible even after thermal treatment. Alternatively, thermal treatment may modify the pea proteins in a manner that renders them less susceptible to proteolytic degradation.

Extrusion treatment significantly increased the digestibility in vitro of pea starch. Measured as liberated maltose, the digestibility increased from 274 ± 1 g maltose kg^{-1} in raw peas to 323 ± 4 g maltose kg^{-1} in extruded peas. The data are consistent with the findings of Asp and Björck (1984). This improved digestibility was not due to elimination of α-amylase inhibitors since raw peas contain little (Grant et al., 1995) or no detectable α-amylase inhibitory activity (Table 5). It was likely to have been a direct effect of the extrusion treatment on starch granules. Previous studies have shown that the effects of extrusion on starch digestibility depend on the severity of the extrusion process (Melcion and van der Poel, 1993). High-temperature (170 °C), high-shear extrusion of starch produced highly digestible carbohydrate while low temperature extrusion and drum drying resulted in a much poorer product (Asp and Björck, 1984). The extrusion treatment in the present study involved relatively high temperatures (145 °C) coupled with high shear. This may account for the improvement in starch digestibility in vitro.

In Vivo Studies. Rats fed diets containing raw peas grew more slowly than pair-fed controls (Table 6). As a result, the food transformation index (FTI) obtained with this diet was high and the protein efficiency ratio (PER) was low. Extrusion treatment of peas did not appear to improve the nutritional quality of the diet. Thus, food intake, growth, FTI, and PER with extruded pea diets were similar to those obtained with raw peas. This apparent failure of extrusion to increase the nutritional value of the pea diet was also noted with faba bean (Fernández et al., 1996; Vidal-Valverde et al., 1997) and was probably the result of the overriding effects of deficiencies in essential amino acids in the diet. The sulfur amino acids were present at around 48% of requirement in raw pea diet and 25% of requirement in extruded pea diet (Table 2).

Supplementation of raw and extruded pea diets with amino acids up to requirements for rats greatly improved food intake, growth, FTI, and PER (Table 6). Furthermore, there were significant differences between the two pea diets. With supplemented extruded pea diets, the weight gain and PER were much higher than those obtained with supplemented raw pea diets and the FTI was lower. Thus, the nutritional quality of supplemented peas was greatly improved as a result of extrusion treatment. This was consistent with the increased in protein and starch digestibility and reduced levels of antinutritional factors (Table 5) observed studies in vitro with the product. However, although peas were far more effectively utilized following extrusion treatment, their nutritional quality remained inferior to that of casein-based (control) diet. Again, this was in agreement with the finding in vitro that extruded pea proteins were less digestible than casein.

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