INFLUENCE OF EXTRUSION ON THE NUTRITIVE VALUE OF PEAS: *IN VITRO* ASSAY

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ABSTRAK

Pengaruh ekstrusi terhadap nilai nutrisi pea: analisa in vitro. *Sampel Pea dianalisis baik dalam bentuk yang tidak diekstrusi maupun yang diekstrusi dengan menggunakan kombinasi dua kondisi kadar air (19 and 22%) dan tiga perbedaan suhu (120, 140 dan 170°C). Hasil penelitian menunjukkan bahwa ekstrusi secara signifikan (P<0,05 – 0,0001) mempengaruhi kandungan protein kasar, polisakarida bukan pati, pati dan trypsin inhibitor (TI), tetapi tidak mempengaruhi (P>0,05) kandungan lemak dan abu. Secara umum, kandungan polisakarida bukan pati yang larut dan TI dari hampir semua sampel pea yang diekstrusi lebih tinggi (P<0,05) daripada sample pea yang tidak diekstrusi, tetapi kandungan total polisakarida bukan pati dan polisakarida bukan pati yang tidak larut mengalami penurunan (P<0,05) dengan ekstrusi. Interaksi antara kandungan air x suhu ditemukan signifikan (P<0,05 – 0,001) pada semua parameter kecuali pada lemak, abu dan pati. Namun demikian untuk protein kasar, tidak adanya pengaruh (P>0,05) dari suhu barrel pada kandungan kadar air pakan yang rendah (19%), tetapi pada kandungan kadar air yang tinggi (22%), kandungan protein kasar dari pea yang diekstrusi meningkat (P<0,05) seiring dengan meningkatnya suhu barrel. Ekstrusi meningkatkan (P<0,05) daya cerna pati secara in vitro dari pea, tetapi menurunkan (P<0,05) daya cerna protein in vitro. Kesimpulannya, ekstrusi merubah secara signifikan kandungan kimia kacang pea, meningkatkan daya cerna pati, tetapi menurunkan daya cerna protein. Penelitian lanjutan diperlukan untuk mengevaluasi pengaruh ekstrusi terhadap ketersediaan nutrisi dari pea.*

Keyword : ekstrusi,suhu, kadar air, in vitro, nutrisi, pea.

INTRODUCTION

Extrusion cooking is a process where the feed is subjected to mixing, shearing, and heating under high pressure before the extrudate is forced through a die (Sørensen *et al.*, 2002). During this process, the feed may undergo reactions that could be beneficial, if nutrient availability is improved or detrimental if nutrients are destroyed or altered to become resistant to digestion.

Extrusion cooking may influence the nature of feed components by changing physical (e.g. particle size), chemical (e.g. starch gelatinization, inactivation of anti nutrients) and nutritional (e.g. nutrient digestibility) properties (Alonso *et al.*, 2000b; El-Hady and Habiba, 2003; Diaz *et al.*, 2006). Camire (2000) reported that five general physicochemical changes can occur during extrusion cooking: binding, cleavage, loss of native conformation, recombination of fragments and thermal degradation. In addition, the composition of feed materials could be altered by physical losses such as leakage of fat and, evaporation of water and volatile compounds at the die.

The degree of change in feed constituents depends on a number of factors such as the type of ingredient or diet, particle size, type of extruder and the extruder conditions (e.g. moisture content, screw speed, barrel temperature, die diameter, feed rate, screw compression ratio, residence time, torque and pressure, energy input and pH) and type of reactants present, such as water, lipids, carbohydrate and proteins (Björk and Asp, 1983; Ilo *et al.*, 1996; Grela *et al.*, 2001; Anguita *et al.*, 2006).

Appropriate processing temperature is critical for the elimination of heat-labile anti-nutritional factors (ANFs) found in legume seeds. In full-fat soybeans, Björk and Asp (1983) reported that the trypsin inhibitor (TI) activity was reduced with increasing extrusion temperature and moisture content. At constant temperature, inactivation increased with the residence time and moisture content. In contrast, some studies have shown that TI activity and some anti-nutrients such as tannins in s peas and lupins were not inactivated, but even increased, after extrusion (Alonso *et al.*, 2001; Masoero *et al.*, 2005; Prandini *et al.*, 2005).

In terms of amino acids, an increase in extruder temperature, screw compression ratio and screw speed has been reported to increase lysine degradation, while an increase in moisture content and die diameter had the opposite effect (Björk and Asp, 1983). Over-processing will also lower amino acid digestibility as amino acids may be destroyed or become unavailable due to the formation of indigestible complexes between reducing sugars and free amino groups in proteins.

On the other hand, extrusion has also been shown to have positive effects on the digestibility of protein *in vitro* (Alonso *et al.*, 2000b; El-Hady and Habiba, 2002), fat (Dänicke *et al.*, 1998; Lichovnikova *et al.*, 2004), amino acids (Lichovnikova *et al.*, 2004) and starch (Alonso *et al.*, 2000b; Diaz *et al.*, 2006) of grain legumes. The enhancement in nutrient digestibility after extrusion was probably due to the inactivation of enzymes and anti-nutritional factors, denaturation of native protein and gelatinisation of starch (Alonso *et al.*, 1998; El-Hady and Habiba, 2003; Sheriff and Sajeev, 2005). In addition, extrusion inactivates or kills the microbes, thus rendering the feed material sterile and stable. The objectives of this study were to examine the effects of extrusion cooking on the chemical composition, nutrient digestibility and apparent metabolisable energy of peas.

MATERIALS AND METHOD

Processing: Round seeded peas, purchased from a commercial supplier, were ground in a hammer mill to pass a 3 mm sieve and extruded in a twin-screw co-rotating self wiping extruder Clextral BC 21 (Firminy Cedex, France) with length/diameter ratio of 25, screw speed up to 600 rpm and outer screw diameter of 25 mm (Figure 1). The screw configuration from feed section to die consisted of three sections with forward elements. The first section had 4 elements (each 50mm length with 3 screw flights and 13 mm pitch); the second zone consisted 5 elements (each 50mm in length having 4 screw flights and 10 mm pitch); and the third zone had 5 elements (each 50mm in length with 6 screw flights and 7 mm pitch) The total length of the screw was 700 mm with 14 elements in three zones. The extruder was equipped with a bulk solids metering feeder (KTRON T20, Switzerland). A round die (2.5 mm diameter), equipped with a cutting device set at 130rpm, was used.

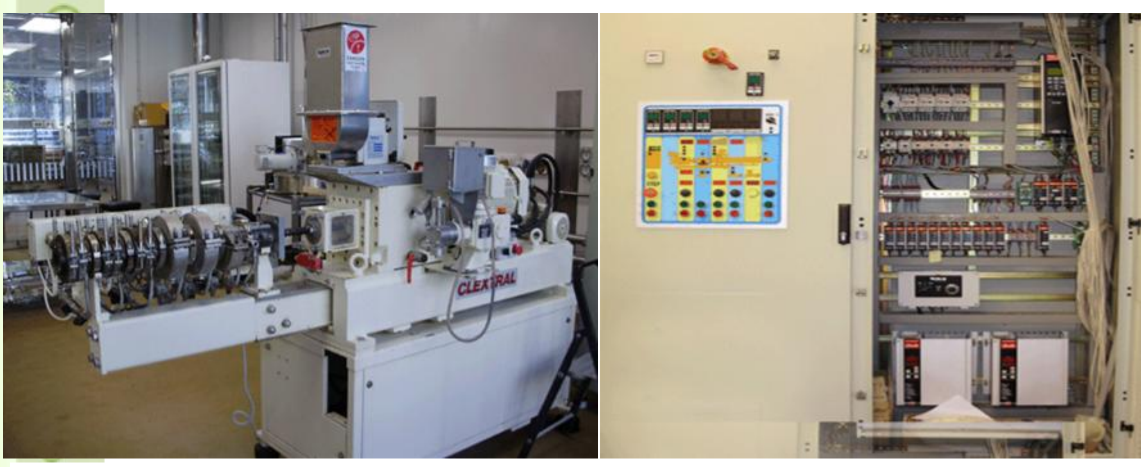

Figure 1. Extruder Clextral BC 21

Extrusion of peas was performed at three temperatures (120, 140 and 170°C) and two moisture levels (19 and 22%) (Figure 2). The desired moisture levels were obtained by adding water prior to the extruder section by means of a pump. The water feed rat for obtaining the final moisture content of 19% was 0.50 kg/h, while 0.75 kg/h was used to achieve 22% final moisture content. The optimum temperatures of the seven extruder sections from the feeder end were 50, 60, 70, 80, 100, 100 and 140°C. The extruded materials were then allowed to cool to room temperature.

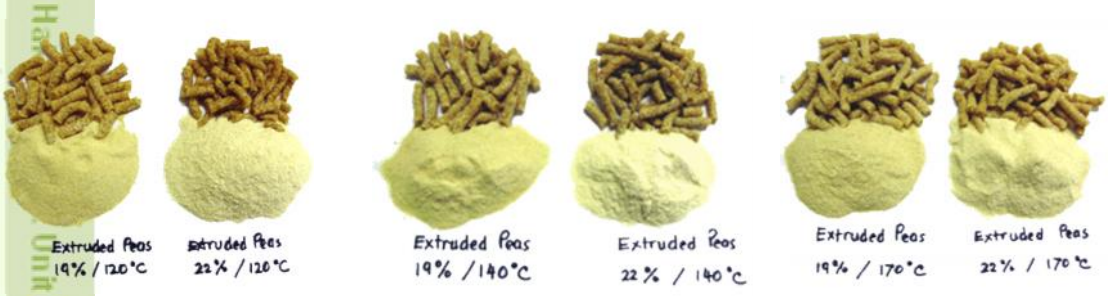

Figure 2. Pea extrudates

Experimental design: Seven treatments consisting of raw-untreated peas and six extruded pea samples were assayed. Each treatment was replicated three times. The extruded materials were ground in a hammer mill to pass through a 0.5 mm sieve, and subjected to *in vitro* protein (Monro, J., Crop and Food Research Inc, New Zealand) and starch digestibility assays (Mishra *et al.*, 2008).

Proximate and fibre composition: The dry matter content of ingredients, diets and excreta was determined in a convection oven at 105°C (AOAC 930.15, AOAC 925.10 AOAC, 2002). Ash was determined as the organic residue present after incineration at 550°C until loss of organic matter (Method 923.03). Ether extract was determined using the Mojonnier method (AOAC 989.05, 2002). Nitrogen content was determined by the Dumas method (Sweeney, 1989) using a CNS-

2000 carbon, nitrogen and sulphur analyser (AOAC 968.06-LECO Corporation, St Joseph, MI, USA). A conversion factor of 6.25 was used to convert N into the crude protein content.

Starch analysis: Starch content was measured using an assay kit (Megazyme, Boronia, Victoria) based on the use of thermostable α-amylase and amyloglucosidase (McCleary *et al.*, 1997).

NSP analysis: Total, soluble and insoluble NSP were analysed using an assay kit (Englyst Fiberzym Kit GLC, Englyst Carbohydrate Services Limited, Cambridge, UK) based on the procedures described by Englyst *et al.* (1994).

Trypsin inhibitor: The procedure to determine trypsin inhibitor was that of Kakade *et al.* (1974) as modified by Vouldebouze *et al.* (1980).

***In vitro* starch digestibility (IVSD):** *In vitro* starch digestibility was determined using the modified method of Mishra *et al.* (2008).

***In vitro* protein digestibility (IVPD):** *In vitro* protein digestibility was determined using the modified method of DR. J. Monro from Crop and Food Research.

Calculations: *In vitro* protein and starch digestibilities were calculated using the following formula:

$$Nutrient\ digestibility\ coefficient = \frac{g\ nutrient\ sample - g\ nutrient\ residue}{g\ nutrient\ sample}$$

Statistical Analysis: The data from *in vitro* study were analysed by both one-way and two-way analysis of variance (ANOVA) using the General Linear Model procedure of SAS (1997). Differences were considered to be significant at P<0.05 and significant differences between means were separated by the Fisher's Least Significant Difference test.

RESULTS AND DISCUSSION

Chemical composition: Chemical composition of peas was significantly (P<0.05) affected by extrusion, except for crude fat and ash (Table 1). Within extruded samples, the main effects (moisture and temperature conditions) and the interaction effect were significant (P<0.05 to P<0.0001) for most parameters, the exceptions being crude fat, ash, and starch contents.

The lack of effect of extrusion on fat and ash contents in peas is in agreement with the findings of Alonso *et al.* (2001). In contrast, Diaz *et al.* (2006) reported that fat and ash contents of peas were increased by 61 and 4%, respectively following extrusion. The observed discrepancy may be due to the differences in extruder type used. In the present study, twin-screw extruder type was used, while in the study by Diaz *et al.* (2006), single-screw extruder type was used. As reported by Björk and Asp (1983), the type of extruder is an important factor affecting the degree of modification in nutritional properties. Extrusion conditions are also important, but it was difficult to compare the effects of this aspect, because Diaz *et al.* (2006) did not clearly describe the conditions used in their study.

The moisture contents of peas extruded with 19%/120^{o}C and 22%/120^{o}C operating conditions were higher (P<0.05) than that of raw peas, while the other extruded peas (19%/140^{o}C; 22%/140^{o}C; 19%/170^{o}C and 22%/170^{o}C) had lower (P<0.05) moisture contents compared to raw peas.

The crude protein contents of extruded pea samples were similar (P>0.05) to that of raw pea meal. A significant decrease (P<0.05) in crude protein content after extrusion was found only in peas extruded at 22%/120^{o}C operating condition. These findings were in general agreement with Alonso *et al.* (2000a). The reason for the decrease in crude protein content in the 22%/120^{o}C treatment was unclear.

The main effects of feed moisture and barrel temperature and the interaction between feed moisture and barrel temperature on crude protein content were found to be significant (P<0.05 to 0.01). The crude protein content was increased (P<0.05) by increasing the barrel temperature in the high moisture level (22%), whereas in the low feed moisture (19%), the crude protein content of peas extruded at 120 and 170^{o}C temperature did not differ (P>0.05) each other.

The effects of extrusion treatments on NSP components were inconsistent, but the general effect of extrusion was to increase soluble NSP and lower insoluble NSP. In general, the total NSP content was not influenced (P>0.05) by the extrusion. Within extruded samples, the main effects (feed moisture and barrel temperature) and the interaction on soluble NSP were significant (P<0.05). The soluble NSP content was increased (P<0.05) by increasing the barrel temperature in the high moisture level (22%); however, the increase was not significant (P>0.05) between peas extruded at 140^{o} and 170^{o}C temperature. In the low feed moisture (19%), the soluble NSP content of peas extruded at 140 and 170^{o}C temperature did not differ (P>0.05) each other.

The increase in soluble NSP with extrusion was in agreement with previous studies (Björk *et al.*, 1983; Østergard *et al.*, 1989; Vasanthan *et al.*, 2002) and this may be attributed to the conversion of part of insoluble NSP to soluble NSP. Lue *et al.* (1991) explained that the changes in dietary fibre profile of grain flours after extrusion occur via the formation of starch resistant to enzymatic attack and macromolecular degradation of fibre increases its solubility.

There was an interaction (P<0.01) between feed moisture x barrel temperature on insoluble and total NSP. The insoluble NSP was decreased by increasing the barrel temperature in the high moisture level (22%), whereas in the low feed moisture (19%), the insoluble NSP of peas extruded at 140 and 170^{o}C temperatures did not differ (P>0.05).

Extrusion cooking had no effect (P>0.05) on the starch content. This finding was in disagreement with the previous studies (Prandini *et al.*, 2005; Diaz *et al.*, 2006) which showed a decrease in starch content of peas extruded with single-screw extruder. This variability was probably due to the difference in methodology, especially the type of extruder used. In the present study, the twin-screw extruder was used, whereas single-screw extruder was used in previous studies. Perez-Navarrete *et al.* (2006) reported that the decrease of starch content of extruded products was probably due to the formation of newly indigestible starch, which makes it difficult to be extracted by enzymes.

Trypsin inhibitor activity was influenced (P<0.05) by extrusion cooking. Contrary to the expectations, the TI activity was increased (P<0.05) by most extrusion treatments. A decrease (P<0.05) of TI activity was observed only in peas extruded at 19%/120°C. The feed moisture x barrel temperature interaction was found to be significant (P<0.01) for TI activity. The TI activity of peas extruded at 120 and 170°C in both low (19%) and high feed moisture (22%) was comparable (P>0.05), but the observed values were higher than that of 140°C.

The improvement of trypsin inhibitor activity of peas after extrusion (except at 22%/140°C) was an unexpected result. These findings were in contrast with those reported by the previous workers (Van der Poel, 1992; Kearns, 1994; O'Doherty and Keady, 2001; Diaz *et al.*, 2006). In the study by Van der Poel (1992), TIA content of pea cultivars (round- and wrinkle-seeded peas) were reduced by extrusion at different processing temperatures (106 to 140°C) and moisture contents (14 to 33%). However, the degree of inactivation was dependent on the processing condition and the cultivar used. The TIA inactivation of round-seeded peas was almost complete under the different processing conditions investigated, whereas the TIA in wrinkle-seeded pea was inactivated only at higher temperature.

The increase of TI activity determined in most extruded samples in our study may be due to the presence of trypsin-like protease activity (Domoney and Welham 1992; Domoney *et al.* 1993; James *et al.*, 2005). It may be that since trypsin cleaves N-α-benzyl-DL-arginine-p-nitroanilide (BAPNA) on the carbonyl side of arginine to render a yellow solution (free p-nitro aniline), the trypsin-like activity observed could be due to a compound that also cleaves on the carbonyl side of the arginine residue (James *et al.*, 2005). The compound responsible for the trypsin-like activity may not be degraded by heat, unlike trypsin inhibitor activity, and thus may appear as an augment in trypsin inhibitor activity in heat-treated or extruded samples.

***In vitro* protein and starch digestibility:** Extrusion resulted in significant (P<0.01) reductions (2.5 to 6.5%) in the *in vitro* protein digestibility (IVPD) of peas. The highest reduction was in treatment 22%/120°C. Feed moisture had no effect (P>0.05) in IVPD, but there was an interaction (P<0.05) between moisture content and barrel temperature on IVPD. The IVPD of peas extruded at 140 and 170°C in high feed moisture (22%) did not differ (P>0.05) each other, but these values were higher than that determined for peas extruded at 120°C. No differences (P>0.05) were found between the IVPD of peas extruded at 120 and 140°C, and between the IVPD of peas extruded at 120 and 170°C in the low feed moisture (19%).

The reduction in *in vitro* protein digestibility values obtained in the present study was in contrast with the evidence shown by Alonso *et al.* (2000b). The variability was probably due to the differences in cultivar and methodology. Several published data have shown that the lack of improvement in protein digestibility of pea protein after heat treatment could be due to protein aggregation (Alonso *et al.*, 2000b; Wang, 2000; Meng *et al.*, 2002; Carbonaro *et al.*, 2005) and Maillard reaction (non-enzymatic browning-thermal cross-linking) (Vasanthan *et al.*, 2002).

Nielsen *et al.* (1988) showed that complete degradation of heated legume proteins (phaseoline, vicilin, glycinin, and beta-conglycinin) did not occur even after 60 minutes of incubation. Unlike phaseolin, the other legume proteins (vicilin, glycinin, and beta-conglycinin) were found to be less completely digested by a variety of proteases in the denatured state than in the native state. Clemente *et al.* (2000) found that the low digestibility of globulins has been related to their compact structure and intracellular location that hinder the susceptibility to proteolysis.

Extrusion cooking, however, improved (P< 0.0001) *in vitro* starch digestibility (IVSD) (Table 1). The magnitude of improvement ranged from 56.9% was in treatment 22%/175°C to 59.5% for treatment 22%/120°C. The main effects of feed moisture and barrel temperature and the interaction between feed moisture and barrel temperature on IVSD were significant (P<0.05 to 0.01). On the high moisture level (22%), there was a downward tendency (P<0.05) of IVSD as the barrel temperature increased, but the IVSD of peas extruded at 120 and 140°C was found to be similar (P>0.05). On the other hand, on the low moisture level (19%), the IVSD of peas at 120, 140 and 170°C did not differ (P>0.05) each other.

The improvement of IVSD of peas after extrusion was in consistent with the published results by Alonso *et al.* (2000a). The improvement of starch digestibility both *in vitro* in peas after extrusion was probably due to gelatinisation which increases the accessibility of starch to endogenous enzymes.

Native granule starch, which consists predominantly of α-glucan in the form of amylose and amylopectin, is hydrolysed slowly by α-amylase and amyloglucosidase compared with gelatinised starch in processed foods. When native starches are heated in excess water, the crystalline structure is disrupted and water molecules form hydrogen bonds to the exposed hydroxyl groups of amylose and amylopectin (Ratnayake *et al.*, 2002; Tester *et al.*, 2004). This causes an increase in granule swelling and solubility. Granule structure is completely lost and a thin paste or gel is formed, which makes the starch completely digestible by starch hydrolysing enzymes

Table 1. The effect of extrusion treatments on the chemical composition (g/kg DM) and *in vitro* nutrient digestibility (%) of peas [1]

Extrusion parameter		Total moisture	Crude protein	Crude fat	Ash	Non-starch polysaccharides			Starch	Trypsin inhibitor (TIU/mg DM)	*In vitro* digestibility coefficient	
Feed moisture (%)	Barrel temperature (°C)					Soluble	Insoluble	Total			Protein	Starch
Raw[2]	Raw	118^{c}	230^{abc}	25	31	23^{b}	177^{a}	200^{ab}	465	0.23^{c}	0.828^{a}	0.547^{d}
19[2]	120	129^{a}	234^{a}	27	32	18^{c}	168^{c}	186^{e}	462	0.29^{b}	0.796^{bc}	0.860^{b}
19[2]	140	99^{g}	226^{c}	25	31	25^{b}	172^{c}	197^{c}	460	0.25^{c}	0.807^{b}	0.862^{ab}
19[2]	170	112^{d}	232^{ab}	26	33	28^{a}	174^{b}	202^{a}	466	0.28^{b}	0.790^{cd}	0.858^{b}
22[2]	120	127^{b}	214^{d}	26	32	24^{b}	174^{b}	198^{bc}	462	0.38^{a}	0.778^{d}	0.872^{a}
22[2]	140	102^{f}	229^{bc}	26	31	28^{a}	166^{d}	194^{d}	461	0.19^{d}	0.794^{bc}	0.864^{ab}
22[2]	170	108^{e}	233^{ab}	26	32	29^{a}	156^{d}	185^{e}	463	0.24^{c}	0.802^{bc}	0.845^{c}
Pooled SEM		0.42	1.28	0.44	0.55	0.96	0.87	0.89	1.73	0.005	0.005	0.003
ANOVA[3]												
Feed moisture (M)		***	***	NS	NS	***	**	**	NS	***	NS	**
Barrel temperature (T)		***	**	NS	NS	*	***	***	NS	***	*	**
M x T		***	***	NS	NS	*	***	***	NS	***	*	*

[a,b,c] Means in a column with different superscripts differ (P<0.05).
*Significant at P<0.05; ** Significant at P<0.01; ***Significant at P<0.001.
[1]Each value represents the mean of three determinations.
[2]Analysed as one-way ANOVA
[3]Analysed as a two-way ANOVA

CONCLUSIONS

In conclusion, extrusion cooking markedly changed the chemical composition of grain legumes. Extrusion increased (P<0.05) the *in vitro* of peas, but decreased (P<0.05) the *in vitro* protein digestibility. The advanced experiment is needed to further evaluate the effect of extrusion on the nutrient availability of peas.

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