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Influence of enzymatic treatment on the nutritional and functional properties of pea flour

Mª Jesús Periago,[a]* Mª Luisa Vidal,[a] Gaspar Ros,[a] Francisco Rincón,[b] Carmen Martínez,[a] Ginés López,[a] Joaquin Rodrigo[a] & Isabel Martínez[a]

[a]*Department of Food Science and Nutrition, Veterinary Faculty, Murcia University, 30071 Murcia, Spain*
[b]*Department of Food Science and Technology, Faculty of Veterinary, Córdoba University, 14014 Córdoba, Spain*

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The effect of enzymatic treatment on the nutritional value and functional properties of pea flour was investigated. Pea flour was hydrolyzed with acid protease from *Aspergillus saitoi*, to give two different hydrolyzed pea flours. This enzymatic treatment led to a significant ($p < 0.05$) decrease in crude and true protein and to an increase of free amino acids and non-protein nitrogen. The nutritional value decreased, but an increase in the avilability of protein was expected as result of lower trypsin inhibitor activity and phytic acid content in hydrolyzed pea flours. The amino acid profile of unhydrolyzed pea flour was slightly modified after enzymatic hydrolysis, increasing (significantly) the isoleucine, leucine, lysine, cystine, phenylalanine, threonine, alanine, arginine and aspartic acid contents as a result of the added enzyme. In addition, enzymatic treatment released hydrophobic amino acids, which significantly improved the protein solubility at acid pH, the oil absorption capacity and the emulsification capacity of pea flours. Protein solubility, foaming capacity, foam stability, water absorption capacity, gelation capacity and green colour decreased. It was thus confirmed that treatment with acid protease improves some functional properties of pea flour, but the effect on nutritional properties was unclear.

INTRODUCTION

Proteins are an essential food component because they are the source of amino acids, needed for growth and maintenance, and provide functional properties to foods (Giese, 1994). Commercially available protein foods are obtained from a range of animal and plant sources and are used as functional ingredients (Amiot and Brisson, 1985). Pea flour and pea protein isolates are examples of protein foods. Pea protein isolates are functional ingredients in terms of water and fat binding, emulsification, and foaming and gelling characteristics (Giese, 1994). They have been used to formulate non-dairy frozen desserts (Chan *et al.*, 1992) and to replace the albumen in sponge cakes (Giese, 1994).

Over the last twenty years, the use of enzymes in the food processing industry has expanded rapidly (Faergeman, 1994). For special foods, such as those destined for children, old people or athletes, protein food has been hydrolyzed (Gottschick, 1994). In general, food proteins are hydrolyzed for many reasons, which range from the improvement of nutritional and functional properties, texture characteristics and for removal of odour, flavour, and toxic or antinutritive components (Lahl and Grindstaff, 1989). The most commonly used proteins in hydrolysis treatments are casein, whey and soya proteins (Lahl and Braun, 1994), but other protein sources, such as legumes, have also been used successfully (Amiot and Brisson, 1985).

Pea flour, obtained from milled seeds, is a good source of protein (around 30% of total composition) (Periago *et al.*, 1996*a*). It also has high levels of non-starch polyssacharides (or dietary fibre) and resistant starch (Periago *et al.*, 1994, 1996*b*) and a high iron content (Periago *et al.*, 1996*a*). However, as in the case of other seed legumes, antinutritive factors such as phytic acid and trypsin inhibitor as well as their colour and flavour, can limit the use of pea flour as an ingredient in bakery products (Nielsen *et al.*, 1980; Repetsky and Klein, 1982), meat products and snack feedstuffs (Owusu-Ansah and McCurdy, 1991). For these reasons, grain legumes were treated enzymatically to improve the nutritional value of the protein (López-Hernández *et al.*, 1977), to remove their beany flavour (Fujimaki *et al.*,

*To whom correspondence should be addressed. Fax: 0034 68 364147; e-mail: mjperi@fcu.um.es

1968) and to reduce the content of antinutritive factors such as polyphenols (Feldman and Vinnikova, 1973), phytic acid (Morehouse and Malzahn, 1976; Li *et al.*, 1989) and trypsin inhibitor (Li *et al.*, 1989).

The aims of the present study were to study the chemical and nutritional properties of pea flour protein after enzymatic treatment, to evaluate the effect of enzymatic hydrolysis on the functional properties of pea flour and to explore the possible uses of the hydrolysed flour to fortify some foodstuffs.

MATERIALS AND METHODS

Samples

Wrinkle pea seeds (*Pisum sativum*, L.) (cultivar *Warindo* with a seed diameter from 8.3 to 8.8 mm) were selected for this study. They were harvested mechanically and the pods removed by a shelling machine. The peas were washed and the whole seeds were frozen and dried in a Virtis Freezer-drier model 10234 (Gardiner, NY, USA) for 48 h. The dry pea seeds were milled in a Moulinex Coffee-grinder (Aleçon, France) with a stainless-steel blade and passed through a US standard 40 mesh sieve.

Enzymatic hydrolysis

Enzymatic treatment of the pea flour protein involved acid protease from *Aspergillus saitoi*, commercially called 'Molsin' (P-2143, Sigma Chemical, St Louis, MO, USA). Trial experiments were performed to determine the best time, temperature and ratio of enzyme to substrate using: temperatures of 40, 60 and 90°C, times of 90, 120 and 180 min, and enzyme to substrate ratios of 0.27, 0.80, 1.62, 2.70, 5 and 10%. Enzyme activity was measured as the amount of solubilized protein in a 3.3% trichloroacetic acid solution and as the free amino acids released after hydrolysis, both quantified spectrophotometrically using Lowry's technique at 710 nm (Lowry *et al.*, 1951) and the ninhidrin method at 580 nm (Awolumate, 1983), respectively. As a result of these trials the following parameters were selected as the best treatment conditions: temperature 40°C, time 90 min and an enzyme/substrate ratio of 1:10. These conditions led to maximum hydrolysis of the protein in pea flour. Prior to enzymatic treatment, a suspension of pea flour was prepared by adding 100 ml of distilled water to 4 g of flour. The suspensions were acidified to pH 2.8 with 3 M HCl solution, and placed in a water bath and the enzyme was added. The enzymatic hydrolysis was conducted at 40°C for 90 min. The reaction was stopped by increasing the pH to 6.0 with 2 N NaOH solution, and the enzyme was inactivated by placing the samples in a boiling water bath for 15 min. The resulting hydrolyzed solution was treated in two differents ways:

Treatment 1

The supension of pea flour was centrifuged at 3500 rpm for 10 min. The superntant was discarded and the pellet was freeze-dried to obtain the hydrolyzed pea flour.

Treatment 2

The suspension of pea flour was directly frozen and then freeze-dried to obtain the hydrolyzed flour.

Chemical analysis

Total nitrogen and crude protein (N×6.25) were determined according to the micro-Kjedahl method (AOAC, 1990). True nitrogen and true protein was analysed following the TCA precipitation method described by Awolumate (1983). Non-protein nitrogen was calculated as the difference between total and true nitrogen contents as recommended by Periago *et al.* (1996*c*). Free amino acids were determined with the ninhydrin reagent (N-1632, Sigma) compared with an amino acid standard (A-9656, Sigma) and measured at 580 nm using a double beam molecular spectrophotometer Hitachi model U-2000 (Hitachi Ltd, Tokyo, Japan).

In vitro protein digestibility

In vitro protein digestibility was determined by the multienzymatic technique (Satterlee *et al.*, 1982) and was calculated from the change in pH of a sample digested within a 20 min period with a mixture of the following enzymes: porcine pancreatic trypsin (Type IX, T-0134, Sigma), bovine pancreatic α-chymotrypsin (Type ×II, C-4129, Sigma), pepsin from porcine stomach mucose (Grade I, P-6887, Sigma) and bacterial protease (Pronase E, P-5147, Sigma). Sodium caseinate (Sigma) was used as reference material.

Amino acid profiles

In order to prepare the sample, the pea flour protein was digested by acid hydrolysis as described by Satterlee *et al.* (1982). The amino acid composition was determined with an amino acid analyser LKB Alpha Plus (Parmacia LKB Ciochrom Ltd, Cambridge, England), comparing the chromatogram of the samples with a standard solution (Part. No. 40 00 9037, Pharmacia LKB Biochrom Ltd). The tryptophan content was analysed by a colorimetric technique (Sastry and Tummuru, 1985) after alkali hydrolysis of the pea flour protein with 5 M NaOH.

Trypsin inhibitor activity assay

Trypsin inhibitor activity was determined using the method of Kakade *et al.* (1974), modified by della Gatta *et al.* (1988). This procedure measures the inhibition by aliquots of pea flour extract, of the enzyme activity of bovine trypsin (T-8003, Sigma) on the synthetic

substrate DL-benzoyl arginine *p*-nitroanilide (DL-BAPNA, B-4875, Sigma), expressing the results as trypsin inhibitor activity units (TIU). One TIU is defined as a decrease in absorbance of the test solution at 410 nm by 0.01 units in 10 min. A sample blank without enzyme was analysed for each sample to correct for any residual turbidity or interaction between substrate and a sample solution.

Phytic acid

Phytic acid was extracted from pea flours with 3% H_2SO_4 solution, and precipitated as phytate-ferric complex, which was converted to ferric hydroxide by adding 1.5 M NaOH solution. After boiling, the phytic acid was released as soluble sodium phytate, which was measured as phosphorus using an Inductively-Coupled Plasma Atomic Emission Spectrometer (ICP-AES) model JY 70 Plus (Jobin Yvon, Paris, France) (Plaami and Kumpulainen, 1991).

Functional properties

Protein solubility
Protein solubility, as a function of pH, was determined by extraction of the protein at different pH values and subsequent determination of the protein in the extract using the Lowry's colorimetric method, as described by Sathe and Salunke (1981).

Water and oil absorption capacities
The water absorption and oil absorption capacities were carried out following the procedure described by Beuchat (1977). One gram of pea flour was mixed thoroughly with 10 ml of distilled water or sunflower oil in a volumetric test tube, and then centrifuged at 55 000×*g* for 30 min. The water absorption and oil absorption capacities were calculated as grams of water or oil absorbed per gram of flour, respectively, considering a density of 1 g ml^{-1} for water and 0.9166 g ml^{-1} for sunflower oil.

Emulsification capacity
The emulsification capacity of pea flour was determined by the method of Beuchat (1977). Two grams of pea flour were mixed with 100 ml of distilled water and blended at low speed (1200 rpm) for 30 s at 25°C, using an Omnimixer homogenizer (Omni International, Waterbury, CT, USA). An aliquot of 5 ml was taken and sunflower oil was added from a burette at a constant rate of 5 ml min^{-1} with continuous blending, until the breakpoint (indicated by separation of the oil from the aqueous phase) was reached. The emulsification capacity was expressed as ml of oil emulsified per g of protein.

Foaming capacity and foam stability
To ascertain the foaming capacity in non-hydrolyzed and hydrolyzed pea flour, 200 ml of distilled water were added to 6 g of sample in a cylinder volumetric flask, and blended with a speed of between 7000 and 8000 rpm with an Omnimixer homogenizer. Foam stability was measured as the percentage increase in volume as recorded before and after blending (Lin and Humbert, 1974). To study the foaming stability, the volume of the mixtures was recorded as function of time over a period from 5 to 120 min (Lin and Humbert, 1974).

Gelation capacity
Gelation capacity was determined with different pea flour suspensions, using the following flour/water ratios: 2, 4, 6, 8, 10, 12, 14, 16% (w/w). 5 ml of these suspensions were introduced into a test tube and placed in a boiling water bath for 1 h, followed by rapid cooling under cold running tap water. The tubes were further cooled for 2 h at 4°C. The gelation capacity was taken to be the concentration which prevented the sample from slipping when the test tube was inverted (Coffman and García, 1977).

Osmolality
The osmotic pressure, measured by freezing point depression, was determined in a suspension of 1 g of pea flour in 10 ml of distilled water using a Micro-Osmometre model 3 MO-Plus (Advanced Instruments Inc. Massachussets, USA).

Colour
Colour was determined according to the 'L' (luminosity), 'a' (greeness) and 'b' (yellowness) values using a colorimeter Minolta Chroma Meter II Reflectance CR-2000 (Minolta Limited, Milton Keynes, UK).

Statistical analyses
The statistical analyses of the data were performed with a SYSTAT program version 5.0 (Wilkinson and Howe, 1992). Results were expressed as the mean values ± standard deviation of three separate determinations. To ascertain the significance among means of the samples, Tukey's means separation test was applied. Unless otherwise stated, $p < 0.05$ was used to establish significant differences.

RESULTS AND DISCUSSION

Effects of the enzymatic treatment on the chemical and nutritional composition of pea flour

Table 1 shows the chemical and nutritive parameters in unhydrolyzed pea flour and in both hydrolyzed pea flours. In hydrolyzed pea flour, the enzymatic treatment led to a significant ($p < 0.05$) reduction in the total and protein nitrogen, and crude and true protein contents, whereas the non-protein nitrogen and free amino acid contents increased significantly ($p < 0.05$). The content of crude protein was 26.1 g 100 g^{-1} in unhydrolyzed pea

Table 1. Effects of enzymatic treatments on the nutritional value of protein and on the content of antinutritive factors of pea flour[1]

Parameters	Unhydrolyzed pea flour	Hydrolyzed pea flours	
		Treatment 1	Treatment 2
Total nitrogen (%)	4.18 ± 0.05^a	3.18 ± 0.15^b	3.59 ± 0.29^b
Crude protein (%)	26.1 ± 0.29^a	19.9 ± 0.30^c	22.4 ± 1.80^b
Protein nitrogen (%)	3.12 ± 0.31^a	1.07 ± 0.12^b	0.92 ± 0.22^b
True protein (%)	19.5 ± 0.43^a	6.65 ± 0.78^b	5.75 ± 1.43^b
Non-protein nitrogen (%)	1.00 ± 0.02^b	2.05 ± 0.14^a	2.50 ± 0.35^a
Free aminoacids ($mg\,g^{-1}$)	0.28 ± 0.01^c	4.45 ± 0.12^b	6.72 ± 0.12^a
In vitro protein digestibility (%)	82.3 ± 0.55^a	71.1 ± 1.43^b	72.9 ± 0.67^b
Trypsin inhibitor ($TIA\,mg^{-1}$)	4.72 ± 1.08^a	2.06 ± 0.37^b	2.11 ± 0.52^b
Phytic acid ($mg\,g^{-1}$)	4.35 ± 0.05^a	1.49 ± 0.33^b	1.73 ± 0.35^b

[1] Mean ± standard deviation of three determinations expressed as dry weight. Different letters within the same row are significantly different at $p < 0.05$.

flour, whereas in hydrolyzed flours, the crude protein content decreased with decreases in the total nitrogen content. In general, the crude protein content in peas varies widely as a result of the variety, size, and genetic and environmental factors (Savage and Deo, 1989; Ros and Rincón, 1990; Periago *et al.*, 1996*a*). The variability observed in the crude protein content was also observed in the true protein content. In peas, true protein is made up of 65–80% globulin and 20–35% albumin (Owusu-Ansah and McCurdy, 1991), increasing with pea size due to the protein synthesis which takes place in the seed kernel during development of the plant, in order to build up a reserve of protein ready for germination (Periago *et al.*, 1996*a*). The true protein content decreased markedly after enzymatic treatment of the pea flour. This effect is mainly due to hydrolysis of the pea protein, since the enzymatic treatment releases peptides and free amino acids from protein, increasing the non-protein nitrogen, which might be solubilized in the NaOH 0.2% and cannot be precipitated by TCA.

In vitro protein digestibility decreased significantly in hydrolyzed flour, from 82.3% to 71.1%, probably due to the fact that the remaining proteins are more resistant to the enzymes hydrolysis. However, López-Hernández *et al.* (1977) have reported that hydrolyzed protein shows better availability since low molecular weight peptides and amino acids are released. These are readily absorbed and available to the human body and could easily fulfil the daily quantities of protein recommended for special groups that require dietetic control (Frøkjaer, 1994). Higher *in vivo* protein digestibility values should therefore be expected in hydrolyzed pea flours, since there is a marked increase in free amino acids after enzymatic hydrolysis. The free amino acid content was significantly ($p < 0.05$) higher in the hydrolyzed pea flour obtained with treatment 2, because, in treatment 1, the supernatant resulting from hydrolysis was discarded, which meant the solubilized free amino acids were removed from the pea flour.

The enzymatic treatment with acid protease led to a considerable reduction in the trypsin inhibitor and phytic acid contents, the former's activity decreasing significantly ($p < 0.05$) from 4.72 to 2.06 $TIU\,mg^{-1}$ and the latter from 4.35 to 1.49 $mg\,g^{-1}$. This reduction in trypsin inhibitor activity might be related to heating during the enzymatic treatment, or due to the action of the enzyme on the protein, leading to denaturation of the protein chains (Vidal *et al.*, 1995), whereas the reduction in the phytic acid content was mainly attributed to the release of phosphorus as orthophosphate (Morehouse and Malzahn, 1976), probably due to the activation of the endogenous phytase. A low antinutritive factor content has an important effect on the nutritional protein value, because trypsin inhibitor and phytic acid significantly reduce the *in vitro* protein digestibility (Carnovale *et al.*, 1988; Al-Wesali *et al.*, 1995). Moreover, a lower phytic acid content has an important effect on mineral bioavailability, since this compound forms an insoluble complex with divalent cations like zinc, copper, iron, manganese and calcium, thus reducing bioavailability (Harland and Oberleas, 1987).

The amino acid composition of pea flours before and after enzymatic treatment are shown in Table 2. In general, the most abundant amino acids were glutamic acid, aspartic acid, lysine, and leucine, whereas the sulphur amino acid content (cystine and methionine) was low compared with that of other protein sources, such as have been described by several authors (Holt and Sosulsky, 1979; Lee *et al.*, 1982; Sosulski and McCurdy, 1987; Savage and Deo, 1989; Periago *et al.*, 1996*a*). However, the amino acid content of peas is known to be affected by cultivar, growing season, and size (Periago *et al.*, 1996*a*). There were no significant differences in the histidine, methionine, tyrosine, valine, triptophan, glutamic acid, proline and serine contents between unhydrolyzed and hydrolyzed pea flours, whereas isoleucine, leucine, lysine, cystine, phenylalanine, threonine, alanine, arginine and aspartic acid increased significantly ($p < 0.05$) after enzymatic treatment of pea flour. The increase in hydrophobic amino acids such as isoleucine, leucine and lysine is important, due to the effects that these have on the physical and functional properties of food proteins (Giese, 1994; Mahmoud, 1994). The hydrolyzed pea flours supplied a higher

Table 2. Effect of enzymatic treatment on the amino acid profiles (g 100 g^{-1} of protein) of pea flour[1]

Amino acid	Unhydrolyzed pea flour	Hydrolyzed pea flours		FAO[2] 'Ideal'	Human requirements
		Treatment 1	Treatment 2		
Essential					
His	0.96 ± 0.13^{a}	1.11 ± 0.28^{a}	1.00 ± 0.10^{a}	—	16
Ile	2.75 ± 0.28^{b}	3.53 ± 0.15^{a}	3.85 ± 0.14^{a}	4	13
Leu	4.76 ± 0.36^{b}	6.74 ± 0.19^{a}	6.20 ± 0.21^{a}	7	19
Lys	4.31 ± 0.42^{b}	3.99 ± 0.69^{ab}	5.66 ± 0.17^{a}	5.5	16
Met	0.83 ± 0.15^{a}	1.03 ± 0.19^{a}	1.04 ± 0.04^{a}	3.5	17*
Cys	0.32 ± 0.04^{b}	0.77 ± 0.11^{a}	0.58 ± 0.15^{ab}	—	17*
Phe	2.53 ± 0.21^{b}	3.30 ± 0.13^{a}	3.10 ± 0.14^{a}	—	19**
Tyr	1.55 ± 0.15^{a}	1.38 ± 0.51^{a}	1.86 ± 0.29^{a}	6	19**
Thr	2.64 ± 0.19^{b}	3.62 ± 0.17^{a}	3.41 ± 0.11^{a}	4	9
Trp	1.03 ± 0.08^{a}	0.87 ± 0.10^{a}	1.02 ± 0.04^{a}	1	5
Val	2.98 ± 0.54^{a}	3.62 ± 0.12^{a}	3.78 ± 0.45^{a}	5	13
Non-essential					
Ala	3.26 ± 0.41^{c}	4.11 ± 0.13^{b}	4.80 ± 0.17^{a}	—	—
Arg	2.55 ± 0.74^{b}	3.51 ± 0.19^{ab}	3.97 ± 0.52^{a}	—	—
Asp	3.28 ± 0.28^{b}	4.77 ± 0.21^{a}	5.08 ± 0.15^{a}	—	—
Glu	10.3 ± 0.94^{a}	10.5 ± 1.91^{a}	12.0 ± 0.23^{a}	—	—
Gly	2.70 ± 0.40^{b}	3.25 ± 0.05^{ab}	3.37 ± 0.09^{a}	—	—
Pro	2.60 ± 0.21^{a}	3.11 ± 0.47^{a}	2.94 ± 0.23^{a}	—	—
Ser	3.00 ± 0.20^{a}	3.46 ± 0.75^{a}	3.82 ± 0.13^{a}	—	—

[1]Mean ± standard deviation of three determinations. Different letters within the same row are significantly different at $p < 0.05$.
[2]FAO 'Ideal', data from FAO/OMS (1973).
[3]Human requirements, data from FAO/OMS (1992).
*Human requirements expressed as Met + Cys.
**Human requirements expressed as Phe + Tyr.

proportion of the amino acid requirements of human than the non-hydrolyzed pea flour, and the quantities of essential amino acids, isoleucine, leucine, lysine, and threonine covered 90.5% to 100% of the FAO 'ideal' (FAO, 1973).

Effects of enzymatic treatment on the functional properties of pea flour

The solubility profiles of protein from unhydrolyzed and hydrolyzed pea flours are shown in Fig. 1. The solubility of pea protein is low at acid pH, but increases in more basic pH conditions (Owusu-Ansah and McCurdy, 1991). The profile of unhydrolyzed pea flour showed a solubility curve with a broad minimum in the pH range of 3–6. Below pH 3, the solubility increased reaching a maximum of 35%. Above pH 6 there was a marked increase in solubility with a maximum of 42.11% at pH 11. Similar solubility patterns were reported in pea protein (Megha and Grant, 1986; Sosulski and McCurdy, 1987), and in other legume seed proteins such as those recovered from cowpea flour (Abbey and Ibeh, 1988) and brown beans (Abbey and Ibeh, 1987). Both hydrolyzed pea flours showed similar protein solubility values in the acid pH range. In general, enzymatic hydrolysis modifies the solubility characteristics of all food proteins, not only those from vegetal sources but also from animal sources (Frøkjaer, 1994). The enhanced solubility of the hydrolyzates is due to their smaller molecular size and the newly exposed ionizable amino and carboxyl groups, that increase the

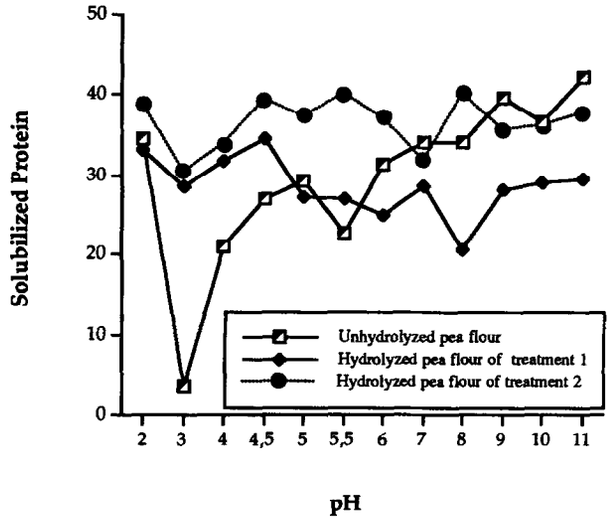

Fig. 1. Protein solubility curves at different pH of unhydrolyzed pea flour and hydrolyzed pea flours.

hydrolyzates' hydrophilicity (Mahmoud, 1994). The additional heat treatment applied during enzymatic treatment might also cause a slight modification in the solubility of proteins from vegetal sources (Megha and Grant, 1986; Abbey and Ibeh, 1987, 1988; Prakash and Ramanatham, 1995).

The foaming capacities and the foam stabilities of protein from unhydrolyzed and hydrolyzed pea flour are represented in Fig. 2. Unhydrolyzed pea flour showed a higher foam capacity, by developing high initial foam volumes and maintaining their relatively

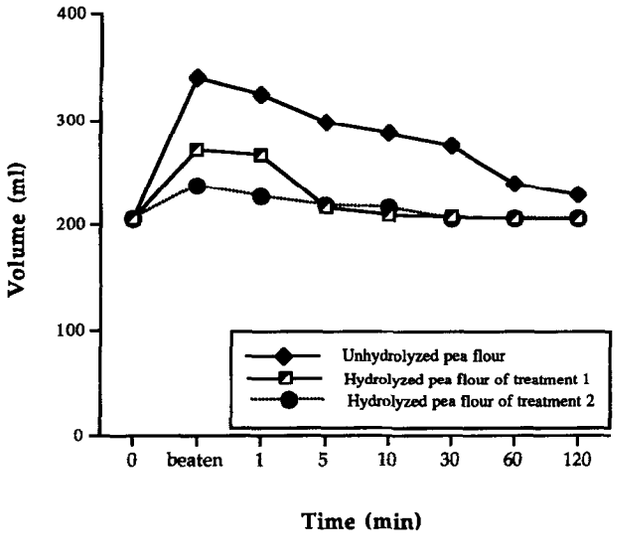

Fig. 2. Foaming capacity and foam stability of unhydrolyzed pea flour and hydrolyzed pea flours.

coarse structure throughout the 2 hour holding period. Hydrolyzed pea flour formed less foam on whipping than the corresponding pea flour, as result of the protein's hydrolysis and also probably due to the loss of soluble low-molecular weight proteins during enzymatic treatment (Sosulski and McCurdy, 1987). The hydrolyzed pea flour of treatment 1, showed a slightly higher foam capacity immediately after whipping than the hydrolyzed pea flour of treatment 2, perhaps due to losses of low molecular weight protein. However, the foam stability of both hydrolyzed pea flours were similar, the foam volume only being maintained for 10 min after whipping.

Table 3 shows the effect of the enzymatic treatment of pea flour on some functional properties. The water absorption capacity of the hydrolyzed pea flour from treatment 2, was significantly ($p < 0.05$) different from that of unhydrolyzed pea flour and hydrolyzed pea flour of treatment 1. The water uptake ranged from 1.5 to 2.9 g of water g^{-1} of sample. This functional property depends on the protein content but mainly on the physical interactions between water and protein (Cheftel *et al.*, 1989). The water absorption capacity in pea protein is significantly correlated with the content of crude protein ($r = 0.87$, $p < 0.01$) (Sosulski and McCurdy, 1987), increasing as the protein content increases (Megha and Grant, 1986). For this reason, the hydrolyzed protein of treatment 2 showed the lowest water absorption capacity, because of its low true protein content (Table 1). As regards oil absorption capacity, the hydrolyzed pea flours showed a significant ($p < 0.05$) increase with respect to that of the corresponding flour (Table 3), suggesting that the protein composition of the fractions was the principal determining factor in the response to these functional tests (Sosulski and McCurdy, 1987). Since lipid binding depends on the surface availability of hydrophobic amino acids, the increased oil absorption capacity could be attributed to an increase in these amino acids during enzymatic treatment (Lahl and Braun, 1994), as mentioned earlier (Table 2). Therefore, the heat treatment applied during enzymatic treatment could lead to some modifications of the pea protein, since heat treatment of pea flour and pea concentrate modified the globulin fraction and increased the oil absorption capacity (Megha and Grant, 1986).

The emulsification capacities of pea flour proteins are shown in Table 3, the enzymatic treatment increasing this capacity significantly ($p < 0.05$) from 35.85 ml of sunflower oil g^{-1} of sample in unhydrolyzed pea flour to 44.52 ml of sunflower oil g^{-1} of sample in hydrolyzed pea flour of treatment 1. It is generally recognised that proteins are improved by enzymatic hydrolysis, and the emulsification capacity of soya protein isolate hydrolyzed by fungal protease and whey protein and casein hydrolyzed with trypsin increased after treatment (Haque and Mozaffar, 1992). However, the extent of hydrolysis could lead to a reduction in the emulsification capacity of proteins, probably due to exposure of the hydrophobic protein interior, which would enhance adsorption at the interface forming a cohesive interfacial film, and the hydrophobic residues interacting with oil and the hydrophilic residues interacting with water (Mahmoud, 1994). The enzymatic treatment of pea flour using acid protease under the conditions selected, led to a substantial enhancement of the emulsifying capacity of the pea protein, which might have

Table 3. Effects of enzymatic treatment on the functional properties of pea flour[1]

Functional properties	Pea flour	Hydrolyzed pea flour	
		Treatment 1	Treatment 2
Water absorption capacity ($g\,g^{-1}$)	2.9 ± 0.10^a	2.9 ± 0.10^a	1.53 ± 0.06^b
Oil absorption capacity ($g\,g^{-1}$)	1.06 ± 0.10^c	1.77 ± 0.10^a	1.38 ± 0.05^b
Emulsification capacity ($ml\,g^{-1}$)	35.85 ± 1.25^b	44.52 ± 1.76^a	42.37 ± 2.05^a
Gellation capacity (%)	8	14	12
Osmolality (m Osm Kg^{-1} H_2O)	63.3 ± 4.71^c	90 ± 14.14^b	253 ± 4.71^a
Colour (coordenadas Lab)			
L	70.93 ± 0.80^a	64.43 ± 0.09^b	55.78 ± 1.30^c
−a	-11.77 ± 0.18^c	$+19.81 \pm 0.05^a$	$+17.14 \pm 0.22^b$
b	26.20 ± 0.43^b	37.22 ± 0.00^b	37.86 ± 0.23^a

[1]Mean ± standard deviation of three determinations. Different letters within the same row are significantly different at $p < 0.05$.

been due to the release of hydrophobic amino acids (Table 2), thus increasing the interactions between oil and proteins.

The gelation concentrations for the unhydrolyzed and hydrolyzed pea flours (treatments 1 and 2), were 8, 14 and 12%, respectively (Table 3). Protein hydrolyzates show a much reduced capacity to form gels, after heating, than the corresponding intact proteins (Mahmoud, 1994). This effect was also observed in the pea flours studied. Unhydrolyzed flour had a higher gelation capacity than the hydrolyzed samples.

Osmolality is an important physical characteristic in hydrolyzed protein when it is used to prepare nutritional formulas for both children and adults. A solution of high osmolality may draw large quantities of water into the small intestine, causing severe diarrhoea, possible dehydration, and disruption of the electrolyte balance, as well as inducing nausea, vomiting and abdominal distension (MacBurney and Young, 1984). Proteins, electrolytes, soluble minerals and simple carbohydrates are the main determinants of this important physical property (Mahmoud, 1994) as can be observed in the pea flour. The osmolality increased from 6.33 m Osm kg^{-1} H_2O in unhydrolyzed pea flour to 253 m Osm kg^{-1} H_2O in hydrolyzed pea flour of treatment 2 (Table 3). This increase is related to the higher content of free amino acids obtained with treatment 2. However, these values should not be considered high, because they are below the physiological osmolality. Thus, neither of the hydrolyzed pea flours will have an adverse effect on the physiological functionality of the small intestine of human.

To complete the study on the functional properties, we evaluated the effect of enzymatic treatment on the colour of the pea flours. Unhydrolyzed pea flour showed a marked green colour determined by the '−a' value (−11.77). Enzymatic treatment caused darkening of the flour, less greenness and more yellowness by decreasing the 'L' and increasing the '−a' and 'b' values, respectively. Thus, the colour of both hydrolyzed pea flours was creamy-yellow, the green color of the initial pea flour being markedly decreased.

CONCLUSIONS

In general, enzymatic treatment of pea flour with acid protease from *Aspergillus saitoi* (Molsin) might lead to an enhancement of nutritional values by increasing the free amino acid content and decreasing the trypsin inhibitor activity and phytic acid content. In addition, such treatment released hydrophobic amino acids, which significantly improve the protein solubility at acid pH, the oil absorption capacity and the emulsification capacity of pea flours. Due to losses of greenness, both hydrolyzed pea flours could be used in acid beverages, bakery and meat products, treatment 1 or 2 being selected according to the osmolality. However, a sensory study should be developed to ascertain the effect of the enzyme on pea flour flavour.

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