Effect of Temperature and Enzyme/Substrate Ratio on the Hydrolysis of Pea Protein Isolates by Trypsin

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Abstract

KARAMAĆ M., AMAROWICZ R., KOSTYRA H. (2002): **Effect of temperature and enzyme/substrate ratio on the hydrolysis of pea protein isolates by trypsin.** Czech J. Food Sci., **20**: 1–6.

Two pea protein isolates, *Pisane* and *Propulse,* were hydrolysed by trypsin. The degree of hydrolysis (DH) was computed using a pH-stat method. Enzymatic treatment of the pea protein isolates was conducted at four different temperatures, namely 35, 40, 45 and 50°C. The relationship between DH and E/S ratio was studied at 50°C and at four different E/S ratios; these were 5, 15, 25, 35 mAU/g (AU – Anson unit). For *Pisane* the highest value of the final DH (10.4%) was obtained at 45°C, whereas for *Propulse* the optimal temperature was 50°C and a DH value of 13.2% was attained. In the case of *Pisane,* the highest DH (11.5%) was recorded if the enzyme/substrate ratio was 35 mAU/g whereas for *Propulse,* the highest DH (13.2%) was observed at an E/S ratio of 15 mAU/g.

Keywords: pea; protein isolates; enzymatic hydrolysis; enzyme/substrate ratio; temperature; trypsin

An increasing interest in the employment of plant proteins for food applications has promoted a search for new protein sources from legumes. Pea (*Pisum sativum* L.) may serve as one of such protein sources. The technology for production of pea protein concentrates and isolates has been well developed and studied (ADSULE *et al*. 1989). The effect of drying techniques on pea and soybean preparations with regard to their functional properties was reported by GWIAZDA *et al.* (1979) and HORVATH *et al.* (1989). PAREDES-LOPEZ *et al.* (1991) showed that the isolation of proteins from chick pea by a micellar technique produced a product with superior functional properties to those from commercial soybean isolates.

One of the ways to modify the functional properties of food proteins is partial enzymatic hydrolysis of the protein. Protein hydrolysates are mixtures of oligopeptides, polypeptides and free amino acids. They may be a source of easily available protein in remedies used to cure metabolic diseases or in dietary/medicinal preparations (SCHMIDL *et al*. 1994). These protein hydrolysates have also been used in "formula" diets for children, the elderly and athletes. On account of their high solubility, protein hydrolysates can supplement beverage or fruit juices (FROKJAER 1994). Additionally, protein hydrolysates were reported to possess antioxidant properties (SHAHIDI & AMAROWICZ 1996; AMAROWICZ & SHAHIDI 1997).

Hydrolysis of a commercial pea protein isolate by the enzyme, Protamex (Novo Nordisk, Denmark), significantly improved the product protein solubility and reduced its pH dependence (SIJTSMA *et al.* 1998). In addition, hydrolysis improved the emulsifying activity and foam expansion. A linear dependence between the degree of hydrolysis and solubility of pea protein hydrolysates was described by SORAL-ŚMIETANA *et al.* (1998).

In our previous paper (KARAMAĆ *et al*. 1998), we reported data on the controlled hydrolysis of pea proteins by trypsin. The aim of the present study was to determine the degree of hydrolysis of pea protein isolates by trypsin as affected by temperature and enzyme/substrate ratio during the enzymatic process.

MATERIAL AND METHODS

Materials: Two pea protein isolates were used for hydrolysis: *Pisane* (Cosucra s.a., Momalle, Belgium) and *Propulse* (Dutch Protein & Services, Tiel, The Netherlands).

Enzymatic hydrolysis: Hydrolysis was carried out in a thermostatted 1 l vessel equipped with a stirrer and pH-meter (Radiometer PHM95); trypsin (Sigma Cat. No T-7409) was added to this vessel. Pea isolates (60 g) were mixed with distilled water (600 ml). pH 8.0 was maintained during hydrolysis by the constant addition of 1M NaOH from a burette. The amount of the base added was recorded in 10 min intervals for a total of 120 min.

Degree of hydrolysis (DH): The degree of hydrolysis (DH) was computed from the following equation (ADLER-NISSEN 1984):

$$\% \text{ DH} = B \times N_B \times \frac{1}{\alpha} \times \frac{1}{MP} \times \frac{1}{h_{tot}} \times 100$$

where: B – base consumption (ml)
N_B – normality of the base
α – average degree of dissociation of α-NH_2
MP – mass of protein (g)
h_{tot} – total number of peptide bonds in the protein
α – substrate (meqv Leu-NH_2/g protein)

The degree of dissociation of α-amino groups was computed from the following equation:

$$\frac{1}{\alpha} = 1 + 10^{pK - pH}$$

By comparing the pairs of hydrolysis at different pH values (pH_1 and pH_2), for which Leu-NH_2 eqv and B eqv are linearly correlated with the slope b, pK was calculated from the following equation (ADLER-NISSEN 1986):

$$pK = pH_2 + \log (b_1 - b_2) - \log (10^{pH_2 - pH_1} \times b_2 - b_1)$$

After acid hydrolysis of the starting material (0.5 g) with 10 ml of 6M HCl at 105°C for 12 h in a flame-sealed glass ampoule (HAJÓS *et al.* 1988), the total number of α-amino groups was determined using a spectrophotometric method with 2,4,6-trinitrobenzene sulphonic acid (PANASIUK *et al.* 1998). The number of free amino groups in the hydrolysis product was assayed in the same fashion.

Dependence of temperature and E/S ratio on DH: The enzymatic process was carried out at four different temperatures (35, 40, 45 and 50°C) with the E/S ratio of 15 mAU per g (AU – Anson unit). The relationship between DH and E/S ratio was studied at 50°C with the following four E/S ratios: 5, 15, 25, 35 mAU/g.

RESULTS AND DISCUSSION

The effect of incubation temperature on the hydrolysis of pea protein isolates is presented in Fig. 1. For *Pisane* the highest DH value, 10.4%, was obtained at 45°C; DH was markedly lower at 35°C (i.e. 7.6%). At 50°C the DH values were highest only during the first 50 min of incubation, then the curve for this temperature was situated below the curve for 45°C and was nearing the curve for 40°C. In the case of *Propulse*, the curve based on data at 50°C was above that at 45°C. At 35°C, the final DH value was 7.4% and it was close to the analogical value for *Pisane*. At temperatures of 40, 45 and 50°C, the DH values were 8.6, 12.2, and 13.2%, respectively. At 40°C, the DH value for *Propulse* was lower than that of *Pisane*, whereas for the temperatures 45 and 50°C this observation was reversed.

At a low temperature, i.e. 35°C for *Pisane* and 35 or 40°C for *Propulse*, the hydrolysis curves can be described by an exponential function $y = a\ x^b$ with the exponent being lower than 1 (Table 1). At higher temperatures, the relationship between DH value and time was described by a logarithmic function $y = a \ln x$. The regression fits

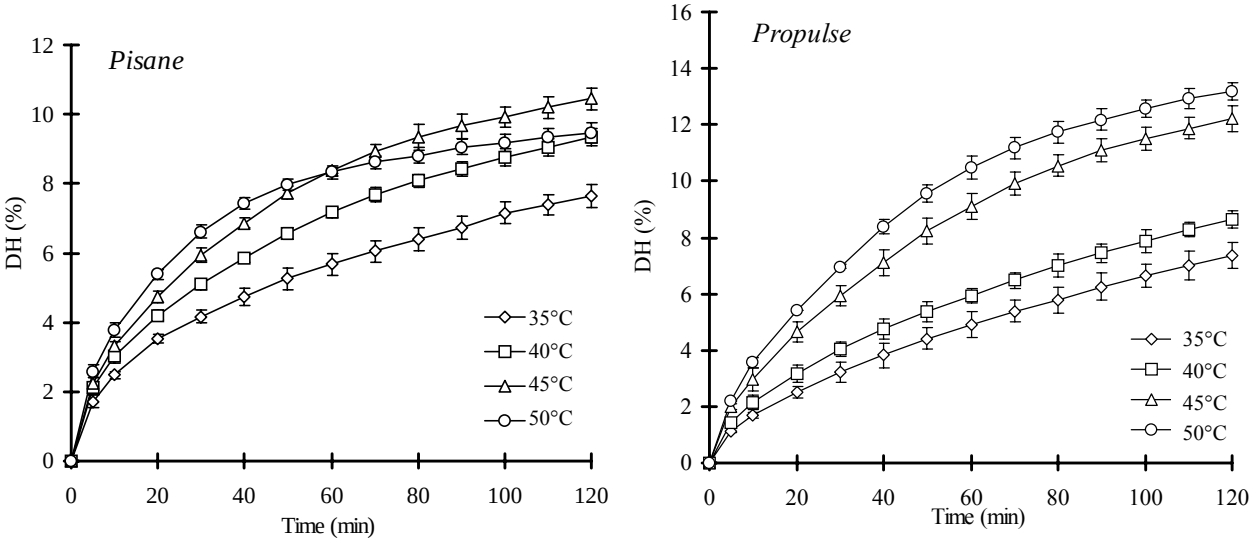

Fig. 1. Kinetics of hydrolysis of pea protein isolates by trypsin in relation to temperature

Table 1. Kinetics of hydrolysis of pea protein isolate *Pisane* and *Propulse* by trypsin in relation to temperature

Temperature (°C)	Time of hydrolysis (min)	DH (%)	Equation	Correlation coefficient *r*
Pisane				
35	0–120	0–7.6	$y = 0.846\ x^{0.464}$	0.999
40	0–60	0–7.2	$y = 0.963\ x^{0.490}$	0.999
	60–120	7.2–9.3	$y = 3.104\ \ln x - 5.531$	0.999
45	0–50	0–7.7	$y = 0.952\ x^{0.537}$	0.999
	50–120	7.7–10.4	$y = 3.059\ \ln x - 4.147$	0.998
50	0–40	0–7.4	$y = 1.146\ x^{0.512}$	0.999
	40–120	7.4–9.5	$y = 1.813\ \ln x + 0.853$	0.996
Propulse				
35	0–120	0–7.4	$y = 0.433\ x^{0.592}$	0.999
40	0–60	0–8.6	$y = 0.590\ x^{0.563}$	0.999
45	0–60	0–9.1	$y = 0.792\ x^{0.617}$	0.999
	60–120	9.1–12.2	$y = 4.447\ \ln x - 9.000$	0.998
50	0–50	0–9.6	$y = 0.817\ x^{0.631}$	0.999
	50–120	9.6–13.2	$y = 4.143\ \ln x - 6.511$	0.997

were well and correlation coefficients ranged from 0.996 to 0.999.

For the hydrolysis of *Pisane* and *Propulse* isolates by trypsin, higher DH values were observed at ascending E/S ratios (Fig. 2). An increase in the E/S ratio to 35 mAU/g gave a DH value of 11.5% (*Pisane*). Use of trypsin at a concentration of 5 mAU/g was ineffective during hydrolysis and the final DH value was only 7.1%. In the case of the *Propulse* pea isolate, the curve generated from data using an E/S ratio of 5 mAU/g was similar to that of *Pisane* (Fig. 2). However at E/S ratios of 15, 25 and 35 mAU/g, the curves were practically identical after 60 min of incubation and the influence of high E/S ratios on the DH value was almost insignificant. The high-

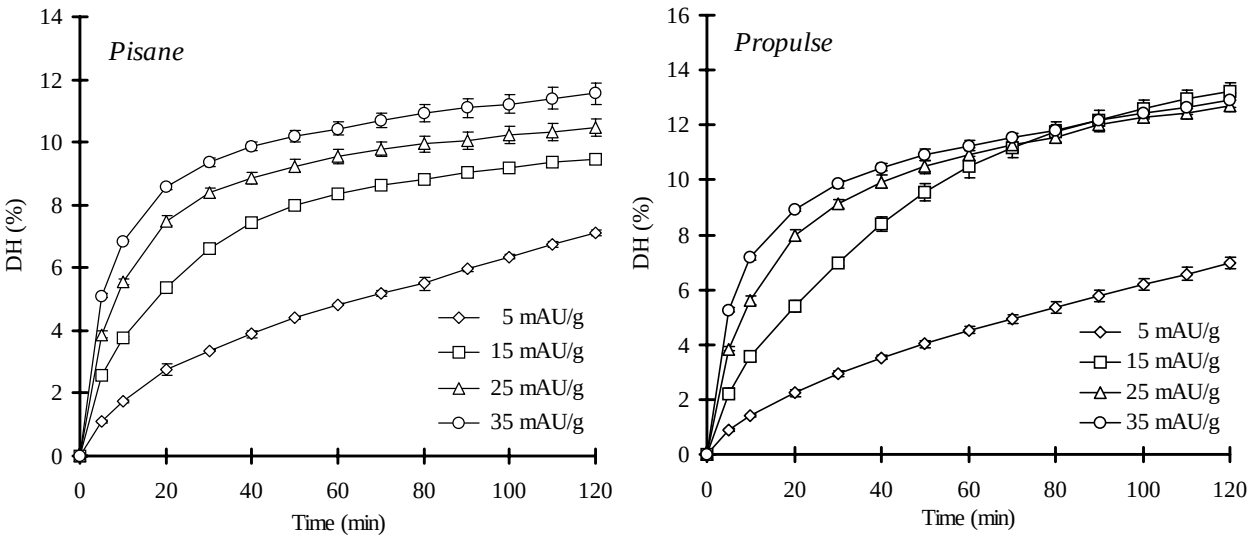

Fig. 2. Kinetics of hydrolysis of pea protein isolates by trypsin in relation to E/S ratio

Table 2. Kinetics of hydrolysis of pea protein isolate *Pisane* and *Propulse* by trypsin in relation to E/S ratio

E/S (mAU/g)	Time of hydrolysis (min)	DH (%)	Equation	Correlation coefficient *r*
Pisane				
5	0–120	0–7.1	$y = 0.469\ x^{0.569}$	0.998
15	0–40	0–7.4	$y = 1.146\ x^{0.512}$	0.999
	40–120	7.4–9.5	$y = 1.813\ \ln x + 0.853$	0.996
25	0–30	0–8.4	$y = 1.971\ x^{0.436}$	0.995
	30–120	8.4–10.4	$y = 1.478\ \ln x + 3.433$	0.997
35	0–20	0–8.6	$y = 2.825\ x^{0.375}$	0.997
	20–120	8.6–11.5	$y = 1.605\ \ln x + 3.871$	0.999
Propulse				
5	0–120	0–7.0	$y = 0.317\ x^{0.648}$	0.999
15	0–50	0–9.6	$y = 0.817\ x^{0.631}$	0.999
	50–120	9.6–13.2	$y = 4.143\ \ln x - 6.511$	0.997
25	0–120	0–12.7	$y = 2.801\ \ln x - 0.593$	0.999
35	0–120	0–12.9	$y = 2.325\ \ln x + 1.761$	0.998

est DH value, 13.2%, was determined for an E/S ratio of 15 mAU/g.

For *Pisane* and *Propulse*, the hydrolysis plots were described by an exponential function only for the E/S ratio of 5 mAU/g (Table 2). In the case of *Pisane* when the E/S ratios were 15, 25 and 35 mAU/g, the relationships between the DH value and time were described as an exponential function, but only for the first phase of enzymatic process. During the later phase of hydrolysis, the aforementioned relationships were observed and accurately described by a logarithmic function. An identical situation was noted for *Propulse* at an E/S ratio of 15 mAU/g. For the same isolate at E/S ratios of 25 and 35 mAU/g, the relationship between the DH value and time was described only by a logarithmic function. For all cases, the fit of equations was accurate and correlation coefficients ranged from 0.995 to 0.999.

Comparison of DH values obtained in this study with those from data in the literature is difficult. The authors use different enzymes and enzymatic preparations in their research and diversification of digested proteins is also high. The results for DH values are obtained by various methods. For example, GWIAZDA *et al.* (1994) using Alcalase and Neutrase for rapeseed proteins obtained DH values in the range of 8 to 10%, whereas if milk proteins were digested by trypsin and chymotrypsin, the DH values so obtained ranged from 6.5 to 7.5% and from 5.5 to 6.0%, respectively (POULIOT *et al.* 1985). After 8 hrs of digestion of soya protein with protease, the DH value ranged from 55 to 75% (SHIH 1990). Use of porcine pancreatin for the enzymatic hydrolysis of casein gave approximately a 70% DH value (MAHMOUD *et al.* 1992); in this work, the α-amino nitrogen was assayed using the formol titration procedure.

Differences in tryptic hydrolysis of the pea isolates from *Pisane* and *Propulse* observed in this study may be caused by a different composition of proteins in the used preparations. VAINTRAUB *et al.* (1976) reported a different efficiency of hydrolysis of 11S and 7S globulins of *Pisum sativum.* BHATTY (1988) found that trypsin hydrolysed the albumin fraction of pea protein much faster than that of the globulin fraction. In the case of *Propulse* at the higher E/S ratios, there appeared to be an inhibition of the enzymatic reaction that was most likely caused by products of the reaction itself (O'MEARA & MUNRO 1985; CONSTANTINIDES & ADU-AMANKWA 1980).

The increase in DH values at a higher temperature, in our work observed for *Pisane* at a temperature of 50°C, was also reported for hydrolysis of wheat proteins by fungal protease (BOMBARA *et al.* 1992). The proportional relation between DH and E/S ratio was recorded for the hydrolysis of whey proteins by trypsin (MARGOT *et al.* 1997) and by Protease 660 L and Alcalase 0.6 L (GONZÁLEZ-TELLO *et al.* 1994). The hydrolysis of rapeseed protein by Alcalase 2.5 L and Neutrase 0.5 L was described by an exponential function (GWIAZDA *et al.* 1994).

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Received for publication June 23, 2001
Accepted after correction November 30, 2001

Souhrn

KARAMAĆ M., AMAROWICZ R., KOSTYRA H. (2002): **Vliv teploty a poměru enzymu k substrátu na trypsinovou hydrolýzu izolátů bílkovin hrachu**. Czech J. Food Sci., **20**: 1–6.

Provedli jsme trypsinovou hydrolýzu dvou izolátů bílkovin hrachu označených jako *Pisane* a *Propulse*. Stupeň hydrolýzy (DH) jsme vypočítali pomocí metody pH-stat. Enzymatická hydrolýza izolátů bílkovin hrachu proběhla při čtyřech rozdílných teplotách – 35, 40, 45 a 50 °C. Závislost mezi DH a poměrem enzym/substrát (E/S) jsme sledovali při teplotě 50 °C a čtyřech různých

poměrech E/S: 5, 15, 25, 35 mAU/g (AU – Ansonova jednotka). U izolátu *Pisane* jsme dosáhli nejvyšší hodnoty konečného DH (10,4 %) při 45 °C, zatímco u izolátu *Propulse* optimální teplota byla 50 °C a bylo dosaženo hodnoty DH 13,2 %. V případě izolátu *Pisane* jsme nejvyšší DH zaznamenali při poměru enzymu k substrátu 35 mAU/g, zatímco u izolátu *Propulse* nejvyšší DH (13,2 %) byl zjištěn pro poměr E/S 15 mAU/g.

Klíčová slova: hrách; izoláty bílkovin; enzymatická hydrolýza; poměr enzymu k substrátu; teplota; trypsin

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