Functional Properties Modification of Extruded Soy Protein Concentrate using Neutrase

Krzysztof SURÓWKA and Daniel ŻMUDZIŃSKI

Department of Refrigeration and Food Concentrates, Faculty of Food Technology, Agricultural University of Cracow, Cracow, Poland

Abstract

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Enzymic hydrolysis of extruded soy protein concentrate with Neutrase was used for the preparation new soy protein products with modified functional properties. After the determination of optimum values of pH as 6.8; temperature as 50°C, and water addition as 13.3 kg/kg protein, the response surface methodology with enzyme:substrate ratio and time as independent variables was used to establish optimal conditions of the process. Solubility, water holding capacity (WHC), back extrusion work (BEW) as well as emulsifying (BAI, ESI) and foaming (FO) properties were taken as optimisation criteria. It was found difficult to obtain an ideal product by combining all the best features due to the multidirectional effect of hydrolysis on the changes of various functional properties. Nevertheless, optimal hydrolysate can be obtained at relatively low additions of Neutrase to the extrudate (e.g., 18 mAU/g protein) and in short time (e.g. 60 min). Under such conditions, the process provides a product containing 0.8 mmol amino nitrogen/g protein, which combines the features of a protein hydrolysate and those of an extrudate. SDS-PAGE of the optimal hydrolysate revealed that proteolysis of the extruded concentrate was more extensive than proteolysis of its counterpart which was not subjected to extrusion.

Keywords: limited proteolysis; Neutrase; soy protein; functional properties; process optimisation; extrusion

Soy and soy products have been for a long time the fundamental source of proteins and the main component of the diet for the inhabitants of the Far East. In the 50s, the cultivation of this plant became so widespread that it achieved the status of one of the most important raw materials for the production of edible oil and fodder protein, worldwide. For many years, soy has also been one of the most economically effective sources of food protein (Deshpande & Desphande 1991). This stems from the fact that, at a relatively low price, soy protein has high nutritional value and, from the technological viewpoint, desirable physico-chemical properties (Stanley 1987). However, nonprocessed soy and traditional oriental soy products do not always meet the taste of consumers in other regions of the world. Hence, in the late 50s, the soy processing industry developed, focusing on converting soy into unstructured, refined products such as flour, protein concentrates and isolates (Wolf 1977). Nowadays, these products are commonly used for enriching foodstuffs in the protein content, as well as substitutes of meat, milk, and eggs. This is possible because, owing to their valuable physico-chemical properties, they can be easily combined with other food components (Furukawa & Ohta 1982). These properties largely depend on the method of production, in

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particular on the degree of protein denaturation and on the presence of non-protein constituents whose effects have not yet been fully explained (Surówka 1997).

The research of the structure and properties of soy protein substances, combined with significant progress in polymer chemistry and technology, has contributed to the development of the production technique of structured soy protein preparations by the extrusion method (Rutkowski & Kozłowska 1981). Extreme conditions of this process, i.e., high temperature, pressure, and shearing forces, with a relatively low water content, lead to inter-protein interactions as well as interactions of proteins with non-protein constituents. As a result, products are formed with modified chemical and functional characteristics (Ledward & Mitchell 1988; Surówka 1997).

In view of continued interest in novel food ingredients, soy extrudates may be an interesting object for enzymic modification in view of the production of new soy products having desirable physico-chemical qualities. Such a modification is often applied to non-extruded soy products like flour (Hrčková *et al*. 2002; Taha *et al*. 2002), concentrates (Bernardi *et al*. 1991) and isolates (Molina-Ortiz & Wagner 2002), thus improving functional properties. The enzymic method is safer than chemical hydrolysis in acidic or basic environments. Namely, in the former case decomposition of certain amino acids (Warchalewski 1985) and the formation of glycerol chlorohydrins with possible toxic effects (Velíšek *et al*. 2002) can occur while in the latter case, potentially dangerous adducts of amino acids can be formed, such as lysine-alanine and lantionine (Lawrence & Jelen 1982). Recently, a method has been patented for enzymic modification of extruded mixtures containing proteins of egg white, milk, cereals, potatoes, beans, and maize, with the aim of achieving nutritionally valuable food components (Cully & Hrčková 2004). In the case of hydrolysed soy extrudates, structure loosening and the transition of certain protein substances into soluble forms, as induced by the process of peptide bonds breaking, may lead to the formation of products combining the qualities of extruded soy protein and those of protein hydrolysate. This was confirmed by preliminary investigation (Surówka *et al*. 2004) into hydrolysis with Alcalase and Esperase of extruded soy protein concentrate, which showed that the kind of enzyme used and the range of proteolysis are the deciding factors determining the functional characteristics of hydrolysates obtained. The aim of the present work is the selection of optimal conditions for enzymic hydrolysis of extruded soy protein concentrate (ExSPC) using Neutrase of *Bacillus* origin as the proteolytic enzyme, and taking the selected physico-chemical and functional properties of the hydrolysates as optimisation criteria.

MATERIALS AND METHODS

Materials. Extruded soy protein concentrate (ExSPC) with 67.6% protein (N × 6.25) in dry matter, obtained in a twin-screw extruder with high shear configuration of the screws, was used as a raw material. The extrudate was dried at 45°C and ground to pass a 0.2 mm screen. The concentrate not subjected to extrusion (SPC) was also used in the investigation. Bacterial neutral protease Neutrase of *Bacillus* origin (Novo Nordisk A/S, Bagsvaerd, Denmark) with activity of 1.5 Anson Units/g was used for hydrolysis.

Determination of optimal conditions of hydrolysis. The ExSPC was mixed with 0.1 mol/l McIlvaine buffer, then the enzyme was added and the mixture was hydrolysed at a specific temperature in a water bath with constant agitation. After the set time of proteolysis (Table 1), an aliquot was taken to assess amino nitrogen, and the remaining material was heated to 90°C for 5 min in order to inactivate the enzyme, after which it was frozen and freeze-dried. The following parameters varied in the hydrolysis process: pH at 0.2–0.4 intervals from 4.4 to 8.0, temperature at 5°C intervals from 35°C to 70°C, and water added to the raw material in a ratio of 11.5, 13.3, 15.7, 19.0, 24.0 and 32.3 kg H_2O/kg protein.

After the initial optimisation of pH, temperature, and water addition, a response surface design was used with enzyme to substrate (E:S) ratio and time as independent variables, and with amino nitrogen, solubility, water holding capacity (WHC), back extrusion work (BEW), emulsifying activity index (EAI), emulsion stability index (ESI), foam overrun (FO), and liquid drainage (LD_5) as dependent variables. A total of 13 different combinations of E:S ratio and proteolysis time were used (Table 1), nine according to principles of a second order central composite design (Cochran & Cox 1957; CSS. Statistica 1991), and four additional ones (combinations 1, 3, 11 and 13). The model was fitted with each data set as follows:

Table 1. The coded and uncoded levels of independent variables of proteolysis in the response surface design

Combination	E:S ratio level		Time level	
	coded	uncoded (mAU/g)	coded	uncoded (min)
1	$-\sqrt{2}$	12	$-\sqrt{2}$	30
2	0	42	$-\sqrt{2}$	30
3	$\sqrt{2}$	72	$-\sqrt{2}$	30
4	–1	20.8	–1	65
5	1	63.2	–1	65
6	$-\sqrt{2}$	12	0	150
7	0	42	0	150
8	$\sqrt{2}$	72	0	150
9	–1	20.8	1	235
10	1	63.2	1	235
11	$-\sqrt{2}$	12	$\sqrt{2}$	270
12	0	42	$\sqrt{2}$	270
13	$\sqrt{2}$	72	$\sqrt{2}$	270

$$Z_i = b_0 + b_1 X + b_2 Y + b_3 X^2 + b_4 Y^2 + b_5 XY$$

where: Z_i – particular dependent variable
b_0–b_5 – regression parameters
b_0 – constant
b_1, b_2 – parameters for linear terms
b_3, b_4 – parameters for quadratic terms
b_5 – parameter for interaction term
X – E:S ratio (mAU/g protein)
Y – time (min)

Multiple regression analysis was completed for each model, and the variance was partitioned into linear, quadratic and interaction components in order to assess the relative significance of these components. The significance of the equation parameters for each dependent variable was assessed using a *t*-test. Coefficients of determination (R^2) were also calculated. To evaluate the relationships between all characteristics examined, the relevant correlation coefficients at $P < 0.05$ confidence level were computed. Statistical analyses and three-dimensional graphs with contour plots were generated from the regression equations over the range of variables tested using the CSS.Statistica package (1991).

Analysis of hydrolysates. The degree of proteolysis was determined by amino nitrogen analysis using trinitrobenzenesulfonic acid (TNBS) (Adler-Nissen 1986). Protein was determined by the Kjeldahl method. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was run under reduced (2-mercaptoethanol) conditions according to the method of Laemmli (1970) using 4% stacking gel and 14% separating gel. To this end, samples of 0.1 g were extracted with 6 ml trifluoroacetic acid (TFA) solution (1 g/l) for 0.5 h in order to remove soluble peptides. After centrifugation (5000 × g, 10 min), the insoluble residue was mixed with 1 ml deionised water. 0.1 ml of the suspension obtained was combined in an Eppendorf test tube with an equal part of 2 × sample treatment buffer (0.125 mol/l Tris-Cl, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol, pH 6.8), and placed in a boiling water bath for 90 s. Aliquots of samples thus prepared were layered into the wells of the gel. Molecular weights of the bands were estimated using MW-SDS-200 and MW-SDS-70L marker kits (Sigma Chemical Co., St. Louis, MO, USA).

Solubility and water holding capacity (WHC) were assessed in parallel. To this end, 2 g of the hydrolysate was weighed in a scaled centrifugal tube and, while stirring, deionised water was added to the total volume of 25 ml. Stirring was continued for 1 hour and afterwards the tube content

was centrifugated (10 000 × g, 10 min). Next, 5 ml of the supernatant was taken, dried in vacuum (70°C, 20 h) and the residue was weighed. The remaining supernatant was discarded and the tube was weighed together with the wet residue. WHC was expressed as the amount of water (in grams) retained by 1 g of hydrolysate, and solubility as the amount of the soluble substance released from 1 g of hydrolysate.

Rheological properties were determined by the back extrusion test using a TA XT2 texture analyser (Stable Micro Systems, Surrey, UK) (Bourne 2002). To this end, in a cylindrical vessel (d = 26 mm, h = 100 mm), 27 ml of the previously prepared 138 g/l water suspension of the hydrolysate was placed, into which a stainless steel cylinder-shaped plunger (d = 25 mm, h = 35 mm) was immersed. The plunger speed was 5 m/s. The values of the back extrusion work (BEW) were calculated from the curves recorded.

Emulsifying properties were investigated by the turbidimetric method (Pearce & Kinsella 1978) with some modifications. To 20 ml of hydrolysate solution (7 g total protein/l) in 0.1 mol/l phosphate buffer having pH 7.0, 6.7 ml soy oil was added and the mixture was homogenised for 1 min in a Waring blender at maximum speed. Immediately after homogenisation, and then after 5 min, portions of 50 µl emulsion were taken, diluted with 10 ml of SDS solution (10 g/l), and turbidance values A_0 and A_5, respectively, were measured at 500 nm relative to the SDS solution used for diluting emulsion, on a Cecil Super Aquarius (UK) spectrophotometer. Emulsifying activity index (EAI) was expressed, according to Pearce and Kinsella (1978) as the area of oil/water interface (m^2) which, under the experimental conditions given, can be formed by 1 g protein of the product. The Emulsifying Stability Index (ESI) was calculated using the formula:

$$ESI = A_0 \times 5_{min}/(A_0 - A_5)$$

Foaming properties were determined by passing argon at a rate of 5.53 cm^3/s through 100 ml of water suspension of the hydrolysate (20 g total protein/l) and measuring the time required to reach 1000 ml level by the produced foam. Immediately afterwards, the remaining suspension was drained off with a syringe and its volume v_1 (ml) was measured. The draining was repeated after 5 min, when a part of the foam was destabilised and released a volume v_2 (ml) of the liquid. Two parameters of the foam were calculated, namely foam overrun (FO) as the ratio of the gas volume in the foam and the volume of the liquid involved in its formation, and foam stability as liquid drainage (LD_5), which is an index expressing, in percent, the ratio of the amount of liquid released from the foam during 5 min since its formation, and the amount of liquid contained in the foam immediately after finishing the aeration (Johnson & Brekke 1983)

$$LD_5 = v_2\ 100\%/(100 - v_1)$$

All determinations were performed in triplicates and the mean values were reported.

RESULTS AND DISCUSSION

First it was shown that Neutrase produces the fastest release of amino nitrogen from the extruded soy protein concentrate (ExSPC) at 50°C and pH within the range 6.7–7.0. At the above values of these parameters, the increase of the water content in the mixture retarded the progress of hydrolysis which resulted from a worse contact of the enzyme with the undissolved substrate. Hence, it was assumed that a technologically sound water addition should be at the level of 13.3 kg/kg protein which corresponds to the concentration of the latter in the hydrolysed mixture equal 70 g/kg, and is equivalent to 103.5 g ExSPC/kg. Such concentration of the substrate was chosen also for another reason, viz., it ensures good hydromechanical conditions of the suspension: easy mixing during the process, and homogeneity.

The changes in amino nitrogen concentration in hydrolysates obtained with various time parameters and with increasing enzyme additions have fairly typical characteristics (Figure 1), which do not differ from those recorded for ExSPC hydrolysis with Alcalase and Esperase (Surówka *et al*. 2004). Both the time and the enzyme concentration increase produce an increment of this form of nitrogen; but while the influence of time is determined mainly by the statistically significant positive linear effect, the E:S ratio influence has a positive linear and negative quadratic character (Table 2). As a result, an excessive prolongation of the process and the use of larger enzyme additions do not lead to a proportional increase in the degree of hydrolysis. Nevertheless, proteolysis of extruded soy raw materials is considered to be easier in comparison with non-extruded ones (Marsman *et al*. 1997).

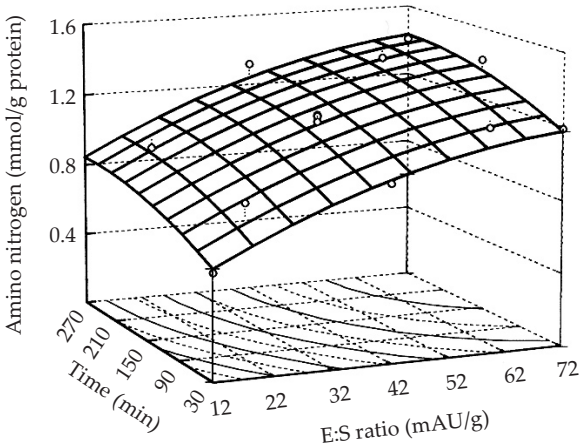

Figure 1. Response surface and contour plot showing the effect of enzyme:substrate ratio and time of proteolysis on amino nitrogen release. Conditions of the process: pH 6.8; temperature 50°C; water added to the raw material 13 kg/kg protein

This follows in part from the denaturing action of high temperatures on soy protein in the extruder, which, together with shearing forces, leads to the formation of laminated structures easily accessible to enzymes (Surówka 1997). Another reason is thermal inactivation of the protease inhibitors (Liener 1994). However, under too drastic extrusion conditions, the conformations of the proteins may refold to give enzyme-resistant structures, possibly stabilised by covalent linkages (Lin & Lakin 1990).

In the next stage of the investigation, the most important functional properties of hydrolysates were taken as optimisation criteria of time and E:S ratio. Mean values of experimental data, response surfaces, and contour plots showing the effect of the above mentioned independent variables on the functional properties are shown in Figure 2. The surfaces presented are a graphical representation of second order equations fitted to each set of data. All equations except for LD_5 had coefficients of determination (R^2) higher than 93% (Table 2).

Hydrolytic processes lead most frequently to an increase of the solubility of the material and enzymic proteolysis of plant proteins is no exception to this rule. This fact gains a special importance for the hydrolysis of extruded raw materials, in which, as a result of extrusion, insoluble structures were formed that were stabilised not only by non-covalent forces (hydrogen bonds, electrostatic and hydrophobic interactions) but also by stronger disulfide and other bonds (Ledward & Mitchel 1988). By transferring some proteins of the extrudate into the solution, limited hydroly-

Table 2. Parameters of the second order polynomial equations developed for the effects of E:S ratio (mAU/g) (b_1, b_3) and time (min) (b_2, b_4) as well as interactions between them (b_5) on amino nitrogen release and functional properties of hydrolysates obtained from ExSPC with Neutrase

Dependent variable	Equation parameters						
	constant	linear		quadratic		interactive	R^2 (%)
	b_0	b_1	b_2	b_3	b_4	b_5	
Amino nitrogen (mmol/g)	3.203×10^{-1}	1.906×10^{-2}**	2.253×10^{-3}*	-1.12×10^{-4}*	4.0×10^{-6}	1.0×10^{-6}	96.8
Solubility (g/g)	2.918×10^{-1}	4.654×10^{-3}**	1.007×10^{-3}**	-3.0×10^{-5}*	-1.0×10^{-6}*	-3.0×10^{-6}	96.9
WHC (g H_2O/g)	3.317	-1.539×10^{-2}*	-5.642×10^{-3}*	3.50×10^{-5}	1.0×10^{-5}*	-2.0×10^{-6}	93.5
EAI (m^2/g)	4.778×10^{-1}	-9.950×10^{-3}***	-2.360×10^{-4}	1.11×10^{-4}***	2.0×10^{-6}	-1.0×10^{-5}*	94.9
ESI (min)	6.881	-3.788×10^{-3}	-5.051×10^{-3}**	-1.68×10^{-4}*	-3.0×10^{-6}	9.1×10^{-5}***	97.3
LD_5 (%)	5.798×10^{1}	-1.879×10^{-2}	-1.740×10^{-2}	9.70×10^{-5}	9.0×10^{-6}	7.1×10^{-4}***	83.1
BEW (J)	5.028	-5.972×10^{-1}**	4.059×10^{-2}	1.99×10^{-3}	-1.7×10^{-4}*	-4.9×10^{-5}	95.8

*$P < 0.05$, **$P < 0.01$,*** $P < 0.001$

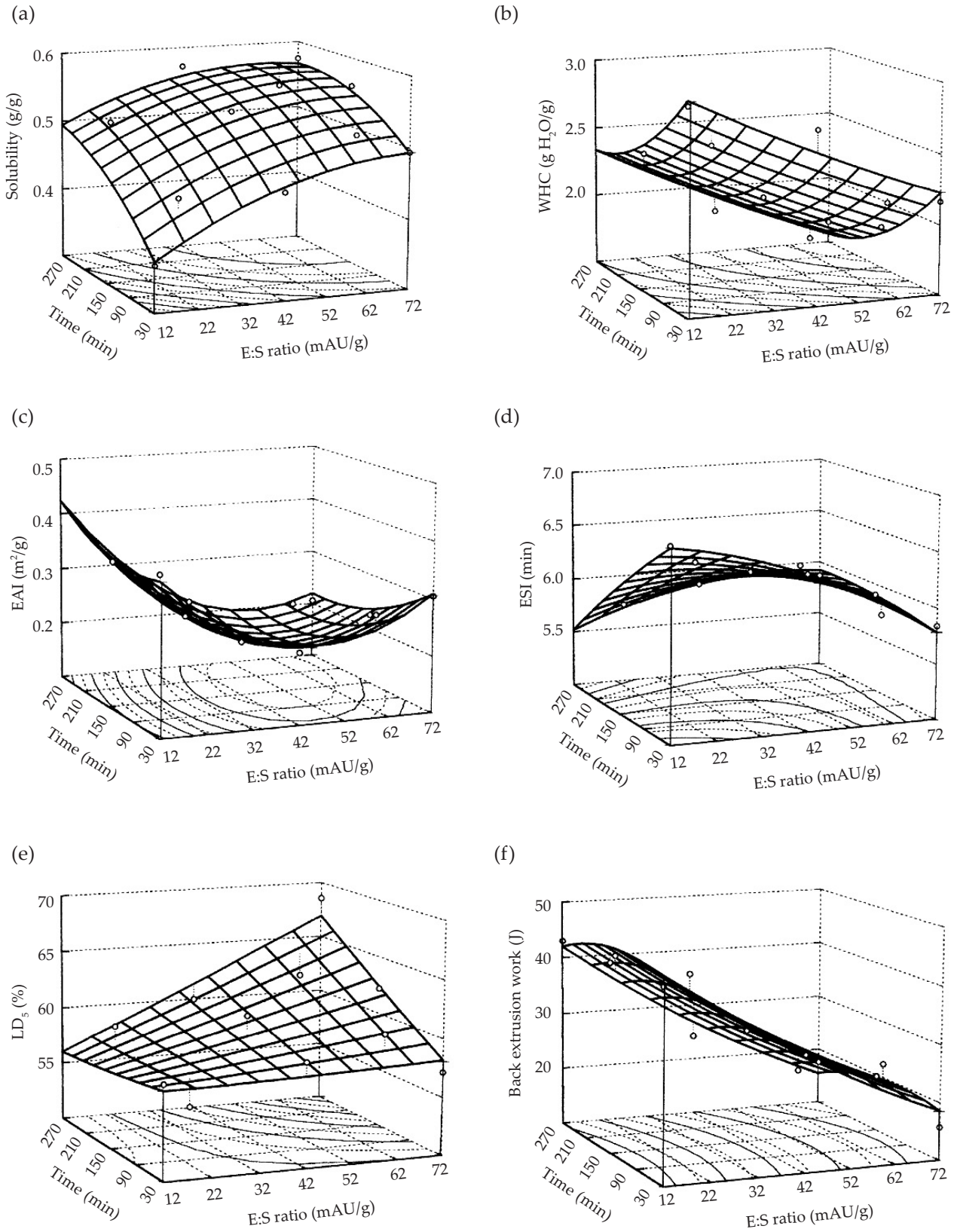

Figure 2. Influence of enzyme:substrate ratio and time of proteolysis on the (a) solubility of hydrolysates, (b) water holding capacity – WHC, (c) emulsifying activity index – ESI, (d) emulsion stability index – ESI, (e) liquid drainage – LD_5, and (f) back extrusion work – BEW. Conditions of hydrolysis as in Figure 1

sis changes its physico-chemical characteristics and, apparently, the increase of solubility is one of the main factors which lead to changes in other functional properties such as WHC, emulsifying, and whipping properties. Solubility increase of the extrudates investigated was most pronounced at the beginning of hydrolysis and required relatively small enzyme additive in order to occur. For example, in order to raise the solubility by a factor of three, starting from the value 0.129 g/g for non-hydrolysed ExSPC, it was sufficient to conduct the process for about 60 min with the smallest enzyme addition out of those applied in the present study (12 mAU/g protein). The shape of the response surface (Figure 2a) is determined by statistically significant positive linear and negative quadratic effect of time and E:S ratio (Table 2); high correlation was recorded between the amino nitrogen content and solubility (Table 3).

One characteristic feature of the soy extrudates is their ability to absorb large amounts of water which occupies the place in the fibrous structure formed by hydrophilic, unfolded protein chains. As shown by Sorgentini *et al*. (1991) in their studies of soy protein isolates, hydration properties are particularly good in products with a high degree of protein denaturation. To this group certainly belong extruded protein concentrates, but their hydration properties are also affected by non-protein constituents, chiefly pectic substances and hemicelluloses, the contents of which may reach in defatted soy flour 12 and 5%, respectively. As was shown by previous studies (Surówka 1997), increased contents of these substances in the raw material for extrusion increase the water uptake of the extrudates obtained. Since hydrolysis leads to a deformation of the protein matrix and, partly, to the transfer of some amount of protein substances into the solution, it should, theoretically, decrease hydration capabilities of the extrudates. In the present studies it was shown that this is in fact the case; however, if the process is conducted for a short time and with a slight enzyme additive, it is possible to maintain WHC at a level characteristic for the raw material (2.56 g H_2O/g), or even to increase somewhat the value of this parameter. The response surface and the contour plot illustrating the influence of the E:S ratio and time on WHC are shown in Figure 2b. The course of the changes observed is manifested by a statistically significant negative linear effect of both independent variables and a positive quadratic effect of time. In the present study, high negative correlations between WHC and amino nitrogen level as well as solubility were found (Table 3).

Rheological properties of dispersive systems are determined by the features of the continuous as well as the dispersed phases. Since 1 kg ExSPC releases only 129 g of dry substance into solution, viscosity of its suspension, which in the present investigation is represented by back extrusion work (BEW), depends chiefly on the characteristics of the dispersed material. This situation changes as a result of the hydrolysis process, which leads to the release, in part, of protein substances into the solution, and to hydrolysis of the already dissolved protein fragments. Owing to the synergy of these two effects, it can be observed, as seen in Figure 2f, for a small addition of the enzyme, a dramatic, more than triple increase of BEW in comparison with the initial value of 13.6 J, typical for a non-hydrolysed product. At higher values of

Table 3. Correlation coefficients ($P < 0.05$) between amino nitrogen content and functional properties of hydrolysates from ExSPC obtained with Neutrase

	Amino nitrogen	Solubility	WHC	EAI	ESI	FO	LD_5
BEW	–0.89	–0.72	0.80	0.61	0.65	ns	ns
LD_5	0.67	0.67	–0.71	ns	ns	ns	
FO	ns	ns	ns	ns	ns		
ESI	–0.76	–0.83	0.77	ns			
EAI	–0.79	–0.78	0.69				
WHC	–0.95	–0.94					
Solubility	0.94						

ns – not significant

the E:S ratio, this increase is not that pronounced – probably because greater saturation with the enzyme is more conducive to the hydrolysis of the already dissolved protein fragments than to their further release from the extrudate matrix into the solution. The shape of the response surface of BEW changes is determined by statistically significant negative linear and quadratic effects of the E:S ratio and time, respectively (Table 2). Moreover, significant correlations were found between this parameter and amino nitrogen, solubility, WHC and emulsifying properties (Table 3).

Good emulsifying properties do not belong to the characteristic features of the extruded proteins; thus, non-hydrolysed ExSPC revealed emulsifying activity index (EAI) as low as 0.210 m^2/g. It is well known, however, that the method of limited proteolysis may improve these properties in the case of protein preparations not subjected to extrusion (Arai & Fujimaki 1991). Hence, an attempt was made to apply this method also to the modification of extrudates. The studies carried out by the present authors revealed that in ExSPC hydrolysed mildly with Neutrase, a marked improvement of EAI occurs (from 0.210 to about 0.410 m^2/g). However, this is followed by a rapid decrease of this parameter with growing E:S ratio in the reaction mixture, whereas the influence of time on the process is much less pronounced (Figure 2c). This phenomenon is connected with statistically highly significant, negative linear and positive quadratic effects of the E:S ratio, and a negative interactive effect of the latter with time (Table 2). It is common knowledge that protein hydrolysates, in order to show good emulsifying properties, should consist of peptide chains of suitable lengths, which ensure proper viscosity of the continuous phase and which will be capable of adsorption at the interface and of forming a strong barrier against coalescence (Cheftel *et al*. 1985). Based on this information and on the shape of the response surface (Figure 2c), a hypothesis can be formulated that such peptides migrate into the solution when proteolysis is conducted even for a prolonged period but at a small enzyme concentration. Hydrolysates obtained under such conditions also have high viscosity which is reflected in high values of back extrusion work (BEW) (Figure 2f). However, in order to make full use of the emulsifying properties, good emulsion stability is required. Initially, the emulsifying stability index (ESI) of ExSPC was equal to 8.0 min. It nevertheless decreased even after a short, 30-min hydrolysis with the E:S ratio (12 mAU/g protein) the smallest out of those employed in the present work. Further drop of this parameter (Figure 2d) was determined by a negative effect of time and E:S ratio, on which a strong, statistically significant positive interactive effect of both these factors was superimposed (Table 2). Consequently, the hydrolysates obtained are not good emulsion stabilisers, and for a practical application of their emulsifying activity it would be advisable to use them in combinations with stabilising substances. Despite the fact that both studied parameters which characterise the emulsifying properties showed negative correlations with amino nitrogen content and solubility, the corresponding response surfaces had different shapes, which contributes to the absence of statistically significant correlation between EAI and ESI (Table 3).

Hydrolysis with Neutrase offers a possibility to obtain, from ExSPC, preparations with good foaming properties. As a result of this process, foam overrun (FO) (response surface not shown), which represents the ratio of the gas volume in the foam and the volume of the liquid involved in its formation, undergoes a significant decrease from the value of 75 for ExSPC to 14–26 for the hydrolysates. This means that the foam produced from the latter has a desirable, finely porous structure with strong walls. This foam is also more stable – provided that the range of proteolysis is not excessive. Namely, after a prolonged proteolysis conducted with a large enzyme addition, it can be observed that liquid drainage value (LD_5), which characterises the rate of the liquid phase release from the foam, comes close to the level typical for non-hydrolysed extrudate, i.e., 68.7%. This dependence is characterised by a statistically highly significant interactive effect of time and the E:S ratio (Table 2). The improvement of foaming properties resulting from limited proteolysis, as observed in this work, can be partly connected with the increase of solubility and with the formation of hydrolysis products having an enhanced ability to occupy a place at the gas-water interface (Yasumatsu *et al*. 1972). In support of this statements, the results of previous studies can be quoted (Surówka 1997) which showed that an addition of soy whey proteins – that is, a fraction containing low molecular weight soy proteins nonprecipitable at the isoelectric point – to soy protein concentrate improves its whipping properties.

As follows from the above considerations, obtaining a hydrolysate which would be characterised by all the best functional properties possible is difficult due to the varied influence of the process parameters. Nevertheless, it can be stated that an optimal product should be obtained at relatively low additions of Neutrase to the raw material and applying short time of proteolysis. In order to delineate this region, such constraints were set that the selected time and E:S ratio would be within the range of acceptable values for each functional parameter of the product. The following values acceptable in the final product were considered: Solubility 0.40 g/g, WHC 2.57 gH_2O/g, EAI 0.29 m^2/g, ESI 6.37 min, LD_5 58% and BEW 39 J. The selected optimal conditions for the process should be based on the region which would satisfy the constraints stated. Superimposing the individual contour plots for the response variables gives a region which satisfies all constraints, as shown in Figure 3. From this region, values were chosen for time 60 min, and E:S ratio 18 mAU/g protein, in order to obtain hydrolysate which was then assigned for mercaptoethanol-containing SDS polyacrylamide gel electrophoresis. Amino nitrogen content in this hydrolysate was equal to 0.80 mmol/g protein. The SDS-PAGE analysis covered also the non-extruded soy protein concentrate (SPC) and the hydrolysate obtained from it with amino nitrogen content equal to 0.79 mmol/g protein which was close to that in the hydrolysate of the extruded material. These electrophoretic investigations were undertaken in order to show that proteolysis of the extruded concentrate (ExSPC) with Neutrase runs differently from that of the non-extruded concentrate (SPC) and, as a result, the products obtained have different compositions. Generally, it can be stated that proteolysis of SPC has a more selective character and, despite reaching nearly the same level of amino nitrogen in the final product as that in ExSPC hydrolysate, the majority of bands typical for soy proteins are still present (Figure 4). It seems that it is compact structure of proteins in SPC which is responsible for the high resistance of the non-extruded raw material toward proteolytic activity (Deshpande & Damodaran 1989). Moreover, as a result of limited proteolysis of the extruded material, total decomposition of the β-conglycinin fraction (subunits α', α and β) and acidic subunits of glycinin (band A) takes place. Basic subunits of glycinin are more resistant and remain visible after hydrolysis at about 21 kDa. Two strong new bands also appeared with molecular weights ca. 29 and 23 kDa. Bands with the same MW emerged also as a result of hydrolysis of soy extrudate with Esperase (Surówka *et al*. 2004). An additional diffuse band of peptides released during proteolysis was visible in the lower part of gel as a dark area.

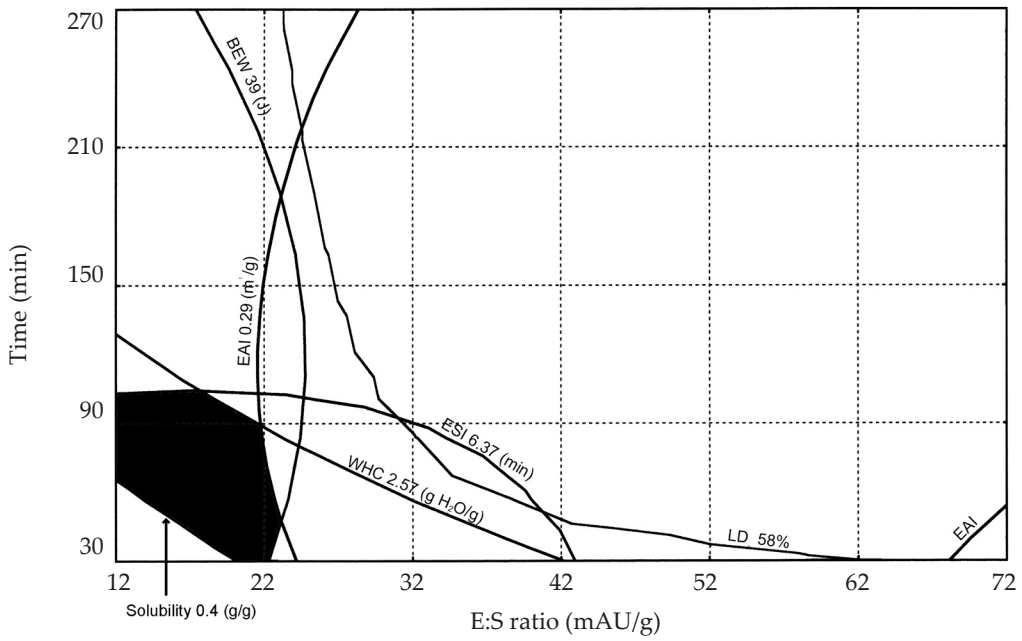

Figure 3. Superimposed contour plots for the response variables showing the region of optimal conditions for the proteolysis of extruded soy protein concentrate (ExSPC) with Neutrase. Abbreviations as in Figure 2

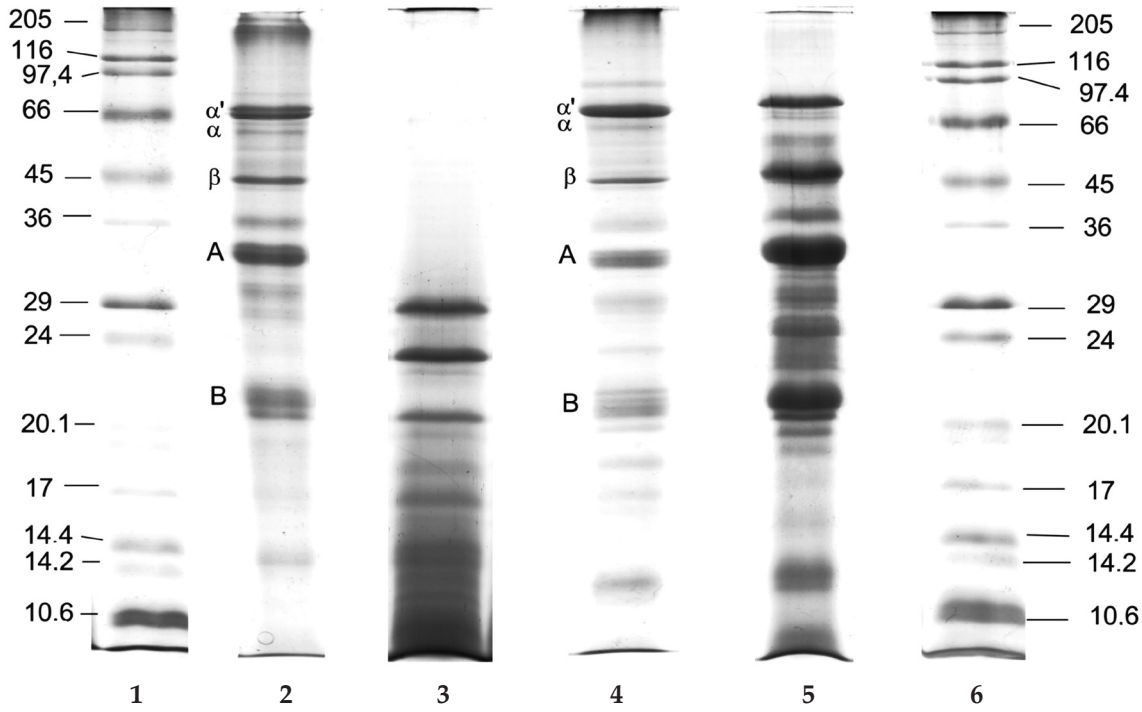

Figure 4. The SDS-PAGE electrophoregrams of Neutrase hydrolysates of extruded (ExSPC) and non extruded (SPC) soy protein concentrates. Lanes; 1,6: molecular weight standards (kDa); 2: ExSPC; 3: ExSPC hydrolysed with Neutrase; 4: SPC; 5: SPC hydrolysed with Neutrase. α, α', β – subunits of 7S globulin; A, B – acidic and basic subunits of 11S globulin

The relatively easy hydrolysis of β-conglycinin and A-polypeptide from glycinin, and higher resistance against proteolytic activity of the basic subunits of glycinin, as noted in the present study, was also reported in the investigations of Marsman *et al*. (1997) and Hrčková *et al*. (2002). This resistance to proteolysis of 11S basic subunits probably results from their tendency to form large complexes, less susceptible to enzymic proteolysis (Yamauchi *et al*. 1991). It should, however, be noted that, although during limited hydrolysis which takes place under conditions optimised in this work the decomposition of the basic subunit of glycinin does not occur, this protein can be further degraded after a longer incubation (Marsman *et al*. 1997).

CONCLUSION

The present study has shown that extruded soy protein concentrates easily undergo enzymic hydrolysis with Neutrase. Provided that such process is conducted under controlled conditions, for example those determined in the present work (pH 6.8, temperature 50°C, water additive 13 kg/kg protein, E:S ratio 18 mAU/g protein, time 60 min), a product is obtained with modified physicochemical properties, combining the features of the extrudate and those of the hydrolysate. Controlling proteolysis by modifying the E:S ratio and the time of the process makes it possible to obtain hydrolysates with differentiated proteolysis advancement and functional properties. The observation of response surfaces and contour plots leads to the conclusion that proteolysis products having the same amino nitrogen content but obtained at different time and E:S ratio can, nevertheless, have different functional properties. This suggests that the modification of these independent variables is an important factor governing the characteristics of the products of protein decomposition. The products obtained under optimised conditions determined in this work can be utilised in various technical fields including foods, beverages, cosmetics and medicins.

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References

Adler-Nissen J. (1986): Enzymic Hydrolysis of Food Proteins. Elsevier Applied Science, London, New York.

Arai S., Fujimaki M. (1991): Enzymatic modification of proteins with special reference to improving their functional properties. In: Fox P.F. (ed.): Food Enzymology Vol. 2. Elsevier, London, New York: 83–104.

Bernardi L.S., Pilosof A.M.R., Bartholomai G.B. (1991): Enzymatic modification of soy protein concentrates by fungal and bacterial proteases. J. Am. Oil Chem. Soc., **68**: 102–105.

Bourne M.C. (2002): Food Texture and Viscosity: Concept and Measurement. Academic Press, San Diego, London.

Cheftel J.C., Cuq J.-L., Lorient D. (1985): Amino acids, peptides and proteins. In: Fennema O.R. (ed.): Food Chemistry. Marcel Dekker Inc., New York, Basel: 245–369.

Cochran W.G., Cox G.M. (1957): Experimental Designs. John Willey and Sons, New York.

CSS. Statistica (1991): Experimental design. In: CSS. Statistica. Vol. 2, Tulsa O.K. USA, StatSoft: 825–880.

Cully Z., Hrčková M. (2004): Verfahren zur Herstellung von Lebensmittelfähigen Produkten mit wertvollen ernährungsphysiologischen Eigenschaften aus selektierten proteinhaltigen Rohstoffen. German Patent DE10230507A1.

Deshpande W.S., Deshpande S.S. (1991): Legumes. In: Salunkhe D.K., Desphande S.S. (eds): Foods of Plant Origin. Production, Technology and Human Nutrition. AVI Book, New York: 137–300.

Deshpande S.S., Damodaran S. (1989): Structure – digestibility relationship of legume 7S proteins. J. Food Sci., **54**: 108–113.

Furukawa T., Ohta S. (1982): Mechanical and water holding properties of heat induced soy protein gels as related to their structural aspects. J. Texture Stud., **13**: 59–69.

Hrčková M., Rusňáková M., Zemanovič J. (2002): Enzymatic hydrolysis of defatted soy flour by three different proteases and their effect on the functional properties of resulting protein hydrolysates. Czech J. Food Sci., **20**: 7–14.

Johnson E.A., Brekke C.J. (1983): Functional properties of acylated pea protein isolates. J. Food Sci., **48**: 722–725.

Laemmli U.K. (1970): Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature, **227**: 680–685.

Lawrence R.A., Jelen P. (1982): Formation of lysinoalanine in alkaline extracts of chicken protein. J. Food Prot., **45**: 923–924.

Ledward D.A., Mitchell J.R. (1988): Protein extrussion – more questions than answers? In: Blanshand J.M.V., Mitchell J.R. (eds): Food Structure – Its Creation and Evaluation. Butterworths, London, Boston, Singapore, Sydney, Toronto, Wellington: 219–229.

Liener I.E. (1994): Implications of antinutritional components in soybean foods. Crit. Rev. Food Sci. Nutr., **34**: 31–67.

Lin S., Lakin A.L. (1990): Thermal denaturation of soy proteins as related to their dye-binding characteristics and functionality. J. Am. Oil Chem. Soc., **67**: 872–878.

Marsman G.J.P., Gruppen H., Mul A.J., Voragen A.G.J. (1997): *In vitro* accessibility of untreated, toasted and extruded soybean meals for proteases and carbohydrases. J. Agric. Food Chem., **45**: 4088–4095.

Molina-Ortiz S.E., Wagner J.R. (2002): Hydrolysates of native and modified soy protein isolates: structural characteristics, solubility and foaming properties. Food Res. Int., **35**: 511–518.

Pearce K.N., Kinsella J.E. (1978): Emulsifying properties of proteins: evaluation of a turbidimetric technique. J. Agric. Food Chem., **26**: 716–723.

Rutkowski A., Kozłowska H. (1981): Preparaty żywnościowe z białka roślinnego. WNT, Warszawa.

Sorgentini D.A., Wagner J.R., Arrese E.L., Añón M.C. (1991): Water imbibing capacity of soy protein isolates: influence of protein denaturation. J. Agric. Food Chem., **39**: 1386–1391.

Stanley D.W. (1987): Food texture and microstructure. In: Moskowitz H.R. (ed.): Food Texture. Instrumental and Sensory Measurement. Marcel Dekker Inc., New York, Basel: 35–64.

Surówka K. (1997): Wpływ składników mąki sojowej na właściwości fizykochemiczne spożywczych preparatów białkowych z soi. Zeszyty Naukowe Akademii Rolniczej w Krakowie, Rozprawy Nr. 232.

Surówka K., Żmudziński D., Surówka J. (2004): Enzymic modification of extruded soy protein concentrates as a method of obtaining new functional food components. Trends Food Sci. Technol., **15**: 153–160.

Taha F.S., Ibrahim M.A., Ismail A. (2002): Effect of partial enzymatic hydrolysis on the molecular weight of some oilseed protein. Egypt. J. Food Sci., **30**: 247–268.

Velíšek J., Doležal M., Crews C., Dvořák T. (2002): Optical isomers of chloropropanediols: mechanisms of their formation and decomposition in protein hydrolysates. Czech J. Food Sci., **20**: 161–170.

Warchlewski J.R. (1985): Produkcja i zastosowanie preparatów amylolitycznych i proteolitycznych w przemyśle spożywczym. PWN, Warszawa, Poznań.

Wolf W.J. (1977): Physical and chemical properties of soybean proteins. J. Am. Oil Chem. Soc., **54**: 112A–117A.

Yamauchi F., Yamagishi T., Iwabuchi S. (1991): Molecular understanding of heat-induced phenomena of soybean protein. Food Rev. Int., **7**: 283–322.

Yasumatsu K., Sawada K., Moritaka S., Misaki M., Toda J., Wada T., Ishii K. (1972): Whipping and emulsifying properties of soybean products. Agric. Biol. Chem., **36**: 719–727.

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Souhrn

Surówka K., Żmudziński D. (2004): **Modifikace funkčních vlastností extrudovaného koncentrátu sójových bílkovin za použití Neutrasy.** Czech J. Food Sci., **22**: 163–174.

Pro přípravu nových sójových produktů s modifikovanými funkčními vlastnostmi byla použita enzymová hydrolýza extrudovaného koncentrátu sójových bílkovin účinkem Neutrasy. Po stanovení optimálních hodnot pH (6,8), teploty (50 °C) a množství vody (13,3 kg/kg bílkoviny) byla pro zjištění optimálních podmínek procesu použita metoda response surface s poměrem enzym : substrát a s časem jako proměnnými. Za kritéria optimalizace byla zvolena rozpustnost, schopnost zadržovat vodu (WHC), práce zpětné extruze (BEW) a dále emulzifikační (BAI, BSI) a pěnicí vlastnosti (FO). Bylo zjištěno, že optimálního hydrolyzátu může být dosaženo při poměrně malém přídavku Neutrasy k extrudátu (např. 18 mAU/g bílkoviny) a při krátké době (např. 60 min). Za takových podmínek vede proces k produktu s obsahem 0,8 mmol aminodusíku na 1 g bílkoviny, který kombinuje charakteristiky jak bílkovinného hydrolyzátu, tak extrudátu. SDS-PAGE optimálního hydrolyzátu ukázala, že proteolýza extrudovaného koncentrátu byla rozsáhlejší než v případě neextrudovaného produktu.

Klíčová slova: částečná hydrolýza; Neutrasa; sójové bílkoviny; funkční vlastnosti; optimalizace procesu; extruze

Corresponding author:

Krzysztof Surówka, PhD., DrSc., Agricultural University of Cracow, Faculty of Food Technology, Department of Refrigeration and Food Concentrates, ul. Balicka 122, 30-149 Cracow, Poland
tel.: + 48 12 662 47 59, fax: + 48 12 662 47 58, e-mail: rtsurowk@cyf-kr.edu.pl