Amino Acid Composition of Enzymatically Hydrolysed Potato Protein Preparations

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Abstract

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We determine the effects of the technology of obtaining potato protein preparation and of different variants of enzymatic hydrolysis on the chemical and amino acid compositions of the hydrolysates obtained. Potato protein concentrates obtained through their thermal coagulation in potato juice with calcium chloride, calcium lactate or without salt addition were subjected to enzymatic hydrolysis using two commercial hydrolytic enzymes: endopeptidase (Alcalase*)* and exopeptidase (Flavourzyme). Chemical (contents of ash, total and coagulable protein) and amino acid compositions of the hydrolysates obtained were determined. On the ground of the findings it was stated that the type of potato protein preparation used and conditions of enzymatic modification influenced on the properties of the hydrolysates obtained. Preparations obtained during the study were characterised by similar chemical and amino acid compositions, whereas the preparation obtained through thermal coagulation with the use of calcium lactate contained insignificantly more protein and essential amino acids. The least liable to enzymatic hydrolysis was the preparation obtained by using calcium chloride, particularly when only endopeptidase was used. The application of endopeptidase enzyme enabled to obtain 60% of proteolysis efficiency and the addition of the second enzyme (exopeptidase) to the protein solution insignificantly increased the proteolysis efficiency (to ca 70%), mainly when the preparation coagulated with the use of calcium chloride was hydrolysed. Proteolysis of the protein preparations obtained with the use of two enzymes was more favourable, particularly due to the quantity of free amino acids in and amino acids composition of the hydrolysates.

Keywords: chemical composition; potato protein hydrolysates; nutritional quality; potato protein isolates

In most starch industries protein is recovered from potato juice by thermal coagulation. Among many protein isolation methods, the most effective and widely used one is that which is carried out under the conditions ensuring the maximum process yield, i.e. high temperature, usually exceeding 90°C, and low pH – in the range of 4–5. On a laboratory scale, potato proteins have been isolated by means of thermal coagulation at varying pH, room temperature and in the presence of HCl, H_2SO_4, $FeCl_3$, $Al_2(SO_4)_3$, citric acid or by salting out with $(NH_4)_2SO_4$ (Ralet & Guéguen 2000; van Koningsveld *et al.* 2001). However, intense heat-coagulation results in complete denaturation of the protein and it also significantly diminishes its functionality (Pęksa *et al.* 2009) as well as digestibility. The protein obtained in this way serves animal feeding purposes. Whereas undenatured potato protein possesses good functional properties, its recovery is too costly since it requires the separation of glycoalcaloids and phenolic compounds together with the removal or inactivation of antinutrients, such as protease inhibitors (Zwijnenberg *et al*. 2002).

A popular and valuable method improving the functional properties of the protein is enzymatic hydrolysis. Several authors underline that hydrolysis (Klompong *et al.* 2007; Thiansilakul *et al*. 2007; Samaranayaka & Li-Chan 2011; Zhao *et al*. 2012) can be a suitable way to increase protein solubility and improve the properties of previously

denatured proteins. Protein hydrolysis has been widely used to facilitate the preparation of protein isolates or concentrates to be used as food and feed. The degree of protein denaturation and the technique of its isolation influence both the process of enzymatic hydrolysis of preparations as well as the degree of protein hydrolysis. The significant factors include the duration of the enzyme action, its ratio to substrate or the types of enzymes used. The most important enzymes accounting for ca. 50% of the total industrial enzymes are proteases (or proteolytic enzymes). Among them, Alcalase is mainly used – it is obtained through fermentation using *Bacillus licheniformis* strains (Rao *et al.* 1998) and it shows high thermostability (optimum temperature is 60°C) as well as a wide range of pH tolerance (pH 5.0–11.0) (Asokan & Jayanthi 2010; Olajuigbe & Ajele 2011). Alcalase is obtained from plants, animal organs, and microorganisms. Enzymatic preparation obtained with the use the selected *Aspergillus oryzae* strains is composed of endo- and exopeptidases. Endopeptidase splits the peptide bonds in the middle of the peptide chains, whereas exopeptidases cut off the individual amino acids beginning at the end of the peptide chain (Kamnerdeptch *et al.* 2007). Enzymatically modified proteins have long been available in many conventional foods such as ripened cheese and fermented soya protein products which have a wide range of applications. Moreover, pure protein hydrolysates have been shown to have valuable dietetic properties and a high nutritional value. Kudo *et al.* (2009) hydrolysed the potato protein with Pancreatin and Amano-P using as the substrate the potato precipitate coagulated at 60°C for 30 minutes. Other authors (Cheng *et al.* 2010) hydrolysed with Alcalase a concentrate which contained 81% of the protein.

As a result of enzymatic modification of plant proteins, some of the authors (Kowalczyk & Baraniak 2005; Yoshie-Stark *et al*. 2006) stated that the effectiveness of hydrolysis is significantly increased after the heat treatment of the respective protein products. The above mentioned authors also emphasised that this process does not cause a decrease in the bioactivity of hydrolysates but it contributes to solubility increase and the improvement on other functional properties. Other authors, who carried out the studies (Kamnerdeptch *et al*. 2007) on the influence of enzymatic hydrolysis on a potato as a compound of waste products in the potato industry (including pulp, juice or potato peels) indicated that using endo- and exopeptidases in a hydrolytic process in different combinations increases the amont of free amino acids in the preparations, particularly aromatic, and generally improves the quality of potato protein. Moreover, they stated that the best effects were obtained using such commercial enzymes as Alcalase and Flavourzyme. In addition, enzymatic hydrolysis of protein which led to catalytic decomposition of polypeptides, peptides, and amino acids, turned out to be a source of easily assimilated nitrogen.

The aim of the present study was to determine the effect of potato protein coagulation in potato juice and to establish the influence of the conditions on the effectiveness of enzyme hydrolysis as well as to verify amino acid composition in the hydrolysates.

MATERIAL AND METHODS

Materials. Potato juice and a commercial potato preparation were provided by the starch factory in Niechlów, Poland in 2010. Both the industrial preparation and the isolates obtained during the experiment were subjected to enzymatic hydrolysis using two commercial hydrolytic enzymes from Novozyme Corp. (Warszawa, Poland): Alcalase (endoprotease) from *Bacillus licheniformis* (A enzyme) with specific activity of ≥ 2.4 AU/g and Flavourzyme (exopeptidase) from *Aspergillus oryzae* (F enzyme) with specific activity of ≥ 500 LAPU/g. Both were purchased from Sigma Chemical Sp. z.o.o.(Poznan, Poland). All chemicals used were of analytical grade.

Preparation of potato protein isolates from potato juice. Potato protein isolates (PPI) were obtained by thermal coagulation in the presence or in the absence of such salts like calcium chloride and calcium lactate. Their concentrations were ca 0.04% in the potato juice which contained 24 mg of total protein per one gram of juice. After being delivered from the starch industry, the potato juice was heated in a vessel to 40°C through the heated coat. The next step was to add suitable quantities of 3% salt solutions (PII and PIII, respectively). When the temperature reached 70°C (PII and PIII) or 80°C (PI), it was maintained for 10 or 20 min, respectively (Table 1). Subsequently, the juice was cooled to room temperature, the coagulated potato protein was separated from the supernatant by centrifugation at 5500 *g* (Contifuge Stratos by Hereaus). The wet protein (paste) was freeze-dried whereas potato protein concentrate was stored at –25°C for further analysis. The conditions of the protein preparations processing are presented in Table 1.

Enzymatic hydrolysis. Potato protein isolates, which were obtained on laboratory scale were hydrolysed together with the commercial preparation,

Table 1. Conditions of recovering the protein preparations used for the experiment

Protein preparation	Type of salt	pH juice	Temperature (°C)	Time (min)
I	without salt addition	6.8	80	10
II	calcium chloride	6.8	70	20
III	calcium lactate	6.8	70	20
IV	industrial potato preparation		thermally coagulated	

with Alcalase as endoprotease and Flavourzyme as exopeptidase, either by individual or sequential treatment. To add to this, the hydrolysis was carried out by means of the following protolols: (a) Alcalase hydrolysis: the quantity of enzyme (ppm) = 5000, temperature = 50°C, pH = 8.5; (b) Alcalse with Flavourzyme hydrolysis: the quantity of each enzyme (ppm) = 5000, temperature = 50°C, pH = 8.5. The whole process, at the end of which the hydrolysate was heated at 85°C for 15 min, was conducted in a reaction vessel. After cooling down, the hydrolysates were centrifuged for 15 min with a Rotofix 32A by Hettich at 3000 *g*. The supernatants containing hydrolysed potato protein were freeze-dried and stored at –25°C for further analysis. Moreover, a control sample without any enzyme addition was obtained during the process.

Chemical analysis. In order to determine the ash content, the selected samples were gradually heated to 550°C and afterwards the residues were weighed (AOAC 1996). Total protein content was established with the use of Kjeldahl method based on AOAC (1996). For nitrogen to protein conversion, is the factor of 6.25 was used in the whole process. In addition, Kjeldahl method was also applied to verify soluble nitrogen concentration in the hydrolysates obtained.

Amino acid analysis. The composition of amino acids was determined after 24-h hydrolysis by means of high-performance liquid chromatography, with the use of 6N HCl and at the temperature of 110°C. The hydrolysed amino acids were separated using AAA-400 (INGOS, Prague, Czech Republic). For the detection, a two wave length photometer (440 and 570 nm) was employed. The column was packed with ion exchanger Ostion LG FA (INGOS) and its length was 200 mm. The column temperature was maintained at 40–70°C and that of the detector – at 121°C. The prepared samples were analysed using the ninhydrine method. Finally, amino acid composition was expressed in g amino acids/16 g N (Spackman *et al.* 1958). In addition, no analysis was carried out in reference to tryptophan.

Quantitative evaluation of protein quality. In order to measure the protein nutritional quality, the chemical score (CS) parameter is commonly used which is defined as follows (FAO/WHO/UNU 2007):

$$CS = \frac{\text{mg of amino acid per g of test}}{\text{mg of amino acid per g of reference}} \times 100$$

The reference used was the FAO/WHO/UNU (2007) amino acid pattern for high-quality protein.

Essential amino acid index (EAA index) was calculated according to the procedure of Oser (1951). In addition, the ratio of EAA in the test protein was taken into account, relative to their respective amounts in the standard protein.

Statistical analysis. The results were submitted to statistical analysis using Statistica 8.0 programme (Statsoft, Warszaw, Poland). Homogenous groups and LSD values were denoted by means of Duncan's multiple comparison test. It concerned the $\alpha = 0.05$ significance level with unidirectional analysis of variation for two variables.

RESULTS AND DISCUSSION

Figure 1 presents the influence of the enzyme that was used for protein hydrolysis on the nitrogen solubility. The analysis concerned potato protein preparations coagulated in different conditions. Looking at the results, it can be seen that the protein preparations used in the experiment differed significantly in terms of nitrogen solubility. After 8 h of heating in the process conditions and in the absence of the enzyme, the most soluble protein turned out to be the one that had been thermally isolated from natural juice in the absence of salt (45.2% solubility). The industrial feed protein preparation proved to be the least soluble one (8.41%). As far as the nitrogen solubility in potato protein isolates is concerned, in the presence of salts (in juice of elevated ionic strength) 30% of nitrogen was precipitated. What is more, the enzyme hydrolysis with Alcalase resulted in an increase of the nitrogen solubility of all potato protein preparations studied to values ranging from 57.69% (PI) to 61.60% (PIV). The most profitable solubility reaching above 60% was found with PII and PIV protein preparations. In the case of hy-

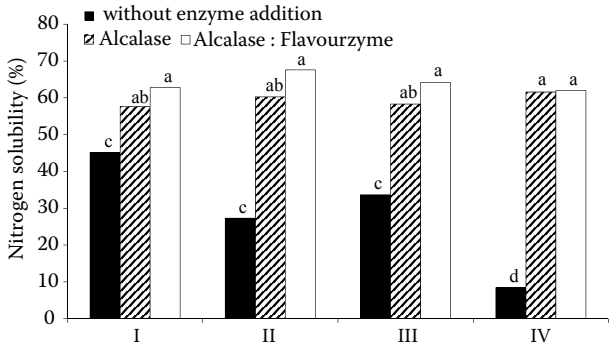

Figure 1. The influence of enzyme used for protein hydrolysis on the nitrogen solubility in potato protein preparations coagulated in different conditions

I – 80°C; II – 70°C with calcium chloride; II – 70°C with calcium lactate; IV – inductrial preparation

drolysis carried out with a mixture of Alcalase and Flavourzyme, it contributed to a further but slight increase in nitrogen solubility (up to 67.60% in PII preparation), particularly in preparations obtained in laboratory conditions.

It was confirmed that the enzyme coming from the *Aspergillus oryzae* strain (enzyme F) is active both as an endo- as well as exopeptidase i.e. it also cuts off individual amino acids from C-and N-terminal peptide chains (Kamnerdeptch *et al.* 2007; Asokan & Jayanthi 2010). This fact explains the increased efficiency of proteolysis with Alcalase and Flavourzyme enzymes combinations (Figure 1). Kołakowski *et al.* (2005) confirmed that the highest degree of proteolysis is efficiency attained when the hydrolysis process is run with two enzymes. Additionally, applying the exopeptidase in proteolysis contributes to the elimination of the bitterness of protein hydrolysates. This may be the result of the high degree of protein substrate degradation (mainly to oligopeptides and free amino acids). Furthermore, the globular structure of the major proteins of PPI was found to be an important limitation of the proteolytic enzymes activity. However, the use of Alcalase facilitated the action of Flavourzyme in the second stage of leading to an extensive hydrolysis (Clemente *et al.* 1999).

Table 2 presents the chemical compositions of the potato protein isolates which underwent thermal coagulation in various conditions. After comparing the preparations obtained in laboratory conditions (in the range of 81.90–80.22%) with the industrial feed potato protein preparation (76.66% in PIV), the higher content of total protein was observed in the former ones (such preparations as those coagulated in natural juice without salt addition (PI), precipitated in

Table 2. Characteristics of potato protein preparations (PP) used for enzymatic hydrolysis (in %)

PP	Dry matter	Total protein	Ash
I	$97.53^{a} \pm 0.38$	$81.90^{a} \pm 1.21$	$0.88^{c} \pm 0.08$
II	$97.00^{a} \pm 1.36$	$80.22^{a} \pm 0.64$	$1.17^{b} \pm 0.01$
III	$96.24^{b} \pm 0.78$	$81.75^{a} \pm 0.21$	$1.17^{b} \pm 0.01$
IV	$94.47^{a} \pm 0.24$	$76.66^{b} \pm 1.02$	$1.98^{a} \pm 0.11$

$^{a–c}$the same letters in column mean homogenous groups

juice at elevated ionic strength by salt addition, using calcium chloride (PII) and calcium lactate (PIII)). In terms of ash content, all studied isolates displayed its low amounts (not more than 1.98% found in PIV). According to some authors (van Koningsveld *et al.* 2001; Miedzianka *et al.* 2012) conducting the thermal coagulation of protein contained in potato juice at increased ionic strength by means of salt addition has several advantages. First of all, it ensures a high protein yield and decreases the temperature of coagulation to 65–70°C. It also contributes to the improvement of such functional properties as water holding capacity, oil binding capacity, water solubility as well as amino acid composition. As far as the increased ionic strength and temperature of denaturation are concerned, they delay irreversible changes in the protein structure. As concerns the calcium chloride use, it ensures the isolation of ca. 80% of the proteins present in potato juice. To sum up, thermally coagulated potato protein preparations can be characterised by a high content of total protein (> 80%), low ash content (1.5–3.0%), light colour and only a slight potato flavour and aroma (Pęksa *et al.* 2009).

Tables 3 (PI and PII) and 4 (PIII and PIV) present amino acid compositions (g/16 g N) and nutritional values of potato protein isolates together with their hydrolysates which underwent the process of proteolysis with Alcalase (A) and Alcalase mixed with Flavourzyme (A : F). The potato protein isolate which precipitated from natural juice by thermal coagulation in 80°C (PI) contained a similar quantity of total amino acids as the thermally denaturated industry feed protein preparation (PIV), i.e. 98.90 g/16 g N and 96.26 g/16 g N, respectively. The preparation produced in the presence of calcium lactate (PIII) was characterised by the highest contents of essential (55.76 g/16 g N) and total amino acids (109.15 g/16 g N). It was compared to several isolates: those obtained with calcium chloride (PII 51.18 g/16 g N and 101.24 g/16 g N, respectively), the ones which precipitated without salt addition (PI 50.06 g/16 g N and 98.90 g/16 g N,

Table 3. Amino acid composition (g/16 g N) and nutritional value of potato protein isolates (PI and PII) and their hydrolysates after hydrolysis with Alcalase (A) and Alcalase in mixture with Flavourzyme (A : F)

Amino acid	Method of protein hydrolysis						Standard (2007)
	PI	enzyme		PII	enzyme		
		A	A : F		A	A : F	
Asp	11.95^{b} ± 0.69	14.95^{a} ± 0.76	13.53^{a} ± 0.34	12.09^{b} ± 0.05	5.46^{c} ± 0.01	13.95^{a} ± 0.12	–
Thr	5.71^{b} ± 0.04	6.87^{a} ± 0.46	6.31^{a} ± 0.23	5.82^{a} ± 0.04	1.84^{c} ± 0.04	6.48^{a} ± 0.09	2.30
Ser	5.24^{c} ± 0.02	6.16^{a} ± 0.04	5.72^{a} ± 0.04	5.37^{b} ± 0.10	2.14^{c} ± 0.02	5.83^{a} ± 0.01	–
Glu	9.57^{b} ± 0.18	13.31^{a} ± 0.16	11.18^{b} ± 0.18	9.91^{b} ± 0.17	3.64^{c} ± 0.06	12.23^{a} ± 0.10	–
Pro	5.41^{b} ± 0.01	6.04^{a} ± 0.03	6.62^{a} ± 0.02	5.62^{a} ± 0.05	5.55^{b} ± 0.29	7.04^{a} ± 0.04	–
Gly	4.68^{b} ± 0.01	5.65^{a} ± 0.08	5.09^{b} ± 0.04	4.76^{b} ± 0.06	2.62^{c} ± 0.02	5.28^{a} ± 0.06	–
Ala	5.01^{c} ± 0.01	6.58^{a} ± 0.03	5.72^{b} ± 0.01	5.17^{b} ± 0.01	2.44^{c} ± 0.08	5.82^{a} ± 0.05	–
Val	6.29^{a} ± 0.04	6.96^{a} ± 0.00	6.70^{a} ± 0.04	6.18^{b} ± 0.05	2.45^{c} ± 0.23	6.48^{a} ± 0.07	3.90
Ile	5.30^{b} ± 0.02	5.26^{a} ± 0.03	5.04^{b} ± 0.03	5.32^{a} ± 0.10	1.70^{c} ± 0.39	5.12^{a} ± 0.04	3.01
Leu	9.89^{b} ± 0.01	11.01^{a} ± 0.09	9.86^{b} ± 0.23	10.10^{b} ± 0.01	5.84^{c} ± 0.10	10.42^{a} ± 0.02	5.30
Phe + Tyr	11.52^{b} ± 0.05	12.25^{a} ± 0.04	11.12^{b} ± 0.01	11.99^{a} ± 1.01	3.37^{c} ± 0.05	11.68^{a} ± 0.57	3.81
His	2.00^{b} ± 0.01	2.21^{a} ± 0.01	2.28^{a} ± 0.04	2.12^{a} ± 0.01	0.83^{c} ± 0.01	2.16^{a} ± 0.32	–
Lys	8.12^{a} ± 0.09	9.95^{a} ± 0.81	8.59^{a} ± 0.74	8.41^{b} ± 0.05	6.05^{b} ± 0.06	8.99^{a} ± 0.13	4.50
Arg	4.98^{b} ± 0.07	5.59^{a} ± 0.10	5.08^{b} ± 0.10	5.04^{b} ± 0.03	1.10^{c} ± 0.03	5.11^{a} ± 0.04	–
Met + Cys	3.23^{a} ± 0.03	2.96^{a} ± 0.03	2.29^{b} ± 0.03	3.36^{a} ± 0.06	0.41^{c} ± 0.03	2.89^{a} ± 0.13	2.21
Σ EAA	50.06^{b} ± 3.02	55.26^{a} ± 2.09	49.91^{a} ± 1.82	51.18^{b} ± 1.30	21.66^{c} ± 1.24	52.06^{a} ± 2.09	25.63
Σ AA	98.90^{c} ± 0.08	115.75^{a} ± 1.39	105.12^{a} ± 1.77	101.24^{b} ± 0.64	45.44^{c} ± 1.88	109.47^{a} ± 1.53	–
$CS_{Met + Cys}$	146.15	133.94	103.62	152.04	18.55	130.77	–
EAAI	167.10	181.54	165.39	171.31	59.91	172.52	–

CS – chemical score of restrictive amino acid; EAAI – essential amino acid index; Σ EAA – sum of essential amino acid content; Σ AA – sum of total amino acid content; $^{a–d}$the same letters in line related mean homogenous groups

respectively), and to an industrial preparation (PIV 48.09 g/16 g N and 92.26 g/16 g N). As to the hydrolysates, they were characterised by high contents of essential amino acids, especially lysine and valine. Those results were independent of potato protein preparation, the conditions of hydrolysis process as well as non essential glutamine and proline (Tables 3 and 4). Simultaneous use of Alcalase and Flavourzyme (A : F) resulted in a better and steadier amino acid profile when compared to the proteolysis conducted only with Alcalase enzyme.

In analysing the data in Tables 3 and 4, it could be noticed that for recovering the majority of amino acids from isolates, usually a mixture of Alcalase and Flavourzyme, was necessary. However, it was not essential in the case of hydrolysates obtained from protein preparation PI (precipitated in natural juice, without salt addition). The use of Alcalase was sufficient to recover all amino acids from that preparation. The enzymatic hydrolysis carried out with the use of Alcalase alone resulted in a decrease in some amino acids (such as methionine with cysteine in PII, PIII, and PIV preparations). To some degree, it also had an influence on the decrease in phenyloalanine with tyrosine and leucine in preparation PIII. The highest losses of methionine with cysteine (about 88%) were observed in the hydrolysates originated from the protein precipitated in the presence of calcium chloride (PII). In the case of hydrolysates obtained as an effect of protein thermal coagulation in the presence of salt, mainly calcium chloride (II), they were characterised by higher contents of all amino acids in comparison with potato protein preparation before hydrolysis. The effects were entirely independent of proteolysis variant. In the studies, the increase was also observed in amino acid content in particular hydrolysates and in the quantity of amino acids found in the supernatants. The above mentioned results were independent of the hydrolysed preparation type. Some authors (Clemente *et al*. 1999; Hrčková *et al*. 2002) who hydrolysed the chickpea proteins and defatted soy flour with Alcalase enzyme observed an increase in tyrosine content in the obtained hydrolysates. Further, Carvalho-Silva *et al.* (2012) who

Table 4. Amino acid composition (g/16 g N) and nutritional value of hydrolysates obtained from preparations precipitated with calcium lactate (PIII) and industrial preparation (PIV) with Alcalase (A) and mixture of enzymes (A : F)

Amino acid	Method of protein hydrolysis						Standard (2007)
	PIII	enzyme		PIII	enzyme		
		A	A : F		A	A : F	
Asp	13.25^{a} ± 0.08	12.91^{b} ± 0.02	13.58^{a} ± 0.10	11.65^{b} ± 0.04	10.69^{b} ± 0.13	12.41^{b} ± 0.22	–
Thr	6.31^{a} ± 0.03	5.92^{b} ± 0.01	6.38^{a} ± 0.11	5.55^{b} ± 0.11	4.83^{b} ± 0.22	5.75^{b} ± 0.13	2.30
Ser	5.71^{a} ± 0.04	5.07^{b} ± 0.03	5.62^{a} ± 0.05	4.98^{c} ± 0.17	4.31^{b} ± 0.04	5.22^{b} ± 0.19	–
Glu	10.41^{a} ± 0.06	10.68^{b} ± 0.07	11.98^{a} ± 0.04	9.87^{b} ± 0.08	8.74^{b} ± 0.05	10.93^{b} ± 0.02	–
Pro	5.47^{b} ± 0.03	5.53^{b} ± 0.01	6.65^{a} ± 0.22	4.83^{c} ± 0.02	4.71^{c} ± 0.08	5.86^{b} ± 0.23	–
Gly	5.12^{a} ± 0.02	5.07^{a} ± 0.04	5.10^{a} ± 0.04	4.53^{c} ± 0.05	4.01^{b} ± 0.19	4.71^{b} ± 0.07	–
Ala	5.64^{a} ± 0.04	5.93^{a} ± 0.01	5.59^{b} ± 0.03	5.32^{b} ± 0.13	4.84^{b} ± 0.23	5.61^{b} ± 0.14	–
Val	6.91^{a} ± 0.03	6.23^{b} ± 0.03	6.14^{b} ± 0.02	6.17^{b} ± 0.17	5.06^{b} ± 0.17	5.95^{c} ± 0.17	3.90
Ile	5.69^{a} ± 0.08	4.81^{b} ± 0.03	4.85^{b} ± 0.11	4.94^{b} ± 0.09	3.99^{b} ± 0.08	4.53^{c} ± 0.21	3.01
Leu	10.73^{a} ± 0.07	10.17^{a} ± 0.03	9.89^{b} ± 0.01	9.55^{c} ± 0.03	7.84^{b} ± 0.03	8.94^{c} ± 0.24	5.30
Phe + Tyr	12.85^{a} ± 0.10	11.03^{a} ± 0.02	11.11^{b} ± 0.06	11.03^{c} ± 0.11	8.47^{b} ± 0.11	10.00^{c} ± 0.30	3.81
His	2.34^{a} ± 0.05	2.15^{a} ± 0.01	2.03^{b} ± 0.04	2.09^{b} ± 0.10	1.72^{b} ± 0.31	2.05^{b} ± 0.21	–
Lys	9.10^{a} ± 0.06	9.36^{a} ± 0.04	8.52^{b} ± 0.04	7.70^{c} ± 0.14	6.86^{b} ± 0.50	7.69^{c} ± 0.08	4.50
Arg	5.44^{a} ± 0.05	5.16^{a} ± 0.03	4.85^{b} ± 0.10	4.91^{b} ± 0.19	3.71^{b} ± 0.03	4.64^{c} ± 0.11	–
Met + Cys	4.17^{a} ± 0.01	1.80^{b} ± 0.08	2.77^{b} ± 0.07	3.15^{b} ± 0.01	1.40^{b} ± 0.21	2.07^{c} ± 0.02	2.21
Σ EAA	55.76^{a} ± 2.11	49.32^{b} ± 1.63	49.66^{a} ± 2.68	48.09^{c} ± 3.22	38.43^{b} ± 1.89	44.93^{b} ± 2.21	25.63
Σ AA	109.15^{a} ± 3.75	101.81^{b} ± 4.05	105.07^{a} ± 3.51	96.26^{c} ± 4.08	81.18^{b} ± 5.67	96.37^{b} ± 4.88	–
$CS_{Met + Cys}$	188.69	81.45	125.34	142.53	63.55	93.67	–
EAAI	188.92	157.84	164.43	162.35	124.35	148.90	–

CS – chemical score of restrictive amino acid; EAAI – essential amino acid index; Σ EAA – sum of essential amino acid content; Σ AA – sum of total amino acid content; $^{a–d}$the same letters mean homogenous groups

hydrolysed the whey protein concentrate noticed a high concentration of glutamine which was derived from L-glutamic acid. On the other hand, Villanueva *et al.* (1999) observed no losses of amino acids as a result of the hydrolytic process of sunflower protein isolate by Alcalase. Kamnerdeptch *et al.* (2007) were the next to hydrolyse the potato protein pulp by using four different enzymes (Alcalase, Novo Pro-D, Flavourzyme, and Corolase). In effect, it was stated that the amino acid concentration in the enzymatic hydrolysates was significantly higher than that in the native potato pulp, especially as concerned aromatic amino acids (histidine, phenyloalanine, tryptophan, and tyrosine) and the sulphur-containing amino acid methionine (methionine).

Such proteases like Alcalase and Flavourzyme hydrolyse peptide bonds in proteins modifying their structure and improving dispersibility and nutritional availability. Hydrolysis is carried out without significantly affecting the flavour, colour, and nutritional value. As far as endoproteases are concerned, they attack peptide bonds in the interior of the polypeptide chain producing a range of polypeptides which differ in molecular weights. This is done without producing significant amounts of free amino acids as end products (Hudson 1993). As for Alcalase, it is able to hydrolyse proteins with a broad specificity for peptide bonds, preferentially those containing aromatic amino acids residues, especially large uncharged residue. Flavourzyme, however, is the endo- and exopeptidase mixture which can produce both amino acids and peptides (Hrčková *et al.* 2002).

The nutritional value of potato protein preparations and their hydrolysates was determined with regard to the chemical score (CS) and essential amino acid index (EAAI) (Tables 3 and 4). All potato protein preparations were characterised by exceeding the contents of essential amino acids established for FAO protein standard (2007). Among them there were in particular leucine, lysine, phenyloalanine, tyrosine, and threonine. Moreover, the highest amount of particular amino acids was contained in the preparations coagulated in the presence of calcium salts, whereas the lowest quantities were determined in the

industrial preparation; they were precipitated in much more drastic temperature condition. Methionine with cysteine occurred as the first limiting amino acids in the hydrolysates obtained from the commercial preparation. Most hydrolysates which were obtained from laboratory isolates contained protein of a high nutritive value: CS in the range from 18.55 to 188.69 and EAAI from 59.91 to 188.92. This refers to hydrolysates in calcium chloride and calcium lactate preparations which were hydrolysed with Alcalase.

Chemical indexes, such as CS or EAAI, determine in a simple and fast way the quality of the studied protein by means of the comparison of its amino acid composition with the standard protein composition. In addition, chemical score also points at the limitation of amino acid. Apart from standard hen egg protein and the one which was modified in 1991 by FAO/WHO Experts Committee, novel protein patterns are suggested. A good example can be the patterns that were developed by WHO/FAO/UNU in 2007. They are of lower amino acids quantities which agrees with current studies on human requirements concerning amino acids.

CONCLUSIONS

The work presents the influence of the technology of potato protein coagulation in potato juice on the effectiveness and amino acid composition of hydrolysates. Another aim of this paper has been to determine the conditions of enzymatic hydrolysis. It turned out that the coagulation method significantly influenced the flexibility of both Alcalase and Alcalase together with Flavourzyme. When both enzymes were used, it was possible to obtain preparations of favourable amino acid composition, similar to that in the raw material subjected to the hydrolysis process. The highest flexibility of Alcalase enzyme was observed in the preparation obtained with no salt addition (PI), whereas the preparation prepared with calcium chloride (PII) displayed the lowest flexibility. What should be emphasised is the fact that it was possible to obtain hydrolysates of profitable amino acid composition from denatured, insoluble feed potato protein preparation with efficiency near 60%, particularly by using both enzymes.

The enzymatic modification of thermally coagulated protein from potato juice had a crucial influence on the increase in its utilisation in food industry as well as in the production of food for special purposes. The newly obtained products were characterised by high nutritional quality and could be alternatively used in many products as functional ingredients which emulsify, bind water or fat, form foams or gels, and alter flavour, appearance, and texture. Such preparations can be applied in the food industry, i.e. in the production of bread. Considering the high content of amino acids in the hydrolysates, they could supplement and enhance the nutritional value of food products or products for different purposea (for sportsmen, convalescents or people suffering from the alimentary tract desease, etc.). However, it is compulsory to check the functional properties of the newly obtained products in order to verify whether they are safe for human consumption by meeting the food additive standards which have been imposed by the United States Food and Drug Administration (FDA).

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