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ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*)

Anne Pihlanto *, Sari Akkanen, Hannu J. Korhonen

MTT Agrifood Research Finland, Biotechnology and Food Research, Et Building, FIN-31600 Jokioinen, Finland

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Abstract

Proteins were isolated from potato tubers (*Solanum tuberosum*) at different physiological states, and by-products from the potato industry were used to evaluate their ACE-inhibitory and radical-scavenging potencies. Protein isolates and by-products were autolysed or hydrolysed by alcalase, neutrase and esperase. Hydrolysis increased the inhibition of the angiotensin-converting enzyme (ACE) and the radical-scavenging activity. The ACE-inhibitory potencies of the hydrolysates were high ($IC_{50} = 0.018 - 0.086$) and the by-product fractions showed ACE-inhibition also before hydrolysis. All samples exhibited low radical-scavenging activity, and hydrolysis for 2 h with proteases was needed to produce an increase in the activity. Ultrafiltration through 10–3 kDa membranes efficiently separated the ACE-inhibitory compounds into permeate fractions. The results of this study suggest that potato is a promising source for the production of bioactive compounds as ingredients for developing functional foods with a beneficial impact on cardiovascular health.

Keywords: Potato; Hydrolysate; ACE-inhibition; Antioxidative; Functional foods

1. Introduction

The possibility of releasing biologically active peptides from food proteins has attracted a lot of interest. Bioactive peptides have been detected in many different food sources, milk proteins being the most commonly known source. Among the different classes of bioactive peptides the best known are the antihypertensive peptides, inhibitors of angiotensin-converting enzyme (ACE). ACE-inhibitory peptides have been purified from diverse protein sources, such as gelatine, blood plasma, fish, wheat, corn, soybean and even mushrooms (Dziuba, Niklewicz, Iwaniak, Darewicz, & Minkiewicz, 2004; Pihlanto & Korhonen, 2003). ACE has an important role in the regulation of blood pressure as well as fluid and salt balance in mammals. It is a dipeptidylcarboxypeptidase, which converts the inactive decapeptide, angiotensin I, into a vasoconstrictor octapeptide, angiotensin II. ACE also inactivates bradykinin, a vasodilator peptide, and, hence, raises blood pressure (FitzGerald, Murray, & Walsh, 2004).

There is strong evidence that reactive oxygen species (ROS) and free radicals play an important part in many degenerative diseases, such as cancer, atherosclerosis and diabetes (Beckman & Ames, 1998). Formation of free radicals, such as superoxide anion radical and hydroxyl radical, is an unavoidable consequence of respiration in aerobic organisms. These radicals are very unstable and react rapidly with other groups or substances in the body, leading to cell or tissue injury. The body has its own defence system against ROS, based on antioxidant enzymes and low-molecular-mass non-enzymatic antioxidant compounds. These defence systems are not effective enough to completely prevent damage, however, and so food supplements containing antioxidants may be used to help the human body to reduce oxidative damage. Several studies have described noteworthy antioxidative activities against the peroxidation of lipids or fatty acids or against free

* Corresponding author. Tel.: +358 3 41883276; fax: +358 3 41883244.
E-mail address: anne.pihlanto@mtt.fi (A. Pihlanto).

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radical-scavenging activity of proteins and peptides from various animal and plant sources, such as milk (Pihlanto, 2006), egg (Davalos, Miguel, Bartolome, & Lopez-Fandino, 2004), barley hordein (Chiue, Kusano, & Iwami, 1997), soybean (Pyo & Lee, 2007) and potato (Wang & Xiong, 2005). Interesting phenomena is that many of the compounds have multifunctional properties, such ACE-inhibitory and antioxidant capacity.

Potato is one of the main vegetables consumed in European and American diets. The content of protein in potato is around 3%; however the nutritional value of proteins is high. Potato is also an important source of vitamins and minerals, such as calcium, potassium and phosphorus, but its value in the human diet, particularly as a source of ascorbic acid, is often underestimated or ignored. Studies have indicated that potato tubers contain phenolic compounds, such as chlorogenic acid, which have been shown to possess free radical-scavenging activity in vitro (Friedman, 1997) and, due to the large amounts consumed, they may be a significant source of health-promoting phenolic compounds. The production of starch from potatoes produces huge quantities of residual by-products. Remnants of the cell walls of potato tubers, including the skin, and residual intact cells containing starch, are mixed with potato fruit liquid. The potato fruit liquid is characterised by a high content of proteins, free amino acids and salts, whereas the particulate fraction, called potato pulp, contains starch, cellulose, hemicelluloses, pectin and proteins, in addition to remnants of fruit liquid and water in intact cells (Kempf, 1980). Potato fruit liquid is primarily used as a source for enriching proteins and amino acids, and also as a fertiliser because of its high nitrogen content. Potato pulp is applied as cattle feed, as a substrate for cultivation of fungi which may be used for degradation of soil contaminants, in an enzymatically treated and condensed form as syrup for crisps and chips, and for the production of cosmetics.

The objective of this study was to evaluate the ACE-inhibitory and antioxidant activity of proteins isolated from potato tubers and by-products from the potato industry. The proteins were isolated from potato tubers at different physiological states (immature, long-term-stored and sprouted tubers). Furthermore, we investigated the impact of enzymatic hydrolysis on the ACE-inhibitory and antioxidant activities.

2. Materials and methods

2.1. Materials

Mature potato tubers (*Solanum tuberosum*) of cv. Lady Christl and Asterix, new-season harvested "immature" tubers of cv. Timo and sprouted tubers of cv. van Gogh cultivars were utilised. The potatoes were purchased from a local supplier. The by-product fractions were obtained from companies producing starch and processed potato products. The fractions were: potato pulp, the fibre material obtained after separating starch, and the liquid part obtained after filtration of the pulp, as well as peel obtained from the manufacture of potato products.

Alcalase ($\geqslant$2.4 U/g, from *Bacillus lobigii*), esperase ($\geqslant$8 U/g, from *Bacillus sp.*) and neutrase ($\geqslant$0.8 U/g, from *Bacillus amyloliguefaciens*) were purchased from Sigma Chemical (St. Louis, MO). ABAP (2,2′-Azobis(2-methylpropionamidine) dichloride) was purchased from Acros Organics (Geel, Belgium). The control peptides, Ile-Pro-Pro and Val-Pro-Pro, were synthesized by GenScript Corporation (Piscataway, NJ, USA). For the HPLC analysis, acetonitrile (HPLC grade, ACN) was purchased from Rathburn Chemicals (Walkerburn, Scotland). The other chemicals were obtained from Sigma Chemicals (St. Louis, MO).

2.2. Preparation of protein isolates

The potatoes were thoroughly cleaned with tap water and then used for protein isolation or peeled in strips about 1 mm thick with a common vegetable peeler. We isolated the proteins from potato tubers by the method described by Ralet and Gueguen (2000). The washed potato tubers, were cut into about 1 cm × 1 cm pieces and ground with a Moulinex blender. Directly after grinding, double-distilled water (200 ml/kg fresh weight) and sodium metabisulfite (1 g/kg fresh weight) were added and stirred thoroughly. The slurry was centrifuged (15 min, 16,300*g*) and the supernatant was filtered with vacuum through Whatman 40 filter paper and frozen to −20 °C. Protein isolation from peels was carried out using the same procedure. The by-products, fruit liquid, potato pulp and peel fraction from industry were used as such for analysis.

The protein contents of the samples were measured by the DC Protein Assay (Bio-Rad Laboratories, USA) using bovine serum as a standard. Dry matter was measured by drying 500 μl of extract in the oven at 102 °C for 20 h. The results were calculated as dry matter % (w/w). The protein content of the by-products was measured by the Kjeldhal method.

2.3. Preparation of potato hydrolysates

The protein isolates were diluted in 0.1 M phosphate buffer (pH 7) to a protein concentration of 0.1% (w/w). Hydrolysis by neutrase was carried out at 50 °C and by alcalase and esperase at 55 °C (E/S 1/100, wt/wt). Incubation was performed for 5 h with shaking (200 rpm). Samples were withdrawn at 0 h, 2 h and 5 h from each mixture, and immediately heated at 85 °C for 10 min, followed by centrifugation at 13,000*g* for 5 min. The resulting clear supernatants were collected and stored at −20 °C until required for analysis of ACE-inhibitory and radical-scavenging activity. A control sample (without added enzyme) was generated using the same procedure.

2.4. Ultrafiltration process

The potato protein isolates hydrolysed by alcalase and esperase, as well as the liquid fraction, were ultrafiltrated

using 10,000, 5000 or 3000 MWCO membranes in order to remove the enzyme and the non-hydrolysed proteins. Ultrafiltration was performed in a one-step process using the three different membranes separately. In a two-step process the hydrolysates were first fractionated through a 10,000 MWCO membrane and the permeate fraction, secondly, through a 3000 MWCO membrane. All fractions were further characterised. The ultrafiltration was conducted using Centriplus YM-10, YM-3 and Amicon ultra-15 centrifugal filter devices (Millipore Corporation, USA).

2.5. Determination of ACE-inhibitory activity by HPLC

The method used for determining ACE-inhibitory activity was that described by Hyun and Shin (2000). In the assay, 225 μl of hippuryl-L-histidyl-L-leucine (HHL) solution (5 mM in 0.1 M borate buffer pH 8.3, containing 0.4 M NaCl) were incubated with 25 μl of sample (1 mg protein/ml) at 37 °C for 5 min, after which 75 μl of ACE solution (60 mU/ml) were added and incubated for 30 min. The reaction was stopped with 20 μl of 5 M HCl. The hippuric acid liberated by ACE was measured by RP-HPLC on a Novapak C8 (3.9 × 150 mm, 4 μm, Waters, Milford, USA) column. The injection volume was 10 μl, the flow rate was 1 ml/min with a linear gradient (0–70% in 24 min) of acetonitrile in 0.1% TFA, and the effluent was monitored at 228 nm. All determinations were carried out in duplicate. ACE-inhibitory activity was calculated according to the following equation: Inhibitory activity $(\%) = [(HA_{control} - HA_{sample})/HA_{control}] \times 100\%$. The IC_{50} value was defined as the concentration of peptide in mg protein/ml required to reduce the height of the hippuric acid peak (50% inhibition of ACE), and determined by regression analysis of ACE inhibition (%) versus peptide concentration.

2.6. Scavenging activity of ABTS radical by spectrophotometry

The scavenging activity against the ABTS radical [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was determined by the decolourisation assay of Re et al. (1999). The ABTS radical cation scavenging assay is applicable to both lipophilic and hydrophilic compounds and has been widely used to assess antioxidant activity (Arnao, 2000). The ABTS radical cation ($ABTS^{\cdot+}$) solution was produced by reacting 2 ml of ABTS solution (7 mM) with 1 ml of K_2SO_4 solution (2.45 mM) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the assay, the $ABTS^{\cdot+}$ solution was diluted with 5 mM phosphate buffered saline pH 7.4 (PBS) to an absorbance of 0.7 (±0.02) at 743 nm. In the assay, a 10 μl of sample (dry matter concentration 6.7–824 μg/ml) was mixed with 1 ml of diluted $ABTS^{\cdot+}$ solution and an absorbance (734 nm) reading was taken at 30 °C exactly 1 min after initial mixing and up to 6 min at 1 min intervals. All determinations were carried out in triplicate, at each separate concentration of the sample. Aqueous phosphate buffer (without ABTS-solution) was used as the blank and trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) as an antioxidant standard. The scavenging activity of ABTS radicals (%) was calculated with the following equation: Scavenging activity $(\%) = [(A_{743,\,blank} - A_{743,\,sample})/A_{743,\,blank}] \times 100\%$. The scavenging % was calculated at 1 min, 4 min and 6 min reaction times and plotted as a function of the concentration of sample dry matter (μg/ml). The IC_{50} value identifies the dry matter concentration (μg/ml) required for half-scavenging, and it was determined by regression analysis of radical-scavenging % versus dry matter concentration.

2.7. Total radical-trapping potential method (TRAP)

The samples with highest radical-scavenging activities were measured by the TRAP method described by Lissi, Salim-Hanna, Pascual, and del Castillo (1995). In the assay, 125 μl of sample or trolox standard were incubated with 25 μl of luminol (20 mM 5-Amino-2,3-dihydro-1,4-phthal-azinedione) and 50 μl of buffer (10 mM sodium phosphate buffer with 0.9% NaCl pH 7.8) in a micro plate for 10 min at 37 °C. After incubation, 50 μl of ABAP solution (90 mM 2,2′-Azo-bis(2-amidinopropane)) were added. A chemiluminescence reading was taken immediately after addition and up to 1 h. The oxidation induction time was plotted as a function of sample dry matter (mg). Total antioxidant potential (TRAP) was calculated as the equivalent trolox concentration by regression analysis of the induction time versus sample dry matter.

2.8. Reversed-phase HPLC of hydrolysates

Analytical reversed-phase high performance liquid chromatography (RP-HPLC) was carried out using a Waters HPLC system, comprising a 2695 separation module, 2996 photodiode array detector, fraction collector III and Empower software. The column was Nucleosil 300-5C-18 (4 × 250 mm, Macherey-Nagel, Düren, Germany). The column was equilibrated with solvent A (0.05% trifluoroacetic acid (TFA) in water) at a flow rate of 1.0 ml/min and the peptides were eluted with an increasing gradient of solvent B (0.05% TFA, 90% acetonitrile, water) from 2% to 60% in 55 min. Detector response was monitored at 214 nm. The hydrolysate samples were filtered through 0.45 μm syringe filters and 100 μl were applied on the column.

2.9. Purification and characterisation of ACE-inhibitory compounds from hydrolysates

The liquid fraction was pre-treated with Sep-Pak cartridges (C_{18}, Waters, Milford, USA) before chromatographic fractionation. The retained compounds were eluted with solvent containing 40% acetonitrile and 0.1%

TFA in water. For further fractionation, the column and solvents were as earlier, but peptides were eluted with a linear gradient from 2% to 60% of solvent B in 45 min. The fractions were collected and dried under a vacuum. This step was repeated several times. When necessary, the fractions were dissolved in solvent A and re-chromatographed as in the first step.

The amino acid composition of the peptides and peptide mixtures was analysed by the Pico-Tag method (Millipore Corporation, 1987). MALDI-TOF mass spectrometry (Biflex, Bruker Daltonic) was used to determine the molecular mass of the isolated compounds. The MS-analysis were done in the Institute of Biotechnology, University of Helsinki.

2.10. Statistical analysis

All the tests were done in triplicate, and data were expressed as means with standard deviation. An independent Student's *t*-test was used to compare the ACE-inhibitory and radical-scavenging activities between treatments and starting material.

3. Results and discussion

3.1. ACE-inhibitory activity

The dry matter (%) and protein content (% of dry matter) were 4.3 and 45.3 for protein isolate, 15.7 and 12.2 for pulp and 4.9 and 48.6 for liquid fractions. Measurements of the ACE-inhibitory activity of the protein isolates and hydrolysates gave the following results: the protein solution isolated from sprouted tubers showed inhibition percentages of 52.5%, whereas those from other tubers showed much lower inhibition percentages (Table 1). In the protein isolates from immature tubers, ACE-inhibitory activity increased during 5 h incubation without added enzyme, whereas exogenous enzymes were essential to

Table 1
ACE-inhibitory and ABTS radical scavenging activity (at 4 min reaction time) of potato protein isolates and by product fractions before and after digestion by proteolytic enzymes or autolysis

Sample and treatment	ACE-inhibition[a]			Radical-scavenging (%)				
	Inhibition (%)		IC_{50} (mg/ml)	Scavenging (%)			IC_{50}-values (μg/ml (dry matter))	
	0	5 h	5 h	0	2 h	5 h	2 h	5 h
Protein isolates								
Mature, stored tubers (cv. *Lady Christl*)	22.4 ± 2.2	21.4 ± 0.9	nd	5.6[b] ± 0.7	nd	4.2[b] ± 0.4	nd	nd
Alcalase		73.7 ± 0.4	0.05		45.2[c] ± 0.7	51.0[c] ± 0.2	29	27
Esperase		60.2 ± 0.2	0.077		36.3[c] ± 0.9	19.9[b] ± 1.1	nd	nd
Neutrase		53.6 ± 0.6	0.086		33.0[c] ± 0.2	16.1[b] ± 0.6	nd	nd
"Immature tubers" (cv. *Timo*)	13.9 ± 0.2	41.4 ± 0.9	0.079	18.2[c] ± 2.2	nd	11.5[c] ± 0.3	nd	nd
Alcalase		49.3 ± 1.9	0.076		nd	39.4[c] ± 0.3	nd	nd
Esperase		47.9 ± 1.1	0.067		nd	nd	nd	nd
Neutrase		44.5 ± 0.7	0.071		nd	nd	nd	nd
Sprouted tubers (cv. *van Cogh*)	52.5 ± 0.3	60.7 ± 0.8	0.686	37.3[c] ± 0.2	nd	25.2[c] ± 0.4	nd	nd
By-products								
Pulp fraction	57.0 ± 0.1	57.4 ± 0.1	0.042	28.3[d] ± 0.8	nd	30.8[d] ± 0.1	nd	nd
Alcalase		50.6 ± 0.8	0.034		53.5[d] ± 1.3	58.9[d] ± 4.0	662	661
Esperase		86.0 ± 0.6	0.023		43.3[d] ± 0.1	54.2[d] ± 1.9	815	785
Neutrase		74.5 ± 1.8	0.027		43.3[d] ± 0.1	49.3[d] ± 1.9	945	815
Liquid	64.4 ± 0.4	49.5 ± 0.6	0.070	31.0[e] ± 1.2	nd	45.3[e] ± 0.2	nd	28
Alcalase		94.2 ± 0.1	0.018		89.4[e] ± 0.7	79.3[e] ± 6.1	12	15
Esperase		93.2 ± 0.2	0.018		85.4[e] ± 0.5	57.5[e] ± 0.4	13	23
Neutrase		93.8 ± 0.2	0.025		63.4[e] ± 2.1	63.6[e] ± 2.5	19	19
Peel fractions (cv. *Asterix*)								
Mature tubers	53.3 ± 0.07	60.0 ± 3.8		41.8[g] ± 3.2				
From industry	75.0 ± 0.8	70.8 ± 2.1		48.0[f] ± 3.1				
Ile-Pro-Pro			0.0019					
Val-Pro-Pro			0.0066					
Captopril			0.15 μM					
Trolox								3

Mean values ± standard deviations is shown.
[a] Protein concentration 0.07 mg/ml.
[b] Dry matter concentration 13.4 μg/ml.
[c] Dry matter concentration 26.8 μg/ml.
[d] Dry matter concentration 824.3 μg/ml.
[e] Dry matter concentration 25.2 μg/ml.
[f] Dry matter concentration 56.4 μg/ml.
[g] Dry matter concentration 38.6 μg/ml.

generate ACE-inhibitory activity in the protein fraction isolated from mature tubers. The ACE-inhibition % of hydrolysates varied from 44% to 94%, and the IC_{50}-values varied from 0.018 to 0.086 mg/ml. The increase in inhibitory activity was observed already after 2 h of incubation, and no further increase occurred during a longer incubation time. There were no remarkable differences among tested proteases ($p = 0.5 - 0.07$). When different starting materials were compared, the test showed that the differences between liquid fraction and protein isolates from mature tubers ($p = 0.006$) and from immature tubers ($p < 0.0001$) were statistically significant.

ACE-inhibitory activities, comparable to those in this study, have been found with whey and pea hydrolysates: 0.048 and 0.076 mg/ml, respectively (Vermeirssen, van der Bent, Van Camp, van Amerongen, & Verstraete, 2004). Li, Liu, Shi, and Le (2005) found that mung bean and rice protein, hydrolysed by alcalase, produced IC_{50}-values of 0.62 and 0.14 mg/ml, while soy protein hydrolysates gave IC_{50}-values from 0.126 to 0.34 mg/ml (Kuba, Tana, Tawata, & Yasuda, 2005).

In addition to peptides, a number of other compounds present in plants have been shown to possess *in vitro* ACE-inhibitory activity, including phenolic compounds, oligosaccharides and amino acid analogues (Actis-Goretta, Ottaviani, & Fraga, 2006; Je, Park, Kim, & Kim, 2006; Liu, Lin, & Hou, 2004). It appeared that the ACE-inhibitory compounds were already present in the by-products. This means that, during processing, compounds such as phenolic acids, alkaloids, amino acids and protease inhibitors are liberated and induce ACE-inhibition. In other words, ACE-inhibitory activity might be due to several components and might be associated with the number of hydroxyl groups available to establish hydrogen bonds with ACE.

3.2. Radical-scavenging activity

The radical-scavenging activity was measured from the protein isolates of mature tubers and their hydrolysates as well as from the by-products, and is presented in Table 1. Before hydrolysis, the scavenging activities varied; the peel fractions had the highest (41.8% and 48.0%) and the protein isolate of mature tubers had the lowest (5.6%). During the 5 h of hydrolysis with added proteases, the scavenging activities increased in most cases and the values varied from 16.1% to 89.4% (scavenging % at 4 min reaction time). The highest increase in radical-scavenging was found during the first two hours of hydrolysis, after which a minor change was observed. There were no statistically significant differences among tested proteases. When different starting materials were compared, the test showed a statistically significant difference between liquid fraction and protein isolates ($p = 0.0196$). The radical-scavenging value expressed as IC_{50} μg/ml of dry matter value was 3 for trolox and 12 for the best hydrolysate, the 2 h alcalase digest of the liquid fraction. The results of the ABTS measurements were confirmed by the TRAP method, which gave values corresponding to those obtained by the ABTS method (oxidation time data not shown). The most active sample was the potato liquid alcalase hydrolysate, which produced an antioxidant capacity of 0.48 g dry matter, equivalent to 1 mmol trolox by the TRAP method.

It is difficult to compare these values with those reported in the literature for potato samples, due to several different methods used for testing antioxidant activity. Furthermore, the results are also expressed in a variety of ways, making comparison difficult. The main tuber storage protein, patatin, and its hydrolysates prepared by Amano P and pancreatin treatments have been found to exhibit antioxidant activity in a series of *in vitro* tests (Kudoh et al., 2003; Liu, Han, Lee, Hsu, & Hou, 2003). Wang and Xiong (2005) observed that hydrolysis of potato proteins by alcalase increases their radical-scavenging activity and that the greatest increase in the TEAC value occurred after 1 h of hydrolysis. In this study, alcalase produced the highest increase in radical-scavenging activity, found during 2 h. During enzymatic treatment, the increased scavenging activity probably resulted in structural changes of proteins and release of peptides. It has been reported that accessibility to the oxidant–antioxidant test system is greater for small peptides and amino acids than for large peptides and proteins (Moosman & Behl, 2002).

Previous research has shown that potato contains antioxidative compounds, such as ascorbic acid (Dale, Griffiths, & Todd, 2003) and phenolic compounds, but fairly low antioxidant activity (4.86 ± 0.2 μmol of vitamin C equiv/g sample) has been obtained by the TOSC assay (Chu, Sun, Wu, & Liu, 2002; Friedman, 1997). During the starch process, the antioxidant compounds were transferred to the liquid fraction since radical-scavenging activity was observed. However, this does not fully explain the

Table 2
ACE-inhibitory activity of liquid fraction and potato protein isolates digested by alcalase and esperase following ultrafiltration through 10, 5 and 3 kDa molecular weight cut-off membranes

Sample	IC_{50} mg protein/ml	Radical scavenging % (10 μl sample)	Protein content (mg/ml)
Liquid fraction	0.37		14.3
10 kDa retentate	0.31		13.8
10 kDa permeate	0.01		0.4
5 kDa retentate	0.86		46.2
5 kDa permeate	0.02		0.8
3 kDa retentate	0.91		43.0
3 kDa permeate	0.05		0.9
10 kDa + 3 kDa retentate	0.08		5.4
10 kDa + 3 kDa permeate	0.05		1.9
Potato isolate + alcalase	0.04	52 ± 0.2	1.0
3 kDa retentate	8.9[a]	63 ± 0.4	nd
3 kDa permeate	20.2[a]	44 ± 0.7	nd
Potato isolate + esperase	0.03	40 ± 1.1	1.0
3 kDa retentate	33.6[a]	89 ± 0.7	nd
3 kDa permeate	30.8[a]	24 ± 0.8	nd
Trolox		62 ± 0.1	

[a] μl/ml.

observed differences. According to literature data, the radical scavenging activity of peel fractions was most likely due to the phenolic compounds (Nara, Miyoshi, Honma, & Koga, 2006).

3.3. Ultrafiltration

The protein isolates hydrolysed by alcalase and esperase from mature tubers, as well as the liquid fractions, were subjected to ultrafiltration in order to assess the partitioning of active compounds through membranes with defined molecular mass cut-off values (Table 2). The potato liquid fraction showed the lowest IC_{50} value for the 10 kDa permeate, indicating that the ACE-inhibitory compounds were partitioned into that fraction. The potato isolate hydrolysed by alcalase showed a low IC_{50}-value for the 3 kDa retentate fraction, but in the esperase hydrolysate the two ultrafiltration fractions exhibited almost the same activity. It should be taken into account that the IC_{50} values of alcalase and esperase hydrolysates are calculated on the basis of sample volume and therefore not comparable to the IC_{50} values of the liquid fraction. When the liquid fraction was subjected to ultrafiltration, the permeate fractions showed higher ACE-inhibitory potencies than the original or retentate fractions. One-step ultrafiltration proved suitable in this study, since no remarkable increase in ACE inhibition was observed in combined ultrafiltration. Similar results have been reported earlier with whey hydrolysates (Pihlanto-Leppälä, Koskinen, Piilola, Tupasela, & Korhonen, 2000).

Radical-scavenging capacities were measured from alcalase and esperase hydrolysates ultrafiltrated using a 3000 MWCO membrane. Similar results were obtained for these two hydrolysates. The scavenging capacities increased in the retentate and decreased in the permeate fraction by ultrafiltration as compared to the original hydrolysate (Table 2). It should be taken into account, however, that there is more dry matter in the retentate than in the permeate.

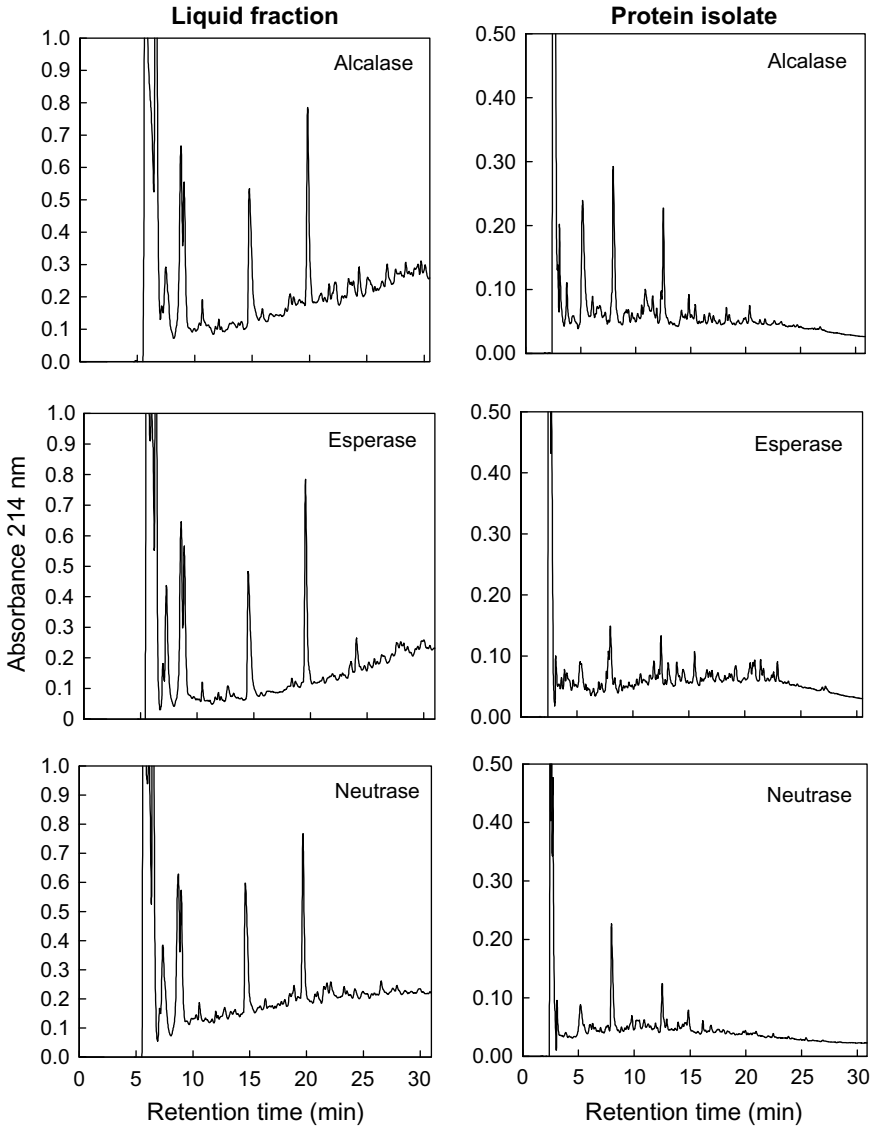

Fig. 1. RP-HPLC profiles of alcalase, esperase and neutrase digests of liquid fraction (A) and protein isolates (B).

3.4. HPLC profiles

The HPLC profiles of the samples are compared in Fig. 1. There were differences between the chromatograms of the digests from protein isolates. However, the profiles from liquid fractions were quite similar. Alcalase seemed to be most effective in hydrolysing potato proteins, as several degradation products were observed

3.5. Fractionation of ACE-inhibitory components

The potato fruit liquid and alcalase-hydrolysed liquid fractions were selected for the purification of active components. The fruit liquid sample was first concentrated with SepPak cartridges and, after drying, fractionated by reversed-phase chromatography. The alcalase-hydrolysed liquid fraction was fractionated into four fractions (10 ml), and ACE-inhibitory activity was found in the first three fractions. The fraction eluted from 10 to 20 min was further fractionated to 1.5 min fractions. Rather low ACE inhibition was observed for all of the collected fractions, with ACE inhibition varying from 19.7% to 29.8% and protein concentration from 0.7 to 1 mg/ml. These fractions were not purified further. The concentrated liquid fraction was divided similarly as the hydrolysate, which is shown in Fig. 2A. Of the four collected fractions, the first three possessed ACE-inhibitory activity. Fraction 3, corresponding to peptides eluting between 20 and 30 ml, was selected for further purification of the ACE-inhibitory compound. Its elution profile is shown in Fig. 2B. Altogether, 14 fractions were collected and ACE-inhibitory activity was found in three peaks. The most active peaks (peaks 8 and 12) were purified further, and peak 8 was split into two peaks (8A and 8B). Table 3 describes the characteristics of these fractions. All three fractions exhibited similar ACE inhibition. Alanine, proline, glutamine and glycine were the most common amino acids in peaks 8A and 8B. The mass spectrometer analyses showed that peaks 8A and 8B contained more than one compound, and so the peaks needed to be purified further for specific identification of the active compounds. The amino acid composition and size of the identified ACE-inhibitory peptides are known to vary, and several peptides with similar mass have been purified from other sources (Pihlanto & Korhonen, 2003). However, the potential impact of other compounds on the observed ACE inhibition should be taken into consideration.

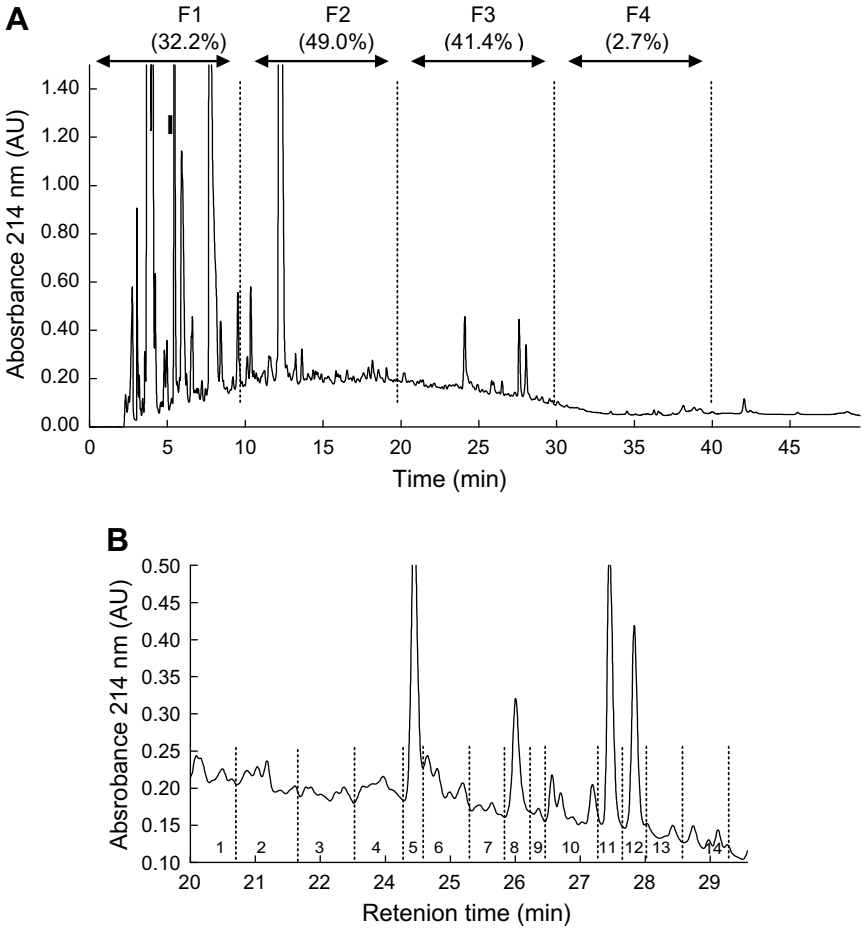

Fig. 2. RP-HPLC chromatogram of potato fruit liquid fraction. Fractions collected are numbered and the percentages of ACE-inhibitory activity are presented. The lower chromatogram is part of fraction 3 under different chromatographic conditions. Each peak of this chromatogram was collected separately and the ACE-inhibitory activity was tested.

Table 3
Characteristics of the most active peaks collected from the second RP-HPLC

Fraction	Asp	Glu	Ser	Gly	Thr	Ala	Pro	Val	Ile	Leu	Phe	Lys	Observed mass	ACE-inhbition (%)
F8A	0.19	0.36	0.26	0.52	0.22	3.70	0.33	0.24	0.21	0.26	0.14	0.20	704.3 850.5	25.8
F8B	0.15	0.31	0.22	0.41	0.17	3.63	0.31	0.23	0.15	0.19	0.12	0.13	704.2 850.5	24.3
F12	0.35	0.47	0.38	0.74	0.42	3.89	0.65	0.37	0.29	0.27	0.22	0.27		15.6

4. Conclusions

Our findings imply that potato isolates and by-products from the potato industry comprise a source of bioactive compounds with ACE-inhibitory and antioxidant activities. The bioactivities of protein hydrolysates were most likely related to peptides and /or free amino acids liberated during digestion. However, the possibility of other unknown compounds should also be kept in mind. The phenolic compounds presumably induce the observed ACE-inhibitory and radical-scavenging potencies of the by-products – the pulp, liquid and peel fractions. The results of this study indicate that potato proteins are a promising source for the production of bioactive compounds as materials for developing functional foods with a positive impact on cardiovascular health. Moreover, food by-products have the additional advantage of representing a value-added outlet for inexpensive starting material. Further studies are still required to identify the active compounds and to investigate the *in vivo* antihypertensive activity of the hydrolysates. An economic analysis for the whole process from the collection of by-products to the production of the powered peptide mixture would provide valuable information for the food industry.

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