Effect of heat treatment on re-solubility of potato proteins isolated from industrial potato fruit juice

V. Bártová, J. Bárta

Biotechnological Centre, Department of Plant Production, Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic

Abstract: The contribution deals with thermal stability and re-solubility of potato tuber proteins isolated by ethanol precipitation from industrial potato fruit juice. The protein isolate was exposed to the temperatures ranging from 25°C to 70°C. Patatin, the tuber protein with a high nutritious value, was detected by SDS-PAGE in the region of 39–43 kDa. Patatin was evaluated as thermal sensitive – temperatures above 30°C caused its strong insolubility. Potato protease inhibitors were detected in the region from 4.3 to 24 kDa. Thermal stability of potato protease inhibitors (region from 25 to 14 kDa) was higher, although the temperatures above 45°C caused denaturation and insolubility of most of the protease inhibitors. Extremely thermo-stable was potato carboxypeptidase inhibitor with molecular weight of 4.3 kDa that remained soluble even after having been exposed to the highest temperatures.

Keywords: potato proteins thermo-stability; potato fruit water; patatin; protease inhibitors

The subject of food proteins, originating in plants, is dominated by the use and processing of soya proteins. However, other plant proteins are of topical interest, for example those derived from other legume seeds or even from the waste of plant processing (Márquez *et al.* 1998). Such a waste by-product is potato fruit juice (PFJ) remaining after starch manufacture, which is currently used as animal feed or low-quality fertiliser. PFJ contains 2–5% of solids, of which the protein represents about 25% (Knorr 1978; Koningsveld *et al.* 2001). Potato protein is well known particularly for its high digestibility and favourable amino acid composition (Kapoor *et al.* 1975; Bárta & Čurn 2004), being thus suitable for human nutrition. The potato proteins present in PFJ have been tentatively classified into three groups (Pots 1999). Patatin, the major potato tuber protein, comprises 38% of the proteins in PFJ. The protease inhibitors make up from 45 to 50%, and other proteins represent from 12 to 15% of total protein in PFJ (Pouvreau *et al.* 2001; Bárta *et al.* 2008). Protein recovery from industrial PFJ has been recently achieved by heat coagulation, which is an efficient method, however, the resulting product has unacceptable flavour and functionality (Zwijnenberg *et al.* 2002). Several alternatives have been reported for the recovery of native protein from industrial PFJ (Lindner *et al.* 1981; Gonzales *et al.* 1991; Koningsveld *et al.* 2001; Zwijnenberg *et al.* 2002; Bárta *et al.* 2008), however these methods have not yet been incorporated in the process of industrial production of potato protein from waste PFJ, because of the reasons such as financial costs, high contents of glycoalcaloids and potassium or partial denaturation of the isolated protein. The effect of the heat treatment on the potential food proteins needs also to be known, because that treatment is often a necessary processing step in the food manufacture (heat drying, heat sterilisation, concentrating potato fruit juice prior to protein precipitation). In addition, the heat treatment may prove to be unavoidable for diminishing the activity of potato proteinase inhibitors. The purpose of this study was to examine the effect of the heat treatment on the re-solubility of potato protein fractions isolated from acidified industrial potato fruit juice by using ethanol precipitation.

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MATERIALS AND METHODS

Potato fruit juice (PFJ) was provided from a potato starch manufacture (Lyckeby Amylex a.s, Horažďovice, Czech Republic). PFJ was centrifuged (15 min, 3600 rpm, 4°C) and the supernatant was filtered. 30 ml of PFJ was freeze-dried, using freeze drier Alpha 1-4 (Martin Christ, Osterode am Harz, SRN), to constant weight for the gravimetrical determination of PFJ dry matter in four replications. The dry matter obtained was subsequently used for total N content determination using modified Dumas method and nitrogen/protein analyzer Flash EA 1112 (ThermoQuest, Italy). The average weight of the analysed sample was 50 mg. The analysis of nitrogen content was made in duplicates. The resulting PFJ characteristics were: pH 5.81; dry matter content 5.55%, and the content of N 3.76 mg/ml.

On the basis of the previously presented results of Koningveld *et al.* (2001) and Bárta *et al.* (2008), potato protein precipitation was performed using absolute ethanol at 0°C. The precipitation was performed in 20 replications (variants of heat treatment plus their replications) and was initiated by adjusting the pH of stirred PFJ samples in 50 ml Fisher tubes to the final pH of 5.0. 10 ml of undercooled absolute ethanol was added to 30 ml of acidified PFJ. The precipitation was performed for 1 hour on ice. The samples were centrifuged (15 min; 4°C; 3600 rpm) and the precipitates formed were washed twice by suspending them in 5 ml of 0.1M sodium-acetate buffer, pH 5.0, containing the equivalent amount of ethanol. After each washing step, the samples were centrifuged (15 min; 4°C; 3600 rpm).

The washed protein concentrates in Fisher tubes were used for the evaluation of the effect of the heat treatment on potato proteins re-solubility. The heat treatment was performed in Binder incubator and dryer (Binder Inc., New York, USA). The variants temperatures used were the following: freeze-drying (control variant of native protein), 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, and 70°C. Next, the remaining moisture in the protein concentrates was removed by freeze drying and the re-solubility was evaluated in the following way. Two samples each of the respective variant in original tubes were used for the determination of the re-solubilisation of the precipitated protein. The precipitates were suspended in 20 ml of 100mM sodium phosphate buffer pH 7.0, then thoroughly shaken in the buffer and incubated for 1 hour at 30°C. The tubes were centrifuged (15 min; 22°C; 3600 rpm) and the supernatant was sampled for the determination of the re-soluble protein composition with SDS-PAGE. The non-resoluble part of the precipitates was freeze-dried and analysed for nitrogen content as described above. Protein N of PFJ was determined as N recovered by TCA precipitation (Bollag *et al.* 1996). The average N content in PFJ was 3.76 mg/ml, 65.16% was precipitated with TCA; this N is determined as protein N. The protein content (N × 6.25) was 15.3 mg/ml. The re-soluble part of the precipitated protein was determined as the precipitated protein minus non-resoluble protein.

The composition of the re-soluble parts of the protein precipitates was determined by SDS-PAGE (Laemmli 1970). 100 µl of the collected re-soluble protein samples was mixed with 25 µl of loading buffer (5 ml 1.25M Tris-HCl, pH 6.8, 2.3 g SDS, 10 ml glycerol, 5 mg Bromophenol Blue; to 500 µl of this buffer 170 µl 2-mercaptoethanol was added). The protein separation was performed using the standard cooled dual vertical slab units SE 600 (Hoefer Scientific Instruments, San Francisco, USA). The discontinuous gel system (Hames & Rickwood 1987) was used (4% stacking gel and 10% separating gel. The gel processing after the protein detection with Coomassie Brilliant Blue R-250 was performed after Hames & Rickwood (1987). The individual protein components were detected on electrophoretic profiles by digital image analysis using special software BioProfil 1D++ – measuring of absorbance profiles and computation of individual portions (BioProfile software package, Vilbert-Lourmat, Marne la Vallée, France).

RESULTS AND DISCUSSION

The basic property necessary for the utilisation of protein concentrates in food and feed industry is the re-solubility of the precipitated and dried protein powder, suitable for use from the health point of view and possessing suitable nutritional and biological values. The re-solubility is a value that can be directly connected with the degree of denaturation of the protein isolates studied and this value can be suitable for the presentation of the biological value (native properties) of the potato protein powder obtained (Bárta *et al.* 2008). The used term solubility issues from the methodology of Koningsveld *et al.* (2001). The solubility data presented were not really solubility, since this should be expressed as the amount per unit volume. The proportion of the total protein that became insoluble was used as an index of solubility. We kept the re-solubilisation volume constant and the re-solubility was expressed as the proportion of the total protein originally present in PFJ. The re-solubility data indicated the differences

Table 1. Amount of protein (mg/ml of PFJ) remaining re-soluble or insoluble after treatment under different temperature regimes

Temperature of protein isolate treatment (°C)	Amount of protein (mg/ml of PFJ)			
	re-soluble		insoluble	
Freeze-drying	10.1	e	5.2	a
25	7.9	de	7.5	ab
30	5.9	cd	9.4	bc
35	5.2	bc	10.2	cd
40	3.8	abc	11.6	cde
45	3.2	ab	12.1	de
50	2.7	a	12.7	de
55	3.3	ab	12.1	de
60	2.3	a	13.1	e
70	1.8	a	13.6	e

Levels followed by the same letter are not significantly different (HSD test at 5% level)

in denaturation of the potato protein that was exposed to different temperature regimes.

As can be seen from the data presented in Table 1, the amount of totally precipitated protein was approximately 15 mg from 1 ml of industrial PFJ. The amount of the re-soluble protein was naturally highest when using freeze-drying; when using the high temperatures (60°C, 70°C) for the protein treatment, the protein powders obtained were almost absolutely insoluble. Figure 1 shows the relative abundance of re-soluble protein in total protein precipitated from potato fruit juice. As can be seen, maximal temperature usable for the potato protein treatment is 40°C; the temperatures above this point caused that almost 90% of the total precipitated protein remained insoluble. The results obtained indicated differences in precipitation and thermal stability of the protein components forming the total tuber protein of PFJ. Approximately 20% of the PFJ protein seems to be very sensitive, losing the native properties just before, during, or immediately after the precipitation process because this part of the precipitated PFJ protein remained insoluble just after freeze drying. Freeze drying is generally used for biological products that have a high sensitivity to heat and most proteins fall into this category (Harrison *et al.* 2003); Freeze drying should not cause denaturation of the treated protein. The remaining proteins precipitated from PFJ are less or more thermal sensitive which can be explained by high heterogeneity of the PFJ proteins. When using the minimal temperature (25°C), protein denaturation and the following insolubility of the precipitated protein could be rather explained by partial degradation of the potato protein by proteases that should be inactivated at this temperature only by protease inhibitors. When using higher temperatures, thermal stability of the protein components and their isoforms differ considerably,

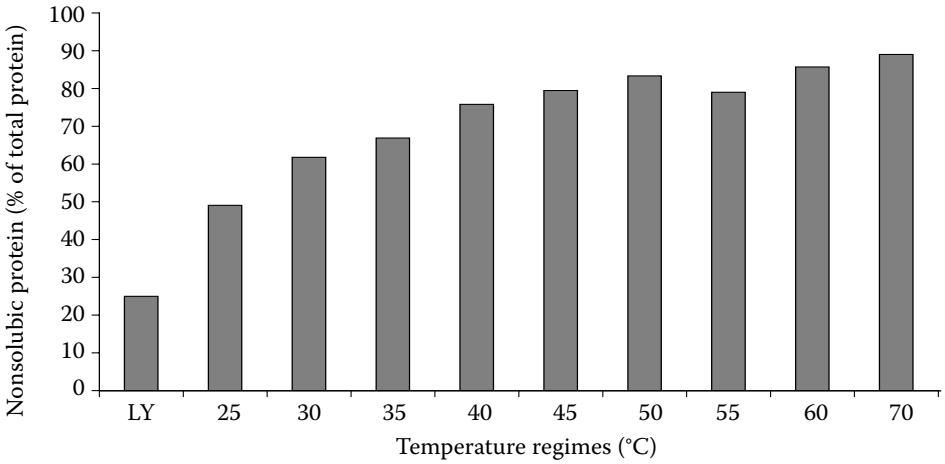

Figure 1. Increasing of relative amount of protein remaining insoluble after heat treatment of protein concentrates under different temperatures regimes

Temperature regimes of protein isolates treatment ranging from freeze-drying (LYO) to 70°C

Table 2. Representation of the main protein components remaining re-soluble after exposure of the potato protein isolated from industrial PFJ to different temperature regimes

Temperature (°C)	Representation of the main protein components in total protein (%)		
	patatin proteins 39–43 kDa	protease inhibitors 25–14 kDa	protease inhibitors 14–4.3 kDa
Freeze-drying	39.3 ± 0.82	43.5 ± 0.61	16.2 ± 0.43
25	34.7 ± 0.35	45.8 ± 0.54	18.6 ± 0.87
30	32.0 ± 1.17	45.6 ± 0.46	22.4 ± 0.71
35	17.5 ± 0.98	52.9 ± 1.30	29.6 ± 0.32
40	13.4 ± 0.41	54.6 ± 0.17	31.1 ± 0.23
45	12.6 ± 0.55	46.5 ± 1.23	40.9 ± 0.96
50	13.9 ± 0.62	43.2 ± 1.32	41.3 ± 0.47
55	12.0 ± 1.06	44.1 ± 2.16	43.8 ± 3.22
60	4.6 ± 0.08	13.7 ± 1.55	81.6 ± 1.46
70	0.0 ± 0.00	0.0 ± 0.00	100 ± 0.00

as can be seen in Figure 2. In this figure (above all re-soluble potato protein after freeze-drying) protein bands are recognisable in two molecular mass regions – the main bands in the region approximately from 39 to 43 kDa, and the minor bands found in the region approximately from 4.3 to 25 kDa. The potato proteins present in PFJ have been tentatively classified into three groups (Pots 1999). No overall quantitative data on the protein composition of the potato varieties are available, except those from the cultivars Elkana (Pouvreau *et al.* 2001) and Tomensa (Bárta *et al.* 2008). Patatin, the major potato tuber protein (39–43 kDa), comprises approximately 38% of protein; protease inhibitors (25–4.3 kDa) make up about 50% and other proteins up to 12% of total protein in PFJ (Pouvreau *et al.* 2001; Bárta *et al.* 2008). Patatin represents the most important protein component of the tuber protein for its high nutritional quality (Bárta & Čurn 2004), foaming and emulsifying properties (Ralet & Guégen

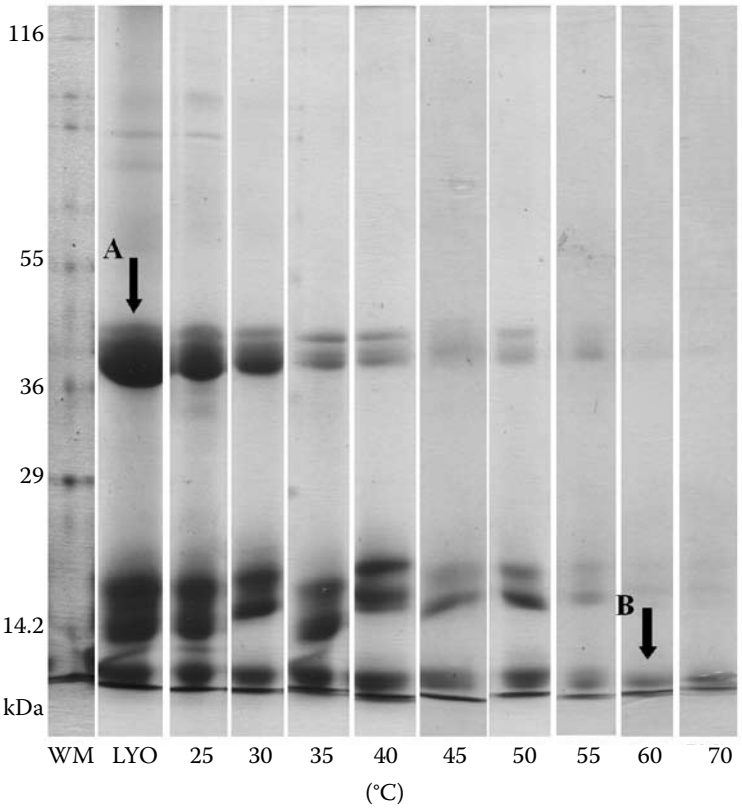

Figure 2. SDS-PAGE spectra of tuber protein components isolated from industrial PFJ remaining re-soluble after heat treatment under various temperature regimes

Temperature regimes of protein isolates treatment ranging from freeze-drying (LYO) to 70°C

Letter A indicates the region of patain proteins (39–43 kDa) in re-soluble part of tuber protein isolated from industrial potato fruit juice; Letter B indicates extremely thermostable carboxypeptidase inhibitor with molecular mass of 4.3 kDa

2001), specific enzymatic activity (Andrews *et al.* 1988; Senda *et al.* 1996; Jimenez *et al.* 2001), or antifungal activity (Tonón *et al.* 2002). In contrast to patatin, the protease inhibitors are a more heterogeneous group of the tuber proteins. They differ with respect to molecular mass, amino acids sequence, and inhibitory activity (Pouvreau *et al.* 2001). The general characterisation of the protease inhibitors is that they are small, cysteine-rich, and heat-resistant proteins of 3–23 kDa (excluding tandemly repeated inhibitors domains resulting in inhibitors of 36 to 85 kDa). According to Figure 2 and Table 2, a lower thermal stability of patatin proteins is obvious. The fault point was detected at 30°C and all the temperatures above this point caused strong denaturation and insolubility of patatin proteins. This evaluation confirms the data of Pots (1999) who established that patatin is thermally destabilised at temperatures exceeding 28°C, as indicated by near-ultra violet circular dischroism. It was shown that parts of the α-helical contribution unfold in the 45°C to 55°C region, whereas the β-stranded parts unfold gradually at temperatures from 50°C to 90°C. The observed unfolding of the protein coincides with the inactivation of the patatin enzyme activity and with the precipitation which occurs in the potato fruit upon heating. At high temperatures, patatin still contains some helical and stranded structures. Upon cooling, the protein partly refolds; it was observed that mainly α-helical structures were formed. Thermal stability of potato tuber protease inhibitors has been discussed previously by Pouvreau (2004) in view of protease inhibitors of the cultivar Elkana, although there is no information about protease inhibitors thermo-stability in the case of PFJ which represents a specific cultivar mixture. Thermal unfolding and a significant reduction of the inhibition activity of PSPI (Potato Serine Protease Inhibitors) and PCPI (Potato Cystein Protease Inhibitors), which together represent 40% of the total protein content in potato juice, were observed by Pouvreau (2004) in the case when the point of 40°C was exceeded. These data are entirely confirmed by our data of thermal stability of PFJ potato protease inhibitors as can be seen in Figure 2 and Table 2. The exception in thermal stability is represented by potato carboxypeptidase inhibitor (PCI) that is the only one protein that remains soluble even when the high temperatures of 60°C and 70°C are used. PCI is the smallest of the protease inhibitors present in potato, with the molecular weight of 4.3 kDa, and is known to be extremely stable upon heating (Huang *et al.* 1981).

The results obtained during the experiments may be concluded by stating that the treatment (drying, concentration, protease inhibitors inactivation) of potato proteins isolated from industrial PFJ at temperatures above 40°C caused intensive degradation of most of the proteins present in the protein isolate obtained from industrial potato fruit juice.

CONCLUSIONS

Patatin, the major storage tuber protein with a high nutritious value, was detected in protein isolates by SDS-PAGE in the region of 39–43 kDa and was evaluated as thermal sensitive because temperatures above 30°C caused its strong insolubility.

Potato protease inhibitors were detected in protein isolates in the region from 4.3 to 24 kDa. Thermal stability of potato protease inhibitors was higher, although the drying temperatures above 45°C caused denaturation and insolubility of most of the protease inhibitors.

Potato carboxypeptidase inhibitor with molecular weight of 4.3 kDa was extremely thermo-stable as if remained soluble even when the high temperatures had been used.

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Abstrakt

Bártová V., Bárta J. (2008): **Vliv tepelného ošetření na zpětnou rozpustnost hlízových proteinů brambor izolovaných z průmyslové hlízové vody.** Res. Agr. Eng., **54**: 170–175.

Tato práce se zabývá termální stabilitou izolátů hlízových proteinů získaných z průmyslové hlízové vody, která vzniká jako vedlejší produkt při zpracování brambor na škrob. Patatin, hlízový protein s vysokou nutriční hodnotou, byl pomocí techniky SDS-PAGE detekován v oblasti 39–43 kDa. Patatin byl vyhodnocen jako termolabilní protein, neboť použití teplot nad 30 °C způsobilo jeho silnou nerozpustnost. Bramborové inhibitory proteas byly detekovány v oblasti 4,3 až 24 kDa. Termální stabilita inhibitorů byla vyšší než patatinu, avšak teplota nad 45 °C způsobila denaturaci a nerozpustnost většiny inhibitorů proteas. Extrémní termostabilita byla zjištěna u karboxypeptidasového inhibitoru s hmotností 4,3 kDa, jenž si zachoval zpětnou rozpustnost i při použití nejvyšších teplot (70 °C) pro ošetření proteinového izolátu.

Klíčová slova: termální stabilita hlízových bílkovin brambor; hlízová voda brambor; patatin; inhibitory proteas

Corresponding author:

Ing. Veronika Bártová, Jihočeská univerzita v Českých Budějovicích, Zemědělská fakulta, katedra rostlinné výroby, sekce rostlinné biotechnologie, Studentská 13, 370 05 České Budějovice, Česká republika
tel.: + 420 387 772 441, fax: + 420 387 772 431, e-mail: vbartova@zf.jcu.cz