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ARTICLE

Chemical Composition and Nutritional Value of Protein Concentrates Isolated from Potato (*Solanum tuberosum* L.) Fruit Juice by Precipitation with Ethanol or Ferric Chloride

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Effects of protein precipitators, ethanol and ferric chloride, on yield, resolubility, chemical composition and nutritional value of protein concentrates isolated from industrial potato fruit juice (PFJ) were studied. Optimum precipitating concentrations of ethanol and ferric chloride in PFJ were 4 M (23.1% v/v) and 20 mM (2% w/v), resulting in yield of 69% and 86.5% of total protein, respectively. Contents of total glycoalkaloids and potassium in both protein concentrates were significantly lower ($P < 0.05$) as compared with contents in PFJ dry matter. Both protein concentrates exhibited high nutritional value; values of essential amino acid index (EAAI) were 81.7% and 82.7%, respectively. Fraction of patatin proteins (39−43 kDa) represented with EAAI value of 86.1% the nutritionally improving protein component. Lipid acyl hydrolase activity of patatin family was not negatively affected by cooled ethanol precipitation. It can be thus suggested that biological and enzymatic activities of this protein family are utilizable after this type of precipitation.

KEYWORDS: Potato; *Solanum tuberoslum* L.; potato tuber proteins; potato fruit juice; potato protein precipitation; protein concentrates composition; patatin family

INTRODUCTION

Potato tubers comprise about 2% of nitrogen compounds on a fresh basis, of which proteins represent 35−75% (*1*). The content of total potato protein is affected by many extrinsic and intrinsic factors, such as are cultivar, location, fertilization, year and system of crop management (*2*). Potato fruit juice (PFJ) is an aqueous byproduct of the potato starch industry containing most of the tuber protein released after grinding of potato tubers in a rasping machine. PFJ dry matter content varies between 20 and 50 g/kg, of which weight proportion of crude protein represents approximately one-third. The PFJ crude protein contains proteins, peptides, amino acids and amides (*3*, *4*). Three protein groups can be specified. Patatin, the major potato tuber protein (39−45 kDa), protease inhibitors (4−25 kDa) and the other proteins of higher molecular weight comprise 30−40, about 50 and 10−20% of total protein weight, respectively (*3*, *5*). In addition to easily soluble fraction of protein components, PFJ contains carbohydrates, lipids, organic acids, polyphenols, minerals, fiber and glycoalkaloids. All of these components affect the isolation process as well as the functional properties and qualitative parameters of obtained protein isolates.

The process of PFJ protein recovery from such a dilute aqueous system is a challenge in terms of expenses. The most common system of protein recovery from industrial PFJ is through heat coagulation and acid precipitation, which however results in products with unacceptable flavor and functionality and is usable only as a feed (*6*). Potato protein recovered from PFJ in a non-denaturized form under the conditions of a careful isolation has satisfactory solubility and significant foaming and emulsifying properties, and the enzymatic activities of individual potato tuber proteins are preserved (*3*, *4*, *7*). Furthermore, the protein of potato tubers has also high nutritive value (*1*), and therefore, the potato protein concentrates can be an interesting commodity potentially usable in food and feed industry as well as in biotechnological applications. Several less or more successful alternatives have been found for recovering of native potato protein from industrial PFJ (*3*, *4*, *8*−*10*). Limiting factors in each of the "native" protein isolation method there are the yield of isolation process, degree of isolated protein denaturation (*4*), functional properties of yielded protein and its individual fractions (*11*), and also the content of accompanying components that cause the considerable decrease of the protein qualitative parameters and of its potential for food application (*7*). For instance, application of carboxymethyl cellulose was reported to be successful in recovering potato proteins from PFJ (*8*). However, since the binding takes place at low pH (1.5−4.0), the resulting product can be expected to be at least partially denatured. Ion exchange, using the expanded bed adsorption technique, is well-suited method for the recovery of protein from PFJ. This method makes it possible to remove antinutritional factors and low-molecular weight components (*7*). However, such a process would be too expensive for industrial applications.

Precipitation of potato tuber protein from PFJ with low-molecular additives has been previously described as a promising method for isolation of non-denatured potato protein from PFJ (*3*, *12*). The protein concentrates prepared by the ethanol

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precipitation technique were evaluated from the point of view of structure, solubility and foaming properties of the obtained protein isolates (*13*, *14*). On the base of presented data (*3*, *4*), there were selected the most promising precipitation additives, ethanol and ferric chloride, that have a potential for the isolation of maximum amount of native protein from industrial PFJ. The present article deals with (1) the effect of increasing concentration of precipitation agents ethanol or $FeCl_3$ on yield and resolubility of protein isolated from PFJ; (2) the chemical composition of protein concentrates obtained by ethanol or $FeCl_3$ precipitation; (3) proportion of the individual protein components (especially patatin and protease inhibitors) in obtained concentrates; (4) purification of patatin from protein concentrates and analysis of patatin lipid acyl hydrolase activity and nutritional value as a reference standard with the precipitates.

MATERIALS AND METHODS

Preparation of Protein Concentrates with Different Concentrations of Ethanol and $FeCl_3$. Potato fruit juice (PFJ) was provided from a potato starch manufacturer (Lyckeby Amylex, Horažd'ovice, Czech Republic). PFJ was centrifuged (15 min, 1600*g*, 4 °C) and filtered. Thirty milliliters of PFJ was freeze-dried (freeze-dryer Alpha 1-4, Martin Christ, Germany) to constant weight for gravimetrical determination of dry matter. The dried material was subsequently used for the analysis of chemical composition. The precipitation was initiated by adjusting the pH of PFJ samples in 50 mL Fisher tubes to a final value of 5.0 using 0.5 M H_2SO_4. Absolute and cooled ethanol (0−2 °C) and a 1 M solution of $FeCl_3$ were added to 30 mL of acidified PFJ in increasing amounts from 1.5 to 18 mL and from 30 to 600 μL, respectively. The detailed design of the used concentrations of precipitation additives is shown in **Table 1**. The precipitation took 1 h (at 4 °C), and subsequently the samples were centrifuged (15 min; 4000*g*, 4 °C). The formed protein precipitates were washed twice by suspending in 20 mL of 0.1 M sodium-acetate buffer of pH 5.0 containing the equivalent amount of relevant additive. The precipitates were freeze-dried and weighed, and nitrogen contents were determined.

Protein Concentrate Resolubility. Duplicates of each of the variants were resuspended in 20 mL of 100 mM sodium phosphate buffer of pH 7.0. In the variant using $FeCl_3$, the buffer contained 30 mM EDTA. The precipitates were thoroughly shaken in the buffer and incubated for 1 h at 30 °C. After centrifugation (15 min, 4000*g*, 4 °C), the supernatants were sampled and analyzed for the resoluble protein composition using SDS−PAGE technique. The nonresoluble parts of the precipitates were freeze-dried, and dried pellets were analyzed for nitrogen contents as described in the section Nitrogen Content. The content of PFJ-protein nitrogen was determined as nitrogen recovered by trichloroacetic acid (TCA) precipitation (*15*). The precipitated protein is expressed as the proportion of the TCA-precipitable nitrogen content in PFJ. The resoluble part of the precipitated protein was calculated as the precipitated protein minus nonresoluble protein.

Preparation of Protein Concentrates for Determination of Chemical Composition. Chemical composition was determined in protein concentrates isolated by ethanol or $FeCl_3$ precipitation with optimum concentration of precipitating agents and for heat coagulate (see below). The protein precipitates were obtained according to the method mentioned above using ethanol variant G (4.4 M) and $FeCl_3$ variant H (20 mM) (see **Table 1**). Protein concentrates were freeze-dried, and dry matter was analyzed. Heat coagulate was prepared from the same industrial PFJ as used for the precipitation by adjusting pH of stirred PFJ samples in 50 mL Fisher tubes to a final value of 5.0 using 0.5 M H_2SO_4. Acidified PFJ samples were heated (10 min, 100 °C) in a water bath (ShakeTemp, Julabo, Germany). Coagulated protein samples were washed twice by suspending in 20 mL of distilled water, centrifuged (15 min, 4000*g*, 4 °C) and subsequently freeze-dried and used for determination of chemical composition.

Nitrogen Content. Nitrogen content was determined in freeze-dried matter of ethanol and $FeCl_3$ precipitates, heat coagulate, PFJ and freeze-dried pellets of nonresoluble parts of ethanol and $FeCl_3$ precipitates. Nitrogen content was determined in duplicate by the elemental analyzer FLASH EA 1112 (ThermoQuest, Italy). Protein nitrogen (crude protein) was calculated as nitrogen content multiplied by factor 6.25.

Minerals Content. Content of calcium, potassium, magnesium and sodium in dry matter of ethanol and $FeCl_3$ precipitates, heat coagulate and PFJ freeze-dried matter were determined by atomic absorption flame spectrometry (Aurora Instruments, Vancouver, Canada) after digestion with concentrated nitric acid (*16*). Phosphorus was determined spectrophotometrically at 355 nm using KH_2PO_4 as standard (*17*).

Glycoalkaloid Content. Content of potato glycoalkaloids was determined in dry matter of ethanol and $FeCl_3$ precipitates, heat coagulate and PFJ freeze-dried matter. Determination was performed using the HPLC−MS/MS method (*18*). The samples were extracted using methanol, and extracts were filtered and filled to the volume of 100 mL with methanol. HPLC separation was carried out with a column Hypurity Aquastar 100 × 3 mm, 3 μm (Thermo Electron Corporation, USA) and mobile phases: phase A, H_2O:CH_3CN:CH_3OH:0.1 M CH_3COONH_4:CH_3COOH (22:8:4:2:1), and phase B, CH_3CN. Ionization of the HPLC effluent components was carried out in the positive mode. There were determined α-solanin, β_2-solanin, solanidin, α-chaconin, β_1-chaconin, β_2-chaconin and γ-chaconin contents.

Amino Acids Composition. Contents of amino acids were evaluated in dry matter of ethanol and $FeCl_3$ precipitates, heat coagulate and PFJ freeze-dried matter. Amino acids were determined after hydrolysis of the samples with 6 M HCl at microwave field (acid hydrolysis 40 min at 140 °C; oxidative hydrolysis 40 min at 150 °C) followed by separation of the amino acids by ion-exchange chromatography (column AminoPac PA1, Dionex, Sunnyvale, CA) on an HPLC system (Dionex DX-600, Sunnyvale, CA) using postcolumn derivatization. For the determination of methionine and cystine an initial oxidation to methionine sulfone and

Table 1. Concentrations of Precipitation Agents (Ethanol or $FeCl_3$) Used for Precipitation of Potato Tuber Protein from an Industrial Potato Fruit Juice (PFJ)

(**a**) Ethanol

variant	A	B	C	D	E	F	G	H	I	J	K	L
ethanol (M)	1.0	1.6	2.3	2.9	3.4	4.0	4.4	4.9	5.3	5.7	6.1	6.4
ethanol (mL)	1.5	3	4.5	6	7.5	9	10.5	12	13.5	15	16.5	18
PFJ (mL)	30	30	30	30	30	30	30	30	30	30	30	30
ethanol concn in PFJ (% v/v)	4.8	9.1	13.0	16.7	20.0	23.1	25.9	28.6	31.0	33.3	35.5	37.5

(**b**) $FeCl_3$

variant	A	B	C	D	E	F	G	H
$FeCl_3$ (mM)	1	2	4	6	8	10	15	20
μL 1 M $FeCl_3$	30	60	120	180	240	300	450	600
PFJ (mL)	30	30	30	30	30	30	30	30
$FeCl_3$ concn in PFJ (% w/v)	0.1	0.2	0.4	0.6	0.8	1	1.5	2

cysteic acid was carried out with a performic acid/phenol mixture before hydrolysis. To evaluate the quality of isolated protein concentrates, amino acid scores (AAS) and essential amino acid indices (EAAI) were used. Amino acid composition of whole egg protein was used for EAAI estimation (*19*, *20*).

Estimation of Relative Abundance of Individual Protein Components. Proportions of individual protein fractions were estimated in ethanol and $FeCl_3$ precipitates, heat coagulate and PFJ freeze-dried matter using the SDS−PAGE method (*21*). The freeze-dried samples (50 mg) were extracted (4 h, 4 °C) in 1 mL of SDS-extraction buffer: 0.065 M Tris-HCl, pH 6.8, 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol. Protein separation was performed by standard cooled dual vertical slab units SE 600 (Hoefer Scientific Instruments, San Francisco, CA) with a discontinuous gel system (4% stacking and 10% resolving gel) and protein detection with Coomassie Brilliant Blue R-250. Protein fractions were detected on electrophoretic profiles by digital image analysis using software Bio-1D++, Version 99 (Vilber Lourmat, Germany) measuring the absorbance of profiles and computation of individual protein bands proportions according to the software manual.

Purification of Patatin Proteins. Patatin was isolated from ethanol-precipitated protein concentrate using a three-step chromatography procedure. Ethanol-precipitated protein was dissolved in 25 mM Tris HCl buffer (pH 7.4), pH of the protein solution was adjusted to a value of 8 and the solution was loaded on an equilibrated (25 mM Tris-HCl, pH 7.4) anion exchange column, diethylaminoethyl (DEAE) 52-Cellulose SERVACEL (Serva, Germany). After sample application, the column was washed using 25 mM Tris-HCl, pH 7.4. The bound protein fraction was eluted with 25 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl. The collected protein pool was subsequently loaded on an affinity column, concanavalin A (ConA) Sepharose 4B (Pharmacia Biotech, GE Healthcare, Uppsala, Sweden). Equilibration and washing steps of the affinity column were performed with 25 mM Tris-HCl, pH 7.4 + 0.5 M NaCl. The bound protein fraction was eluted using 25 mM Tris-HCl (pH 7.4) + 0.5 M NaCl + 100 mM α-methyl-D-glucoside. The collected patatin fraction was desalinated on Sephadex G-25 gel filtration column.

Determination of Lipid Acyl Hydrolase Activity. Lipid acyl hydrolase activity was determined in extractable supernatants (Tris-HCl, pH 7.4) of freeze-dried PFJ, ethanol and $FeCl_3$ precipitates, heat coagulate and in purified patatin solution. LAH activity is the main enzyme activity of patatin, and we used this enzyme activity as an indicator of preservation of the native form of the isolated tuber protein. Three replicates of each sample were used for enzyme specific activity determination. Enzyme reaction was performed in a 1.5 mL reaction tube, and the working solution consisted of a preincubated mixture of 180 μL of Tris-HCl buffer, pH 7.4 + 10 μL of substrate solution (10 mM *p*-nitrophenyl butyrate). Reaction was started by adding of 10 μL of extracted supernatant. After incubation at 37 °C for 10 min, the reaction was stopped by sample heating (100 °C, 30 s.). The absorbance was measured at the wavelength of 410 nm using a spectrophotometer BioMate 5 (ThermoElectron, U.K.). BCA Protein Assay Kit (Pierce, USA) was used for determination of pure protein content in extractable supernatants. Bovine serum albumine (BSA) was used as standard for protein determination. The absorbance was measured at the wavelength of 405 nm.

Statistics. Data were subjected to analysis of variance by the factorial ANOVA method and means comparison by Tukey HSD test. Differences among samples were considered significant at $P < 0.05$ unless stated otherwise. The program Statistica 6.0 (StatSoft, USA) was used for data analysis.

RESULTS AND DISCUSSION

Yield and Resolubility of Protein Concentrates Isolated from PFJ. As can be seen from **Figure 1A**, yield of potato protein precipitated from industrial PFJ ranged, in dependence on different saturation with ethanol, from 0.55 to 2.02 mg/mL of PFJ, corresponding to the proportion from 21.3 to 77.9% of total protein, respectively. Ethanol concentration in PFJ was performed stepwise: the lowest value was 1.0 M, the highest one 6.4 M. The ethanol concentration increasing gradually by 0.6 M caused a mean increase of protein yield by about 0.26 mg/mL. The increasing yield of the precipitated protein was linear for 1−4 M ethanol, and then followed a decline.

Resolubility of precipitated protein ranged, in dependence on ethanol concentration, from 0.35 to 1.73 mg/mL. A maximum protein yield and resolubility was observed between 4.0 and 4.4 M saturation with ethanol, and this range of concentration should be thus considered to be optimum for potato protein isolation from PFJ. However, using optimum ethanol concentration, approximately 22% of precipitable protein remains in solution and, as can be seen from the SDS−PAGE analysis (**Figure 2C**), the remaining protein consisted of both patatin isoforms and proteins with M_r below 25 kDa (probably protease inhibitors). It should be considered that precipitation of proteins or protein isoforms sensitive to ethanol precipitation was preferred and filled the precipitation capacity of ethanol. On the other hand, we used for the experiments the "real" PFJ from starch manufacture with the possibility of partly denatured protein that was difficult to precipitate. Differences in PFJ used might explain differences in our results and results of van Koningsveld et al. (*4*), who prepared PFJ from cultivar Elkana and found the optimum concentration of ethanol at 4.9 M, yielding 83% of total protein as resoluble proportion.

Yield of protein precipitated from industrial PFJ ranged in our experiments, in dependence on different saturation with $FeCl_3$, from 0.48 to 2.25 mg/mL of PFJ, corresponding to the proportion from 18.6 to 86.5% of total protein, respectively. As can be seen from **Figure 1B**, no maximum point with following decline point was found for protein yield and resolubility. Ferric chloride concentration of 20 mM can be considered as the optimum for

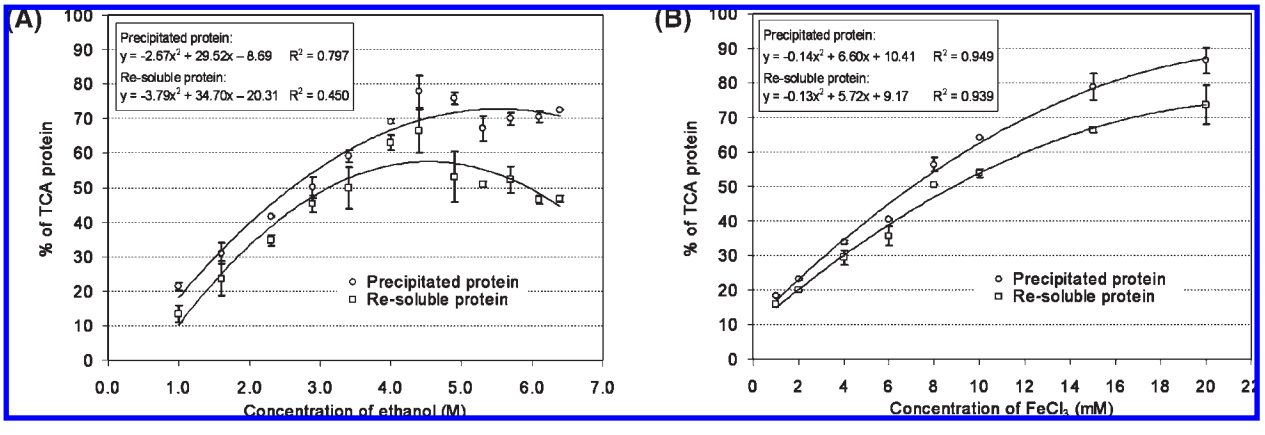

Figure 1. (**A**) Effect of increasing concentration of ethanol in PFJ on yield and resolubility of precipitated protein. (**B**) Effect of increasing concentration of ferric chloride in PFJ on yield and resolubility of precipitated protein.

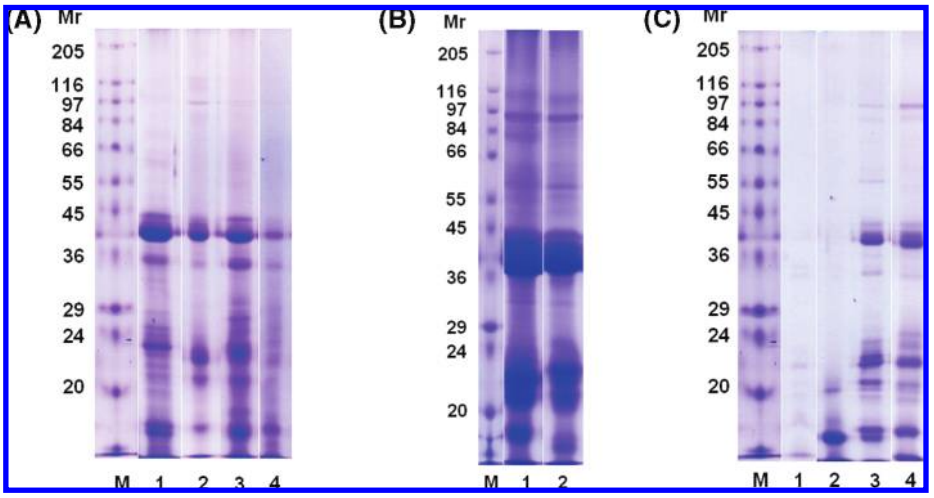

Figure 2. (**A**) SDS−PAGE of protein samples derived from SDS-extraction buffer; extraction was made from the following materials: (1) PFJ dry matter, (2) heat coagulate, (3) ethanol concentrate, (4) ferric chloride concentrate; M, protein marker in kDa. (**B**) SDS−PAGE of resoluble protein fraction of precipitates isolated from PFJ by (1) ethanol and (2) ferric chloride precipitation; M, protein marker in kDa. (**C**) SDS−PAGE of protein fraction remaining in solution after precipitation with (1) TCA, (2) heat coagulate, (3) ethanol, (4) ferric chloride; M, protein marker in kDa.

Table 2. Estimation of Proportion of Extractable Protein Components of Protein Concentrates Isolated from Potato Fruit Juice and Determined after SDS−PAGE[a]

		proteins with majority of protease inhibitors		
type of protein concentrates	patatin 39−43 kDa	25−21 kDa	20−15 kDa	14−0 kDa
PFJ	30.7 a	25.4 b	4.8 b	22.5 b
heat coagulate	32.3 a	33.0 a	3.3 c	3.2 d
ethanol precipitate	25.6 b	29.2 ab	7.2 a	14.5 c
$FeCl_3$ precipitate	20.3 c	18.3 c	7.9 a	32.3 a

[a] Means followed by the same letter in a column are not significantly different (HSD test at 5% level).

protein isolation from PFJ, because it is improbable to precipitate more than about 85% of total PFJ protein with high resolubility. Effects of metal ions on protein precipitation were described in previous publications (*3*, *4*, *22*). Our present results are in general agreement with refs *2* and *3*. These authors used three types of inorganic salts ($FeCl_3$, $FeSO_4$ and $ZnCl_2$), and only $FeCl_3$ gave promising results for its usage in practical applications. Maximum concentration of $FeCl_3$ used by other researchers (*4*) was 16 mM, resulting in yield of approximately 45% of precipitated potato tuber protein. Lower yield could be explained by differences in PFJ used, particularly by contents of polyphenols, because metal salts could form complexes with proteins as well as with polyphenols, which could influence yield of precipitated protein (*23*).

Estimation of Relative Abundance of Protein Components in Potato Protein Concentrates. Table 2 shows estimation of relative abundance of individual protein components presented in extract derived from SDS-extraction of dry matter of PFJ, heat coagulate and protein concentrates. The used industrial PFJ was a mixture of several unidentified cultivars, and it is thus impossible to compare the relative abundance of individual protein components with other authors because these types of quantitative data were not published. As mentioned previously, potato protein of PFJ could be classified into three groups: patatin, protease inhibitors and other proteins (*3*, *5*, *24*). Patatin, the major potato protein comprised 38%, protease inhibitors make up about 50% and other proteins up to 12% of total protein in PFJ prepared from cultivar Elkana (*5*). Protein composition of industrial PFJ used in our experiments approximately corresponds with these data. However, the difference was obvious in patatin content that comprised only 30.7% of the PFJ total protein (**Table 2**, **Figure 2A**). Protein composition of heat coagulate differs significantly from ethanol and $FeCl_3$ precipitates in patatin proportion. Patatin, which represents a nutritionally and functionally very interesting protein, is thermally sensitive according to the literature data (*24*, *25*). Heating of industrial PFJ caused total isolation of this protein, but the heat coagulate was almost completely insoluble including the patatin fraction. Thus, these types of protein isolates are applicable only in the feed industry and are not suitable for food or biotechnological applications. The finding of significantly lower proportions of patatin in ethanol and $FeCl_3$ protein concentrates (25.6 and 20.3%, respectively) was connected with lower extractability in SDS-extraction buffer and higher proportion of protein fraction (probably protease inhibitors) with molecular weight below 25 kDa. These proportional changes could be given by different thermostability of PFJ protein components. Pots et al. (*25*) found that temperatures above 28 °C caused patatin unfolding and irreversible changes. Protease inhibitors with low-molecular weight are more thermostable (*26*), and their proportion in heat coagulate is thus lower. The remarkably heat stable potato protein is carboxypeptidase inhibitor, the smallest protease inhibitor of potato tubers, for which inhibiting activity decreased at pH 7.0 after treatment with temperatures above 150 °C (*26*). Molecular weight of carboxypeptidase inhibitor was determined around 8 kDa using SDS−PAGE analysis (*27*) and ranged from 4.0 to 4.6 after MALDI-TOF MS analysis (*26*, *27*). This protein is difficult to isolate from PFJ by heat coagulation, and the majority probably remains in the solution (**Figure 2C**). The higher heat stability of carboxypeptidase inhibitor and alternatively also other proteins with lower molecular weight is suggested also from the difference in proportion of the protein fraction of 0−14 kDa shown in **Table 2**. **Figure 2B** shows SDS−PAGE of resoluble protein fraction prepared by resuspension in sodium phosphate

buffer, pH 7.0 (when using $FeCl_3$ the buffer contained EDTA). Relative abundances of patatin proteins in these resoluble parts of ethanol and $FeCl_3$ concentrates were 32.5 and 35.2%, respectively (data not shown). Relative abundance of patatin and protease inhibitors in protein concentrates obtained by various precipitation additives (organic solvents, inorganic salts) were previously reported in ref *12*. However, ref *12* expressed the relative abundances as the intensity (markers +/−) of the protein bands obtained via SDS−PAGE and could not be considered as really quantitative data. According to the data of ref *12*, patatin dominated in the isolates obtained by ethanol and $FeCl_3$ precipitation. Unfortunately, protein composition of heat coagulate was not studied. Løkra et al. (*7*) isolated potato proteins from PFJ by expanded bed adsorption chromatography and found that the obtained protein isolates contained two major fractions (patatin 40−42 kDa and protease inhibitors 20−23 kDa), and several minor bands of higher molecular weight. Proportion of determined protein bands was not calculated in their publication.

Table 3. Glycoalkaloid Content (mg/kg) in Dry Matter of Freeze-Dried Potato Fruit Juice, Heat Coagulate and Precipitates Obtained by Precipitation with Ethanol or $FeCl_3$

type of glycoalkaloids	PFJ	heat coagulate	ethanol precipitate	$FeCl_3$ precipitate
α-solanin	559	414	342	291
β_2-solanin	3.9	4.7	3.7	4.3
solaninidin	113	366	151	229
α-chaconin	777	494	312	408
β_1-chaconin	280	323	203	254
β_2-chaconin	14.3	7.4	4.2	10
γ-chaconin	ND[a]	3.4	ND	ND
total glycoalkaloids[b]	1747 a	1612 a	1016 c	1196 b

[a] Not detectable. [b] Data represent the mean of duplicate with average analysis error of 2%. Total contents of glycoalkaloids with different letters are significantly different ($P < 0.05$).

Chemical Composition and Nutritional Value of Potato Protein Concentrates. The freeze-dried PFJ and protein concentrates obtained by heat coagulation, ethanol and $FeCl_3$ precipitation were analyzed for nitrogen, glycoalkaloids and mineral content and amino acid composition (**Tables 3−5**). Dry matter of PFJ contained 7.27% of total nitrogen, of which 69.2% (w/w) was TCA-precipitable and could be considered as protein nitrogen. Dry matter of heat coagulate and ethanol protein concentrate contained 84.9% and 82.4% (w/w) of protein, respectively. The processes of heat coagulate and protein precipitate production included two washing steps that should remove the nonproteinaceous nitrogen compounds and determination of nitrogen content by elemental analyzer should express the real content of protein nitrogen. The standard methods for analysis of "real" protein contents such as Bradford or BCA protein assay are difficult to utilize. The ferric chloride-precipitated protein

Table 4. Contents of Nitrogen, Crude Protein and Minerals in Dry Matter of PFJ, Heat Coagulate and Protein Concentrates Obtained by Precipitation with Ethanol or $FeCl_3$

	components (% of dry matter)[a]						
type of protein concentrates	nitrogen	protein (N × 6.25)	calcium	magnesium	sodium	phosphorus	potassium
PFJ	7.3 b	45.5 b	0.1 a	0.7 a	0.1 b	1.1 a	12.1 a
heat coagulate	13.6 a	84.9 a	0.1 a	0.1 b	0.1 b	0.4 c	1.6 bc
ethanol precipitate	13.3 a	82.9 a	0.2 a	0.1 b	0.6 a	0.4 c	1.3 c
$FeCl_3$ precipitate	11.1 ab	69.4 ab	0.1 a	0.1 b	0.7 a	0.9 b	1.8 b

[a] Means followed by the same letter in a column are not significantly different (HSD test at 5% level).

Table 5. Amino Acid Composition of PFJ and Protein Precipitates (Grams of Amino Acids per 16 g of Nitrogen)

amino acid	PFJ	heat coagulate	ethanol precipitate	$FeCl_3$ precipitate	purified patatin
aspartic acid [a]	24.19	15.63	15.36	15.51	14.32
threonine	3.39	4.57	4.47	4.42	5.56
serine	3.54	5.11	5.10	4.89	5.03
glutamic acid	11.65	9.77	9.76	9.93	10.97
proline	3.11	4.10	4.13	3.89	3.56
glycine	4.80	6.10	6.15	6.10	5.04
alanine	4.48	5.17	5.47	5.87	6.87
cysteine	1.04	1.21	1.25	1.30	0.89
valine	4.46	4.49	4.22	4.46	3.81
methionine	1.08	1.35	1.33	1.24	1.02
isoleucine	3.06	3.41	3.14	3.32	3.04
leucine	5.24	6.36	6.22	6.48	7.80
tyrosine	2.26	2.83	2.73	2.69	3.21
phenylalanine	2.87	3.68	3.49	3.47	3.60
histidine	4.27	4.92	5.20	5.10	8.21
lysine	7.75	9.19	9.41	9.58	8.21
arginine	12.80	12.10	12.56	12.26	8.86
total AA g/kg of dry matter	406.6	837.9	789.1	691.8	564.8
total EAA g/kg of dry matter[b]	174.0	417.6	487.3	343.6	292.7
average AAS[c]	79.5	93.8	93.9	94.4	111.2
% AAS of limited amino acid	37.7 cysteine methionine	45.5 cysteine methionine	45.9 cysteine methionine	45.2 cysteine methionine	34.0 cysteine methionine
% EAAI[c]	70.6	83.3	81.7	82.7	86.1

[a] Mean value (gram of amino acid per 16 g of N) of measurements for triplicate. [b] Total content of essential amino acids (g of amino acids per kg of dry matter): threonine, methionine, cysteine, valine, isoleucine, leucine, phenylalanine, tyrosine, lysine and histidine. [c] AAS and EAAI (%) were computed from the whole egg standard.

concentrate is insoluble without a chelating agent (e.g., EDTA) addition, which in connection with high concentration of ferric ions causes incompatibility with using of the protein assay kits. The surprisingly low concentration of protein N determined in $FeCl_3$ protein concentrate could be explained by high concentration of Fe^{3+} ions (in average 43.9 mg Fe^{3+}/g of dry matter, data not shown) that lower relative proportion of other components. The calculated values of approximately 80% (w/w) of total protein in dry matter of both ethanol protein concentrate and heat coagulate correspond with the results of Løkra et al. (*7*), who determined 83.1% (w/w) of total protein in protein powder prepared from PFJ using expanded bed adsorption chromatography technique.

Low quality of potato protein concentrates currently produced in starch manufactures from waste PFJ by preconcentration and subsequent coagulation of the protein is mainly connected with the low functionality and salty, bitter taste that makes it inapplicable as a food commodity (*6*). Negatively there have been perceived components such as phenolic compounds that have been rapidly oxidized to brown pigments (*7*), glycoalkaloids that are toxic for human and animals and moreover can cause a bitter taste of the final protein concentrate (*6*, *7*, *28*) and some of the minerals, especially potassium, that are connected with the bitter and salty taste of the products (*7*). **Table 3** shows the effect of a different way of protein concentrate production on the content of total glycoalkaloids and their individual forms. Two washing steps with 0.1 M sodium-acetate buffer (pH 5.0) included in the procedure of protein precipitation were able to decrease significantly ($P < 0.05$) the level of total glycoalkaloids in protein concentrates obtained by ethanol and $FeCl_3$ precipitation as compared with content of glycoalkaloids in dry matter of industrial PFJ and in the heat coagulate (washed with distilled water). However, the content of glycoalkaloids in ethanol and $FeCl_3$ protein concentrates (**Table 3**) seems to be still too high, particularly as compared with data of Løkra et al. (*7*). They isolated potato proteins from PFJ using adsorption chromatography on a large-scale expanded bed and removed most of the glycoalkaloids originally present in PFJ. However, they presented only data on α-solanine and α-chaconine contents and, as can be seen from **Table 3**, the content of other potato glycoalkaloids is significant and should not be omitted. On the other hand, ref *29* revealed that a presently produced commercial potato protein concentrate contains significant amounts of glycoalkaloids about 2000 mg/kg which is approximately two times higher than the glycoalkaloid content of ethanol and $FeCl_3$ protein concentrates. The methods how to minimize the presence of glycoalkaloids are mostly based on lowering of pH. For instance, ref *28* presented the method of Backleh et al. (*30*), who devised an adsorption bubble separation method with a pH gradient. However, extreme pH changes might cause potato protein (especially patatin) denaturation and loss of their solubility and biological activities. On the other hand, the content of approximately 1000 mg/kg of glycoalkaloids in dry matter of protein concentrates prepared by ethanol and $FeCl_3$ precipitation is comparable with about 200 mg/kg of total glycoalkaloids on fresh weight basis of potato tubers. Next, reduction of glycoalkaloid content in potato tubers could be the second way to influence the quality of the final protein products. Total content of glycoalkaloids in potato tuber is influenced by factors such as crop management, cultivar, environmental factors, postharvest exposure to light and heat, or mechanical injury. Production of high quality potato protein concentrates should be for the future connected with both an improved management of processing potato production and new breeding programs aimed at increasing of tuber protein content and quality, and minimization of glycoalkaloid content.

Potassium represents other problematic component of PFJ that could be limiting for food usage of the protein concentrates causing their salty and off-flavor taste. Contents of selected minerals are shown in **Table 4**. In contrast to glycoalkaloids, the washing steps included in the precipitation process were able to lower the contents of potassium in a very satisfactory manner.

High nutritional value of potato tuber protein that has been previously documented (*1*, *28*) was confirmed. The mean nutritional value of potato tuber protein occurring in PFJ was calculated as essential amino acid index (EAAI) related to whole-egg standard. A value of 70.6% was determined for total tuber protein present in PFJ, being higher than that presented for potato tubers by Mitrus et al. (*31*) (at average 54.6%) and slightly higher than the EAAI of potato granules (EAAI 63%) reported by Eriksen (*32*).

The nutritional values of the heat coagulate, ethanol protein concentrate and $FeCl_3$ concentrate were comparable and achieved 83.3, 81.7 and 82.7% of whole egg standard, respectively. Higher nutritive quality of the protein concentrate is given particularly by a decrease of free amino acid content (aspartic and glutamic acids and their amides) that usually represent 6.6−9.6% of PFJ dry matter (*12*). Excellent nutritional value (EAAI 86.1%) of patatin protein purified from ethanol protein concentrate confirms our hypothesis that the patatin family represents a nutritionally improved factor of potato tuber protein. This finding indicates the importance of careful selection of potato cultivars appointed for starch production with high relative abundance of patatin protein, which would provide protein concentrates with a higher nutritional quality. Significant cultivar variability in patatin content and its relative abundance in extractable total tuber protein was reported in previous publication of ref *24*.

Patatin Purification and Its Lipid Acyl Hydrolase Activity in Differently Prepared Potato Protein Concentrates. In addition to nutritional quality, patatin proteins dispose with enzymatic, foaming and emulsifying properties (*7*, *11*−*13*). Lipid acyl hydrolase (LAH) activity of this protein family was used for the detection of conditional changes in patatin functional properties as an indicator of retained functionality after the precipitation procedure. As can be seen from the data of **Table 6**, enzyme activity of patatin proteins was preserved particularly in ethanol-precipitated protein concentrate. The low patatin LAH activity in heat coagulate was presumed, and the very low value of 0.0108 μmoL/min/mg protein represents the LAH activity of the minimum rest of patatin proteins that remained resoluble. The low LAH activity of patatin protein present in $FeCl_3$-precipitated protein concentrate is explainable by the dependence of the resolubility and extractability on the use of a chelating agent (EDTA here). Extraction of this concentrate by the enzymatic extraction with Tris-HCl buffer resulted in low resolubility and subsequently low activity of LAH. Protein concentrate prepared by ethanol precipitation was easily resoluble in Tris-HCl buffer (pH 7.4) with corresponding higher value of patatin LAH activity. LAH activity determined for the purified patatin was

Table 6. Patatin Lipid Acyl Hydrolase Activity of Freeze-Dried PFJ, Heat Coagulate, Patatin Purified from Ethanol-Precipitated Protein Concentrate and Precipitates Obtained by Precipitation Agents Ethanol or $FeCl_3$

samples	specific protein activity (μmoL/min/mg)	change in specific protein activity *x*
PFJ	0.03 b	1.0
heat coagulate	0.01 b	0.3
ethanol precipitate	0.07 b	2.1
$FeCl_3$ precipitate	0.03 b	1.0
purified patatin	0.50 a	14.7

approximately 15 times higher than that determined for PFJ. Specific LAH activity of patatin isolated from potato tubers of three cultivars ranged from 0.7 to 8.4 μmoL/min/mg of protein (*33*). These results indicated the importance of selection of suitable cultivars for appropriate simultaneous production of starch and native protein concentrate. Moreover, these results indicated the importance of careful manipulation with PFJ and production of protein concentrate, because the decrease of patatin LAH activity could be caused by partial denaturation of patatin proteins during PFJ processing, protein precipitation and patatin isolation.

The study suggested that protein with acceptable solubility, nutritional quality and enzyme activity might be obtained from industrial potato fruit juice by protein precipitation with optimized concentration of ethanol and ferric chloride. Protein concentrate prepared by ethanol precipitation was considered as more promising for industrial use for its simplicity of preparation, easy removal of precipitation additive, high nutritional value and preservation of patatin LAH activity. The level of total glycoalkaloids in protein concentrates obtained by ethanol and $FeCl_3$ precipitation was decreased significantly. Excellent nutritional value of isolated protein concentrates and purified patatin proteins indicated the importance of careful selection of processing potato cultivars with respect not only to starch content but also to content of total protein and patatin relative abundance.

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