EFFECT OF LOW-MOLECULAR ADDITIVES ON PRECIPITATION OF POTATO FRUIT JUICE PROTEINS UNDER DIFFERENT TEMPERATURE REGIMES

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Accepted for Publication July 19, 2007

ABSTRACT

The effects of two mineral and two organic acids, four organic solvents (methanol, ethanol, 2-propano and acetone) and three inorganic metal salts in combination with temperature regimes 0 and 22C on the yield and resolubility of potato tuber proteins were studied. Using acids, the yield of precipitated protein ranged from 22.3% (citric acid, 0C) to 54.5% (acetic acid, 22C) of total protein; however, the resolubility was generally very low. The precipitation with organic solvents resulted in significantly higher yield as well as resolubility ($P < 0.05$) when precipitated at low temperatures. The yield ranged from 23.4% (ethanol, 22C) to 64.5% (2-propanol, 0C) of total protein. The use of the salts resulted in precipitates with high resolubility regardless of the temperature regimes. The yield of precipitated protein ranged from 25.8% ($ZnCl_2$, 0C) to 86.4% ($FeCl_3$, 0C) of total protein.

PRACTICAL APPLICATIONS

The work studied the possibility of isolation of native potato proteins from potato fruit juice (PFJ) resulting from starch manufacturing process. Ethanol and $FeCl_3$ were evaluated as the most promising precipitators for the recovery of potato tuber proteins from PFJ. However, ethanol usage for industrial isolation of potato proteins is strongly limited by the temperature regime in contrast to $FeCl_3$, which could be used in a much wider range of temperature regimes without significant difference in protein yield and resolubility.

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Journal of Food Process Engineering **31** (2008) 533–547.

DOI: 10.1111/j.1745-4530.2007.00167.x

INTRODUCTION

Potato tubers comprise about 2% (w/w) of nitrogen compounds on a fresh basis, of which protein represents 35–75% (Bárta and Čurn 2004). The easy soluble protein fractions get into an aqueous by-product called potato fruit juice (PFJ), which remains in potato starch processing plants. PFJ contains 2–5% of solids, of which crude protein represents about 35% (Knorr 1978; Koningsveld Van *et al.* 2001). Potato proteins have been classified by Pots *et al.* (1999) into three groups. Patatin (43 kDa) comprises 38% in PFJ, protease inhibitors (4.3–25.0 kDa) 50% and other proteins up to 12% (Pouvreau *et al.* 2001). Properties and possibilities of usage of tuber proteins have been discussed (Pots *et al.* 1998a,b; Tonón *et al.* 2001). Potato protein is well known particularly for its high digestibility and favorable amino acid composition (Kapoor *et al.* 1975; Bárta and Čurn 2004), being thus proper for human nutrition.

PFJ has been traditionally used as a low-quality fertilizer, but new environmental regulations and content of valuable nutrients are the reasons for searching new possibilities of PFJ processing. Protein recovery from industrial PFJ has recently been achieved by heat coagulation, which is an efficient method, but the resulting product has unacceptable flavor and functionality (Zwijnenberg *et al.* 2002). Several alternatives have been reported to recover native protein from industrial PFJ. Lindner *et al.* (1981) used bentonite, but removing the adsorbent was difficult. Using carboxymethylcellulose has been successful in protein recovery; however, low binding pH has caused partial denaturation (Gonzales *et al.* 1991). The use of membrane techniques (e.g., ultrafiltration) has given promising results in laboratory studies (Wojnowska *et al.* 1981; Zwijnenberg *et al.* 2002); however, on a large scale, considerable membrane fouling may occur and the protein concentrate contains glycoalcaloids (Wojnowska *et al.* 1981). Expanded bed adsorption technique has been used for the isolation of patatin; however, the technique is too expensive for industrial application (Straekvern *et al.* 1999). Several methods of precipitation at acidic pH or with precipitation additives have also been tested (Meister and Thompson 1976; Knorr 1978, 1982; Koningsveld Van *et al.* 2001).

Koningsveld Van *et al.* (2001) tested the possibility of using various additives such as acids, organic solvents and inorganic salts for potato protein precipitation. The best results were obtained using ethanol at 0C. However, an important complication of using organic solvents or other effective additives could be the temperature regime of the precipitation, regarding the fact that cooling of a large volume of industrial PFJ would be energy and financially demanding. The effect of precipitation temperature in combination with an additive on yield and resolubility of potato protein precipitated from PFJ has not yet been studied.

Regarding the above-mentioned information, our work aimed to study the effects of two temperature regimes (0 and 22C) in combination with various precipitation additives on the qualitative and quantitative parameters of the precipitation process, namely, the yield of the precipitated protein, its resolubility and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) spectra composition. The group of additives used in this study follows the work of Koningsveld Van *et al.* (2001) for possible comparison.

MATERIALS AND METHODS

Preparation of PFJ

Twenty tubers of average size were used for PFJ preparation. Potatoes (processing cultivar Tomensa) were washed thoroughly and cut into large pieces which were crushed up in a domestic juice extractor (AEG, Nurenberg, Germany). Approximately 1,500 mL of PFJ was prepared from the used 20 tubers (2,400 g). A 2% (w/v) solution of $NaHSO_3$ was added to the juice in the amount of 50 mL/kg to prevent enzymatic browning. The resulting liquid was centrifuged (15 min; 9,000 × g; 4C), and the supernatant was filtered through a paper filter (KA 1, Fisher Scientific, Pittsburgh, PA). The clear filtrate is known to be a good imitation of industrial PFJ (Koningsveld Van *et al.* 2001) and is denoted henceforth as PFJ. Properties of PFJ were determined before protein precipitation: pH 5.89; volume density, 1.013 kg/L; dry matter content, 5.65%; and nitrogen content, 4.17 mg/mL of PFJ. The volume density was determined gravimetrically by weighing 30 mL of PFJ in four replications; 30 mL of PFJ was freeze-dried using freeze drier ALPHA 1-4 (Martin Christ, Osterode am Harz, Germany) to the constant weight for gravimetrical determination of PFJ dry matter in four replicates. The obtained PFJ dry matter was subsequently used for the determination of total N content by modified Dumas method, using a nitrogen/protein analyzer Flash EA 1112 (ThermoQuest, Milan, Italy). The average weight of the analyzed sample was 50 mg, and the analysis of the nitrogen content was made in duplicate (Bárta 2002).

Protein Precipitation

Protein precipitation was performed by the method of Koningsveld Van *et al.* (2001), with a modification of additive concentration and temperature regimes on the basis of the conclusions of Koningsveld Van *et al.* (2001, 2002), our own observations and known properties (particularly isoelectric point [pI] and thermal stability) of main tuber proteins (Pouvreau *et al.* 2001; Rydel *et al.* 2003; Pots *et al.* 1998a). The used additives and their concentrations are given in Table 1.

TABLE 1.
LIST OF ADDITIVES USED FOR POTATO FRUIT JUICE (PFJ) PROTEIN PRECIPITATION AND CONCENTRATION OF USED SOLUTIONS AND AMOUNT OF ADDITIVE ADDED TO PFJ

Additive	Final concentration of additive (w/w) in PFJ	Added to 8 mL of PFJ
HCl	3.5*	1 M (up to final pH)
H_2SO_4	3.5*	0.5 M (up to final pH)
Acetic acid	3.5*	Concn. acetic acid (up to final pH)
Citric acid	3.5*	25% (up to final pH)
Ethanol	20%	2-mL absolute ethanol
Methanol	20%	2-mL absolute methanol
Acetone	20%	2-mL absolute acetone
2-propanol	20%	2-mL absolute isopropanol
$FeSO_4$	15 mM	1.42 mL of 100-mM solution
$FeCl_3$	15 mM	1.42 mL of 100-mM solution
$ZnCl_2$	15 mM	1.42 mL of 100-mM solution

* Final pH.

The precipitation was performed in four replications and was initiated by adjusting the pH of the stirred PFJ samples in 15-mL Fisher tubes to a final pH of 5.0. The additives were added to 8 mL of acidified samples in 15-mL Fisher tubes according to the instructions in Table 1. The precipitation for all additive variants was performed for 1 h at two temperature regimes: on ice (0C) or at room temperature (22C). Then, the samples were centrifuged (15 min; 4 or 22C; 3,600 × g) until clear supernatants were obtained. Precipitates formed were washed twice by suspending in 5 mL of 0.1-M sodium acetate buffer pH 5.0 that contained the equivalent amount of relevant additive for maintenance of the precipitated state. When using acids, the samples were washed with the same buffer with pH 3.5. After each washing step, the samples were centrifuged (15 min; 4 or 22C; 3,600 × g). Then, the precipitates were freeze-dried, and dry matter and nitrogen contents were determined.

Protein Resolubilization

Two samples of each variant in original tubes were used for determination of the resolubilization of the precipitated protein. The precipitates were suspended in 10 mL of 100-mM sodium phosphate buffer pH 7.0. When using inorganic salts, the buffer contained 30-mM ethylenediamine tetraacetic acid (EDTA). The precipitates were thoroughly shaken in the buffer by using a vortex mixer (2,000 × g, 2 min) and incubated for 1 h at 30C. Then, the tubes were centrifuged (15 min; 22C; 3,600 × g) and the supernatant was sampled for determination of the resoluble protein composition with SDS-PAGE. The

nonresoluble part of the precipitates was freeze-dried and analyzed for nitrogen content.

Protein Content Analysis

Nitrogen content was determined by the elemental analyzer Flash EA 1112 (ThermoQuest) in duplicate. Protein N of PFJ was determined as N recovered by trichloroacetic acid (TCA) precipitation (Bollag *et al.* 1996). Average N content in PFJ was 4.17 mg/mL; 63.7% was precipitated with TCA, and this N was referred to as protein N. Protein content (N × 6.25) was 16.60 mg/mL. Protein precipitated with additives was 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 less nonresoluble protein.

SDS-PAGE

The resoluble part of the precipitates was determined for protein composition using SDS-PAGE, according to Laemmli (1970). Standard discontinual conditions in polyacrylamide gel (focusing gel, 4%; separation gel, 10%) were used for protein sample separation. Gel processing after protein detection with Coomassie brilliant blue R-250 was performed by Hames and Rickwood (1987). Quantification of protein regions by molecular weight was performed from electrophoretic profiles by digital image analysis and by special software BioProfil 1D++ – measuring of absorbance profiles and computation of individual portions (Bioprofile software package, Vilbert-Lourmat, Marne-la-Vallée, France).

Statistical Analysis

Data were analyzed using the statistical program Statistica 6.0 (StatSoft Inc., Tulsa, OK). The file of data referring to precipitation yield and protein resolubility was evaluated in the analysis of variance (ANOVA) (factorial ANOVA method). Distribution of data file to the homogeneous groups was carried out using Tukey's honestly significant difference test ($P < 0.05$). Evaluation of the effect of the observed factors (temperature and various precipitation additives) was made by the relative variance component method (ANOVA method, type I SS).

RESULTS AND DISCUSSION

Proteins are used in many food products as emulsifiers, emulsion stabilizers or foaming agents. Potato protein has high nutritional quality (Bárta and Čurn 2004), and it thereby has a good potential for utilization in foods. The

emulsifying and foaming properties of undenatured potato proteins have only been studied in a limited extent (Wojnowska *et al.* 1981; Ralet and Guéguen 2000, 2001; Koningsveld Van *et al.* 2006). Emulsifying properties were observed to be inferior to those of whey proteins (Jackman and Yada 1988) but were superior to those of commercial soy isolate (Holm and Eriksen 1980) and casein. Koningsveld Van *et al.* (2006) prepared emulsions using various potato protein preparations. It was concluded that only trace amounts of patatin, the lipolytic activity of which has been strongly underestimated, sufficed to release considerable amounts of surfactants such as fatty acids and monoglycerides. The foaming properties of potato protein obtained by ultrafiltration were shown to be very good in a number of food systems, being at least comparable to those of casein and egg albumin (Ralet and Guéguen 2001). According to the results of Ralet and Guéguen (2001) and Koningsveld Van *et al.* (2001), especially the patatin fraction showed to be a very promising foaming agent with high foamability and foam stability. However, the basic circumstance for utilization potato tuber protein's foaming and emulsification properties is recovery of the potato protein from industrial PFJ in native, soluble form with retaining biological activities.

The used term solubility issues from the methodology of Koningsveld Van *et al.* (2001). The presented solubility data were not really solubility, because these should be expressed as amount per unit volume. The proportion of total protein that became insoluble was used as an index of solubility. We kept the resolubilization volume constant, regardless of the amount of precipitate, and the resolubility was expressed as the proportion of total protein originally present in PFJ. The precipitation and resolubility data indicated the possibility of PFJ protein mixture isolation and differences in their solubility depending on the precipitation additive and temperature regime.

Characteristics of PFJ

The used PFJ (cultivar Tomensa) contained (mean $\pm$ SD) 4.17 ± 0.09 mg nitrogen/mL. On average, 63.7% of this nitrogen could be precipitated with TCA. The TCA-precipitated nitrogen was therefore assumed to be of protein origin, which means an average protein ($N \times 6.25$) concentration of 16.60 ± 0.4 mg/mL PFJ. Nitrogen as well as protein contents were slightly higher than those reported by Koningsveld Van *et al.* (2001); however, the PFJ used in their work was prepared from the cultivar Elkana.

Precipitation Yield and Resolubility

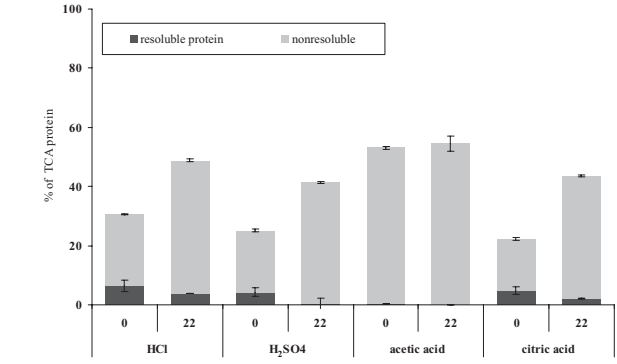
Acids. Four acids issued from isoelectric points of the majority of PFJ proteins, which range between pH 4.5 and 6.5, were used (Pots *et al.* 1998a;

Pouvreau 2004). Isolation of proteins from industrial PFJ by precipitation at acidic pH was previously reported by Knorr (1982) and Koningsveld Van *et al.* (2001).

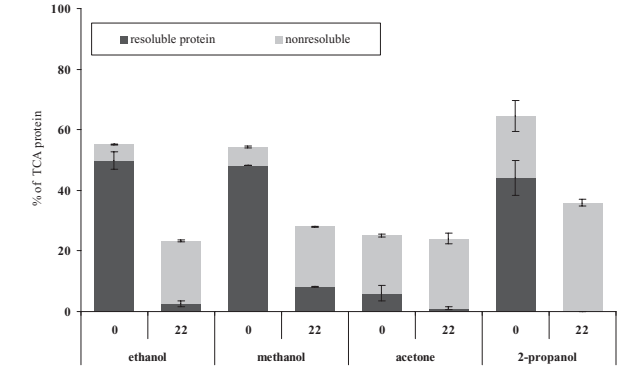
As can be seen in Fig. 1a, the amount of precipitated protein was higher for all the additives at 22C than at 0C. Statistically significant differences ($P < 0.05$) between the temperature regimes were found for citric acid, H_2SO_4 and HCl. At 0C, the yield ranged from 22.3% (citric acid) to 53.1% of total protein (acetic acid). At 22C, the minimum yield was achieved with H_2SO_4 (41.5% of total protein), the maximum with acetic acid (54.5% of total protein). Resolubility of precipitates was in general low. Slightly higher resolubility was observed at the processing temperature 0C than at 22C; however, no statistically significant differences were observed. Using acetic acid at 22C, the resulting precipitate was quite insoluble. At 22C, the highest resolubility was recorded for HCl (4.0% of total protein). At 0C, the lowest resolubility was determined for acetic acid precipitate (0.3% of total protein), while the maximum solubility was reached with HCl (6.4% of total protein). Table 2 shows the percentage effect of the studied factors (temperature and additive) on the precipitation yield and resolubility. Temperature was the most significant factor for yield (44.5%), while resolubility was affected first by the factor of additive (49.2%).

From the results, it may be concluded that using acids for PFJ protein isolation would not be successful for industrial application. The yield was higher at ambient temperature; however, the ambient temperature lowered the resolubility, probably because of protein denaturation. The best resolubility (6.44% of total protein) observed with HCl at 0C was much lower than the resolubility of 56% obtained by Knorr (1982) or the resolubility of 17% obtained by Koningsveld Van *et al.* (2001). However, their experiments were carried out at 22C. The differences in resolubility observed were likely due to differences in the PFJ used.

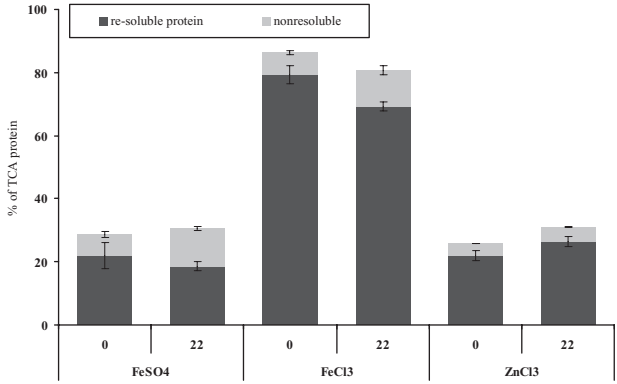
Organic Solvents. The amount of precipitated and resoluble protein is shown in Fig. 1b. The yield of precipitation was considerably higher at 0C. Except for acetone, the differences between temperature regimes were statistically significant ($P < 0.05$). At 0C, the minimum yield was achieved with acetone (25.06% of total protein), the maximum with 2-propanol (64.52% of total protein). The respective yields at 22C ranged from 23.4% when using ethanol to 28.0% of total protein when using methanol. The effect of temperature was demonstrated mainly on the resolubility of the precipitates obtained. The statistically significant differences between the temperature regimes in resolubility were observed with ethanol, methanol and 2-propanol. The resolubility at 22C ranged from 1.1% (acetone and 2-propanol) to 8.2% of total protein (methanol). Resolubility was much higher when precipitated at 0C. In this regime, maximum resolubility was achieved by ethanol and methanol,



a) effect of acids



b) effect of organic solvents



c) effect of metal ions

FIG. 1. PROPORTIONS (%) OF RESOLUBLE AND NONRESOLUBLE PROTEIN AT pH 7.0 EXPRESSED AS PROPORTION OF PROTEIN PRECIPITATED BY TRICHLOROACETIC ACID (TCA PROTEIN), PRECIPITATED BY TESTED ADDITIVES UNDER TEMPERATURES 0 AND 22C

TABLE 2.
INFLUENCE OF STUDIED FACTORS (TEMPERATURE AND PRECIPITATION ADDITIVES) ON THE YIELD AND RESOLUBILITY OF PRECIPITATED PROTEIN

Factors effect %	Various acids		Organic solvents		Metal salts	
	Yield	Resolubility	Yield	Resolubility	Yield	Resolubility
Temperature regime (1)	44.5	30.9	56.7	69.9	0.0	0.0
Precipitation additive (2)	36.3	49.2	16.5	4.4	98.2	96.8
Interaction 1×2	18.3	8.2	26.0	24.9	1.4	2.5
Error	0.9	11.6	0.8	0.8	0.4	0.6

Relative variance components; analysis of variance method.

both resulting in 49.8% of total protein. The minimum solubility was seen with acetone precipitate (5.9% of total protein). From Table 2, it is evident that temperature was the most important factor for both precipitate yield and resolubility.

Organic solvents have been previously used at ambient temperature without lowering pH of PFJ (Wilhelm and Kempf 1977), and also at 0C with lowering pH (Koningsveld Van *et al.* 2001). Wilhelm and Kempf (1977) found 2-propanol to be the most effective and acetone to be the least effective at 22C, causing 25 and 21% of total protein precipitate, respectively. According to our results, the yield resulting with 2-propanol (24.1%) approximately agreed with the conclusions of Wilhelm and Kempf (1977). The solubility of precipitates was very low when precipitated at 22C, which could be explained by the high denaturation effect of organic solvents when used at ambient temperature. According to Koningsveld Van *et al.* (2001), ethanol and 2-propanol seem to be the most promising for industrial application, when used at 0C. When using these additives, about 90% of total protein was reported to be precipitated and about 80% of total protein was resoluble. Our conclusions indicate the same trend, but both yield and resolubility were lower. In our study, the maximum yield was achieved at 0C by 2-propanol (64.5%), but resolubility was higher when using ethanol, almost 50% of total protein.

Inorganic Salts. The amount of yielded protein (Fig. 1c) was higher when using $FeSO_4$ and $ZnCl_2$ at 22C than at 0C; however, the differences between temperature regimes were statistically significant only when using $ZnCl_2$. $FeCl_3$ caused slightly higher yield at 0C than at 22C; however, the difference was not significant. At 22C, the lowest yield was when using $FeSO_4$ (30.7%) and the highest when using $FeCl_3$ (80.6% of total protein). At 0C, the highest yield was $FeCl_3$ (86.4% of total protein), and the lowest was $ZnCl_2$ (25.8% of total protein). As regards resolubility, the more efficient temperature

was 0C for $FeCl_3$ and $FeSO_4$, compared with 22C for $ZnCl_2$. However, a significant difference between the temperature variants was observed only in resolubility of $FeCl_3$ precipitates ($P < 0.05$). The highest resolubility was achieved when using $FeCl_3$ at 0C (79.3% of total protein). The least resoluble precipitate was produced by $FeSO_4$ at 22C (18.5% of total protein). Table 2 confirms the above-mentioned results regarding the main effect of additives on precipitation yield as well as on resolubility.

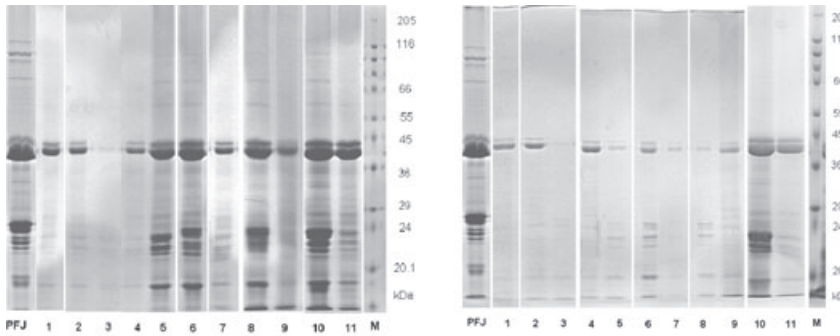
Metal ions have been used for protein precipitation for a long time. The ions (Fe^{3+}, Zn^{2+} and Fe^{2+}) are able to form stable complexes with specific amino acids (Zachariou and Hearn 1996; Koningsveld Van *et al.* 2001). These noncovalent bonds are easily broken at neutral pH, which could be the reason for high solubility of metal salt precipitates. Alternatively, proteins are able to form complexes with polyphenols (McDonald *et al.* 1996; Friedman 1997). The addition of salts may prevent the formation of complexes between polyphenols and proteins (Koningsveld Van *et al.* 2001). As regards our results with salts, the important finding was the minimum effect of temperature on the protein yield as well as on resolubility. $FeCl_3$ was evaluated as the most effective additive resulting in 79.3 and 69.2% of resoluble protein at 0 and 22C, respectively. $FeCl_3$ was evaluated as the most effective of metal salts also by Koningsveld Van *et al.* (2001); however, the presented results were not so optimistic – the yield was 46% and resolubility 41% of total protein. The differences may be caused by differences in the PFJ used.

Protein Compositions of the Resolubilized Protein Fractions

The presence of protein classes and changes in their composition depending on additive and temperature were studied in the resoluble part of precipitates. Protein classes presented in PFJ have been classified into three groups (Pots *et al.* 1999): patatin (43 kDa), comprising 38%; protease inhibitors (4.3–25.0 kDa), making up about 50%; and other proteins up to 12% of total protein in PFJ of cultivar Elkana (Pouvreau 2004). The composition of protein fractions of PFJ is shown in Fig. 2. The PFJ used (cultivar Tomensa) comprised 38.0% of patatin (43 kDa) and 45.6% of the inhibitor proteases (0–25 kDa), which is approximately in agreement with results reported by Pouvreau (2004).

Acids. As can be seen in Fig. 2, when acetic acid was used, both on ice as well as at 22C, no protein bands were detected, which corresponded with the low yield of resoluble protein. In the case of other acids, an interesting finding is a deficiency of the protease inhibitors in both temperature regimes. The SDS-PAGE showed that using acids resulted mainly in the precipitation of

a



proteins in kDa at „ice cooling“ proteins in kDa at 22°C

b

			proteins in kDa at “ice cooling”				proteins in kDa at 22C			
additives	**code**	**pH**	**40-43**	**21-25**	**14-20**	**0-14**	**40-43**	**21-25**	**14-20**	**0-14**
potato fruit juice	***PFJ***		***38.0***	***36.2***	***4.6***	***4.8***	***38.0***	***36.2***	***4.6***	***4.8***
HCl	1	3.5	74.4	13.9	0.0	10.2	63.1	19.9	6.6	10.4
H_2SO_4	2		54.9	34.3	0.0	8.1	65.6	14.8	8.8	10.9
acetc acid	3		-	-	-	-	-	-	-	-
citric acid	4		61.2	24.4	0.0	5.5	60.6	13.2	8.0	7.9
methanol	5	5.0	34.5	40.2	2.6	12.8	18.3	39.7	20.3	17.5
ethanol	6		37.9	35.6	2.8	13.0	27.4	40.4	19.6	12.6
acetone	7		42.4	23.7	6.1	26.4	31.2	41.3	12.9	14.6
2-propanol	8		31.1	39.3	4.5	18.7	23.8	46.1	17.5	12.7
$ZnCl_2$	9		34.4	39.0	11.1	11.7	26.5	46.1	12.1	6.3
$FeCl_3$	10		44.1	22.7	8.2	10.3	45.9	29.3	7.7	10.0
$FeSO_4$	11		45.1	19.2	5.7	10.9	34.5	7.1	28.0	18.7

FIG. 2. QUANTITATIVE YIELDS OF INDIVIDUAL POTATO PROTEIN CLASSES IN RESOLUBLE PARTS OF PRECIPITATES OBTAINED VIA SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (a) AND THEIR ESTIMATION BY SOFTWARE BIO-PROFIL 1D++ (VILBERT-LOURMAT, MARNE-LA-VALLÉE, FRANCE) (b).

resoluble patatin, while the group of protease inhibitors (0–25 kDa) was eliminated during precipitation or remained nonresoluble. However, patatin bands also seem to be very weak, especially when precipitated at 22C. These results agree with those of Koningsveld Van *et al.* (2001), demonstrating that using acids resulted mainly in precipitation of patatin, whose resolubility was higher when obtained at pH 5.0 than at pH 3.0.

Organic Solvents. The effect of temperature on the resolubility of precipitates was marked also on the basis of SDS-PAGE protein spectra analysis. When precipitated at 0C, protein spectra were, with the exception of acetone,

more similar to the original than when precipitated at 22C. At 22C, the resolubility was minimal, which corresponded with SDS-PAGE protein spectra analysis in terms of the protein composition varying significantly from the original. The proportion of patatin and protease inhibitors with molecular mass of 20–25 kDa was lower in favor of smaller-mass proteins (14–20 kDa). The low protein yield and resolubility were likely caused by the denaturing effect of organic solvents when used at ambient temperature, which is a generally known fact (Fennema 1996; Harrison *et al.* 2003). At 0C, the resoluble protein comprised both patatin and protease inhibitors with the exception of those of weight 14–20 kDa, which is in agreement with the conclusions of Koningsveld Van *et al.* (2001).

Inorganic Salts. Addition of metal salts at 0C resulted in precipitation of soluble protein comprising the whole protein spectrum with higher proportion of patatin. An exception represented $FeCl_3$ that produced precipitate where soluble was mainly patatin (43 kDa) and small proteins (<14 kDa). Metal salts were the additives with minimum effect of the temperature on protein bands spectra. Precipitation at 22C resulted in partial lowering of patatin proportion ($ZnCl_2$ and $FeSO_4$) and an increased proportion of proteins with molecular weight 14–25 kDa. The precipitation with $FeCl_3$ was more stable at both temperatures and the protein spectra were very similar.

Industrial Applicability of the Tested Additives for Protein Precipitation from PFJ

Ethanol (at 0C) and $FeCl_3$ (in both temperature regimes) were evaluated according to the obtained results as the most promising precipitation additives for potato tuber protein isolation from PFJ. The positive result of ethanol at 0C was in agreement with Koningsveld Van *et al.* (2001), although the positive result of $FeCl_3$ was surprising. The significant advantage of $FeCl_3$ is the possibility of using it without the necessity of precipitation in the low temperature regime. However, the temperature handicap in the case of ethanol may be partly resolved by concentrating PFJ using membrane techniques, e.g., reverse osmosis or ultrafiltration (Wojnowska *et al.* 1981; Zwijnenberg *et al.* 2002). These techniques can reduce the large volume of PFJ and thus reduce the expenses for cooling during the precipitation with ethanol. PFJ concentration has been used, e.g., in starch manufacture of Avebe (Foxhol, the Netherlands), before heat coagulation to improve the effectiveness of the coagulation process. The practical application of ethanol or $FeCl_3$ would be mainly dependent on the interest of food industries and the financial expensiveness of the precipitation process.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Agriculture of the Czech Republic, project NAZV QF 4030.

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