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Cultivar Variability of Patatin Biochemical Characteristics: Table versus Processing Potatoes (*Solanum tuberosum* L.)

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ABSTRACT: Biochemical characteristics of patatin proteins purified by ion-exchange and affinity chromatography from tubers of 20 potato cultivars were studied to evaluate their genotype differences with respect to utility groups, table potato cultivars (TPCs) and processing potato cultivars (PPCs). Both groups of cultivars showed similar values of protein content in dry matter (3.98−7.39%) and of patatin relative abundance (5.40−35.40%). Three mass levels (~40.6, 41.8, and 42.9 kDa) of purified patatins were found by MALDI-TOF MS within all cultivars. Differences among mass levels corresponding with the mass of sugar antenna (~1.2 kDa) confirmed the previous concept of different glycosylation extentsin patatin proteins. It was showed that the individual types of patatin varying in their masses occur in the patatin family in a ratio specific for each of the cultivars, with the lowest mass type being the major one. Electrophoretic analyses demonstrated wide cultivar variability in number of patatin forms. Especially 2D-PAGE showed 17−23 detected protein spots independently on the utility group. Specific lipid acyl hydrolase (LAH) activity of purified patatins from the individual tested cultivars varied between 0.92 and 5.46 μmol/(min mg). Patatin samples within most of the TPCs exhibited higher values of specific LAH activity than samples of PPCs. It may be supposed that individual patatin forms do not have similar physiological roles.

KEYWORDS: *table and processing potatoes, Solanum tuberosum, cultivar variability, patatin, isoforms, glycosylation, lipid acyl hydrolase activity*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's fourth most important crop after rice, wheat, and corn,[1] and it is considered as an important source not only for human consumption[2] but also for the starch industry.[3] Its tuber protein has high nutritive quality.[4] During tuber processing in starch manufacture it is, because of its high solubility, released to the potato fruit juice (PFJ), which is a waste product containing approximately 1.5% (w/v) of soluble protein.[3,5−7]

The major protein of potato tubers, called patatin, is a family of immunologically identical isoforms of glycoproteins, comprising up to 40% of the total protein in tubers.[3,8−12] The molecular mass of patatin monomer ranges between 39 and 43 kDa.[11,13−16] It was determined that one to three glycosylations (by sugar antenna with molecular mass of about 1.1−1.2 kDa) have the main effect on the mentioned molecular mass range.[17,18] Patatin appears to serve as a storage protein, but unlike most other plant storage proteins, it has also surprising enzymatic activities of nonspecific lipid acyl hydrolase (LAH),[19,20] phospholipase A2,[21] β-1,3-glucanase,[22] and β-1,2-xylosidase.[23] These findings have supported the concept that patatin is not only a storage protein but could also be a part of the potato defense mechanism.[24] However, the real physiological role of patatin in potato tubers has not yet been completely established.[4,11,20] The mentioned enzymatic activities of patatin and, furthermore, its characteristics such as solubility,[7] high foaming activity,[25] antioxidative potential,[26] and high level of essential amino acid index with value of about 86.1%,[27] make patatin an interesting protein source for use in food and biotechnological applications. Some of these promising applications have been just reported, for example, synthesis of special monoacylglycerols,[28] production of food-stable foams and emulsions[25,29] production of food gels,[30] use as antioxidative additives[26] or as an agent with biocide effects reported for its antifungal activity against plant pathogen *Phytophthora infestans*,[31] or its effect on the reduction of larval growth rate of pollen beetle larvae (*Meligethes* spp.)[32] and inhibition of larval growth of *Diabrotica* spp.[33] Thus, the search for large-scale production methods of native protein recovery from PFJ retaining its biological activities became the priority in this field of research during the past decade.[34−39]

Variability of protein content in potato tubers, patatin relative abundance in total protein, and also biochemical and other properties of patatin proteins are influenced particularly by genotype (cultivar).[40] Lachman et al.[41] demonstrated that genotype features of the tested potato tubers are substantial for the nutritive value and content of potato protein. Growing regions, cultivars, and year of cultivation showed statistically significant effects on potato protein content, whereas the effect of crop management was less pronounced. Potato breeding is mainly focused on the formation of new cultivars for two utility types—table and starch-processing potatoes.[42,43] In general, the groups differ each from other in their basic parameters such as

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Table 1. Evaluated Processing and Table Potato Cultivars

table potato cultivars (TPCs)			processing potato cultivars (PPCs)		
cultivar	country of origin	maturity[a]	cultivar	country of origin	maturity
Adora	Netherlands	9	Asterix	Netherlands	3
Agria	Netherlands	5–4	Fresco	Netherlands	9
Bionta	Austria	2	Javor	Czech Republic	4
Filea	Germany	6–7	Kuras	Netherlands	2
Impala	Netherlands	8	Merkur	Austria	2
Karin	Czech Republic	7	Ornella	Czech Republic	3
Laura	Germany	5	Sibu	Germany	2
Marabel	Germany	7–8	Tomensa	Germany	6
Rosara	Germany	8	Vaneda	Czech Republic	7
Symfonia	Netherlands	4–5	Westamyl	Czech Republic	4

[a]Maturity: relative expression from 1 (latest) to 9 (earliest) according to the Central Institute for Supervising and Testing in Agriculture of the Czech Republic.[53]

tuber yield potential, duration of growing season, tuber dry matter and starch content, and only potato cultivars with the highest performance in production are cultivated for starch-processing use. As reported above, a lot of information about patatin, as a protein with interesting properties, was given, but most information about patatin is based on research of one or a few genotypes. Current information about patatin cultivar variability with respect to cultivar use is absent or only insufficiently available.

The aim of the presented work was to evaluate the variability of modern table and processing potato cultivars in (i) tuber protein content and patatin relative abundance, (ii) basic biochemical characteristics of purified patatins, and (iii) representation of patatin mass isoforms and electrophoretic isoforms in purified patatin family proteins.

MATERIALS AND METHODS

Potatoes Used, Sample Preparation, and Dry Matter Determination. Mature tubers of 20 potato cultivars were used for analyses included in this work. Cultivars were selected and divided into two model utility groups, table potato cultivars (TPCs) and processing potato cultivars (PPCs) (see Table 1), according to official data of registration procedure of the Central Institute for Supervising and Testing in Agriculture of the Czech Republic, which is based on utility information given by individual cultivar law owners. Potatoes were grown under conventional crop management at the Experimental Station of the University of South Bohemia in České Budějovice, Czech Republic (48° 59′ N, 14° 28′ E). Cultivar samples were represented by 10 mature and healthy tubers of average size (4–7 cm). Each sampled tuber was taken from a different plant. In each cultivar sample, potato tubers were washed thoroughly in tap water and subsequently in distilled water and cut longitudinally into identical halves (the tuber halves were carefully compared visually). Half of each of 10 tubers was cut into 2 mm thin slices. Some of the slices were immediately frozen to −80 °C, later freeze-dried (−50 °C, 0.040 mbar, 48 h, freeze-dryer Alpha 1-4 LSC, Martin Christ, Germany), and finally homogenized using a laboratory grinder to dry potato meal prepared for analysis of total tuber protein content and patatin relative abundance. Dry matter was determined in duplicate in the remaining part of the slices by drying in an oven at 105 °C until constant weight. Each of the second halves of 10 tubers was ground with 20 mL of the 20 mg/mL solution of sodium bisulfite in a household-type juice extractor ESF 103 (AEG, Germany). The obtained potato juice was centrifuged (10 min, 6000*g*, 4 °C) and then filtered through a paper filter KA1 (Fisher, U.K.). The resulting clear yellowish filtrate was used as a starting material for patatin purification.

Protein Extraction and Protein Content Determination. Protein from potato meal was extracted with 0.0625 M Tris-HCl buffer, pH 6.8, with 2% SDS (200 mg of meal + 2 mL of buffer) for 4 h at 4 °C. The mixture was then centrifuged (10000*g*, 3 min), and the obtained supernatant was divided into two 250 μL parts. One part was used for protein content determination and the second part for the estimation of patatin relative abundance. The analysis of protein content was performed from a prepared protein extract using the BCA protein assay kit (Pierce, Rockford, IL, USA). All steps were performed according to the manufacturer's instructions. Colorimetric measurement was performed in triplicate using a spectrophotometer BioMate 5 (ThermoElectron, U.K.) at a wavelength of 562 nm.

Estimation of Patatin Relative Abundance (PRA) by Chip Electrophoresis Experion. PRA in total tuber protein and proportion of patatin mass isoforms in purified patatin were quantified using automated electrophoresis Experion, protein kit Experion Pro260, and special software (all from Bio-Rad, Hercules, CA, USA) as protein peaks corresponding with molecular mass of patatin proteins. Analysis was performed in triplicate according to the manufacturer's instructions.

Patatin Purification. Chromatographic purification of patatin was performed in three steps.

1. Ion-Exchange Chromatography. Ion-exchange chromatography was performed on 50 mL DEAE 52-Cellulose Servacel (Serva, Germany). The DEAE column was first equilibrated with 600 mL of starting buffer (25 mM Tris-HCl, pH 7.4). Fifty milliliters of potato juice sample was again centrifuged (15 min, 3600*g*, 4 °C) prior to the application on column, and the pH value of the supernatant was adjusted to 7.4 using 1 M Tris. After sample application, the column was washed with 300 mL of starting buffer. Isocratic elution of bound proteins was performed twice with 50 mL of elution buffer (25 mM Tris-HCl, pH 7.4 + 0.5 M NaCl). Eluents were collected, and the protein profile was tested on SDS-PAGE.[44]

2. Affinity Chromatography. A 30 mL concanavalin A Sepharose 4B (GE Healthcare, Piscataway, NJ, USA) column was equilibrated with 300 mL of 25 mM Tris-HCl, pH 7.4 + 0.5 NaCl (starting buffer). All DEAE eluent from the previous step was adjusted to pH 7.4 and loaded on column, which was consequently washed with 300 mL of starting buffer. Elution of bound patatin was performed twice with 30 mL of elution buffer (25 mM Tris-HCl, pH 7.4 + 0.5 M NaCl + 100 mM α-methyl-D-glucoside). Eluents were collected.

3. Desalting Protein Eluents by Gel Filtration. Protein eluents after affinity chromatography were desalted on PD-10 desalting columns (GE Healthcare, USA) as prescribed in the manufacturer's instructions. The purity of the obtained proteins was verified by SDS-PAGE[44] and patatin identity using MALDI TOF MS after previous trypsin digestion of samples with subsequent peptide mass fingerprinting analysis using Mascot software (http://www.matrixscience.com) and information in the NCBI (National Center for Biotechnology Information, Bethseda, MD, USA) database. Peptide mass fingerprinting was accomplished as described elsewhere.[45]

MALDI TOF MS: Determination of the Protein Mass. Mass spectra of positively charged ions were recorded with a Bruker Reflex

IV (Bruker Daltonics, USA) operated in the linear mode for intact protein mass measurements and in the reflectron mode for measurements of protein digests. XMASS 5.1.5 and MS Biotools 2.0 (Bruker Daltonics, USA) software were used for data processing. Aqueous solutions of the purified patatin proteins (2 mg/mL) were mixed with DHB matrix solution (90% 2,5-dihydroxybenzoic acid and 10% 2-hydroxy-5-methoxybenzoic acid; 40 mg/mL in 20% acetonitrile and 1% trifluoroacetic acid) in a 1:4 v/v ratio. The mixture in a volume of 0.6 μL was pipetted on the MALDI target ("dried-droplet" sample preparation technique). Spectra were externally calibrated using the BSA standard. The reproducibility of the measurements was better than 0.1%.

Native PAGE: Detection of Native Proteins and Lipid Acyl Hydrolase (LAH) Activity on the Gels. Run sample was composed from 5 μL of desalted purified patatin solution plus 1 μL of loading buffer: 40% w/v sucrose, 0.03% w/v bromophenol blue (3′,3″,5′,5″-tetrabromophenolsulfonephthalein) in deionized water. PAGE of proteins and isozymes was performed by standard cooled dual vertical slab units SE 600 (Hoefer Scientific Instruments, USA) under conditions of a 0.031 M Tris−0.074 M boric acid (pH 7.9) buffer system. The continuous 6% gel system was utilized (the same buffer system as above was used). The proportion between acrylamide and bisacrylamide was 20:1. Native proteins were detected by staining the gels overnight in a staining solution (1 g of Coomassie Brilliant Blue R-250 was dissolved in 500 mL of methanol + 100 mL of acetic acid + 400 mL of deionized water) with following processing of the gels in destaining solution (250 mL of ethanol + 100 mL of acetic acid + 650 mL of deionized water). LAH activity on the gel was visualized by gel incubation (in the dark, at a temperature of 37 °C) in 100 mL of 100 mM Tris-HCl, pH 7.2, containing 50 mg of 2-naphthyl butyrate (dissolved in 5 mL of acetone) and 100 mg of Fast Blue RR Salt (modified by Bárta et al.).[46]

Native-IEF. Native isoelectric focusing of purified patatin samples was performed using instrument model 111 Cell (Bio-Rad) with an ampholyte pH 4−6 gel system. Analysis was performed per the manufacturer's instruction manual.

2D-PAGE Analysis. Purified patatin samples were diluted in an equivalent amount of thiourea/urea lysis buffer containing 8 M urea, 4% (w/v) CHAPS, carrier ampholytes, and 40 mM Trizma base. Protein concentrations of the total protein extract were measured according to the method of Bradford.[47] Bovine serum albumin was used as a standard. Thirty-five micrograms of purified patatin was separated using gel strips forming an immobilized nonlinear pH gradient from 4 to 7 (Immobiline DryStrip, pH 4−7 NL, 7 cm; Bio-Rad). Strips were rehydrated for 12 h at 22 °C with the thiourea/urea lysis buffer. Isoelectrofocusing was performed with the IEF100 First-dimension Isoelectric Focusing Unit (Hoefer Scientific Instruments, USA). Prior to the second dimension, the gel strips were equilibrated for 2 × 15 min in 2 mL of equilibration solution containing 6 M urea, 20% (v/v) glycerol, 2% (w/v) SDS, and 1.5 M Tris-HCl, pH 8.8. DTT (130 mM) was added to the first equilibration solution, and iodoacetamide (2.5% w/v) was added to the second. The second-dimension electrophoresis was performed using a Bio-Rad Mini-PROTEAN Tetra Cell on a continuous system of 10% SDS−polyacrylamide gels described by Laemmli.[44] Coomassie Brilliant Blue (R-250) staining of gels was performed. Each patatin sample was analyzed in triplicate. Obtained data were processed using PDQuest software (Bio-Rad).

Determination of Specific LAH Activity of Purified Patatin. LAH activity of patatin was determined according to a modified method of Pots.[11] Patatin solutions (in 10 mM Tris-HCl, pH 7.4) with a given concentration (0.10 mg/mL) were used for analysis. Enzyme reaction was performed in 1.5 mL reaction tubes, and the working volume of reaction consisted of a preincubated mixture of 180 μL of 10 mM Tris-HCl, pH 7.4, buffer plus 10 μL of substrate (10 mM 4-nitrophenyl butyrate). Reaction was started by the addition of 10 μL of patatin solution. After incubation at 37 °C for 10 min, the reaction was finished by immersion of the reaction tubes in a boiling water bath for 30 s. The absorbance at 410 nm was measured using a spectrophotometer BioMate 5 (ThermoElectron, U.K.).

Data Processing. Data were subjected to analysis of variance by the one-way ANOVA method and means comparison by Tukey HSD test. Differences among samples were considered to be significant at $P < 0.05$ unless stated otherwise. The software Statistica 6.0 (StatSoft, Tulsa, OK, USA) was used for data analysis.

RESULTS AND DISCUSSION

Tuber Protein Content and Patatin Relative Abundance. In both evaluated groups of potato cultivars (TPCs and PPCs), a wide range of protein content in tuber dry matter (DM) was found (Table 2), which confirmed supposed differences among potato genotypes described in previous studies.[5,40] A similar range of protein content in potato tuber dry matter was found for both utility potato groups. Content of tuber protein of TPCs and PPCs ranged from 3.85% (cv. Bionta) to 6.23% (cv. Marabel) and from 3.98% (cv. Fresco) to 7.39% (cv. Javor) of tuber dry matter, respectively. However, mean tuber protein content was statistically significantly ($P < 0.05$) higher for PPCs (5.36% DM) than for TPCs (4.79% DM). The difference in protein content between these two potato utility groups was much more obvious when calculated on a fresh weight (FM) basis, being 1.44% FM in PPCs versus 1.02% FM in TPCs ($P < 0.001$) (data not shown). This fact could be explained by dry matter content in potato tubers of PPCs (Table 2). The obtained results could be important both in the future use of processing potato cultivars and in specific potato breeding programs. Most of the cultivars within the PPC group (Table 1) are assigned for potato starch production. Mean starch and dry matter contents in potato tubers of PPC group were higher in comparison with cultivars of the TPC

Table 2. Protein Content, Patatin Relative Abundance in Total Tuber Protein, and Proportion of Patatin Mass Isoforms in Tubers of Table and Processing Potato Cultivars[a]

cultivar	dry matter in tubers (% of fresh matter)	protein content in tubers (% of dry matter)	patatin relative abundance (% of total tuber protein)
TPCs			
Adora	19.13 l	4.54 hi	25.60 ef
Agria	21.97 gi	4.69 gh	24.90 f
Bionta	25.42 cd	3.85 j	5.40 j
Filea	21.40 ij	4.83 fg	20.30 h
Impala	17.62 m	4.39 i	30.00 bc
Karin	22.67 fgi	4.66 ghi	27.50 de
Laura	20.52 jk	5.00 ef	31.20 b
Marabel	20.32 jkl	6.23 b	23.90 fg
Rosara	19.26 kl	4.55 hi	15.70 i
Symfonia	24.93 de	5.20 de	27.40 de
PPCs			
Asterix	24.50 de	5.35 d	24.90 f
Fresco	22.93 fg	3.98 j	15.80 i
Javor	26.36 c	7.39 a	24.40 fg
Kuras	27.77 b	4.49 hi	16.55 i
Merkur	29.05 b	4.54 hi	22.65 g
Ornella	27.95 b	6.24 b	35.40 a
Sibu	28.08 b	4.54 hi	18.70 h
Tomensa	28.35 b	6.29 b	35.15 a
Vaneda	23.73 ef	5.88 c	30.75 b
Westamyl	31.06 a	4.90 fg	28.30 cd

[a]Different letters in the individual columns indicate significant differences based on Tukey HSD ($P < 0.05$).

Table 3. Molecular Mass Analysis of Purified Patatins from 20 Potato Cultivars by MALDI-TOF MS

			molecular mass (Da)			differences among peaks (Da)		
	cultivar	no. of detected peaks	peak 1	peak 2	peak 3	Δ 1−2	Δ 1−3	Δ 2−3
TPCs								
	Adora	3	40594	41824	42917	1230	2322	1093
	Agria	2	40597	41794		1197		
	Bionta	2	40575	41702		1127		
	Filea	3	40655	41814	42975	1159	2320	1161
	Impala	3	40557	41796	42886	1239	2329	1090
	Karin	3	40666	41849	42913	1183	2247	1064
	Laura	3	40563	41802	42942	1239	2379	1140
	Marabel	2	40600	41822		1222		
	Rosara	2	40571	41804		1233		
	Symfonia	2	40585	41804		1219		
PPCs								
	Asterix	2	40579	41819		1239		
	Fresco	3	40639	41884	42988	1244	2349	1104
	Javor	1	40539					
	Kuras	3	40557	41768	42930	1211	2373	1162
	Merkur	2	40655	41791		1135		
	Ornella	3	40513	41798	42953	1285	2440	1155
	Sibu	3	40601	41799	42950	1198	2349	1151
	Tomensa	2	40551	41751		1200		
	Vaneda	3	40617	41745	42884	1128	2267	1139
	Westamyl	3	40579	41791	42891	1211	2312	1100

group. Mean starch contents in tubers of TPCs and PPCs were 15.78 and 20.32% FM, respectively. Respective DM contents were 21.32 and 26.98%. Seven cultivars from the PPC group had starch content higher than 20% FM. The existence of cultivars with high production potential of starch as well as tuber protein gives positive preconditions for parallel isolation of potato starch and tuber protein in starch manufacture.[3,6]

Estimation of PRA in spectra of total extractable tuber protein was performed using a system of automated chip electrophoresis Experion (Bio-Rad) on the base of sum quantification of proteins detected in molecular mass ranging from 39 to 43 kDa. This interval of molecular mass corresponded with those of patatin proteins given by other authors.[16,18] Table 2 shows that within the 20 analyzed potato cultivars was found a noticeable range of PRA from 5.4% (cv. Bionta) to 35.4% (cv. Ornella). Levels of PRA ranged from 15.7% (cv. Rosara) to 31.2% (cv. Marabel) and from 15.8% (cv. Fresco) to 35.40% (cv. Ornella) within the TPC and PPC groups, respectively. However, within the group TPCs was not taken into consideration cv. Bionta, which exhibited extremely low values of PRA.

PRA in the range from 20 to 40% was reported in previous studies.[3,8−11] However, with the exception of one work,[8] the other studies analyzed only a limited number (from one to four) of potato genotypes. The genotype variability was not thus ensured sufficiently, and the range of PRA values could be considerably higher for a larger group of potato genotypes. This hypothesis was confirmed by low PRA determined in our study for the cv. Bionta (5.4% from total extractable protein). The low PRA value was found in this cultivar repeatedly during different experimental years as reported in our previous study.[40] In general, the family of patatin proteins is considered to be the main storage protein of potato tubers.[9,12] Significant variation of PRA within analyzed potato cultivars should be explained by different numbers of patatin genes in monoploid potato genome. For instance, it was presumed there are approximately 10−18 copies of patatin genes for each potato monoploid genome, created by 12 chromosomes.[48] The tetraploid configuration of *S. tuberosum* L. genotypes gives a precondition for PRA high genotype variability.

Analysis of Molecular Mass Isoforms and Their Proportion in Purified Patatin Proteins. Patatin proteins were isolated from potato tubers of both utility groups using chromatographic techniques. The purity of the obtained patatin proteins was verified using a SDS-PAGE technique; the identity of the purified patatin proteins was verified using peptide mass fingerprinting.

The molecular masses of patatin isoforms were assessed in the previous studies[11,13,14,17] on a model set of genotypes (no more than three cultivars) using electrophoretic techniques, calculation from primary patatin amino acid sequence, and finally mass spectrometry. It can be deduced from these studies that the molecular mass of patatin ranges from 39.5 to 43.3 kDa. In our study, MALDI TOF MS analyses of purified patatin proteins of both potato utility groups (TPCs and PPCs) revealed a range of molecular mass from 40513 to 42988 Da (Table 3) that confirmed data of the above-mentioned studies. Three levels of molecular mass of purified patatin proteins were found in dependence on analyzed cultivars. The first level of molecular mass was created by patatin mass isoforms with molecular mass between 40513 and 40666 Da. This level of patatin molecular mass was found in all 20 analyzed potato cultivars. The second level of molecular mass was created by patatin isoforms with molecular masses ranging from 41702 to 41884 Da. This level was found in 19 analyzed potato cultivars, whereas in the starch-processing cv. Javor there was found only one patatin mass isoform with a molecular mass of 40539 Da. The third level was created by patatin mass isoforms with molecular mass ranging between 42884 and 42988 Da. This level was found in 11 analyzed potato cultivars. Three patatin mass isoforms of the analyzed potato cultivars were characterized by mean molecular masses of 40590, 41798,

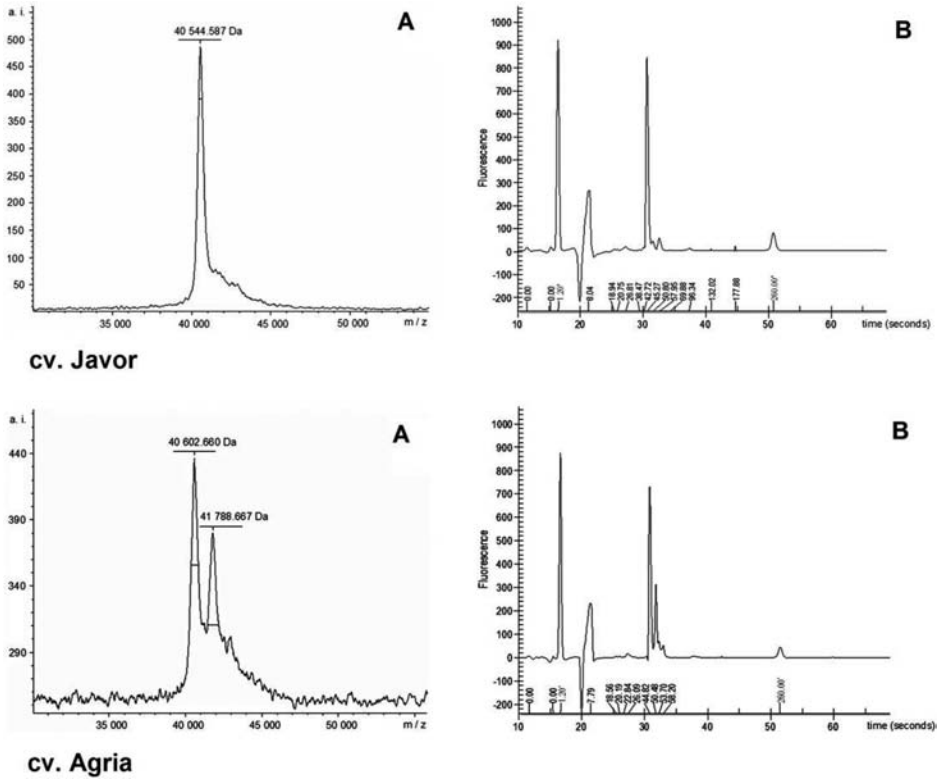

Figure 1. Demonstration of differences in mass spectra of patatin isoforms obtained by MALDI TOF MS (A) and chip electrophoresis Experion (B) in patatins purified from tubers of cv. Javor and cv. Agria.

and 42930 Da. The difference between first and second level of patatin molecular mass ranged from 1127 and 1285 Da; the difference between first and third level ranged from 2247 to 2440 Da.

These results confirm the conclusions of Pots,[11] who analyzed patatin proteins of three potato cultivars and found also three molecular mass isoforms with similar molecular mass; the differences between individual molecular mass levels were approximately 1.2 kDa. A further study[18] dealt with detail isolation and characterization of patatin isoforms obtained from cv. Bintje. The authors concluded that such relatively high differences could not be explained by changes in amino acid sequence of patatin proteins. They agreed with a previous study[17] that the patatin protein chain of about 363 amino acids (without a sequence of 23 amino acids of signal peptide, which is split during post-translation processing) had up to three sites for N-glycosylation (asparagine at positions 60, 90, and 202 in the original amino acid sequence of patatin). For this reason, it is assumed that three levels of patatin molecular mass are caused by one, two, or three glycosylations of the patatin protein chain. Also, the differences found in our study between the molecular mass levels (1.1−1.2 kDa) were close to the molecular mass of patatin glycans. Patatin glycans are characterized by a molecular mass of 1169 Da with the structure Man(α1−3)[Man(α1−6)][Xyl(β1−2)]Man(β1−4)-GlcNAc(β1−4)[Fuc(α1−3)]GlcNAc.[17]

The purified patatin proteins were in addition to MALDI TOF MS also analyzed using chip electrophoresis Experion for verification of the number and quantification of relative abundance of detected patatin mass isoforms. Analysis by chip electrophoresis ensures that a similar separation of purified patatins as from MALDI TOF MS analysis will be obtained. The results obtained by Experion and MALDI TOF MS differed mainly in the detected molecular mass of patatin proteins—the results of chip electrophoresis showed a mild shift of measured values. In general, the phenomenon of molecular mass differences detected by chip electrophoresis Experion was just described in previous studies.[49,50] Figure 1 shows that both methods were able to give similar character of mass spectra in patatin region.

Because MALDI TOF MS is unsuitable for the quantification of intact protein,[51] peak proportion data were calculated only via Experion system (see Table 4). In some cultivars, the evaluation by Experion software revealed the existence of additional peaks (of three mentioned mass levels) remaining in the patatin region after affinity chromatographic purification. Their occurrence should be explained by a mutation in the primary structure of the patatin protein chain or by deviation in patatin glycan structure. The first hypothesis was supported by the results of Mignery et al.,[13] who found the homology of the primary structure of patatin proteins isoforms between 94 and 100% that could cause weight differences of 100−200 Da or more.[13,14] However, these deviations in patatin primary structure cannot explain molecular mass differences between the three molecular mass levels of patatin isoforms even when all of the changed amino acids would have the lowest weight as reported in the study of Pots et al.[18] Both presented explanations of the existence of different patatin molecular mass isoforms have to be further studied and verified.

Table 4 shows relative quantification of patatin mass isoforms using analysis by Experion system and also differences in the number of detected patatin bands. Patatin mass isoforms detected with a molecular mass of around 40.6 kDa were found again in all analyzed cultivars. The mean relative abundance of this type of patatin mass isoform ranged between 37.1 and 88.6%. The second level of patatin mass isoforms (around 41.8

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Table 4. Proportion of Patatin Mass Isoforms in Purified Patatin from 20 Potato Cultivars Determined by Chip Electrophoresis Experion

	proportion of mass isoforms in purified patatin (%)			
cultivar	peak 1[a] (~40.6 kDa)	peak 2[a] (~41.8 kDa)	peak 3[a] (~42.9 kDa)	additional peaks
TPCs				
Adora	**47.6**	**46.5**	**5.9**	
Agria	**64.7**	**26.7**	4.1	4.5
Bionta	**80.9**	**11.5**	7.6	
Filea	**60.6**	**29.7**	**9.7**	
Impala	**57.1**	**36.6**	**6.3**	
Karin	**61.8**	**27.1**	**5.9**	5.2
Laura	**39.3**	**30.3**	**21.9**	8.5
Marabel	**71.4**	**21.5**	5.2	1.9
Rosara	**55.4**	**38.9**	5.7	
Symfonia	**58.6**	**41.4**		
PPCs				
Asterix	**62.3**	**32.4**	5.3	
Fresco	**34.9**	**48.0**	**17.1**	
Javor	**88.6**	4.6	6.8	
Kuras	**50.7**	**19.0**	**23.7**	6.6
Merkur	**73.2**	**20.7**	6.1	
Ornella	**48.2**	**47.2**	**4.6**	
Sibu	**80.3**	**9.3**	**10.4**	
Tomensa	**70.9**	**29.1**		
Vaneda	**64.6**	**23.5**	**11.9**	
Westamyl	**55.5**	**32.3**	**12.2**	

[a]Proportion of peaks corresponding to individual mass level of patatin (mass isoforms that were detected as well as by MALDI-TOF are printed in bold).

kDa) was by Experion system detected again in all of the analyzed cultivars, and the mean relative abundance of this type of patatin mass isoform ranged from 4.6 to 48.0%. The third level (around 42.9 kDa) was detected in 18 cultivars, but according to relative abundance of this patatin isoform level (from 4.1 to 23.8%), this type of isoform could be considered as a minor fraction. The additional patatin peaks outside the three main isoform levels were detected in relative abundance of no more than 8.5% (cv. Marabel). The average relative abundances of patatin molecular mass isoforms were similar for both potato utility groups (TPCs and PPCs).

The obtained results indicate that most of the potato cultivars dispose with three levels of patatin molecular mass isoforms; however, these isoforms have different relative abundances. In all, the 20 studied potato cultivars were found to have patatin isoform of about 40.6 kDa (MALDI TOF MS and Experion analysis). Pots[11] did not find this molecular isoform in a group of patatin proteins purified from cv. Desireé. Our results indicate that patatin isoforms with one and two glycosylations seem to be the most frequent.

Charge Heterogeneity in Purified Cultivar Patatin Proteins. The purified patatin proteins isolated from potato cultivars of both utility groups were analyzed using electrophoretic analyses for the characterization of patatin charge isoforms. The characterization was performed using native PAGE with parallel detection system (first gel for Coomassie Blue staining, second gel for detection of patatin LAH activity), native IEF, and 2D-PAGE analysis with modification agents thiourea, dithiothreitol, and iodacetamide.

Figure 2 shows bands of patatin samples analyzed by native-PAGE. These bands were detected in a region of relative

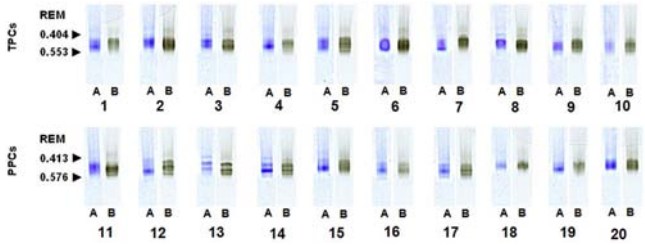

Figure 2. PAGE patterns of purified patatins from table potato cultivars (TPCs) and processing potato cultivars (PPCs) detected as native protein by Coomassie Brilliant Blue (A) and as lipid acyl hydrolase by 2-naphthylbutyrate and Fast Blue RR Salt (B). TPCs: 1, Adora; 2, Agria; 3, Bionta; 4, Filea; 5, Impala; 6, Karin; 7, Laura; 8, Marabel; 9, Rosara; 10, Symfonia. PPCs: 11, Asterix; 12, Fresco; 13, Javor; 14, Kuras; 15, Merkur; 16, Ornella; 17, Sibu; 18, Tomensa; 19, Vaneda; 20, Westamyl. REM, relative electrophoretic mobility.

electrophoretic mobility (REM) of 0.400−0.600. The number of protein bands (Table 5) detected after staining with Coomassie Blue ranged from two to three and from one to four in TPCs and PPCs, respectively. The number of patatin bands with LAH activity was in range of two to four for both potato utility groups. As can be seen from Figure 2, most of the bands with LAH activity are matched to the bands detected by Coomassie Blue, but not in all cases. For example, one band was examined after Coomassie Blue staining and four bands with LAH activity for patatin proteins purified from cv. Fresco. The REM value of the most intensive patatin band with LAH activity corresponded with the patatin band detected after Coomassie Blue staining. Similarly, in purified patatin of cv. Javor there were detected four protein bands, but only three bands with LAH activity.

A high number of protein bands and spots detected using the method of native IEF and 2D-PAGE is logically given by substantially higher differentiation ability of these analytical methods. The native IEF revealed from 7 to 12 and from 7 to 14 bands of patatin proteins for TPCs and PPCs, respectively (Table 5). Isoelectric bands of detected proteins ranged between pH 4.45 and 5.14 and between pH 4.48 and 5.07 for TPCs and PPCs, respectively. These results confirm the conclusions of other authors. Racusen and Foote[8] reported that patatin could be resolved into 6−10 ionic forms, and Park et al.[52] reported that most potato cultivars contain 12−15 "patatin species", which are immunologically identical glycoprotein isoforms. Pots et al.[11] analyzed isoforms of patatin proteins purified from cv. Bintje and found two bands after native PAGE analysis and called these bands "upper" and "lower". Moreover, the same study described six bands with p*I* 4.6−5.2 after IEF analysis. Detail separation of the purified patatin proteins by anion-exchange chromatography (Source-Q) resulted in four fractions. Pots et al.[18] studied in detail three major fractions (A, B, D). They reported that fraction A showed two bands with p*I* 5.0 and 5.2 after native PAGE and IEF analyses, whereas fractions B and D showed one lower peak after PAGE and two or one peak with p*I* 4.6−4.7 after IEF analysis.

2D-PAGE analysis of patatin protein isoforms combines two separation systems (denaturation IEF and SDS-PAGE) and therefore exhibits considerable success in the number of detected patatin proteins. The total number of purified patatin

Table 5. Number of Bands in Patatins Isolated from 20 Potato Cultivars after PAGE and Isoelectric Focusing Analyses

	band no. after PAGE and detection by		isoelectric focusing		
cultivar	Coomassie Blue	LAH activity	band no.	range of p*I*	spot no. after 2D-PAGE and detection by Coomassie Blue
TPCs					
Adora	2	2	10	4.50−5.01	19
Agria	2	3	12	4.50−5.09	19
Bionta	3	2	11	4.51−5.14	17
Filea	2	2	9	4.48−4.91	19
Impala	2	4	11	4.47−4.90	17
Karin	2	3	10	4.49−4.92	18
Laura	3	2	10	4.45−4.85	18
Marabel	3	3	11	4.50−4.99	20
Rosara	2	3	7	4.52−4.99	23
Symfonia	3	3	10	4.49−4.83	18
PPCs					
Asterix	2	3	12	4.55−5.07	19
Fresco	3	4	7	4.49−4.79	21
Javor	4	3	9	4.58−4.82	18
Kuras	4	4	14	4.49−4.81	21
Merkur	3	3	9	4.57−4.79	19
Ornella	3	2	7	4.53−4.82	21
Sibu	3	3	13	4.48−4.79	21
Tomensa	1	2	7	4.61−4.84	22
Vaneda	2	3	10	4.49−4.85	19
Westamyl	2	3	12	4.48−5.03	23

spots detected by Coomassie Blue ranged from 17 to 23 and from 18 to 23 for TPCs and PPCs, respectively (Table 5). Thus, our results presented higher numbers of spots than the conclusions of other studies; for example, Lehesranta et al.[15] identified 9 patatin isoforms in cv. Desireé, and Bauw et al.[16] reported 17 patatin isoforms in cv. Kuras. This fact may be explained by the uniqueness of the patatin family in cv. Desireé (absence of 40.6 kDa mass level) and also by different conditions used for analysis and spot detection.

Figure 3 shows 2D-PAGE profiles of patatin proteins of analyzed potato cultivars. The obtained profiles showed partly

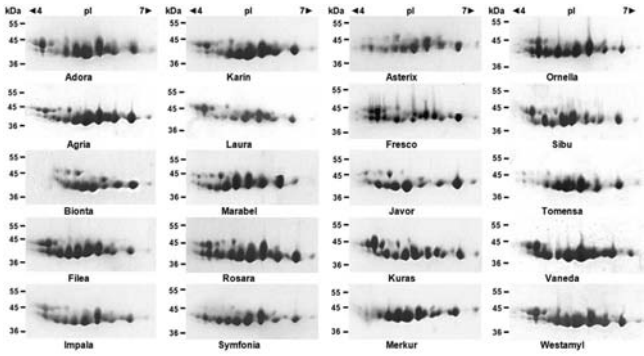

Figure 3. 2D-PAGE profiles of purified patatins from 20 potato cultivars stained by Coomassie Brilliant Blue. For more information on the individual cultivars, see the caption to Figure 2.

spots with common character for all evaluated cultivars and partly spots with cultivar-specific character. Interesting variability was found in the group of not so much noticeable spots with molecular mass around 43−44 kDa (probably a higher degree of glycosylation) and p*I* close to a value of 4. These spots were very apparent in patatin profiles of some of the analyzed cultivars (e.g., Kuras, Fresco, Filea, and Agria), whereas they were weak in another cultivars (e.g., Asterix, Tomensa, and Symfonia).

The studies mentioned in the Introduction characterized patatin as a storage tuber protein[20] with interesting enzymatic activities[19,21−23] that can be probably connected with defense mechanisms of potato tubers.[24] There should be thus answered several questions. What are the factual functions of individual patatin isoforms (spots), and what is the variability of these functions between cultivars? Does the contact of potato tubers with various pathogens induce an expression of specific patatin isoforms? What is the role of various glycosylation degrees in the function of patatin isoforms? To answer these questions detailed studies of patatin need to be evaluated.

LAH Activity in Purified Cultivar Patatin Proteins. The samples of patatin proteins obtained from both potato utility groups were analyzed for LAH activity using 4-nitrophenylbutyrate as a substrate. Within 20 analyzed cultivars, specific LAH activity ranged from 0.92 (cv. Rosara) to 5.46 (cv. Filea) μmol/(min mg) (Table 6). These values also express the range of LAH activity within the TPC group. Values of LAH activity within the PPC group were narrower, ranging between 1.27 and 4.41 μmol/(min mg) in cv. Kuras and cv. Merkur, respectively. Patatin proteins isolated from tubers of TPCs exhibited significantly higher mean specific LAH activity (3.34 μmol/(min mg); $P < 0.001$) in comparison with the PPC value of 2.22 μmol/(min mg). The obtained results confirm the supposed importance of genotype differences in specific patatin LAH activity. However, the differences found in our study were not so noticeable as these reported by Racusen;[19] the patatin LAH activity of cv. Desireé (substrate PNP laurate) was only 0.66% of the LAH specific activity found for cv. Kennebec. However, when Racusen[19] used another substrate (α-naphthyl acetate), the LAH activity of cv. Desireé was 2.8 times higher in comparison with cv. Kennebec.

The native-PAGE analysis in our study showed different LAH activities using 2-naphthyl butyrate as substrate (Figure

Table 6. Lipid Acyl Hydrolase Activity of Purified Patatins (Expressed as Specific Activity) Isolated from Tubers of 20 Potato Cultivars[a]

table potato cultivars		processing potato cultivars	
	specific activity μmol/(min mg protein)		specific activity μmol/(min mg protein)
Adora	2.37 gh	Asterix	1.41 jk
Agria	4.00 cde	Fresco	1.72 ij
Bionta	3.36 f	Javor	1.96 hi
Filea	5.46 a	Kuras	1.27 kl
Impala	2.52 g	Merkur	4.41 bc
Karin	3.69 def	Ornella	1.70 ij
Laura	2.53 g	Sibu	2.57 g
Marabel	4.06 bcd	Tomensa	3.62 ef
Rosara	0.92 L	Vaneda	1.67 ijk
Symfonia	4.48 b	Westamyl	1.88 i

[a]The different letters in both columns indicate significant differences based on Tukey HSD ($P < 0.05$).

2). These results indicate that LAH activity values determined with this substrate exhibit different activities for individual patatin isoforms within one genotype. On the contrary, Pots et al.[18] did not find significant differences in LAH activity between the patatin isoforms of cv. Bintje.

We determined substantial differences in patatin LAH activity both between cultivars and between individual patatin isoforms. Genotype differences can be important for an explanation of the speculated connection between enzyme activities of patatin and different resistances of potato cultivars against tuber diseases and pests. Previous studies[24,31,33] indicated an effect of patatin enzyme activities in potato tuber physiological functions. Strickland et al.[33] revealed cultivar-dependent ability of patatin proteins to inhibit the growth of corn rootworm. Cultivar-specific patatin esterase and phospholipase activity was not found to be correlated with insect growth inhibition, whereas galactolipase activity correlated significantly. The phospholipase activity of patatin was found to be pH dependent and to increase when the cell is disrupted, which supports the theory of the patatin role during plant tissue membrane autolysis and suggests participation of patatin in the defense mechanism.[24,33] Sharma et al.[31] isolated from potato somatic hybrids patatin proteins with the ability to inhibit *P. infestans* spore germination. However, the accurate mechanisms and role of patatin enzyme activities in insecticide and antifungal impacts are not known, and it may be very difficult to reach conclusions regarding patatin variability of esterase activities and its physiological role. The final insecticide and antifungal activities may be the result of a combined effect of patatin enzyme activities, its relative abundance in potato tuber protein, the capability of patatin and its isoforms with variable acyl side chains, the presence of key patatin isoforms, and their features. Answers to these questions were not the task of our study and require deep physiological studies.

The importance of cultivar differences in patatin enzyme activity is clear also from a practical point of view. Cultivar seems to be the predominant parameter of enzyme activities of patatin proteins obtained by native isolation process from PFJ in starch manufacture[34−39] and assigned in immobilized form for use in the food industry or biotechnological applications.[25,28−32] Potato tubers are mostly processed in industry as a cultivar mixture, and therefore it will be necessary to know the parameters of patatin protein activities of the used potato cultivars. The results confirmed the assumption of higher mean concentration of tuber proteins in potato dry matter as well as on fresh weight basis in the group of processing potato cultivars. Patatin proteins isolated from tubers of table potato cultivars exhibited higher LAH activity. The obtained information about patatin proteins from tubers of potato cultivars of both utility groups could be utilized in breeding programs for the production of new cultivars of processing potatoes with high concentrations of starch, protein, and patatin proteins and LAH activity of patatin proteins or other tuber proteins. Moreover, the increase of patatin relative abundance in potatoes of table cultivars should have potential for an increase of their nutritional value and protein quality for high patatin EAAI value as indicated in the previous work of Bártová and Bárta.[27] Next, the cultivar variability of LAH and selectivity of patatin LAH activity give presumption of cultivar-specific patatin exploitation for the synthesis of monoacylglycerols from fatty acids and glycerol in microaqueous reaction systems. Oleic, linoleic, linolenic, capric, lauric, and myristic acids can be used as reactants for the production of moanoacylglycerols of 95% purity.[28]

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Notes

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