# Patatin, the Major Protein of Potato (*Solanum tuberosum* L.) Tubers, and its Occurrence as Genotype Effect: Processing Versus Table Potatoes

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**Abstract**

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Patatin relative abundance in SDS-extractable protein and patatin content in dry matter were evaluated in tubers of forty processing and table potato cultivars usually cultivated in the Czech Republic, Germany, and the Netherlands. The patatin characteristics were evaluated over three experimental years. Patatin relative abundance in the processing cultivars achieved on average a significantly higher value ($P < 0.001$; Tukey HSD test) than patatin relative abundance in the table cultivars, resulting in average values of 25.80% and 21.59%, respectively. A high patatin relative abundance (over 30% in extractable protein) was determined only in the case of two cultivars: Vaneda (average 31.29%) and Tomensa (average 31.24%). Patatin content in tuber dry matter was significantly higher in the processing potato cultivars in all three experimental years ($P < 0.001$), attaining a mean of 1.28% with the processing cultivars and 1.03% with the table cultivars. The direct effect of the cultivar on patatin relative abundance in SDS-extractable protein was higher (33.1% for processing potato cultivars and 48.1% for table potato cultivars) than the effect of the growing year (15.6% for processing potato cultivar and 22.8% for table potato cultivars).

**Keywords**: *Solanum tuberosum* L.; potato tuber proteins; patatin; patatin relative abundance; patatin content

Patatin represents a group of immunologically identical glycoprotein isoforms with molecular mass ≈ 40–43 kDa (native conformation is a dimer). The presence of molar mass differences of individual isoforms is caused by different numbers of glycosylation sites in combination with mutations in the primary sequence of the patatin protein chain (Pots 1999). Patatin genes are mainly expressed in tubers, with a significantly lower amount of transcripts in other tissues (Prat *et al.* 1990). It has been estimated that 10–18 copies of patatin genes are present in each haploid (12 chromosomes) of the potato genome (Twell & Ooms 1988).

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) (Andrews *et al.* 1988), phospholipase A2 (Senda *et al.* 1996), β-1,3-glucanase (Tonón *et al.* 2001) , acyl transferase (Jimenez *et al.* 2001), and β-1,2-xylosidase (Peyer *et al.* 2004).

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This finding supports the concept that patatin is not only a storage protein, but can also be part of the potato defence mechanism. However, the real physiological role of patatin in potato tubers has not yet been established (Paiva *et al.* 1983; Pots 1999; Shewry 2003; Bárta & Čurn 2004). Patatin can be considered as a nutrition-improving protein in the spectrum of total tuber protein (Bárta & Čurn 2004).

Patatin relative abundance can vary considerably in tuber extractable protein, ranging from 20 to 40% (Racusen & Foote 1980; Rosahl *et al*. 1986). Patatin is considered to be present in all genotypes of cultivated potato (Lee *et al.* 1983). Only few studies have reported on the effect of genotype (cultivar) and agro-ecological conditions on patatin proteins accumulation in potato tubers. Both the total content of protein in potato tubers and patatin relative abundance in extractable tuber protein are dependent on several factors, such as the cultivar (Hannapel 1991), degree of tuber development (Hannapel 1991), storage duration and influence of agro-ecological conditions (Kumar *et al.* 1999; Pots *et al.* 1999; Lachman *et al.* 2005). However, the most relevant data of Hannapel (1991) presented only patatin relative abundance in SDS-extractable tuber protein of four potato cultivars without annual repetitions.

Potatoes are important root-crops of the temperate zone (especially Europe and North America) where this crop is cultivated in two utility groups: table potatoes (food exploitation) and starch processing potatoes (starch production). The guarantee of the product quality in both groups is affected by the genetic potential of the cultivars, which is often considerably different with table and processing potatoes. The table potato cultivars are characterised by a shorter growing season, and properties corresponding with the cooking type; the nutritionally-favourable tuber protein represents a welcome component. The processing genotypes are characterised by a longer growing season, and high a starch concentration; the tuber protein was considered to be an unwanted by-product of the starch production rather than a valuable raw material for further processing. However, because of the above mentioned nutritional and biochemical potential of potato tuber proteins and especially patatin (Strickland *et al.* 1995; Macrae *et al.* 1998; Ralet & Guéguen 2000; Tonón *et al.* 2001; Åhman & Melander 2003; Wang & Xiong 2005), efforts exist to isolate tuber proteins from potato fruit water for utilisation in feed, food, and biotechnological applications (Straetkvern *et al.* 1999; Koningsveld *et al.* 2001). Information describing the genotype and annual stability of patatin presence in potato tubers as well as correlations between patatin abundance and the selected characteristics (tubers size, pure protein content, starch content), is basic for the production and future utilisation of this protein component in various branches. The aim of the presented contribution was to evaluate the cultivar effect (processing versus table potato cultivars) and annual stability of the relative abundance and patatin content. The work also determined the correlations between patatin relative abundance and the selected potato tuber characteristics.

## MATERIAL AND METHODS

### Field growing of experimental material

Plants of 40 potato (*Solanum tuberosum* L.) cultivars were grown over three years (2003–2005) at the Experimental Station of the University of South Bohemia in České Budějovice, Czech Republic (48°58'N, 14°27'E). The soil at the experimental station has the following characteristics: sandy loam texture, pH 5.72, 0.17% N, 0.0117% P, 0.0103% K, 0.0101% Mg, and 1.93% of organic carbon. The climatic conditions of the site during the experimental years are presented in Table 1.

Two groups of cultivars, processing potatoes (PPCs) and table potatoes (TPCs), were tested (Table 2). Seed tubers of propagation grade C1 were provided by the Central Institute for Supervising and Testing in Agriculture in Brno.

Fifteen potato plants from each cultivar were grown in 4.5 m long rows (planting space 300 × 750 mm), with blocks of PPCs and TPCs placed next to each other with a space of 1 m. The total experimental area (10 × 15 m) was located inside a protective potato plantation (cv. Ditta).

Oats was always used as the foregoing crop, and manure (40 t/ha) was applied on the experimental area after its harvest in September. NPK fertilisation was used before planting, consisting of 100 kg N/ha (ammonium sulphate), 35 kg P/ha (Hypercorn), and 60 kg K/ha (60% potash salt). Planting was performed by hand, and the crop was cultivated 4× during a season by ridging. Chemical protection against late blight (*Phytophthora infestans* (Mont) de Bary) and Colorado beetle

Table 1. Rainfall and average daily temperature at the site České Budějovice during 2003–2005

| Month | Long-term mean (1960–1990) | | Departure from long-term mean | | | | | |
|---|---|---|---|---|---|---|---|---|
| | rainfall (mm/month) | average daily temperature (°C) | 2003 | | 2004 | | 2005 | |
| | | | (mm/month) | (°C) | (mm/month) | (°C) | (mm/month) | (°C) |
| January | 23 | –1.8 | 27 | 0.4 | 23 | –0.2 | 9 | 2.9 |
| February | 23 | –0.3 | –19 | –2.9 | 25 | 2.6 | 32 | –2.2 |
| March | 32 | 3.4 | –10 | 1.8 | 35 | –0.1 | –11 | –0.6 |
| April | 47 | 8.1 | –29 | 0.6 | 36 | 1.5 | 19 | 1.8 |
| May | 70 | 13.0 | –7 | 3.4 | –4 | –0.5 | –5 | 1.4 |
| June | 93 | 16.2 | –7 | 4.6 | 8 | 0.1 | –25 | 1.5 |
| July | 78 | 17.7 | –21 | 2.1 | –26 | 0.6 | 85 | 1.3 |
| August | 79 | 17.1 | –64 | 4.4 | –31 | 2.1 | 79 | –0.3 |
| September | 48 | 13.5 | –7 | 0.6 | 1 | 0.2 | 51 | 1.3 |
| October | 32 | 8.4 | 48 | –2.5 | 11 | 1.5 | –24 | 1.3 |
| November | 35 | 3.3 | –19 | 1.6 | 14 | 0.8 | 1 | –0.4 |
| December | 25 | –0.3 | 14 | 0.1 | –20 | 0.4 | 7 | –0.2 |
| Mean | 583 | 8.2 | –94 | 1.2 | 73 | 0.7 | 215 | 0.6 |

(*Leptinotarsa decemlinata* Say) was applied. In 2003, the first application was done on 10th June using tank-mix of Ripost M + Vaztak 10 SC; the next applications were: Tattoo + Nurelle D, Tattoo, Acrobat® MZ and Acrobat® MZ. In 2004, the first application was carried out on 22nd June using tank-mix of Casoar® + Mospilan 20 SP; the next applications were: Casoar®, Acrobat® MZ, Acrobat® MZ and Altima 500 SC. In 2005, the first application was carried out on 20st June using tank-mix of Casoar® + Mospilan 20 SP; the following applications were: Casoar®, Acrobat® MZ, Acrobat® MZ and Altima 500 SC. The applied doses were used according to the instructions of the producers. The terms of the subsequent applications were chosen according to the pressure of late blight and Colorado beetle. Potato tubers of each cultivar were harvested from each plant independently as a replication. The tubers were analysed after wound-healing period (3 weeks, 15°C).

## Chemical analyses

***Sample preparation and dry matter determination***. The cultivar samples for chemical analyses consisted of 2 mm wide slices from ten whole tubers (each tuber for the sampling was taken from a different plant). After sampling, the tuber slice samples were immediately frozen at –80°C, later dried by freeze drying (–50°C, 0.040 mBar, 48 h), and finally they were homogenised with a laboratory grinder to dry potato meal. The evaluation of dry matter representation in tubers was performed gravimetrically in two replications. Starch content determination was performed by the underwater weight method using the special Hošpes-Pecold scales ESPRA.

***Crude protein and pure protein contents determination***. Crude protein content was determined as total nitrogen content in dry matter of potato tubers multiplied by a factor of 6.25. Total nitrogen content was determined by a modified Dumas method on a nitrogen (protein) analyser Flash EA 1112 (ThermoQuest, Italy/USA). Two 100 mg samples of dry potato meal were analysed.

Pure protein from potato meal was extracted using 0.0625M Tris-HCl buffer, pH 6.8, with 2% SDS (200 mg meal + 2 ml buffer) at 4°C for 4 hours. After extraction, the mixture was centrifuged (10 000 rpm, 3 min) and the supernatant obtained was divided into two 250 µl parts. The first part was used for net protein determination and the second part was analysed by SDS-PAGE for patatin determination. The analysis of pure protein content was performed on the prepared protein

Table 2. List of evaluated processing and table potato cultivars

| Cultivar | Processing potato cultivars (PPCs) | | | Cultivar | Table potato cultivars (TPCs) | | |
|---|---|---|---|---|---|---|---|
| | origin | earliness (points*) | usage | | origin | earliness (points*) | cooking type |
| Asterix | NL | SL (3) | F | Adéla | CZ | E (7) | B |
| Delikat | D | E (7) | F | Adora | NL | VE (9) | B-BC |
| Fresco | NL | VE (9) | F | Agria | NL | SE (5–4) | B |
| Innovator | NL | SE (6) | F | Bionta | A | SL (2) | BC |
| Javor | CZ | SL (4) | S | Bolesta | NL | SE (5–4) | C |
| Krumlov | CZ | SL (3) | S | Cicero | NL | E (7) | BC |
| Kuras | NL | SL (2) | S | Cinja | D | E (7) | BA |
| Merkur | A | SL (2) | S | Colette | D | VE (8) | BA |
| Morene | NL | SL (2–3) | F | Dali | NL | E (7) | BA |
| Ornella | CZ | SL (3) | S + F | Filea | D | SE (6–7) | BA |
| Pacov | CZ | SL (3) | S + F | Impala | NL | VE (8) | B |
| Producent | NL | SL (2) | S | Karin | CZ | E (7) | BA |
| Saturna | NL | Sl (4–5) | S + F | Laura | D | SE (5) | B-BC |
| Sibu | D | SL (2) | S | Ditta | A | SE (5) | AB |
| Tábor | CZ | SL (3) | S | Marabel | D | E (7–8) | BA-B |
| Tegal | CZ | E (7) | S + F | Milva | D | SE (5–4) | AB |
| Tomensa | D | E (6) | S + F | Rosara | D | VE (8) | BA |
| Vaneda | CZ | E (7) | S + F | Rosella | D | SE (5–4) | B |
| Vladan | CZ | SE (6) | S + F | Santé | NL | SE (6) | B |
| Westamyl | CZ | SL (4) | S | Symfonia | NL | SL (4–5) | BC |

A – Austria, CZ – Czech Republic, D – Germany, NL – the Netherlands; VE – very early, E – early, SE – semi early, SL – semi late; *scale of earliness – 9 = the earliest, 1 = the latest; F – fried products, S – starch production

extract using the BCA protein assay kit (Pierce, USA). This method is based on the reduction of $Cu^{2+}$ to $Cu^{+}$ by protein in alkaline medium and selective colorimetric detection of the cuprous cation ($Cu^{+}$) by bicinchoninic acid. All steps were performed according to the manufacturer's instructions. Colorimetric measurement was performed at the wave length of 562 nm using a spectrophotometer BioMate 5 (ThermoElectron, UK).

***SDS-PAGE and determination of patatin relative abundance (PRA) and patatin content.*** After centrifugation (10 000 rpm, 3 min), the mixture of 10 µl of the supernatant + 2.5 µl of the loading buffer (5 ml 1.25M Tris-HCl, pH 6.8, 2.3 g SDS, 10 ml glycerol, 5 mg Bromophenol Blue; to 500 µl of this buffer to add 170 µl 2-sulphanylethanol) was used as a run sample for electrophoretic analysis. SDS-PAGE of denatured proteins was performed using standard cooled dual vertical slab units SE 600 (Hoefer Scientific Instruments, San Francisco, USA) under the conditions of the 0.025M Tris, 0.192M glycine, 0.1% SDS (pH 8.3) buffer system. The discontinuous gel system (Hames & Rickwood 1987) was used in the following way: 4% stacking gel (0.125M Tris-HCl, pH 6.8) and 10% separating gel (0.375M Tris-HCl, pH 8.8). Proteins were detected by staining the gels overnight in the staining solution (1 g Coomassie Brilliant Blue R-250 was dissolved in 500 ml methanol + 100 ml acetic acid + 400 ml distilled water), gel processing after protein detection was performed based on Hames and Rickwood (1987). The determination of the relative abundance of patatin proteins, with a molecular weight range from 39–44 kDa, was performed from electrophoretic profiles by digital

image analysis and the special software Bio-1D++ (Vilber Lourmat, France), which measures the absorbance profiles and computes individual protein portions. Patatin content in the dry matter of tubers was calculated as the percentage of patatin (patatin relative abundance) from pure protein content.

***Data processing***. Statistical analyses were performed using factorial analysis of variance (as the basic evaluation for a two-factorial experiment), or Tukey's HSD test and correlation analysis. All statistical analyses were conducted using software Statistica, Version 6 (StatSoft, Inc., 2001).

## RESULTS AND DISCUSSION

Considering that patatin consists of a family of about 43 kDa glycoproteins (Racusen 1983; Pots 1999), patatin protein bands were detected in the patatin area between 39–44 kDa on the obtained gel after SDS-PAGE. The patatin area was recorded in the case of all 40 potato cultivars analysed, which confirmed the assumption about patatin existence in all cultivars of cultivated potatoes (Prat *et al.* 1990; Rajapakse *et al.* 1991; Jimenez *et al.* 2001).

### Patatin relative abundance and patatin content

Patatin relative abundance achieved significantly higher average values ($P < 0.001$) in PPCs than in TPCs, being 25.80% and 21.59%, respectively (Tables 3 and 4). However, this does not apply uniformly to all three experimental years. Significant between-

Table 3. Patatin relative abundance (%) in total tuber protein of processing potato cultivars (2003–2005)

| Cultivar | 2003 | 2004 | 2005 | 2003–2005 |
|---|---|---|---|---|
| Asterix | 19.72$^{l}$ | 28.84$^{de}$ | 26.66$^{cd}$ | 25.07$^{e}$ |
| Delikat | 23.69$^{gh}$ | 29.86$^{cd}$ | 26.94$^{cd}$ | 26.83$^{c}$ |
| Fresco | 21.47$^{jk}$ | 28.86$^{de}$ | 24.61$^{ef}$ | 24.98$^{e}$ |
| Innovator | 24.35$^{fg}$ | 26.45$^{f}$ | 23.79$^{fg}$ | 24.86$^{e}$ |
| Javor | 22.27$^{hij}$ | 28.56$^{de}$ | 12.65$^{k}$ | 21.16$^{g}$ |
| Krumlov | 22.25$^{hij}$ | 31.66$^{ab}$ | 26.07$^{de}$ | 26.66$^{c}$ |
| Kuras | 21.69$^{ijk}$ | 23.10$^{gh}$ | 15.03$^{j}$ | 19.94$^{h}$ |
| Merkur | 27.31$^{e}$ | 26.07$^{f}$ | 25.84$^{de}$ | 26.41$^{cd}$ |
| Morene | 22.76$^{ghij}$ | 28.79$^{de}$ | 22.48$^{gh}$ | 24.68$^{e}$ |
| Ornella | 27.61$^{de}$ | 32.95$^{a}$ | 28.29$^{c}$ | 29.61$^{b}$ |
| Pacov | 20.08$^{kl}$ | 30.53$^{bc}$ | 15.81$^{j}$ | 22.14$^{f}$ |
| Producent | 30.71$^{abc}$ | 33.09$^{a}$ | 24.65$^{ef}$ | 29.49$^{b}$ |
| Saturna | 23.28$^{ghi}$ | 22.33$^{h}$ | 21.02$^{hi}$ | 22.21$^{f}$ |
| Sibu | 25.45$^{f}$ | 26.74$^{f}$ | 24.43$^{ef}$ | 25.54$^{de}$ |
| Tábor | 22.11$^{hij}$ | 25.98$^{f}$ | 20.04$^{i}$ | 22.71$^{f}$ |
| Tegal | 29.33$^{cd}$ | 29.60$^{cd}$ | 30.24$^{a}$ | 29.73$^{ba}$ |
| Tomensa | 32.30$^{a}$ | 27.57$^{ef}$ | 33.85$^{a}$ | 31.24$^{a}$ |
| Vaneda | 31.15$^{ab}$ | 31.19$^{bc}$ | 31.51$^{a}$ | 31.29$^{a}$ |
| Vladan | 25.91$^{ef}$ | 23.52$^{gh}$ | 24.63$^{ef}$ | 24.69$^{e}$ |
| Westamyl | 30.07$^{bc}$ | 24.33$^{g}$ | 25.98$^{de}$ | 26.79$^{c}$ |
| Mean | 25.18 | 28.00 | 24.23 | 25.80 |

Within column values followed by the same letter are not significantly different at $P < 0.05$ (Tukey HSD test); the letters are given in alphabetical order with decreasing level of a parameter

Table 4. Patatin relative abundance in total tuber protein (%) in table potato cultivars (2003–2005)

| Cultivar | 2003 | 2004 | 2005 | 2003–2005 |
|---|---|---|---|---|
| Adéla | 13.63$^{i}$ | 15.67$^{i}$ | 3.01$^{k}$ | 10.77$^{l}$ |
| Adora | 26.12$^{e}$ | 23.63$^{e}$ | 23.08$^{b}$ | 24.28$^{bcd}$ |
| Agria | 28.28$^{bc}$ | 24.55$^{cde}$ | 21.27$^{cde}$ | 24.70$^{bc}$ |
| Bionta | 8.28$^{j}$ | 12.87$^{j}$ | 0.33$^{l}$ | 7.16$^{m}$ |
| Bolesta | 29.18$^{ab}$ | 25.38$^{abcd}$ | 19.95$^{ef}$ | 24.84$^{b}$ |
| Cicero | 28.13$^{bcd}$ | 21.08$^{fg}$ | 22.36$^{bc}$ | 23.86$^{de}$ |
| Cinja | 27.21$^{cde}$ | 25.10$^{abcde}$ | 18.03$^{g}$ | 23.45$^{ef}$ |
| Colette | 26.48$^{de}$ | 24.39$^{de}$ | 21.31$^{cd}$ | 24.06$^{bcde}$ |
| Dali | 23.89$^{f}$ | 24.25$^{de}$ | 23.36$^{b}$ | 23.83$^{de}$ |
| Filea | 30.21$^{a}$ | 26.00$^{abc}$ | 22.65$^{b}$ | 26.29$^{a}$ |
| Impala | 25.81$^{e}$ | 25.06$^{bcde}$ | 17.15$^{hg}$ | 22.67$^{f}$ |
| Karin | 21.38$^{g}$ | 25.57$^{abcd}$ | 6.56$^{j}$ | 17.84$^{k}$ |
| Laura | 23.10$^{f}$ | 26.66$^{a}$ | 16.24$^{h}$ | 22.00$^{g}$ |
| Lenka | 22.48$^{fg}$ | 25.37$^{abcd}$ | 12.43$^{i}$ | 20.09$^{i}$ |
| Marabel | 26.70$^{cde}$ | 24.45$^{cde}$ | 20.93$^{de}$ | 24.03$^{cde}$ |
| Milva | 23.55$^{f}$ | 19.72$^{g}$ | 25.37$^{a}$ | 22.88$^{f}$ |
| Rosara | 21.05$^{hg}$ | 21.87$^{f}$ | 20.53$^{def}$ | 21.15$^{h}$ |
| Rosella | 23.12$^{f}$ | 25.18$^{abcde}$ | 25.62$^{a}$ | 24.64$^{bc}$ |
| Santé | 19.59$^{h}$ | 17.49$^{h}$ | 20.51$^{def}$ | 19.20$^{j}$ |
| Symfonia | 26.28$^{e}$ | 26.62$^{ab}$ | 19.61$^{f}$ | 24.17$^{bcde}$ |
| Mean | 23.72 | 23.04 | 18.01 | 21.59 |

Within column values followed by the same letter are not significantly different at $P < 0.05$ (Tukey HSD test); the letters are given in alphabetical order with decreasing level of a parameter

group differences in PRA were found in 2004 (PPCs 28.00% versus TPCs 23.04%) as well as in 2005 (PPCs 24.26% versus TPCs 18.01%). However, PRA did not show any statistically significant difference in 2003 (PPCs 25.18% versus TPCs 23.72%). The average PRA in the PPCs ranged from 19.94% (cultivar Kuras) to 31.29% (cultivar Vaneda). The range of PRA was wider in the case of TPCs, from 7.16% (cultivar Bionta) to 26.29% (cultivar Filea). This wider range was affected mostly by two table cultivars that stably showed low PRA. These cultivars were Adéla and Bionta with average PRA of 10.77% and 7.16%, respectively. The trend of significantly different PRA in these cultivars was greatest in 2005; PRA in the cultivar Adéla was only 3.01%, and only 0.33% in the cultivar Bionta. On the contrary, high patatin relative abundance (> 30% of patatin in SDS-extractable tuber protein) was found in the Vaneda (average 31.29%) and Tomensa (average 31.24%) cultivars.

The actual patatin amount in potato tubers is dependent not only on the patatin relative abundance in SDS-extractable tuber protein, but also on the amount of total tuber protein in dry matter. There was a considerable range of patatin in potato tuber dry matter for both processing and table potato cultivars (Tables 5 and 6). Patatin content was significantly higher in PPCs in all three experimental years ($P < 0.001$). The average patatin content was 1.28% of dry matter in PPCs and 1.03% of dry matter in TPCs. Patatin content in PPC tuber dry matter ranged from a minimum value of 0.52% (cv. Kuras, year 2005) to a maximum value of 2.19% (cv. Tomensa, year 2003). Patatin content in TPC dry matter ranged from the minimum value of 0.01% (cv. Bionta, year 2005) to

Table 5. Patatin content in tuber dry matter (%) in processing potato cultivars (2003–2005)

| Cultivar | 2003 | 2004 | 2005 | 2003–2005 |
|---|---|---|---|---|
| Asterix | $0.71^{l}$ | $1.54^{cd}$ | $1.17^{efg}$ | $1.14^{ihg}$ |
| Delikat | $1.21^{def}$ | $1.67^{bc}$ | $1.25^{cde}$ | $1.38^{d}$ |
| Fresco | $0.96^{hjk}$ | $1.15^{efg}$ | $1.11^{fgh}$ | $1.07^{ijk}$ |
| Innovator | $1.32^{d}$ | $1.49^{d}$ | $1.08^{fghi}$ | $1.30^{de}$ |
| Javor | $1.06^{fgh}$ | $2.11^{a}$ | $0.54^{kl}$ | $1.24^{ef}$ |
| Krumlov | $0.96^{hjk}$ | $1.51^{cd}$ | $1.19^{efg}$ | $1.22^{efg}$ |
| Kuras | $0.89^{jk}$ | $1.04^{fg}$ | $0.52^{l}$ | $0.81^{l}$ |
| Merkur | $1.25^{de}$ | $1.18^{ef}$ | $1.07^{ghi}$ | $1.17^{fgh}$ |
| Morene | $1.04^{ghj}$ | $1.56^{cd}$ | $1.02^{hi}$ | $1.20^{fg}$ |
| Ornella | $1.55^{c}$ | $2.06^{a}$ | $1.34^{cd}$ | $1.65^{bc}$ |
| Pacov | $0.87^{k}$ | $1.54^{cd}$ | $0.68^{k}$ | $1.03^{k}$ |
| Producent | $1.75^{b}$ | $1.83^{b}$ | $1.21^{def}$ | $1.60^{c}$ |
| Saturna | $1.13^{efg}$ | $1.00^{g}$ | $1.02^{hi}$ | $1.05^{jk}$ |
| Sibu | $1.13^{efg}$ | $1.21^{e}$ | $1.17^{efg}$ | $1.17^{fgh}$ |
| Tábor | $0.96^{hjk}$ | $1.19^{ef}$ | $0.98^{hi}$ | $1.04^{jk}$ |
| Tegal | $2.07^{a}$ | $1.67^{bc}$ | $1.38^{c}$ | $1.71^{b}$ |
| Tomensa | $2.19^{a}$ | $1.73^{b}$ | $2.18^{a}$ | $2.03^{a}$ |
| Vaneda | $1.50^{c}$ | $1.84^{b}$ | $1.59^{b}$ | $1.64^{bc}$ |
| Vladan | $1.15^{efg}$ | $1.24^{e}$ | $0.95^{i}$ | $1.11^{hij}$ |
| Westamyl | $1.32^{d}$ | $1.19^{ef}$ | $1.22^{def}$ | $1.24^{ef}$ |
| Mean | 1.25 | 1.49 | 1.13 | 1.29 |

Within column values followed by the same letter are not significantly different at $P < 0.05$ (Tukey HSD test); the letters are given in alphabetical order with decreasing level of a parameter

1.52% (cv. Marabel, year 2004). The table cultivar Bionta showed the lowest patatin content in all three experimental years. High patatin contents (> 2% in tuber dry matter) were found only in the processing potato cultivars Tomensa (years 2003, 2005), Tegal (year 2003), Ornella (year 2004), and Javor (year 2004).

The data of patatin accumulation in potato tubers confirmed the significance of the cultivar variability that had been previously suggested (Hannapel 1991; Pots 1999). The variability should be connected with the differences in patatin gene copy numbers, which are responsible for patatin expression. Patatin is encoded by a gene family with ~10–18 copies per haploid genome (Bánfalvi *et al.* 1994), which would mean about 40–72 patatin gene copies for a tetraploid. A higher patatin content in the tubers of PPCs was probably connected with the considerable genetic potential of these cultivars to accumulate indigenous tuber matter such as starch and proteins. However, our data of PRA were surprisingly lower in comparison with the values of 20–40% that had been previously presented (Racusen & Foote 1980; Paiva *et al.* 1983; Prat *et al.* 1990; Jørgenson *et al.* 2006). Pots (1999) even presented PRA ranging from 30% up to 60% in buffer-extractable tuber protein. These differences in calculated PRA are explainable by the low number of potato cultivars that had been so far analysed. Pots (1999) analysed three cultivars, Paiva *et al.* (1983) one cultivar and detected 40–45% of patatin in soluble protein, and Hannapel (1991) analysed mature tubers of four cultivars with PRA ranging from 24% to 30%. The cited author analysed tubers from one-year experiment without annual rep-

Table 6. Patatin content in tuber dry matter (%) in table potato cultivars (2003–2005)

| Cultivar | 2003 | 2004 | 2005 | 2003–2005 |
|---|---|---|---|---|
| Adéla | $0.66^{k}$ | $0.90^{jk}$ | $0.12^{k}$ | $0.56^{j}$ |
| Adora | $1.43^{abc}$ | $1.07^{ghi}$ | $0.98^{e}$ | $1.16^{d}$ |
| Agria | $1.41^{abcd}$ | $1.15^{efgh}$ | $0.86^{fg}$ | $1.14^{de}$ |
| Bionta | $0.41^{l}$ | $0.50^{l}$ | $0.01^{l}$ | $0.31^{k}$ |
| Bolesta | $1.37^{abcde}$ | $1.50^{ab}$ | $1.08^{cd}$ | $1.32^{ab}$ |
| Cicero | $1.34^{bcdef}$ | $0.88^{k}$ | $0.98^{e}$ | $1.07^{fg}$ |
| Cinja | $1.21^{fg}$ | $1.21^{def}$ | $0.71^{h}$ | $1.04^{fg}$ |
| Colette | $1.28^{def}$ | $1.08^{ghi}$ | $1.09^{c}$ | $1.15^{d}$ |
| Dali | $1.11^{gh}$ | $1.02^{hij}$ | $1.28^{a}$ | $1.14^{de}$ |
| Filea | $1.48^{a}$ | $1.26^{cde}$ | $1.10^{c}$ | $1.28^{bc}$ |
| Impala | $1.27^{ef}$ | $1.10^{fghi}$ | $0.87^{fg}$ | $1.08^{efg}$ |
| Karin | $1.02^{hij}$ | $1.19^{efg}$ | $0.33^{j}$ | $0.85^{i}$ |
| Laura | $1.09^{gh}$ | $1.33^{cd}$ | $0.87^{fg}$ | $1.10^{def}$ |
| Lenka | $0.94^{ij}$ | $1.16^{efg}$ | $0.48^{i}$ | $0.86^{i}$ |
| Marabel | $1.47^{ab}$ | $1.52^{a}$ | $1.15^{bc}$ | $1.38^{a}$ |
| Milva | $1.30^{cdef}$ | $0.87^{k}$ | $1.24^{ab}$ | $1.14^{de}$ |
| Rosara | $0.91^{j}$ | $1.00^{ijk}$ | $0.83^{fg}$ | $0.91^{ih}$ |
| Rosella | $1.05^{hi}$ | $1.09^{fghi}$ | $0.92^{ef}$ | $1.02^{g}$ |
| Santé | $0.97^{hij}$ | $1.00^{ijk}$ | $0.82^{g}$ | $0.93^{h}$ |
| Symfonia | $1.34^{bcdef}$ | $1.39^{bc}$ | $0.98^{ed}$ | $1.24^{c}$ |
| Mean | 1.15 | 1.11 | 0.84 | 1.03 |

Within column values followed by the same letter are not significantly different at $P < 0.05$ (Tukey HSD test); the letters are given in alphabetical order with decreasing level of a parameter

etition. The variation could be also explained by the chosen procedures of tuber protein extraction and quantification as evident from the results of Pots (1999) who extracted only the easily soluble fraction and performed the quantification using Bradford assay after partial purification of patatin proteins on DEAE sepharose anion exchanger. Paiva *et al.* (1983) and Hannapel (1991) used a more suitable rocket immunoelectrophoresis and ELISA analysis for patatin quantification.

### Year effect on cultivar stability in patatin relative abundance and patatin content

The direct effects of the cultivar, year, and their interaction on patatin relative abundance and patatin content in tuber dry matter can be seen in Table 7. In the case of both evaluated characteristics, the direct effect of cultivar was greater (33.1% for PPCs and 48.2 for TPCs) than the effect of the growing year (15.6% for PPCs and 22.8% for TPCs). However, the interaction of the year and cultivar was important especially for PPCs, explaining 50.7% of the total variability of PRA and 40.5% of the variability of patatin content in tuber dry matter.

The annual variability of patatin relative abundance and patatin content should be connected particularly with the differences in temperature and rainfall during the experimental years and their impact on the potato plants. The climatic conditions in 2003 were most critical for potato cultivating (Table 1). The absence of rainfall especially in the months July and August, in combination with high temperatures in the period from June to August, negatively affected the production ability of the

Table 7. Summary of ANOVA and variance components (Mixed Model ANOVA) for patatin relative abundance in total tuber protein and patatin content in tuber dry matter

| Cultivar group | Factor | Patatin relative abundance | | | Patatin content | | |
|---|---|---|---|---|---|---|---|
| | | df | MS | % TV | df | MS | % TV |
| PPCs | year (1) | 2 | 154.25** | 15.6 | 2 | 1.3074** | 18.3 |
| | cultivar (2) | 19 | 64.02** | 33.1 | 19 | 0.5185** | 40.2 |
| | 1 x 2 | 38 | 21.76** | 50.7 | 38 | 0.1313** | 40.5 |
| | error | 60 | 0.14 | 0.6 | 60 | 0.0014 | 0.9 |
| TPCs | year (1) | 2 | 389.21** | 22.8 | 2 | 1.1862** | 24.6 |
| | cultivar (2) | 19 | 139.50** | 48.2 | 19 | 0.3855** | 46.9 |
| | 1 x 2 | 38 | 23.26** | 28.8 | 38 | 0.0642** | 27.7 |
| | error | 60 | 0.10 | 0.3 | 60 | 0.0009 | 0.8 |
| All cultivars | year (1) | 2 | 421.7** | 14.4 | 2 | 2.0746** | 16.3 |
| | cultivar (2) | 39 | 126.4** | 49.1 | 39 | 0.5420** | 48.2 |
| | 1 x 2 | 78 | 25.1** | 36.2 | 78 | 0.1060** | 34.7 |
| | error | 120 | 0.1 | 0.3 | 120 | 0.0012** | 0.8 |

MS – mean square; % TV – percentage of total variability; $^{**}P < 0.01$

evaluated potato cultivars, especially in PPCs. This relationship could be explained by the fact that most of the PPCs had a longer growing season, with a later tuber formation, than TPCs. PPCs tubers are mostly formed during July and August. The damage of the photosynthetic apparatus by high temperatures and water deficit resulted in the production of lower weight tubers, limiting the creation of the storage matter, including patatin. Apparently this was the reason why the difference in patatin relative abundance between PPCs and TPCs was low and statistically inconsistent in 2003 (Table 8). The climatic conditions in 2004 should be considered as the most favourable for the content matter accumulation, as demonstrated especially in the case of PPCs, because 15 PPCs reached their maximum patatin contents in this year. Low patatin content in 2005 could be characterised as opposite to 2003, because extraordinary rainfalls occurred during July and August, which caused an accrual of the tuber weight (Table 8) and dilution of tuber dry matter, including the storage matter.

Table 8. Yearly means of patatin relative abundance and patatin content and selected tuber parameters in PPCs and TPCs

| Year | Cultivar group | Patatin relative abundance | Patatin content | Selected tuber parameters | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | average tuber weight | dry matter content | starch content | crude protein content | pure protein content |
| 2003 | PPCs | 25.18$^{ab}$ | 1.25$^{b}$ | 74.85$^{c}$ | 24.34$^{b}$ | 16.25$^{bc}$ | 9.27$^{b}$ | 4.88$^{ab}$ |
| | TPCs | 23.72$^{b}$ | 1.15$^{b}$ | 86.10$^{bc}$ | 21.08$^{c}$ | 13.30$^{d}$ | 10.93$^{a}$ | 4.86$^{ab}$ |
| 2004 | PPCs | 28.00$^{a}$ | 1.49$^{a}$ | 78.90$^{c}$ | 26.56$^{a}$ | 20.03$^{a}$ | 8.94$^{b}$ | 5.28$^{a}$ |
| | TPCs | 23.04$^{b}$ | 1.11$^{b}$ | 84.20$^{bc}$ | 21.34$^{c}$ | 15.41$^{c}$ | 9.80$^{b}$ | 4.81$^{b}$ |
| 2005 | PPCs | 24.23$^{b}$ | 1.13$^{b}$ | 101.20$^{ab}$ | 24.00$^{b}$ | 16.74$^{b}$ | 7.41$^{c}$ | 4.60$^{b}$ |
| | TPCs | 18.01$^{c}$ | 0.84$^{c}$ | 115.13$^{a}$ | 19.22$^{d}$ | 12.50$^{d}$ | 9.02$^{b}$ | 4.57$^{b}$ |

Different letters in a block mean significant difference at $P < 0.05$ (Tukey HSD test). The letters are given in alphabetical order with decreasing level of a parameter

Patatin relative abundance and patatin content were lower in 2005 than in 2003 and 2004, especially in TPCs. The results (Tables 3–6 and 8) demonstrated differences in physiology of both utility groups and different strategies of PPCs and TPCs in the accumulation of tuber matter including patatin protein. The year ability to significantly modified patatin content, thus confirming the presumption about the storage function of patatin in tubers (Pots 1999). However, a discussion is difficult because the influence of annual ecological factors on PRA as well as patatin contents in potato tubers of TPCs and PPCs, have not been studied. On the other hand, the significant positive correlation found between protein and patatin contents could be indirectly used for the determination of environmental conditions effect on patatin content because some factors (genotype, environment, tillage) which modified tuber protein content are known (Leszkowiat *et al.* 1991; Debre & Brindza 1996; Mitrus *et al.* 2003; Lachman *et al.* 2005). Lachman *et al.* (2005) determined statistically the significant effect of the growing region, varieties, and years on nitrate and protein contents, e.g. higher regions (probably higher rainfalls and lower temperatures) showed lower average protein contents than lower regions.

### Correlations between patatin characteristics and selected parameters

The correlations between the selected parameters (Table 9) and patatin relative abundance and patatin content were evaluated on three levels: the data of all cultivars obtained over the whole experimental period; separate data of PPCs and TPCs obtained over the whole experimental period (both presented in Table 9); and separate data of both cultivars groups obtained in individual years.

A statistically significant positive correlation was found between tuber protein content and patatin content on all three data levels, with the range of correlation coefficients being from +0.25 ($P < 0.05$) to +0.92 ($P < 0.001$), which again confirmed the presumption about patatin storage function as previously suggested (Hannapel 1991; Pots 1999; Bauw *et al.* 2006). The relationships between the factors studied were closer for the PPCs ($r = 0.923$, $P < 0.001$; $r = 0.878$, $P < 0.001$; and $r = 0.835$, $P < 0.001$ for 2003, 2004 and 2005, respectively) than for the TPCs ($r = 0.400$, $P < 0.05$; $r = 0.616$, $P < 0.001$; and $r = 0.579$, $P < 0.001$ for 2003, 2004 and 2005, respectively). Positive correlations were also found between patatin relative abundance in SDS-extractable protein and patatin content in tuber dry matter, however, a positive correlation in all three experimental years was found only in the processing potato cultivars ($r = 0.665$, $P < 0.001$; $r = 0.409$, $P < 0.01$ and $r = 0.598$, $P < 0.001$ for 2003, 2004 and 2005, respectively).

There were no significant correlations between starch and patatin contents in any year, with the exception of 2005 when a negative correlation ($r = -0.496$, $P < 0.01$) was found between the above-mentioned parameters. However, when the three experimental years were evaluated together, a small positive correlation was found between patatin relative abundance and starch content ($r = +0.329$, $P < 0.001$). A strong relationship between starch and protein contents was presented by Hunnius *et al.* (1976), however, the relationship between starch and patatin contens has not yet been studied. Potatoes are an important industrial starch source, as in the Netherlands, where about 50% of the potato production is destined for the starch industry (Bradshaw & Mackay 1994). During processing, it is possible to recover protein in some starch manufactures as a valuable by-product for use as feed supplements (denatured protein) or as, a component for food and biotechnological applications. Patatin proteins with high nutritious quality (Bárta & Čurn 2004) and specific functional properties (Ralet & Guéguen 2000; Lyn & Youling *et al.* 2005; Koningsveld *et al.* 2006) present the key protein component of the tuber protein and high patatin content in tuber guarantees high quality of the protein concentrate produced in starch manufacture. Nevertheless, starch contents in potato tubers remain the basic qualitative parameter for the starch industry and the determination of indifferent (or even small positive) correlation between starch content and PRA presumes that it is possible to produce potato tubers with high contents of starch, protein, and patatin. When the three experimental years were evaluated together, statistically significant correlations were found between patatin content and dry matter content ($r = +0.318$, $P < 0.001$), and also between patatin content and average potato tubers weight ($r = -0.298$, $P < 0.001$). The positive correlation between dry matter and patatin was expected confirming the correlation with starch and storage function of patatin proteins. Negative correlation between average tuber weight

Table 9. Correlation between patatin relative abundance or patatin content and selected qualitative parameters of potato tubers

| | All cultivars | | Processing potato cultivars | | Table potato cultivars | |
|---|---|---|---|---|---|---|
| | PRA | PAT-C DM | PRA | PAT-C DM | PRA | PAT-C DM |
| SE | 0.031$^{ns}$ | 0.055$^{ns}$ | 0.220* | 0.242** | 0.251** | 0.240** |
| ATW | –0.291*** | –0.301*** | –0.187* | –0.211* | –0.263** | –0.298** |
| DM | 0.320*** | 0.318*** | 0.066$^{ns}$ | 0.139$^{ns}$ | 0.131$^{ns}$ | 0.103$^{ns}$ |
| SC FM | 0.320*** | 0.329*** | 0.142$^{ns}$ | 0.179$^{ns}$ | 0.096$^{ns}$ | 0.108$^{ns}$ |
| CPC DM | 0.204** | 0.354*** | 0.452*** | 0.594*** | 0.355*** | 0.505*** |
| PC DM | 0.418*** | 0.756*** | 0.589 *** | 0.889*** | 0.255** | 0.565*** |

PRA – patatin relative abundance; PAT-C DM – patatin content in dry matter; SE – scale of earliness in points declared by central Institute for Supervising and Testing in Agriculture in Brno; ATW – average tuber weight; SC FM – starch content in fresh matter; CPC DM – crude protein content in dry matter; PC DM – protein content in dry matter; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $^{ns}$not significant

and patatin content could be explained by similar results of relationship between protein content and average tuber weight presented by Snyder and Desborough (1978).

The presented results showed the importance of the cultivar type with regard to the ability of patatin proteins accumulation in potato tubers. These also indicated that, in the case of future exploitation of potato tuber protein potential in food or other applications, it will be necessary to choose optimal processing cultivars not only according to the starch content, but also on the gasis of the pure protein content and especially the main storage protein, patatin.

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