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THE EFFECT OF STORAGE ON THE AMINO ACIDS COMPOSITION IN POTATO TUBERS

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Abstract

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The aim of this study was to investigate the effect of storage duration on amino acids content in potato tubers. Tubers of six cultivars were stored for 16 weeks. Crude protein was determined by the Kjeldahl method. The amino acid content was identified after acid hydrolysis; sulphur amino acids were oxidized with the mixture of hydrogen peroxide, formic acid and phenol. The essential amino acids index and chemical score was calculated. The whole egg protein was used as reference protein. The effect of storage duration and cultivar on the total amino acids content was found to be significant in all potato varieties. The level of the total amino acids contents was determined within the range of 80–87%. Similar downward trend was observed in crude protein. The essential amino acids index varied considerably among the potato cultivars, ranging from 42 to 57%. Sulfur amino acids and isoleucin were the limiting amino acids in all the investigated tubers.

Solanum tuberosum L., storage, amino acid, protein, essential amino acids index

Potato (*Solanum tuberosum* L.) is a typical crop grown in many countries in Europe. In the Czech Republic it is one of the most important horticultural crops. More than 753 thousand tons of potatoes were harvested in 2009 (ČSÚ, 2010). Potatoes are often perceived as a mere starch source, but they are also rich in minerals, vitamins and protective substances. Potatoes are used in diets, namely for their good digestibility and a low allergen potential. Food intolerance can be triggered only by potato products like chips and crisps. It is a well known fact that potato crops produce a higher quantity of protein per hectare than cereals (FAO, 2009). Potatoes contain about 2% of crude protein in fresh matter. Because of relatively higher potato consumption (annually approx. 70 kg per capita in the Czech Republic), potato tubers are an important source of protein in a human diet. However, the amount of crude protein depends on cultivar, agricultural practices and agroclimatic factors. Crude protein includes a number of nitrogen fraction, amides and free amino acids (Eppendorfer *et al.*, 1979). Potato protein is valuable for a high biological value. The biological value of potatoes mixed with eggs reaches to the amount of about 140% of standard protein (Zrůst, 2004). The basic role of potato protein in nutrition is the contribution to sufficient amounts of essential amino acids for protein endogenous synthesis by human body. It is essential to take the difference between nutritional and biological aspects in consideration. Therefore as far as biological aspect is concerned, nonessential amino acids are more important than the essential ones (Murray *et al.*, 1998). Chemical score obtained by comparing the limiting amino acids in relation to the reference protein is a chemical method which reflects the protein quality (Friedman, 1996).

Total amino acids content mainly depends on cultivar (Zrůst, 2004). Previous studies report variable trends in free amino acids contents in potato during the storage (Talley *et al.*, 1984). Certain studies on the free amino acid content in stored potato tubers have shown high values of amide asparagine and glutamine in tubers (Brierley *et al.*, 1997). Amino acids content is associated with Millard reaction and acrylamide formation. Several studies have shown that acrylamide can be formed in Millard reaction from asparagine with carbonyl compounds (Stadler *et al.*, 2002; Zyzak *et al.*, 2003; De Wilde *et al.*, 2005). The relationship among the quantity of reducing sugars, amino acids content and typical co-

lour of potato products was investigated by many researches (Roe *et al.*, 1990; Brierley *et al.*, 1996). Most studies in the field of amino acids content in potato have only focused on the amount of free amino acids. So far, there have been only a few discussions about the total amino acids content in stored potato tubers.

The purpose of the present study was to determine the influence of storage duration on the amino acids content, amino acids profile and amino acid score in six potato varieties.

MATERIALS AND METHODS

Storage and sampling of potato tubers

Potatoes (*Solanum tuberosum* L.) were grown in five locations in the Czech Republic on the premises of AGRO-MĚŘÍN, a.s. Six potato cultivars with different maturity class were selected for the experiment. Characteristics of the cultivars are shown in Tab. I. Winter barley (*Hordeum vulgare*) was used as a fore crop. In the autumn, manure was applied (45 t.ha^{-1}) together with nitrogen (40.5 kg N.ha^{-1} for straw decomposition) and potassium fertilization. Plants were grown using a standard commercial practice and a uniform fertilization regime. Nitrogen, potassium and phosphorus fertilization, in doses 131 kg N.ha^{-1} (UREA Stabil), 162 kg K.ha^{-1} (K_2O) and 72 kg P.ha^{-1} (P_2O_5), were used in the experiment. Tubers were harvested in the period from August till October 2008.

Randomly selected tubers were stored in a storage house (Beskyd Fryčovice, a.s., CZ) with an automatic air-conditioning control. Potatoes were kept at 3–6 °C and 80% RH in 20-kilogram boxes. Sprout formation during the storage was suppressed by NEO-STOP L 300 treatment in the form of gas. Tubers were sampled for analysis at 4-week intervals, in total period of 16 weeks. Each month a sample consisting of two 20-kilogram boxes was sent to laboratory analysis.

About two kilograms of healthy tubers of each delivery were selected, thoroughly washed to remove soil particles, peeled and ground. All the samples were kept at –80 °C and then lyophilized at –4 °C, 12.156 Pa for 48 h (Christ Alpha1–4 LSC, Labicom, CZ) before the analysis.

Total nitrogen content

Total nitrogen was determined by the micro of Kjeldahl method by an automatic Pro-Nitro 130. Conversion factor of 6.25 was used for the calculation of the crude protein content. Chemical analyses were repeated five times and the results were expressed on 100% dry matter basis (DM).

Amino acids analysis

To determine the fifteen amino acids (aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, phenylalanine, tyrosine, histidine, lysine and arginine), 0.1 g of lyophilized tubers was weighted into screw-capped vial. Amount 15 ml of 6 mol.l^{-1} HCl was added into a vial and the solution was purged by argon for 1 min. The vial was consequently placed in a term-block (Labicom, CZ) which was heated to 115 °C and a sample was hydrolyzed for 23 hours. Then the vial was cooled down, HCl was removed by a rotary vacuum evaporator (RVO 400a, Ingos, CZ) and the ropy residue was quantitatively transferred into the 25 ml volumetric flask with sodium-citrate buffer (pH 2.2). For the assessment of the sulphur amino acids (methionine and cysteine), 0.7 g of lyophilized tubers was oxidized in hydrogen peroxide, formic acid and phenol. Oxidation was accomplished at 2 °C for 16 hours (Official Journal of the European Union, 2009). The following procedure was the same as that of acid hydrolysis. The sample was filtered through a 0.45 μm filter before using the analyzer. For the assignment of amino acids, the ionic liquid chromatography with sodium-citrate buffers, post-column ninhydrin derivatization and spectrophotometric detection (Amino Acid Analyzer AAA 400, Ingos, CZ) was applied. Each sample was repeated six times and each hydrolysate was analyzed twice. The results were expressed in 16 g N.

I: *Characteristics of potato samples*

Variety	Maturing	Boiling type	Locality	GPS	Planting	Harvest
Angela	VE	B	Žďár/Sázavou	49°33´17´´N; 15°54´20´´E	17/05	29/08
Princess	VE	A	Krmelín	49°44´07´´N; 18°14´03´´E	30/05	10/09
Marabel	E	B	Měřín	49°25´01´´N; 15°54´41´´E	20/05	15/08
Laura	E	B	Paskov	49°43´50´´N; 18°17´51´´E	15/05	09/10
Milva	SE	AB	Paskov	49°43´50´´N; 18°17´51´´E	10/05	04/09
Belana	SE	AB	Petřvald	49°50´04´´N; 18°17´51´´E	12/05	04/10

VE – very early; E – early; SE – semi-early

All results were evaluated using the variation statistics, correlation matrices and regression functions were calculated according to Snedecor and Cochran (1967) using the statistical package Unistat (v. 5.5).

RESULTS AND DISCUSSION

The changes in the sum of nonessential (ΣNEAA), essential amino acids (ΣEAA) and total amino acids (ΣAA) in potato tubers of different varieties during the 16-week storage are presented in Tab. II. The results demonstrate that the storage duration has a significant influence on the amino acids content in all potato varieties. At the beginning of the storage, the value of ΣAA moved in the range of 79.9–87.2 g/16 g N. The results have shown that Angela variety contained the maximum of total amino acids. After 16-week storage of the potatoes, the total quantity of amino acids decreased approximately by 20%. The highest loss in the total amino acids content was observed in the potato variety Belana (28.4%), whereas the lowest reduction of ΣAA (8.7%) was recorded in Marabel variety. Both varieties belong to early potato varieties. A considerable fall (27%) in ΣNEAA was found in samples of Princess variety, which was stored for 4 months. Minor reduction in ΣNEAA resulting from the storage duration was observed in Marabel variety. The highest amount of essential amino acids was found in tubers of early variety Angela. Semi-early varieties Marabel and Belana had comparable values of ΣEAA. From the data in Tab. II, it is apparent that the amino acid composition is significantly influenced by potato variety. There exists a certain evidence that the storage time slightly effected the amino acids content in tuber of Marabel variety, whereas the value of the amino acids in Milva variety was markedly influenced by the storage duration. Tab. III shows the mean value of chemical score (CS) and essential amino acids index (EAAI) of the tested potato varieties. The chemical score with respect to the reference pattern (whole egg protein) was calculated for each cultivar. At the beginning and at the end of the storage, the mean value of the EAAI (considering all the essential amino acids except tryptophan) ranged from 54–66 and 42–57%, respectively. These results appear to be consistent with the previously published data (Sotelo *et al.*, 1998; Galdón *et al.*, 2010). The researches who used a reference protein (WHO/FAO) found the value of chemical score of amino acids between 42–53%. Further, it is known that potatoes contain a high amount of lysine which occurs rarely in plant proteins. The highest amount of lysine was determined in Marabel and Angela. At the beginning of the experiment, sulphuric amino acids (Cys+Met) were found as limiting amino acids in all the samples except Milva. It this variety, isoleucine was found as the first amino acid limiting the protein nutrition value. After the 16-week storage, Cys and Met were repeatedly detected as limiting acids in the tested varieties except Angela variety which had isoleucin as the firth and Cys and Met as the second limiting amino acids. Leucine was the second limiting amino acid for Milva and isoleu-

II: *Effect of 4-month storage on the sum of nonessential, essential and total amino acids in potato tubers in g/16 g N*

Variety	Angela		Princess		Marabel		Belana		Milva		Laura	
Storage (month)	1	4	1	4	1	4	1	4	1	4	1	4
ΣNEAA	51.30	41.96	53.78	39.17	49.08	43.50	49.07	37.77	46.71	36.77	50.18	40.30
ΣEAA	35.87	29.60	29.56	24.89	34.75	33.02	34.89	22.43	33.21	24.40	32.61	26.82
ΣAA	87.16	71.56	83.34	64.06	83.83	76.52	83.96	60.20	79.92	61.17	82.79	67.18

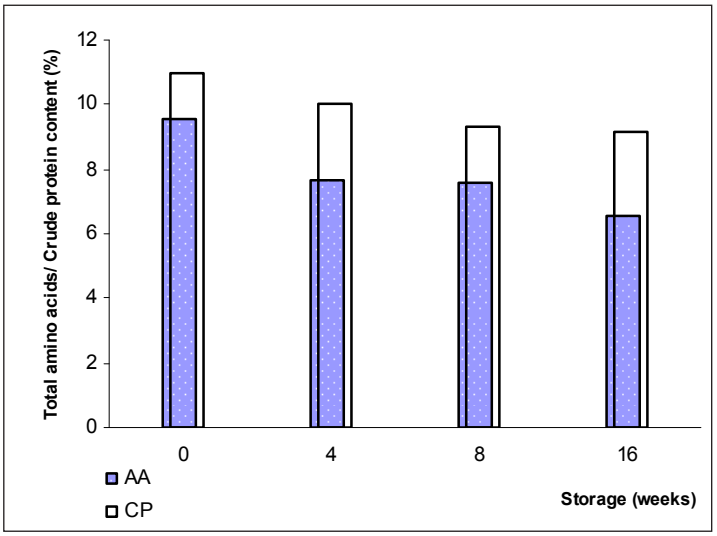
III: *Changes of Chemical score and Essential amino acids index in potato tubers during 4-month storage*

Index	Variety	Angela		Princess		Marabel		Belana		Milva		Laura	
	EAA	1	4	1	4	1	4	1	4	1	4	1	4
	Thr	69.2	54.6	64.3	41.2	69.2	60.8	61.1	54.6	64.3	41.6	60.3	41.9
	Val	80.5	63.2	68.5	61.0	74.9	71.4	74.5	63.2	68.5	53.4	74.9	59.9
	Leu	63.8	52.8	49.3	43.3	61.0	66.3	65.1	52.8	49.3	43.7	63.2	43.9
	Ile	55.4	44.6*	44.9	40.8	50.6	50.8	53.6	44.6	44.9*	39.0	51.3	41.8
CS	Lys	75.4	58.8	61.5	47.0	74.0	72.9	66.0	58.8	61.5	51.1	67.5	50.7
	Arg	67.1	57.4	51.1	52.2	67.7	51.3	80.1	57.4	51.1	48.7	54.0	68.9
	His	63.1	53.2	61.7	53.5	66.6	64.5	62.7	53.2	61.7	50.2	61.2	58.9
	Phe	67.7	54.9	55.8	45.5	66.1	65.6	65.7	54.9	55.8	45.6	62.5	48.1
	ΣCys+Met	52.2*	52.4	41.0*	37.1*	50.1*	43.1*	49.1*	38.4*	50.1	34.4*	44.7*	38.8*
EAAI		66.1	53.8	54.1	44.4	61.0	56.9	60.5	55.8	54.1	42.4	57.5	47.6

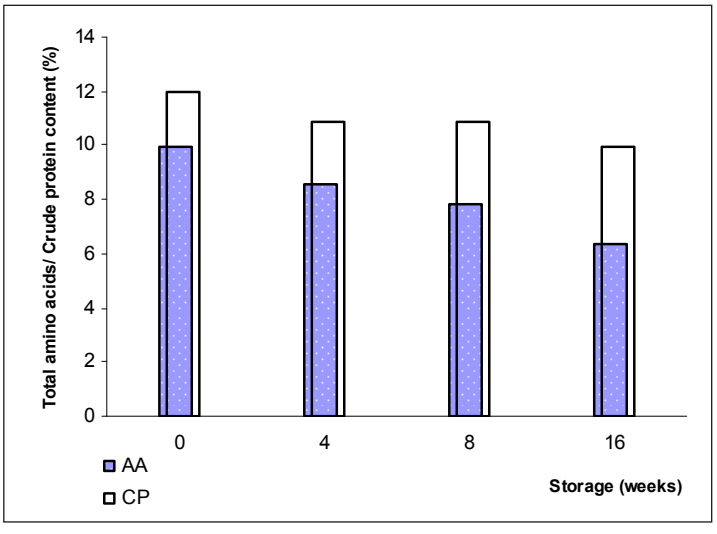
CS – Chemical score
EAAI – Essential amino acids index
* – The first limited amino acid

cine for the rest of the varieties. Suárez-López *et al.* (2006) have also found a very low level of sulfuric acids in the research that potato proteins content.

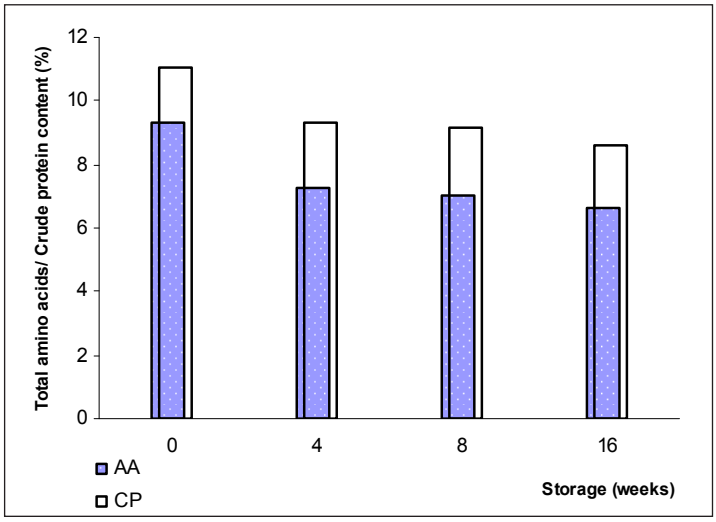
Figures 1–6 compare the content of total amino acids (AA) and crude protein (CP) in different potato varieties during the storage. At the beginning of the storage, the amount of CP was in the range from 8.9–11.9% of 100% DM. This finding is in an agreement with Galdón's *et al.* (2010) results, where it is mentioned that crude protein content ranged from 1.46–2.38% of the fresh weight. Similar results are mentioned by other investigators (Blenkinsop *et al.*, 2003). De Wilde's *et al.* (2005) research shows that crude protein was fairly constant in tubers stored for 24 weeks. According to our results, longer storage time results in a decrease of the content of amino acids and the similar downward trend can be seen in crude protein. After the 16-week storage, the amount of CP and AA decreased approx. by 35 and 18%, respectively. A rapid change in CP value (24%) was found in the samples of Belana variety. This variety showed the highest drop of total amino acids content (46%). On the contrary, a slight change in the amount of CP (10%) was observed in the tubers of Milva variety and in the lowest fall of AA content (29%) was found in Marabel variety. There exists certain evidence that the amino acids content and the quantity of crude protein are affected by the storage time and by the variety, but not the same way. Bártová *et al.* (2009) studied the effect of cultivar, locality and nitrogen fertilization on crude protein content in tubers of starch processing potato. Bártová *et al.* show that the most important factor affecting CP is the cultivar that participated in the total variability with approximately 34%.



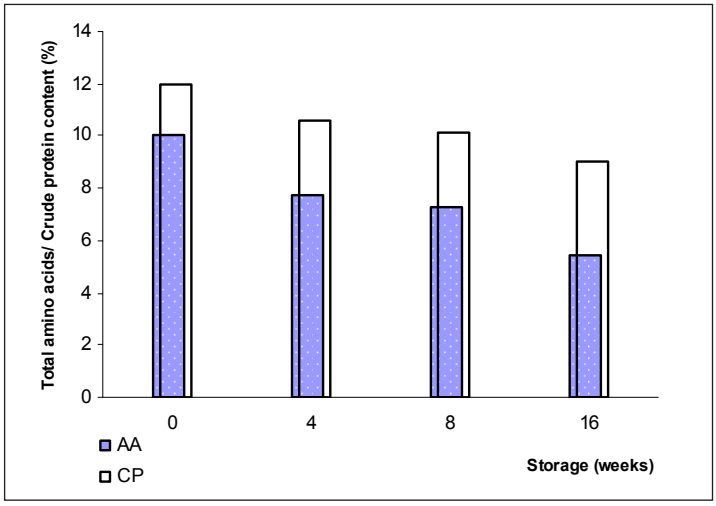
1: *Effect of storage on value of AA and CP (%) in tubers of Angela variety*



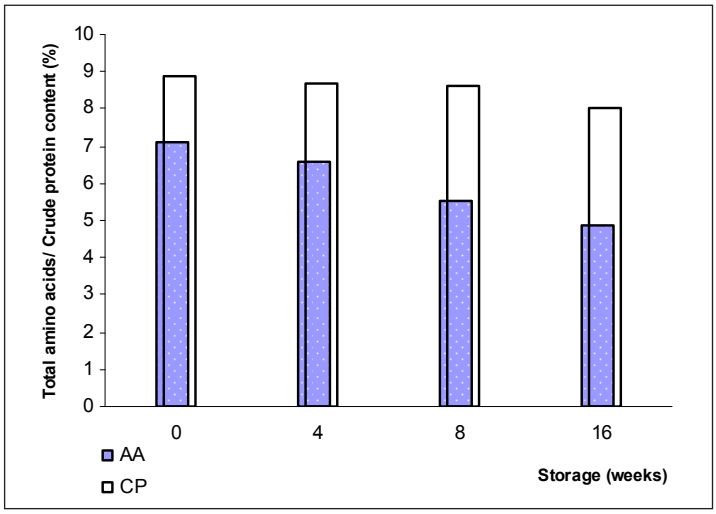
2: *Effect of storage on value of AA and CP (%) in tubers of Princess variety*



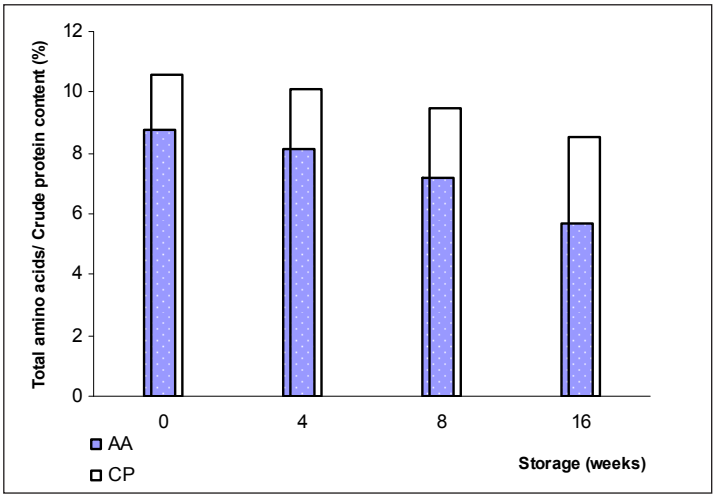
3: *Effect of storage on value of AA and CP (%) in tubers of Marabel variety*



4: *Effect of storage on value of AA and CP (%) in tubers of Belana variety*



5: *Effect of storage on value of AA and CP (%) in tubers of Milva variety*



6: *Effect of storage on value of AA and CP (%) in tubers of Laura variety*

CONCLUSION

This study has investigated the influence of storage and cultivars on the amino acids content. The results of this investigation demonstrate that the genetic characteristics of potato cultivars influence amino acids content and therefore their nutrition value. Furthermore, longer time of storage caused a reduction of amount of crude protein and consequently the total amino acids content. Angela and Belana varieties showed the best amino acid profiles. In general, in case of amino acids amount, Marabel variety appears to be the most resistant to the storage time. This study has shown that all potato varieties had methionine, cysteine and isoleucine as limiting amino acids. Nevertheless, any quality evaluation of protein has to take into account also digestibility of that protein and thus subsequently correct also its chemical score.

SOUHRN

Vliv skladování na obsah aminokyselin v hlízách brambor

Práce sleduje vliv doby skladování na obsah aminokyselin v hlízách brambor. Šest odrůd brambor bylo skladováno za standardních podmínek po dobu 16 týdnů. Hlízy byly analyzovány vždy po čtyřech týdnech. Byl zjištěn negativní vliv doby skladování na obsah aminokyselin. Na počátku skladovacího pokusu se celkový obsah aminokyselin pohyboval v rozmezí 80–87 g/16 g N. Po čtyřměsíčním skladování bylo zaznamenáno snížení v obsahu aminokyselin v průměru o 20 %. Podobný trend byl shledán i v případě hrubého proteinu, jehož množství se v hlízách skladovaných 16 týdnů snížil v průměru o 17 %. Jako první limitující aminokyseliny byly v bramborách detekovány sirné aminokyseliny cystein a methionin a dále isoleucin, který byl zároveň i druhou limitující aminokyselinou. Po použití Scheffého metody bylo zjištěno v rámci každé odrůdy, že skladování ovlivňovalo index esenciálních aminokyselin, jehož průměrné hodnoty dosahovaly na počátku skladování 58 % a na konci skladovacího pokusu 51 %.

brambory, skladování, aminokyseliny, protein, index esenciálních aminokyselin

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REFERENCES

BÁRTOVÁ, V., BÁRTA, J., DIVIŠ, J., ŠVAJNER, J., PETERKA, J., 2009: Crude protein content in tubers of starch processing potato cultivars in dependence on different agro-ecological conditions. *Journal of Central European Agriculture*, 10: 57–65. ISSN 1332-9049.

BLENKINSOP, R. W., COPP, L. J., YADA, R. Y., MARANGONI, A. G., 2003: A proposed role for the anaerobic pathway during low-temperature sweetening in tubers of *Solanum tuberosum*. *Physiologia plantarum,* 118: 206–212. ISSN 1399-3054.

BRIERLEY, E. R., BONNER, P. L. R., COBB, A. H., 1996: Factors influencing the free amino acid con-

tent of potato (*Solanum tuberosum* L.) tubers during prolonged storage. *Journal of the Science of Food and Agriculture*, 70: 515–525. ISSN 0022-5142.

BRIERLEY, E. R., BONNER, P. L. R., COBB, A. H., 1997: Aspects of amino acids metabolism in stored potato tubers (cv. Pentland Dell). *Plant science*, 127: 17–24. ISSN 0168-9452.

ČSÚ: Sklizeň zemědělských plodin v roce 2009. [online]. [Cit. 2010-01-02.] Available on: Český statistický úřad. Web site: < http://www.czso.cz/csu >.

DE WILDE, T., DE MEULENAER, B., MESTDAGH, F., GOVAERT, Y., VANDEBURIE, S., OOGHE, W., FRASELLE, S., DEMEULEMEESTER, K., VAN PETEGHEM, C., CALUS, A., DEGROODT, J. M., VERHÉ, R., 2005: Influence of storage practices on acrylamide formation during potato frying. *Journal of Agricultural and Food Chemistry,* 53: 6550–6557. ISSN 1520-5118.

EPPENDORFER, W. H., EGGUM, B. O., BILLE, S. W., 1979: Nutritive value of potato crude protein as influenced by maturing and amino acid composition. *Journal of the Science of Food and Agriculture*, 30: 361–368. ISSN 0022-5142.

FAO, 2009: Statistics Division. [online]. [Cit. 2010-01-02.] Available from: FAOSTAT. Web site: <http://faostat.fao.org>.

FRIEDMAN, M., 1996: Nutritional value of proteins from different food source. *Journal of Agricultural and Food Chemistry*, 44: 6–29. ISSN 1520-5118.

GALDÓN, B. R., MESA, D. R., RODRÍGUEZ, E. M. R., ROMERO, C. D., 2010: Amino acid content in traditional potato cultivars from the Canary Island. In: *Journal of food composition and analysis.* [online], Available from: http://dx.doi.org/10.1016/j.jfca.2009.08.009. ISSN 0889-1575.

MURRAY, R. K., GRANNER, D. K., MAYES, P. A., RODWELL, V. W., 1998: Harper's Biochemistry. 24th ed. Appleton & Lange. 848 p. ISBN 978-0838536117.

COMMISSION REGULATION (EC) No. 152/2009 of January 17, 2009 laying down the methods of sampling and analysis for the official control of feed, February 26, 2009, *Official Journal of the European Union* L 54, p. L 54/12–L 54/14.

ROE, M. A., FAULKS, R. M., BELSTEN, J. L., 1990: Role of reducing sugars and amino acids in fry colour of chips from potatoes grown under different nitrogen regimes. *Journal of the Science of Food and Agriculture*, 52: 359–364. ISSN 0022-5142.

SNEDECOR, G. W., COCHRAN, W. G., 1967: *Statistical Methods. Iowa: 6th ed.* Iowa State University Press, 1967, p. 534.

SOTELO, A., CONTRERAS, E., SOUSSA, H., HERNÁNDEZ, V., 1998: Nutrient composition and toxic factor content of four wild species of Mexican potato. *Journal of Agricultural and Food Chemistry*, 46: 1355–1358. ISSN 1520-5118.

STADLER, R. H., BLANK, I., VARGA, N., ROBERT, F., HAU, J., GUY, P. A., ROBERT, M. C., RIEDIKER, S., 2002: Acrylamide from Millard reaction products. *Nature*, 419: 449–450. ISSN 1476-4687.

SUÁREZ-LÓPEZ, M. M., KIZLANSKY, A., LÓPEZ, L. B., 2006: Evaluación de la calidad de las proteínas en los alimentos calculando el escore de aminoácidos corregido por digestibilidad. *Nutrición hospitalaria,* 21: 47–51. ISSN 0212-1611.

TALLEY, E. A., TOMA, R. B., ORR, P. H., 1984: Amino acid composition of freshly harvested and stored potatoes. *American potato journal,* 61: 247–29. ISSN 0003-0589.

ZRŮST, J., 2009: Faktory ovlivňující obsah nutričně významných a škodlivých látek v hlízách a výrobcích z brambor [online]. [Cit. 2009-05-03.] Available from: Vědecký výbor fytosanitární a životní prostředí. Web site: <http://www.fytosa-nitary.org/projekty/2004/vvf-05-04.pdf>.

ZYZAK, D. V., SANDERS, R. A., STOJANOVIC, M., TALLMADGE, D. H., EBERHART, B. L., EWALD, D. K., GRUBER, D. C., MORSCH, T. R., STROTHERS, M. A., RIZZI, G. P., VILLAGRAN, M. D., 2003: Acrylamide formation mechanism in heated foods. *Journal of Agricultural and Food Chemistry,* 51: 4782–4787. ISSN 1520-5118.

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