

**POTATO GENETICALLY MODIFIED BY 14-3-3 PROTEIN REPRESSION IN GROWING RAT DIETS.
PART I: CHEMICAL COMPOSITION AND DIGESTIBILITY OF NUTRIENTS***Iwona Kosieradzka¹, Ewa Sawosz¹, Jan Szopa², Vitali Vasko¹**¹Department of Animal Nutrition and Feed Science, Warsaw University of Life Sciences; ²Institute of Biochemistry and Molecular Biology, Wrocław University*

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A study was undertaken to assess the nutritional value of tubers of transgenic lines of potato cv. Desiree with repression of a and c isoforms of 14-3-3 proteins obtained by means of antisense transformations. Isoforms of 14-3-3 protein are responsible for metabolism of carbohydrates, amino acids and calcium. Repression of that protein, *i.e.* reduction of its synthesis in a potato plant, results in: a decrease in total protein content of tuber with a slight but favourable change in the amino acid profile, enhanced synthesis of starch, small differentiation in contents of minerals – diminished concentrations of Fe, P, and Ca. The 30% addition of dried transgenic potato tubers with repression of isoform a, c as well as a and c of 14-3-3 protein was found not to affect values of digestibility coefficients of nutrients. Repression of 14-3-3 protein was demonstrated to affect diversification of the chemical composition of tuber and contents of nutrients only to a small extent, without diminishing the nutritional value of potato tubers.

INTRODUCTION

Intensive advance in techniques of genetic engineering has enabled the improvement of agrotechnical characteristics of one of the most popular in Poland crop used for feed and food purposes, namely: potato. Genetic modification of plants of that species usually concerns characteristics that determine their resistance to pathogenic fungi, bacteria and viruses [Gazendam *et al.*, 2004; Flis & Zimnoch-Guzowska, 2000; Missiou *et al.*, 2004]. Potato plants are also subjected to transformations aimed at changing the nutritional value and quality attributes of tubers. Such an effect has been obtained by *e.g.* expression of soybean glycinin in a transgenic plant [Hashimoto *et al.*, 1999a,b] or by strengthening inulin synthesis and reducing the concentration of starch [Broll *et al.*, 2005]. A change of the nutritional value of the edible part of a plant may result from the introduction of genes encoding the synthesis of regulatory proteins that alter plant's metabolism. That group of proteins included isoforms of 14-3-3 protein subjected to modifications in the reported study. The 14-3-3 protein functions as an adapter of multiple enzymes. Its isoforms are responsible for the course of carbohydrate metabolism in a plant through the regulation of synthesis processes of catecholamines and other metabolic processes – among others: activation of tyrosine hydroxylase and tryptophan, kinase of C proteins, endonuclease and phospholipase. The 14-3-3 protein inhibits nitrate reductase and sucrose phosphate synthase as well as affects assimilation of nitrogen and carbon. In addition it is a significant factor regulating, among others, the length of the vegetative season of a plant, and affects as-

similation of phosphorus. Isoforms of 14-3-3 protein control the synthesis of amino acids and metabolism of calcium in a plant. Repression of a and c isoform of 14-3-3 protein evokes changes in the activity of nitrate reductase, sucrose phosphate synthase and starch synthase in transgenic plants. Plants with the repression of 14-3-3 protein are characterised by a reduced number of tubers, yet the tubers are bigger and crop of fresh matter of tuber is higher [Szopa, 2002].

Genetic modification of potato plant consisting in the repression of 14-3-3 protein is likely to lead to significant changes in the chemical structure of nutrients, micro- and macrocomponents as well as to changes in the concentration of biologically-active substances. An undesired and unexpected effect of transgenesis may also be a change in bioavailability of metabolites. Safety tests recommended by the European Food Safety Authority (EFSA) preceding commercialization of genetically-modified organisms designed for feedstuffs or foodstuffs, apart from changes in the chemical composition include also the stage of the assessment of digestibility of nutrients of the transformers [EFSA, 2004]. Thus, the undertaken research was aimed at evaluating the effect of genetic modification of potato plant, leading to repression of 14-3-3 protein, on the chemical composition and digestibility of nutrients of tubers as well as their nutritional value.

MATERIAL AND METHODS

The experimental material were tubers of potato *Solanum tuberosum L.cv. Desiree* subjected to genetic transformation of a regulatory protein P14-3-3. Transgenic plants were obtained

by means of bacterial transformation (with *Agrobacterium tumefaciens*) of potato leaf explants with a construct containing cDNA encoding the 14-3-3 protein in the antisense orientation. Use was made of a cellularly non-specific vector BinAR. A selective marker of GMO was kanamycin (npt II – genes of neomycin transferase), not applied in the prophylaxis nor treatment of humans and animals. Transformation was carried out at the Department of Genetic Biochemistry of Wrocław University.

In a nutritional experiment, use was made of tubers of three transgenic lines of plants: J4 – with repression of isoform a of 14-3-3 protein, J5 – with repression of isoform c of 14-3-3 protein, and G1 – with repression of isoform a and c of 14-3-3 protein.

Transformation of protein isoforms 14-3-3a and 14-3-3c in a potato plant, namely repression of genes responsible for the extent of synthesis of that protein, was carried out at the Department of Genetic Biochemistry of Wrocław University. Potato lines with repression of a and c isoforms of 14-3-3 proteins were obtained by means of antisense transformations.

Potato plants (both transgenic and non-transgenic ones) were cultivated in a greenhouse under conditions of the following light regime: 16 h of light (temp. 22°C) and 8 h of darkness (temp. 15°C) in individual containers. The plants were watered each day. Tubers were collected after 3-month cultivation in the greenhouse. Until being prepared for chemical and nutritional analyses, tubers cleaned off the soil were stored at a temperature of ca. 5°C in a dark room.

Comminuted and dried (at a temp. of ca. 50°C) tubers of transgenic and non-transgenic potatoes were subjected to chemical analyses. Contents of dry matter, ash, total protein, crude fat, crude fibre and starch of the dried tubers were determined according to AOAC procedures [1996]; energy value (gross energy) – heat of combustion of a dried plant tissue was determined with the use of a calorimetric bomb KL10. The concentration of minerals (Ca, Mg, Cu, Zn, K and Fe), after microwave mineralization of the sample (Milestone 1200 Mega) was assayed with the method of flame atomic absorption spectrometry (FAAS) using a Shimadzu AA 660 apparatus. The content of phosphorus (P) was determined by means of atomic emission spectrometry, ICP-AES; whereas amino acid composition, after protein hydrolysis, with the HPLC method for ion-exchange analysis of amino acids (Beckman 6300). The samples were hydrolysed with 6N HCl at a temperature of 110°C for 22 h, and the hydrolysate was determined for all amino acids except for methionine and cystine that were assayed after preliminary oxygenation of the samples with performic acid according to the method of Moore [1963], and for tryptophan that was assayed after basis hydrolysis with barium hydroxide according to the method of Eggum [1968]. The sum of glycoalkaloids (aglycones – alpha, beta and gamma forms of solanine and chaconine) was determined with the colorimetric method of Bergers [1980].

Digestibility of nutrients was evaluated in a 7-day (+5 days of preliminary period) experiment carried out on male Wistar rats from the outbred IF₂Jaz. The animals with a body weight of ca. 150 g (10 rats in each group), kept in individual balance cages in an air-conditions room with a constant temperature of ca. 22°C and 12-h dark/light periods, were fed with iso-

protein diets containing transgenic potatoes with repression of 14-3-3 proteins or non-transgenic potatoes of the same cultivar. The composition of diets balanced according to Nutrient Requirements of Laboratory Animals (NRC 1996) was provided in Table 1. Over the experimental period, diet intake was monitored and complete faeces were collected that were next determined for the contents of major components [AOAC, 1996].

RESULTS AND DISCUSSION

Tubers of transgenic potatoes with the repression of a and c isoform of 14-3-3 protein contained less ash, fat and crude fibre, but a 30% higher concentration of starch as compared to the non-transgenic Desiree cv. (Table 2). This was probably due to increased activity of starch synthase confirmed in a study by Žuk *et al.* [2005]. A reduction on the level of 14-3-3a and 14-3-3c protein synthesis in a potato plant resulted in a decreased content of total protein in tuber (Table 3) and

TABLE 1. Composition of experimental diets (g/kg) and their nutritional value (% dry matter).

Components	Group-diet			
	Desiree cv.	Transgenic		
		J4	J5	G1
Potatoes	300.0	300.0	300.0	300.0
Casein	106.3	117.5	122.9	120.2
Cellulose	30.2	30.2	31.4	32.5
Soybean oil	40.0	40.0	40.0	40.0
Mineral mix*	35.0	35.0	35.0	35.0
Vitamin mix**	10.0	10.0	10.0	10.0
Choline chloride	2.0	2.0	2.0	2.0
DI-met	1.0	1.0	1.0	1.0
Maize starch	475.5	464.3	457.7	459.3
Determined nutritional value				
Crude ash	4.21	5.11	4.61	4.97
Total protein	14.72	14.30	12.91	13.94
Crude fat	4.67	4.75	4.18	4.49
Crude fibre	2.88	4.24	3.13	2.52

*AIN-93G-MX **AIN-93G-VX,

TABLE 2. Contents of nutrients (g/kg dry matter) and energy value (cal/100 g) of potato tubers.

	Desiree cv.	Transgenic		
		J4	J5	G1
	Crude ash	57.0	53.5	42.2
Total protein	142.8	113.3	98.2	105.1
Crude fat	3.4	2.9	2.9	2.6
Crude fibre	35.6	35.7	31.5	27.1
Starch	527.0	618.2	568.7	685.5
Soluble saccharides	37.8	18.3	80.0	21.7
Gross energy	77	80	83	82

TABLE 3. Amino acid profile of protein of transgenic and non-transgenic potatoes (g/16 gN).

Specification	Desiree cv.	Transgenic		
		J4	J5	G1
Nitrogen (%)	2.106	1.656	1.434	1.544
Lys	4.73	5.06	5.34	4.78
Met	1.12	1.07	1.11	1.07
Cys	0.90	0.83	0.94	0.79
Thr	2.39	2.24	2.57	2.19
Trp	0.88	1.05	1.08	0.98
Val	3.86	3.62	4.38	3.91
Ile	2.85	2.7	3.15	2.82
Leu	3.95	3.67	4.08	3.49
Tyr	4.11	4.63	4.36	4.5
Phe	2.91	2.85	3.25	3.5
His	1.63	1.68	1.65	1.6
Arg	2.98	3.40	4.24	3.62
Asp	15.45	19.88	23.3	21.37
Glu	26.04	22.06	17	20.51
Ser	2.37	2.26	2.71	2.26
Pro	4.19	4.01	3.8	3.55
Gly	2.33	2.16	2.41	2.04
Ala	3.62	2.96	2.58	2.61
Sum of AA*	86.31	86.13	87.95	85.56
Sum of EAA**	32.31	32.8	36.15	33.22
EAA /AA	37.43	38.08	41.10	38.82

*AA—total amino acids; **EAA—total essential amino acids

in slight changes in the amino acid composition of protein (Table 4). The transgenic potatoes were observed to be characterised by negligibly enhanced synthesis of tryptophan and lysine. The value of a coefficient determining the ratio of exogenous amino acid to a sum of amino acids appeared to be higher, which may point to a slightly higher nutritional value of protein in tissue of tubers of transgenic potatoes as compared to the non-transgenic ones.

Tubers of transgenic potatoes contained lower amounts of nitrogen compounds (total protein), but the protein was characterised by slightly better amino acid composition (it contained more exogenous amino acids), (Tables 2 and 3). Potato tubers of lines J4 and G1 contained higher concentrations of starch than the tubers of parental line, whereas tubers of line J5 contained higher amounts of monosaccharides (Table 2).

Changes in the metabolism of plants upon genetic modification of 14-3-3 protein (repression) were accompanied by negligible changes in the concentration of minerals. Tubers of transgenic potatoes were characterized by relatively lower contents of Ca, Fe and P (Table 4).

An expected effect of potato plant transgenesis was modification of resistance to stress, which, among other things, was obtained owing to the intensification of synthesis of flavonoid group compounds, confirmed with HPLC analysis of peridermal layer of tuber [Szopa, 2002; Szopa *et al.*, 2001].

TABLE 4. Contents of minerals (mg/kg dry matter and anions) of potato tubers examined.

Minerals	Desiree cv.	Group-diet		
		Transgenic		
		J4	J5	G1
Ca	414.5	235.4	260.9	206.9
Mg	1390.1	1247.0	1238.5	1271.9
Zn	34.5	27.2	26.5	19.9
Mn	12.6	15.0	9.6	10.4
Cu	6.6	4.5	2.5	3.1
Na	37.6	23.7	16.6	15.3
K	31000.0	30000.0	24000.0	27000.0
P	2908.3	694.1	340.9	185.1
Fe	334.2	178.5	101.6	212.0
Cl ⁻	1646.2	1196.6	1576.1	1445.1
NO ³⁻	110.7	64.9	281.7	183.0
SO ₄ ²⁻	4896.4	4617.9	3544.5	4643.4
HPO ⁴⁻	3326.1	5594.5	3582.9	3189.6

Plants with the repression of 14-3-3 protein were also characterised by diminished accumulation of glycoalkaloids in the tubers [Szopa, 2002]. However, the evaluation of the chemical composition of dried tubers used as a dietary component in the nutritional experiment demonstrated slightly higher content of glycoalkaloids (Figure 1) in dried whole tubers of plants displaying repression of 14-3-3 protein, especially of its isoform a.

Digestibility of all basic nutrients of iso-protein diets with 30% addition of dried transgenic and non-transgenic potatoes (Table 5) was very similar. Taking into account negligible changes in the content of selected metabolites and minerals as

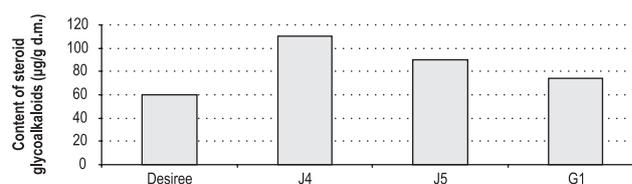


FIGURE 1. Content of the sum of steroid glycoalkaloids – alpha, beta and gamma forms of aglycones of solanine and chaconine.

TABLE 5. Digestibility of nutrients (% dry matter) with experimental diets.

Components	Desiree cv.	Group-diet			SEM	p
		Transgenic				
		J4	J5	G1		
Total protein	82.39	75.66	78.13	80.12	2.0834	0.1718
Crude fat	93.87	94.07	93.06	95.11	0.7373	0.2818
Crude fibre	19.22	21.51	10.56	20.23	2.9143	0.0656
N-free extractive compounds	93.67	93.91	95.06	94.19	0.5323	0.3085

well a comparable value of digestibility coefficients of nutrients, the nutritional value of the compared potatoes determined with those parameters might be acknowledged as equivalent.

Ample experiments aimed at evaluating the equivalence of the chemical composition of plants that were made, by means of genetic modification, resistant to herbicides, insects and viruses (the so-called first generation of GMOs), have confirmed the equivalence of the chemical composition and nutritional value of transgenic lines and their corresponding conventional lines [Hashimoto *et al.*, 1999a,b; Zduńczyk *et al.*, 2005; Aulrich *et al.*, 2001; Aumaitre *et al.*, 2002].

In turn, significant differences in the chemical composition of tubers of genetically-modified potatoes resistant to insects, displaying an expression of lectin gene (of snow-drop bulb lectin) and their corresponding non-transgenic lines were reported by Ewen & Pusztai [1999]. None of the published research on the composition of potato tubers subjected to various genetic modifications, however, have demonstrated the occurrence of an unexpected effect of transgenesis leading to increased concentrations of toxic substances or anti-nutrients, though contents of some metabolites were reported to vary [Novak & Halsberger, 2000; Ewen & Pusztai, 1999].

A few-year investigations into the chemical composition of potatoes with modified protein 14-3-3, conducted at the Department of Genetic Biochemistry of Wrocław University and co-operating units, have yielded relatively diversified results that were only in part consistent with results presented in this manuscript [Świądrych *et al.*, 2002; Szopa *et al.*, 2001]. This may result from natural variability of plant composition, independent of modification, or suggest a considerable effect of cultivation conditions on the chemical composition – the value of the assayed parameters. According to Matthews *et al.* [2005], changes in the concentration of secondary metabolites in a plant may occur both in transgenic and non-transgenic lines of potatoes as a result of introducing a transgene or as a consequence of interactions with environmental factors.

In a study by Catchpole *et al.* [2005] metabolic changes in plants originating from conventional cultures appeared to be more significant than those being an undesired effect of transgenesis.

CONCLUSIONS

Repression of 14-3-3 protein (a and c isoforms) in genetically-modified plants of potato affected a decrease in the content of total protein in tubers and a slight improvement of its amino acid composition, evoked an increase in starch synthesis, but also a negligible increase in the concentration of glycoalkaloids. Diversified contents of minerals were reported in tubers of both transgenic and non-transgenic potato lines, and the repression of 14-3-3 protein resulted mostly in diminished concentrations of Fe, P, Ca. The administration of diets with 30% addition of dried transgenic or non-transgenic potato tubers had no significant effect on the growth of animals. No statistically significant differences were either observed in the values of digestibility coefficients of nutrients. The results obtained indicate that the nutritional value of the examined transgenic potatoes with repression of a and c isoform of 14-3-3 protein is equivalent with that of the non-transgenic potatoes.

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ZIEMNIAKI GENETYCZNIE MODYFIKOWANE PRZEZ REPRESJĘ BIAŁKA 14-3-3 W DIETACH ROSNĄCYCH SZCZURÓW. CZĘŚĆ I: SKŁAD CHEMICZNY I STRAWNOŚĆ SKŁADNIKÓW POKARMOWYCH

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Oceniano wartość pokarmową bulw transgenicznych linii ziemniaka odmiany Desiree z represją izoform a i c białka 14-3-3 uzyskanych poprzez transformacje antysensowe. Izoformy białka 14-3-3 są odpowiedzialne za metabolizm węglowodanów aminokwasów i wapnia. Efektem represji tego białka, redukcji poziomu jego syntezy w roślinie ziemniaka jest: zmniejszenie zawartości białka ogólnego w bulwie z niewielką ale korzystną zmianą profilu aminokwasowego, wzrost syntezy skrobi, niewielkie zróżnicowanie zawartości składników mineralnych – zmniejszenie koncentracji Fe, P, Ca. Nie stwierdzono wpływu 30% dodatku suszu z bulw ziemniaków transgenicznych z represją izoformy a, c oraz a i c białka 14-3-3 na wartość współczynników strawności składników pokarmowych. Represja białka 14-3-3 wpłynęła w niewielkim stopniu na zróżnicowanie składu chemicznego bulwy i zawartość składników pokarmowych nie zmniejszając wartości odżywczej bulw ziemniaków.