Food ©2009 Global Science Books

Nutritional Properties of Tubers of Genetically Modified Potatoes Cultivated in Poland - A Review

Zenon Zduńczyk[1*] • Iwona Kosieradzka[2]

[1] Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland
[2] Department of Animal Nutrition and Feed Science, Warsaw University of Life Sciences, Ciszewskiego 8, 02-786 Warsaw, Poland
Corresponding author: * **zez@pan.olsztyn.pl**

ABSTRACT

This review addresses *in vivo* experiments concerning nutritional and health properties of tubers of transgenic potatoes cultivated in a field experiment in Poland. Genetic modification was conducted with three aims: (1) to improve resistance to a necrotic strain of *Potato virus Y* (transgenic lines with a truncated gene coding PVY^N polymerase in the sense or antisense orientation and lines with a non-translated region of the PVY^N genome in the sense or antisense orientation), (2) to modulate the content of flavonoids through overexpression of 14-3-3 protein from *Cucurbita pepo* or to repress the gene encoding protein ADP-ribosylation factor, and (3) to enhance flavonoids synthesis through overexpression of the enzymes of the flavonoid synthesis pathway, i.e. chalcone synthase, chalcone isomerase and dihydroflavonol dehydrogenase. Special attention is focused on differentiated chemical composition and nutritional properties of diets. Investigations were conducted on rats fed for 3-5 weeks diets with a high content (20-40%) of autoclaved and dried potato tubers. The *in vivo* experiments indicate that tubers of genetically modified potato are a substantial and nutritional equivalent to the non-transgenic cultivars, although some indices of the physiological response of animals pointed to the need for conducting longer investigations.

Keywords: modified potato, nutritional value, health status, rats
Abbreviations: ΣEAA, total essential amino acids; **2'dG**, 2'deoxyguanosine; **8-oxo-2'dG**, 8-oxo-2'deoxyguanosine; **ALT**, alanine aminotransferase; **AP**, alkaline phosphatase; **AST**, aspartate aminotransferase; **BWG**, body weight gain; **CHI**, chalcone isomerase; **CHOL**, total cholesterol; **CHS**, chalcone synthase; **CK**, creatine kinase; **DFR**, dihydroflavone reductase; **DI**, diet intake; **dm**, dry matter; **FCR**, feed efficiency ratio; **GMO**, genetically modified organism; **GPx**, glutathione peroxidase; **GST**, glutathione *S*-transferase; **GT**, glucose transferase; **HDL**, High Density Lipoproteins; **IgE**, E class immunoglobulins; **LDH**, lactate dehydrogenase; **NTR.1.16**, **NTR2.27**, **R1F**, **R2.P**, transgenic clones of 'Irga' potato; **PVY**, *Potato Virus Y*; **PVY^N**, necrotic strains of potato virus; **SCFA**, short chain fatty acids; **SEM**, standard errors of the means; **SGA**, steroid glycoalkaloids; **SOD**, superoxide dismutase; **TAS**, total antioxidant potential; **TBARS**, thiobarbituric acid; **TG**, triglycerides

CONTENTS

INTRODUCTION

For a number of decades, potatoes have been occupying a prominent position in the crop structure and average diet in Poland. Before World War II, in many poor regions of Poland potatoes were the main source of dietary energy, supplemented with cow's milk as a source of deficient animal protein. In addition, potato was a highly important

Received: 29 May, 2008. Accepted: 23 August, 2008.

Invited Review

feedstuff plant extensively applied in the nutrition of pigs. The cultivation of vast, low-efficient plantations of potato was facilitated by overpopulation of the Polish agriculture. A similar situation, which has been confirmed in statistical data of the Central Statistical Office (GUS), occurred in the 20-year interwar period. At the end of the 1970s, the cropping area of potato in Poland was approximately 2.8 million ha – twice as high as the cultivated area of potato in the European Union. Simultaneously, potato yields were nearly two-fold lower than in the EU. In the last 20 years, the cropping area of potato in Poland has been decreasing successively. In 2000-2006 the area of its cultivation diminished from *ca*. 1.3 million ha to ≤600 thousand ha (GUS 2007). The reduced cropping area was however, not accompanied by increased potato yield; in that period the average yield decreased from 180 to 150 dt/ha. This was also the reason for a diminished potato crop, from 23.6 million tons in 1996-2000 to 14.6 million tons in the subsequent 5-year period and finally to 9,000 tons in 2006. In the same period, a decrease was also observed in potatoes' utilization for feedstuff purposes, both in absolute values (from over 12,000 to over 5,000 tons) and in relative values (from over 50% to *ca*. 30% of the total tuber crop).

Despite the above-mentioned unfavorable tendencies, Poland is still one of the countries in which potato is amongst the major crops and constitutes an essential component of the diet of its inhabitants. The number of countries with a similar dependence on potato has been estimated to reach *ca*. 125 (Mullins *et al.* 2006). For this reason, an improvement in potato species improvement is of the greatest importance, whether achieved by conventional methods or by biotechnological tools.

Comprehension of the potato genome would enable locating genes that determine the key properties of this plant (Gebhardt and Valkonen 2001), whereas the available biotechnological tools will allow the improvement of agrotechnical, technological, nutritional and dietetic characteristics of regional cultivars of potato (Mullins *et al.* 2006).

Potato was one of the first crops subjected to successive genetic modification aimed foremost at strengthening its defense mechanisms (Shahin and Simpson 1986; Kaniewski *et al.* 1990). The improvement of resistance to pathogenic factors has been achieved by suppression or enhancement of the expression of genes stimulating defense mechanisms and of genes responsible for the synthesis of substances exerting toxic effects on insects and microorganisms (Missiou *et al.* 2004). The genetic modification of regional cultivars of potatoes has resulted in an increase in their resistance to fungal pathogens (Chye *et al.* 2005), insects (Gatehouse *et al.* 1997; Davidson *et al.* 2006), and stress (Matthews *et al.* 2005). Genetic engineering has been applied to alter the nutritive value of potato tubers, among other ways, by modifying the metabolism of carbohydrates and proteins in plants (Schwall *et al.* 2000) or by inducing gene expression of soybean glycinin (Hashimoto *et al.* 1999). Similar modifications of potato have been conducted in Poland and involved the enhancement of plants' resistance to key viral diseases.

NUTRITIONAL PROPERTIES OF TUBERS OF TRANSGENIC POTATO RESISTANT TO A NECROTIC STRAIN OF *POTATO VIRUS Y* (PVY^N)

The aim and methods of genetic modification

Viral diseases are the most economically important problem in potato crops cultivated in large areas. If pathogens are not properly controlled, the potato yield of infected plants may be reduced by 80%. In Poland, the most important potato pathogens include *Potato Virus Y* (PVY), and particularly a new isolate of PVY from the subgroup of necrotic strains (PVY^N). Even recently, necrotic strains of potato virus were spreading in Poland and were responsible for 80-90% of PVY infections in potato fields (Chrzanowska and Doroszewska 1997). The cultivation of potato involves the application of a number of natural resistance genes to PVY originating from different *Solanum* species. Obtaining new cultivars resistant to viral diseases through classic breeding involves the transfer of desired features from wild potato species, which is a time-consuming process. For this reason, genetic transformation of potato has recently been a focus of interest since it might be a faster and more effective way to prevent pathogen infection (Kaniewski *et al.* 1990; Jondedijk *et al*. 1992).

Transgenic lines of potato were prepared at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Warsaw. Plants of cv. 'Irga', which are relatively important in the Polish market for table potato, were transformed with viral genome sequences in order to improve their resistance to a necrotic strain of *Potato Virus Y* (PVY^N). Two types of constructs were introduced into the 'Irga' genome: R, containing a truncated gene coding PVY^N polymerase (GenBank Acc. No. D00441) and NTR, containing a fragment of cDNA corresponding to 184 nucleotides of the 5′-end of PVY of PVY^{N}Wi isolate (GenBank Acc. No. Z70238). The transformation process, selection and identification of transformed plants were described by Chachulska *et al*. (1997) and field performance of transgenic clones by Flis and Zimnoch-Guzowska (2000). Four clones were chosen for the nutritional study: R1F and R2P (transgenic lines with a truncated gene coding PVY^N polymerase in sense or antisense orientation, respectively) and NTR1.16 and NTR2.27 (transgenic lines with non-translated regions of PVY^N genome in sense or antisense orientation, respectively). Non-transgenic tubers obtained from normally planted 'Irga' were used as a control. Chemical analysis of row tubers was presented earlier by Sadowska *et al.* (2007, 2008), while the composition of autoclaved tubers and the results of *in vivo* evaluation of diets with conventional and transgenic tubers were described in detail by Juśkiewicz *et al.* (2004, 2005) and Zduńczyk *et al.* (2005a, 2005b).

Chemical composition and nutritional value

Results of 3-year investigations show that the contents of starch and protein were similar in tubers of conventional cv. 'Irga' and transgenic clones (Sadowska *et al.* 2007). The average content of L-ascorbic acid in dry matter of transgenic tubers was 27.2 mg/100 g, whereas in tubers of the conventional cultivar it reached 35.6 mg/100 g. The average content of α-solanine (26.6 mg/100 g dm) and α-chaconin (52.6 mg/100 g dm) in tubers of transgenic clones was generally lower than in tubers of 'Irga' (33.2 and 64.5 mg/100 g dm, respectively).

A similar content of the main nutrients was determined in autoclaved (121°C, 1013 hPa, 15 min) and dried at 40°C (Zduńczyk *et al.* 2005a) tubers used in the feeding experiments. The content of starch in tubers of transgenic clones (74.7-76.6% dm) was similar to that determined in dried tubers of cv. 'Irga', i.e. 76.7% dm (**Fig. 1**). The content of crude fiber in transgenic and conventional tubers (8.87-9.78 vs. 8.87% dm) were also similar. The content of resistant starch in autoclaved and dried transgenic tubers was slightly lower (6.80-8.71% dm) than in the conventional tubers (9.59% dm). In other investigations, the analysis of variability of physical and chemical parameters studied in raw and heat-treated potato tubers enabled classifying cv. 'Irga' and its genetically-modified clones as similar, and did not allow distinguishing a clone of special usability for heat processing (Sadowska *et al.* 2008).

Results presented in **Table 1** indicate that the content of crude protein and that of essential amino acids in crude protein of transgenic potatoes was similar or higher than in tubers of conventional cv. 'Irga'. Coefficients of apparent digestibility of protein diet containing 40% of autoclaved and dried potato of different origin were in a very narrow range, i.e. 81.1-81.9% (Zduńczyk *et al.* 2005a). The coefficient of apparent nitrogen retention of diet containing tubers of the transgenic clone NTR1.16 was lower (52.6%)

Table 1 The content of crude protein and essential amino acid in potato tubers of conventional cv. 'Irga' and transgenic clones. (modified from Zduńczyk *et al*. 2005b).

	Irga	Transgenic clones			
		R1.F	R2.P	NTR1.16	NTR2.27
Crude protein (%)	10.00	9.94	9.95	10.25	10.10
Amino acid (g/16 g N)					
Phe + Tyr	6.68	6.84	7.44	6.86	6.71
Iso	3.60	3.74	3.99	3.79	3.70
Leu	5.77	5.94	6.30	6.05	5.82
Lys	4.42	4.45	4.67	4.48	4.46
Met + Cys	3.33	3.42	3.36	3.43	3.46
Thr	3.50	3.60	3.66	3.56	3.44
Val	5.53	5.49	5.85	5.70	5.49
ΣEAA[a]	38.14	38.87	40.88	39.52	38.45

[a]ΣEAA – total essential amino acids
n=15

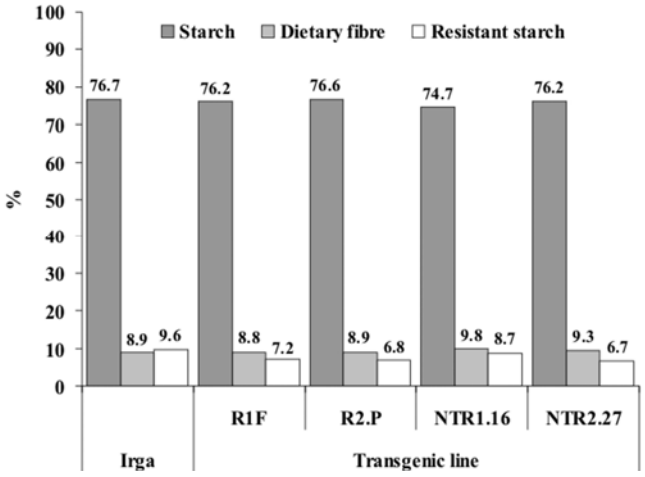

Fig. 1 The content of starch, dietary fiber and resistant starch in autoclaved and dried potato tubers. (modified from Zduńczyk *et al.* 2005a).

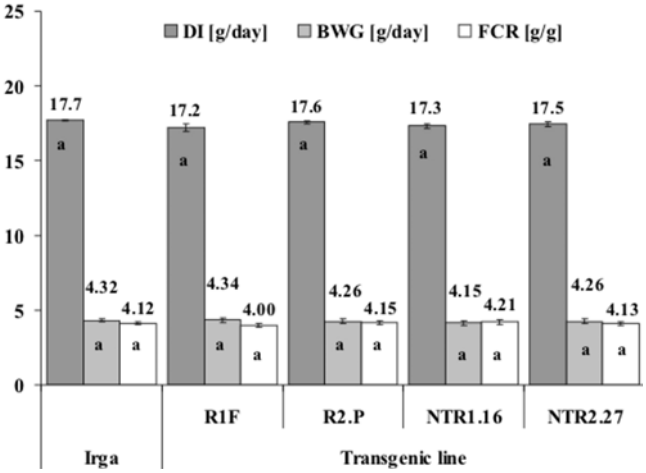

Fig. 2 Diet intake (DI), body weight gain (BWG) and feed efficiency ratio (FCR) in rats fed diet with 40% of autoclaved and dried tubers of different origin. (modified from Zduńczyk *et al.* 2005a).

in comparison with the diet containing tubers of the conventional cultivar.

Fig. 2 indicates that diet supplementation with 40% of autoclaved potato tubers of conventional and transgenic lines did not influence the growth of animals. Feed intake as well as feed utilization were similar in all experimental groups. Lower nitrogen retention in the group with the transgenic clone NTR1.16, which was denoted in a shorter digestibility test, was not confirmed in the entire 3-week experimental feeding. In this group the diet intake, body weight gain of rats and feed efficiency ratio were similar to those reported in the other groups.

Physiological properties of diets containing transgenic potato

In the experiment diets, for rats contained from 25.6 to 28.3% of autoclaved potato starch including from 2.7 to 3.8% of starch resistant to hydrolysis by exhaustive α-amylase and pulanase treatment *in vitro* (resistant starch). Together with dietary fiber the content of ingredients non digestible in the upper gastrointestinal tract ranged from 6.0 to 7.4% of the diet. The relatively few differences in resistant starch and dietary fiber content did not affect the functioning of the bacterial ecosystem in the lower part of the gastrointestinal tract of rats. No significant differences were observed for the activities of bacterial α- and β-glucosidase, α- and β-galactosidase and β-glucuronidase in the caecal digesta of rats fed diets containing tubers of cv. 'Irga' and transgenic lines (Juśkiewicz *et al.* 2004). The relative content of dry matter per cecum of rats was similar in groups fed diet with tubers of conventional cultivar and transgenic clones R1.F, R2.P and NTR.1.16 (0.170-0.178 g), however in the group fed a diet with tubers of the transgenic clone NTR2.27 the content of dry matter in the cecum appeared to be higher (0.204 g). This group was characterized by the highest production of short chain fatty acids (SCFA), including acetate and butyrate (**Fig. 3**). In the other groups, the SCFA pool in the cecum was proportional to the content of resistant starch and dietary fiber in the diet.

The activity of enzymes analyzed in the blood serum of rats was different not only between groups but also within each experimental group (**Table 2**). Comparable activities of aspartate aminotransferase (AST), creatine kinase (CK) lactate dehydrogenase (LDH) and alkaline phosphatase (AP) were measured in all groups. Only the activity of alanine aminotransferase (ALT) in the group fed with the transgenic clone R2.P was higher than that in the 'Irga'-group. In the group fed a diet with transgenic potatoes a distinct humoral response was observed: a higher content of protein and ceruloplasmin, and a higher activity of lysosyme in serum. More differentiated results were observed for indices of non-specific cellular response of rats. In the group fed a diet with tubers of clone R1.F a lower percentage of phagocytic cells and a lower number of bacteria absorbed per cells was observed, however the potential killing activity of macrophages was comparable to that observed in the other groups.

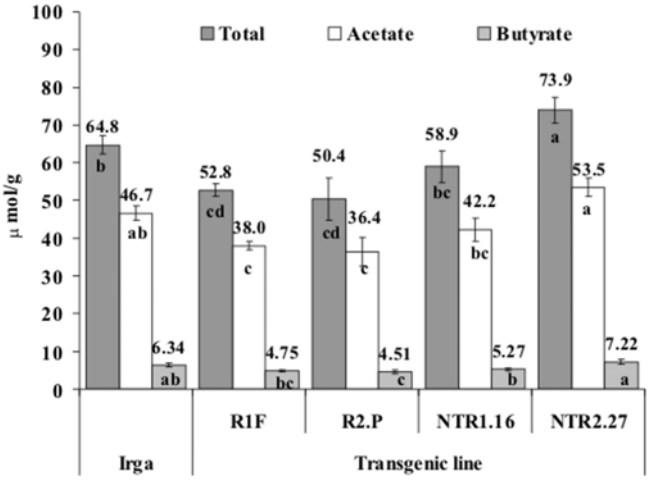

Fig. 3 Short chain fatty acid (SCFA) concentration in cecum of rats fed experimental diets. (modified from Juśkiewicz *et al.* 2005). [a, b, c,...] - Values in series having different superscripts are significantly different at $P \leq 0.05$.

Table 2 Activity of serum enzymes and indices of non-specific humoral and cellular defense of rats. (modified from Zduńczyk *et al*. 2005b)

	Irga	Transgenic clone				SEM
		R1.F	R2.P	NTR1.16	NTR2.27	
Alanine aminotransferase (IU/I)	21.6 b	26.6 ab	28.8 a	26.8 ab	22.4 b	0.78
Aspartate aminotransferase (IU/I)	165	156	161	166	180	4.54
Creatine kinase (IU/I)	1289	1580	1461	1342	1194	73.1
Alkaline phosphatase (IU/I)	589	591	514	502	557	23.9
Lactate dehydranase (IU/I)	1296	1497	1393	1559	1390	70.6
Protein (g/dl)	4.04 b	5.25 a	5.05 a	5.49 a	5.06 a	0.11
Ceruloplasmine (mg/dl)	19.41 c	23.35 ab	20.70 b	23.01 ab	22.50 ab	0.62
Lysosyme (Ug/dl)	3.56 c	4.67 a	4.70 a	3.96 abc	4.31 ab	0.13
Percentage of phagocytic cells	83.3 a	66.7 c	81.7 ab	73.3 bc	73.3 bc	1.49
Potential killing	0.218	0.219	0.214	0.213	0.215	0.01
Number of bacterial absorbed per cell	14.7 a	11.0 b	13.0 a	13.4 a	12.8 ab	0.21

Values in a row with different superscripts are significantly different at $P \leq 0.05$. Duncan's multiple range test was employed and 2 repetitions for all determined values.
SEM – standard errors of the means (standard deviation for all rats divided by square root of rat number, n = 40)

Nutritional and safety aspects of modification

The content of starch (15-18% of fresh weight) and protein (about 2.1% of fresh weight) is of great importance in the evaluation of the nutritional value of potato, as well as the content of glycoalkaloids, mainly α-solanine and α-chaconine (Finottii *et al.* 2006). Results obtained in our study (Zduńczyk *et al*. 2005) indicate that genetic modification of potatoes in order to improve their resistance to a necrotic strain of *Potato Virus Y* (PVY^N) had no effect on the content of starch, dietary fiber, crude protein, nor on the amino acid composition of protein in tubers subjected to hydrothermal treatments. The content of glycoalkaloids in raw tubers was very low compared to of the commonly recommended limit of alkaloids stipulated of 20 mg/100 g of fresh weight of potato (Friedman and McDonald 1997). The results obtained were in accordance with reports of other researchers who worked on plants with genetically-improved tolerance to herbicides and plants resistant to diseases. There were no differences in the chemical composition between genetically-modified potatoes and their conventional ancestor lines (Hashimoto *et al*. 1999; Rogan *et al*. 2000). Results of a later study by Catchpole *et al.* (2005) indicated that the metabolome of field-grown transgenic and conventional potatoes were substantially equivalent, apart from the targeted changes in the GM populations. As indicated in work by Zduńczyk *et al*. (2005a, 2005b), greater differences between the chemical composition (especially in crude protein and starch content) and biological response of rats were observed between tubers from conventional potato cvs. 'Irga', 'Ania' and 'Maryna' than 'Irga' and its genetically-modified clones.

Results of the above-mentioned *in vivo* experiment indicate that tubers of transgenic clones were generally a nutritionally equivalent to their conventional counterpart. However, the results obtained are insufficient to explain some deviations, e.g. higher content of essential amino acids in tubers of line R2.P, an increased bulk of cecal digesta and higher production SCFA in the cecum of rats fed a diet with tubers of the NTR2.27 line and lower indices of non-specific cellular response of rats fed a diet with tubers of the clone R1.F. For this reason, a long-term feeding study is necessary to confirm nutritional properties of tubers of transgenic lines. A long-term feeding experiment should enable the demonstration that genetic transformation of potato (with a truncated gene coding PVY^N polymerase or non-translated regions of the PVY^N genome, both in sense and antisense orientation) is advisable in respect of the nutritional and physiological properties of tubers.

NUTRITIONAL PROPERTIES OF TUBERS OF GENETICALLY MODIFIED POTATO WITH OVEREXPRESSION OF THE ENZYMES OF FLAVONOID BIOSYNTHESIS PATHWAY

The aim and methods of genetic modification

One of the premises of choosing the direction of potato modification was an observation that the content of flavonoids (anthocyanins in particular) in that plant is a significant factor enhancing resistance to bacterial infections (Lorenc-Kukuła *et al.* 2005). An important clue was also the fact that potato plants with overexpression of glucose transferase are characterized by a shorter vegetative season and significantly higher tuber yield (Lorenc-Kukuła *et al.* 2004, 2005). The objective of the reported transformation was to increase the antioxidative capacity of plants and to improve their resistance to stress factors, which may lead to an increase in yield (Łukaszewicz *et al.* 2004). Transformation of potatoes displaying overexpression of enzymes of the flavonoid synthesis pathway was conducted at the Department of Genetic Biochemistry, University of Wrocław. Tubers of the examined transgenic plants were obtained by means of bacterial transformation (Stobiecki *et al*. 2003) with the use of *Agrobacterium tumefaciens* strain C58C1: pGV2260) from potato plants of cv. 'Désirée' with a construct containing cDNA encoding for chalcone synthase – CHS (EMBL/GenBank database Acc. No. X04080); chalcone isomerase – CHI (EMBL/GenBank database Acc. No. X14589); dihydroflavone reductase – DFR (EMBL/GenBank database Acc. No. X15537); and glucose transferase – GT (EMBL/GenBank database Acc. No. AY033489). CHI and CHS genes as well as those of dihydroflavone reductase were isolated from *Petunia hybrida*, whereas that of glucose transferase from *Solanum sogarandinum*. The selective marker of GMOs was the kanamycin-resistant *npt*II gene. The mentioned key enzymes of the flavonoid synthesis pathway – CHI, CHS, DFR and GT may constitute an important source of flavonoids with health-promoting activity. It has become clear that the composition of secondary flavonoid metabolites strongly influences the quality and potential health benefits of food products (Stobiecki *et al*. 2003). They have been suggested to protect against oxidative stress, coronary heart disease, certain cancers and other age-related diseases.

Chemical composition and nutritional value

The induced overexpression of CHI, CHS, DFR and GT enzymes has been demonstrated to increase the concentration of flavonoids (especially of anthocyanins) in tissues of transgenic plants (Stobiecki *et al.* 2003; Kosieradzka *et al.* 2004b; Lorenc-Kukuła *et al.* 2005) and antioxidative capacity of the tissues (Łukaszewicz *et al.* 2004). The presence of cellular structures (vacuoles) containing anthocyanins was observed in the peridermal layer of tubers of transgenic

Table 3 The content of crude protein (% dry matter) and selected amino acid (g/16 g N) in dried potato tubers conventional cv. 'Désirée' and transgenic clones. (modified from Kosieradzka 2008).

	Désirée	Transgenic clone			
		CHI	CHS	DFR	GT
Crude protein (%)	14.28	10.21	9.15	9.20	10.63
Phe + Tyr	7.02	8.24	8.74	7.90	7.19
Iso	2.85	4.43	4.96	4.18	4.06
Leu	3.95	6.78	7.40	6.51	6.26
Lys	4.73	5.66	5.15	5.59	5.03
Met + Cys	2.02	2.79	3.17	2.86	2.93
Thr	2.39	3.82	4.23	3.66	3.42
Val	3.86	5.39	5.85	5.08	4.85
ΣEAA[a]	32.31	43.67	46.14	42.00	39.85

[a]ΣEAA – total essential amino acids
n=15

plants (Kosieradzka *et al.* 2004b). Transgenesis, leading to modification of the flavonoid synthesis pathway, resulted in differentiated content of steroid glycoalkaloids in the peridermal layer of tubers (Stobiecki *et al.* 2003) and dried material from steamed potato tubers (Kosieradzka 2008). Dried material from tubers of transgenic plants with overexpression of CHI, CHS, DFR and GT contained more polyphenolic compounds than that from tubers originating from plants of conventional cv. 'Désirée'. In the case of one line with GT overexpression, the higher content of polyphenolic compounds was accompanied by an increased content of steroid glycoalkaloids (SGA) (Kosieradzka 2008). Changes in SGA in potatoes, triggered by transgenesis, did not depend on the content of flavonoids, yet in some lines a significant positive correlation between contents of both groups of compounds generated in a plant in response to the action of a stress factor, was observed (Stobiecki *et al.* 2003). The concentration of SGA in dried material from whole tubers of transgenic potatoes with overexpressed CHI, CHS, DFR and GT enzymes and from control potatoes of cv. 'Désirée' was not distinctly different from values reported in the literature (Pęksa *et al.* 2002; Machado *et al.* 2007).

An increase in the concentration of polyphenolic compounds (anthocyanins) enhanced the antioxidative potential of tubers, thus improving the plant's protection against stress (Łukaszewicz *et al.* 2004). This, in turn, had a positive effect on the nutritional value and dietetic properties of transgenic potato tubers. The applied recombination of potato DNA, leading to overexpression of enzymes of the flavonoid synthesis pathway (CHI, CHS, DFR and GT), affected the metabolism of carbohydrates, thus resulting in the increased content of starch in tubers of the transformants (by 15 to 17%). The enhanced intensity of starch synthesis in tubers has been shown to be accompanied by a decreased content of reducing sugars (Kosieradzka 2008). The mechanism of correlations between the intensified synthesis of flavonoids and carbohydrate metabolism in transgenic plants has not been elucidated so far (Łukaszewicz *et al.* 2004; Kosieradzka 2008).

Modification of enzymes of the flavonoid synthesis pathway resulted in a diminished content of total protein in tubers of transgenic plants as well as in changes in its amino acid composition that positively affected the nutritional value of tubers (**Table 3**).

The content of minerals in tubers of potatoes with overexpressed CHI, CHS, DFR and GT and in potatoes of the initial cultivar was approximated. An increased concentration of Na in tubers of all genetically-modified lines was accompanied by diminished concentrations of Zn and Mn. In turn, tubers of lines with overexpressed DFR and GT were characterized by an increased concentration of Cr (Kosieradzka 2008).

Health status and metabolism of rats fed diets with 30% addition of tubers of transgenic and non-transgenic potatoes

Results of a 4-week feeding experiment demonstrate that the addition of potatoes with overexpressed enzymes of the flavonoid synthesis pathway (CHI, CHS, DFR, GT) to rats' diets affected the analyzed metabolic indices and their health status only to a slight extent (Kosieradzka 2008). No differences were reported in the growth of animals fed a diet containing tubers of the conventional cultivar and those of transgenic lines (CHI, CHS, DFR), whereas a diet with the addition of GT line tubers (characterized by the highest content of SGA) appeared to decrease the body weight of the animals (**Fig. 4**).

Changes in the composition of transgenic potato tubers did not affect most of the analyzed haematological and biochemical blood parameters. Rats administered a diet containing tubers of CHI, DFR and GT lines were, however, characterized by a low number of white blood cells. The administration of diets with transgenic potatoes of all lines affected the parameters of lipid metabolism, which resulted in decreased concentrations of total cholesterol (CHOL) and triglycerides (TG) in the blood of the animals (**Fig. 5**).

The reported feeding experiment (Kosieradzka 2008) demonstrated also the effect of administering DFR and GT potatoes on the immune system, characterized by para-

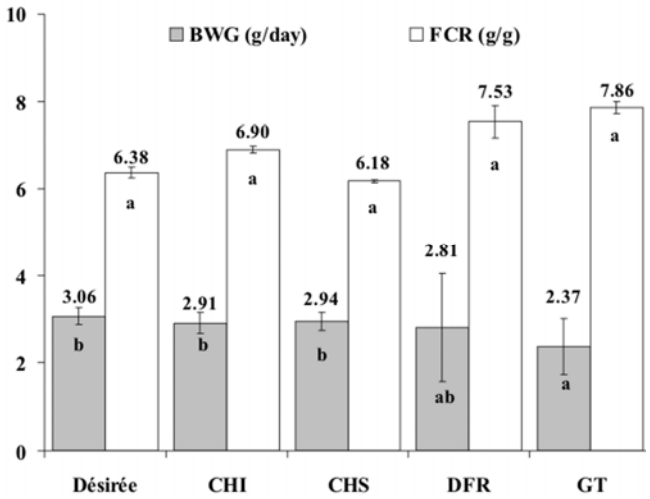

Fig. 4 Body weight gain (BWG) and feed efficiency ratio (FCR) in rats fed diet containing potato tubers of conventional and transgenic origin. (modified from Kosieradzka 2008). [a, b, ab] - Values in series having different superscripts are significantly different at $P \leq 0.05$.

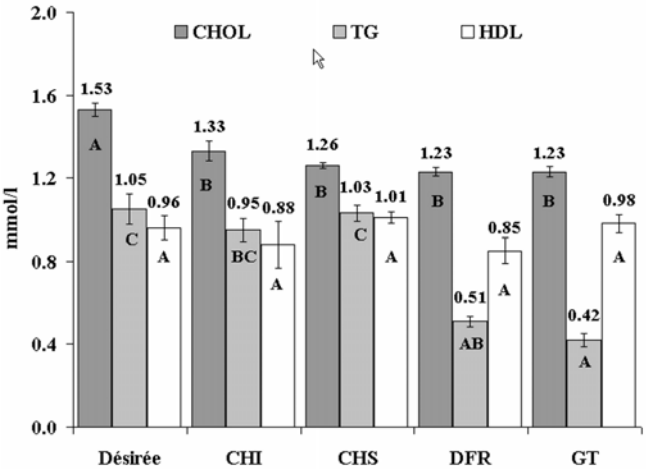

Fig. 5 Cholesterol (CHOL), triglycerides (TG) and HDL-cholesterol concentration in the serum of rats. (modified from Kosieradzka 2008). [a, b, c…] - Values in series having different superscripts are significantly different at $P \leq 0.05$.

Table 4 The activity of blood superoxide dismutase (SOD) and glutathione peroxidase (GPx), and serum Total Antioxidant Status (TAS) in rats fed experimental diets (Kosieradzka 2008).

	Désirée	Transgenic clone				SEM
		CHI	CHS	DFR	GT	
SOD (U/ml)	192.4 a	198.6 ab	202.8 b	207.9 b	201.0 b	3.706
GPx (U/ml)	141.4 a	158.3 a	152.6 a	143.7 a	138.0 a	5.691
TAS (mmol/l)	0.990 a	0.977 a	0.960 a	0.899 a	0.898 a	0.028

Values in a row with different superscripts are significantly different at $P \leq 0.05$. Duncan's multiple rage test was employed and 2 repetitions for all determined values. SEM – standard errors of the means (standard deviation for all rats divided by square root of rat number, n = 60)

meters of non-specific immunity. Enhanced oxidative activity of neutrophiles and phagocytic activity of monocytes was observed in the blood of the experimental animals. In contrast, these diets appeared not to affect the concentration of E class immunoglobulins (IgE-total), i.e. antibodies produced in a response to the presence of a factor inducing allergic reaction.

The elaborated *in vivo* analyses were aimed at evaluating the antioxidative status of the blood of the rats (Kosieradzka 2008). The administration of feed mixtures containing dried tubers of transgenic potatoes or their isogenic equivalent led to an increased activity of superoxide dismutase (SOD). The activity of SOD in the blood of rats fed CHS, DFR or GT potatoes was statistically significantly higher than in the blood of rats receiving the control diet with non-transgenic potatoes. Simultaneously, the total antioxidant potential (TAS) of blood did not differ between the groups (**Table 4**). Transgenic potatoes with enhanced synthesis of flavonoids were observed not to affect the relative weight of selected internal organs of rats (liver, brain, intestine, kidneys). Nor was any effect shown either for transgenic potato content in the diet on intestinal morphology characterized by the length of the jejunum microvilli (Kosieradzka 2008).

Nutritional and safety aspects of modification

Due to the traditionally significant position of potatoes in the diet (according to the International Potato Center in years 1994-96 the average daily intake of potatoes was estimated at 370 g; http://www.cipotato.org/potato/facts/indicators.asp), potatoes may be an important source of a number of compounds, including biologically-active ones. In our study (Kosieradzka 2008), the activity of the flavonoid biosynthetic pathway enzymes may involve beneficial modification of the blood lipid profile in rats fed diets containing transgenic potatos with enhanced synthesis of flavonoids. This was probably due to the presence of anthocyanins in the diet, i.e. substances with antioxidative properties protecting lipid fractions against oxidation (Hollman and Katan 1999). This, in turn, may result from the capability of polyphenols to chelate ions of prooxidative metals and from the formation of complexes with metals and ascorbic acid (Sarma *et al.* 1997; Brown *et al.* 1998).

The increase in the oxidative activity of neutrophiles and phagocytic activity of monocytes observed in our study (Kosieradzka 2008) may point to the immunomodulatory effect of a diet containing potatoes with enhanced synthesis of flavonoids, yet a lack of response of IgE antibodies does not indicate the allergenic character of dietary components.

Although genetic transformation increased the content of biologically-active substances with antioxidative activity, it had no significant effect on the total antioxidative status (TAS) of the blood of rats fed diets containing genetically modified potatoes. The reported enhancement of the activity of SOD was consistent with results of many other experiments which demonstrated that the administration of a diet with an increased concentration of polyphenolic compounds led to an increase in the activity of antioxidative enzymes (SOD, GPx, GST) both in blood and tissues (Lin *et al.* 1998; Augustyniak *et al.* 2005). The activity of antioxidative enzymes, detoxifying reactive oxygen species, may also be stimulated by the presence of free radicals generated as a result of the intake of a substance with toxic activity with diet (Amin and Hamza 2005). Induction of SOD activity assures the protection of cells and tissues by preventing the accumulation of free radicals (Shahjahan *et al.* 2005).

In the reported investigations (Kosieradzka 2008), almost all evaluated parameters of the health status of animals were within values typical of clinically-healthy subjects. Such results were obtained as a consequence of long-term administration of diets with a high content of potatoes (30%), substantially exceeding the average daily intake of potatoes by animals and humans. On that basis, it may be concluded that potatoes with overexpression of enzymes of the flavonoid synthesis pathway are safe for health and may constitute a nutritionally equivalent dietary component, as compared with potatoes of the initial conventional cultivar.

NUTRITIONAL PROPERTIES OF TUBERS OF TRANSGENIC POTATO WITH MODIFICATION OF 14-3-3 PROTEIN

The aim and methods of genetic modification

Genetic transformation was applied to plants of potato *Solanum tuberosum* to induce overexpression of 14-3-3 protein. It is common knowledge that isoforms of this protein regulate processes of catecholamines synthesis (Wilczyński *et al.* 1998b; Kulma *et al.* 2000), affect the activation of tyrosine hydroxylase and tryptophan, C proteins kinases, endonuclease and phospholipase, inhibit nitrate reductase and phosposaccharose synthase, thus affecting the assimilation of nitrogen and carbon as well as controlling the synthesis of amino acids and calcium metabolism in a plant. These are proteins engaged in the regulation of a variety of metabolic processes occurring in a plant and their modification may affect its agrotechnical properties, among others, the length of the vegetative season (Szopa 2002). Repression of genes that regulate synthesis of 14-3-3 protein results in the reduction of a number of tubers, an increase in their size and weight, whereas overexpression yields the opposite effect (Wilczyński *et al.* 1998b; Szopa *et al.* 2001). For these reasons, modification of the expression of the regulatory protein 14-3-3 has been recognized as an effective method of controlling the synthesis of flavonoid-group compounds (Łukasiewicz and Szopa 2005). Yet, that modification may also elicit changes in the course of a number of metabolic pathways as well as diversification of the chemical composition of transgenic potato tubers. It substantiates the need for analyzing the nutritional value and safety of application of such tubers in animal feeding and human nutrition.

Genetic transformation was applied to plants of potato cv. 'Désirée'. It was conducted at the Department of Genetic Biochemistry, University of Wrocław, according to the method described by Szopa and Müller-Röber (1994), Wilczyński *et al.* (1998a) and Szopa *et al.* (2001). Enhanced synthesis of 14-3-3 protein was obtained through the expression of a gene originating from *Cucurbita pepo* var. *Patissonina*, whereas lines of potatoes with the repression of P-14-3-3 protein were achieved by means of antisense transformations.

Chemical composition and nutritional value

Transgenic potatoes displaying overexpression of 14-3-3 protein were characterized by potentially-favorable changes in the chemical composition of tubers. Dry material obtained from steamed tubers of transgenic potatoes had a higher content of total protein (by *ca*. 60%) as compared to potato tubers of the parental line 'Désirée'. Those potatoes were also characterized by a slightly higher content of crude fiber (Kosieradzka *et al.* 2004a). A positive correlation was observed between the enhanced synthesis of 14-3-3 protein and fat content of potato tubers (Prescha *et al.* 2001; Kosieradzka *et al.* 2004a). Overexpression of 14-3-3 protein has been shown to result in an increased concentration of soluble sugars in tubers (Kulma *et al.* 2000). Yet it had a relatively small impact on the mineral composition of potatoes. Only the content of iron in dry tubers of transgenic plants was nearly two-fold higher and that of sodium – considerably lower as compared to dry tubers of non-modified plants of the same cultivar growing under analogous conditions (Kosieradzka *et al.* 2004a).

The repression of 14-3-3 protein resulted in enhanced starch synthesis, a higher ratio of soluble sugars to starch, and a change in the amino acid composition consisting of an increase in the concentrations of methionine, proline, arginine and lysine (Świędrych *et al.* 2002). Dry material obtained from steamed potato tubers with repression of the α and χ isoforms of 14-3-3 protein contained less lipids and crude fiber (by *ca*. 24%), than that obtained from tubers of non-transgenic plants (Kosieradzka *et al.* 2008b). Reduced synthesis of 14-3-3 protein in plants with repression of the α and χ isoforms of 14-3-3 protein resulted in a diminished content of total protein (by *ca*. 26%) in tubers as well as in changes in the amino acid composition of protein of dry material from transgenic tubers in respect of the non-transgenic control potatoes of cv. 'Désirée'. Dry material from potatoes with repression of 14-3-3 protein contained more tryptophan (by *ca*. 11%), phenylalanine (by *ca*. 20%), arginine (by *ca*. 22%), and aspargine (by *ca*. 38%) as well as less glutamine (by *ca*. 22%) and alanine (by *ca*. 28%). Changes in plant metabolism linked with the repression of α and χ isoforms of 14-3-3 protein were found to elicit changes in the mineral composition of dry tubers, i.e. nearly 2–fold lower concentrations of Ca, Zn, Cu, and Na, as well as a considerably lower concentrations of Fe and P (Kosieradzka *et al.* 2008b).

The application of isoprotein diets containing 30% of dry material from steamed potatoes with repression of 14-3-3 protein and administration of diets with 20% addition of potatoes with overexpression of 14-3-3 protein did not affect differentiation of the growth rate of rats in the period of their high susceptibility to the nutritional value of diets, i.e. in the period of 4-5 weeks after weaning. After terminating the feeding experiment, the animals receiving dry material from potatoes of the parental line 'Désirée' or that obtained from transgenic potatoes did not differ significantly in their body weight. Digestibility coefficients of components of the experimental diet were not lower than those reported after administration of a diet containing non-transgenic potatoes (Kosieradzka *et al.* 2004a, 2008b). On that basis it may be concluded that the nutritional value of potatoes with repression and overexpression of 14-3-3 protein was equivalent to the nutritional value of non-transgenic potatoes of the parental line 'Désirée'.

Health status and metabolism of rats fed diets containing tubers of potatoes with genetically-changed expression of 14-3-3 protein and potatoes of the conventional cultivar

In the reported experiments, described in detail by Kosieradzka *et al.* (2004a) and Kosieradzka *et al.* (2008a), analyses were conducted for the physiological response of rats to administration of diets containing 20% of dry material from tubers of potatoes with overexpression of 14-3-3 protein and diets containing 30% of dry material from tubers of potatoes with repression of 14-3-3 protein. No significant differences were observed in the relative mass of organs important for the course of vital functions and metabolic processes, i.e. kidneys, liver, pancreas and heart. Haematological parameters (red blood cell count, hematocrit, mean corpuscular volume, haemoglobin concentration, and white blood cell count) and biochemical parameters (total protein, albumin, globulin, creatinin, asparagine transferase, alanine transferase, glucose, cholesterol, high density lipoprotein, triglycerides) of the blood of rats fed diets containing potatoes with both overexpression and repression of 14-3-3 protein did not differ significantly from analogous parameters determined in the blood of rats fed diets containing potatoes of the control line 'Désirée'. Values of the assayed parameters were within correct values stipulated for that species (Kosieradzka *et al.* 2005a, 2005b, 2008a).

Administration of diets containing transgenic potatoes did not affect the activity of SOD nor GPx. The activity of the antioxidative enzymes was not subject to any significant change upon the administration of potatoes with repression and overexpression of 14-3-3 protein, which may indicate a lack of substances stimulating the generation of reactive oxygen species (**Figs. 6, 7**) in the GMO-containing diet (Kosieradzka *et al.* 2005b, 2008a). The diet containing transgenic potatoes was also found not to affect serum concentrations of products of oxidative degradation of lipids referred to as the sum of substances reacting with thiobarbituric acid - TBARS (**Fig. 8, 9**) (Kosieradzka 2008; Kosie-

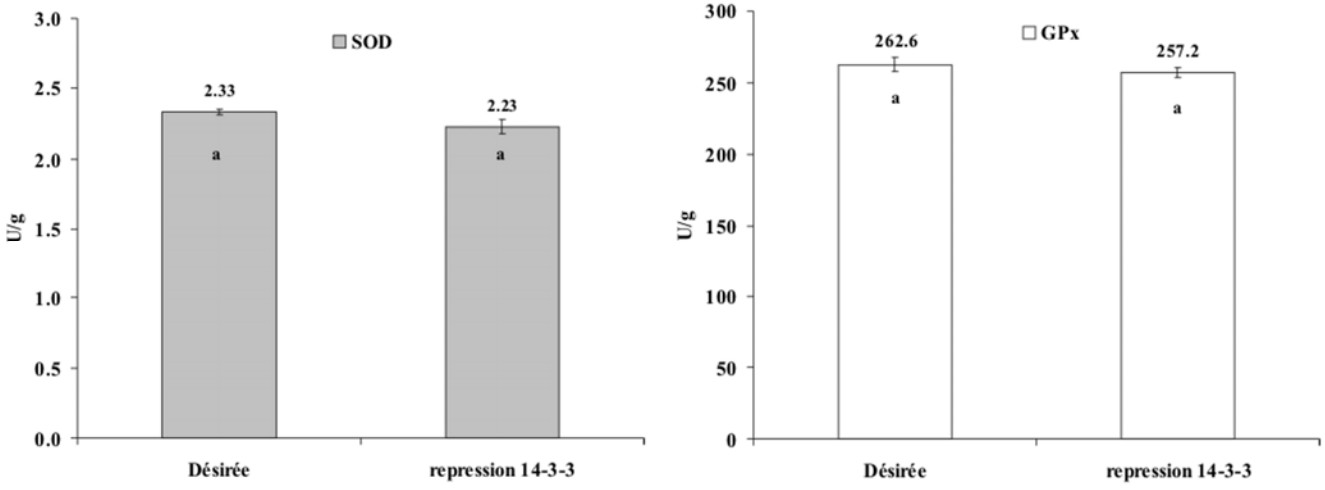

Fig. 6 The activity of blood glutatione peroxidase (GPx) and superoxide dismutase (SOD) in rats fed diets containing the 14-3-3 protein repressed transgenic potato tubers. (modified from Kosieradzka *et al.* 2008b).

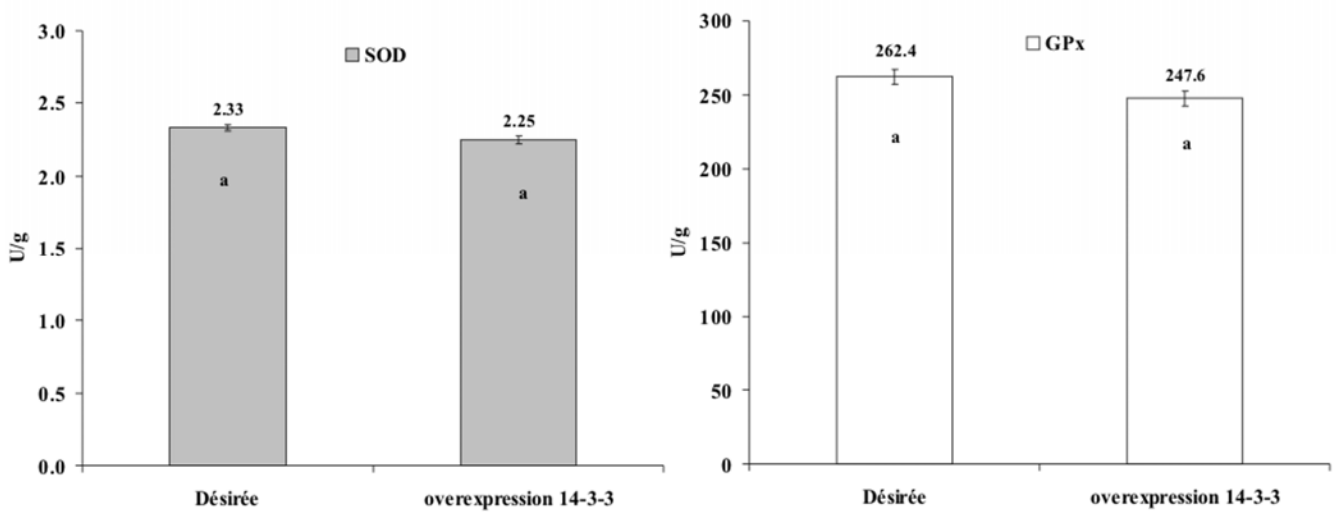

Fig. 7 The activity of blood glutatione peroxidase (GPx) and superoxide dismutase (SOD) in rats fed diets with transgenic tubers with overexpression 14-3-3 protein. (modified from Kosieradzka *et al.* 2008b).

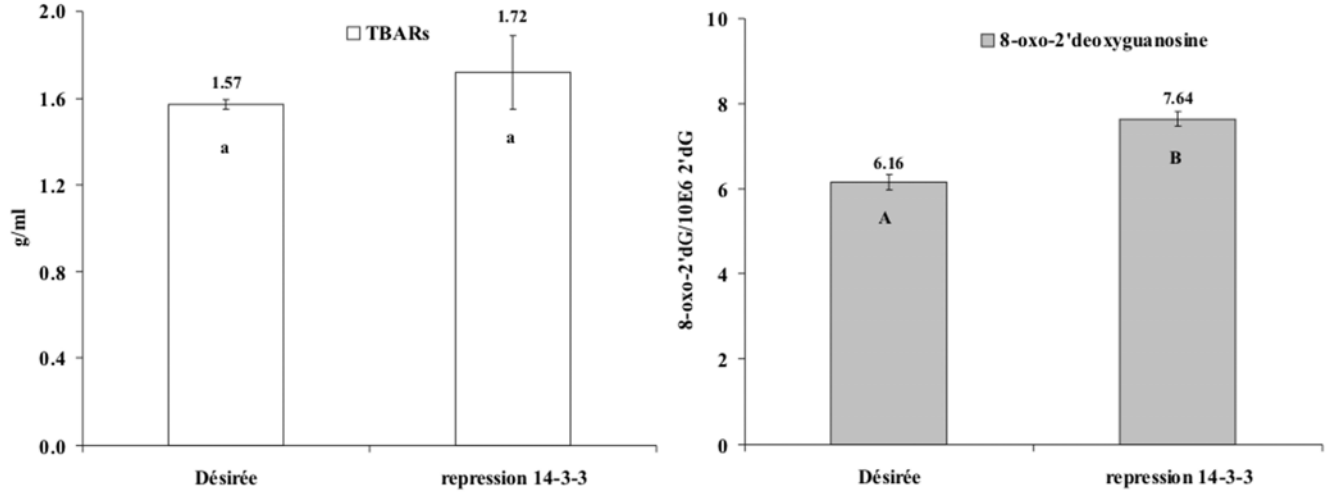

Fig. 8 Thiobarbituric acid-reactive substances (TBARs) and 8-oxo-2'deoxyguanosine in liver of rats fed diets containing the 14-3-3 protein repressed transgenic potato tubers. (modified from Kosieradzka *et al.* 2008b). [a, b] - Values in series having different superscripts are significantly different at $P \leq 0.05$.

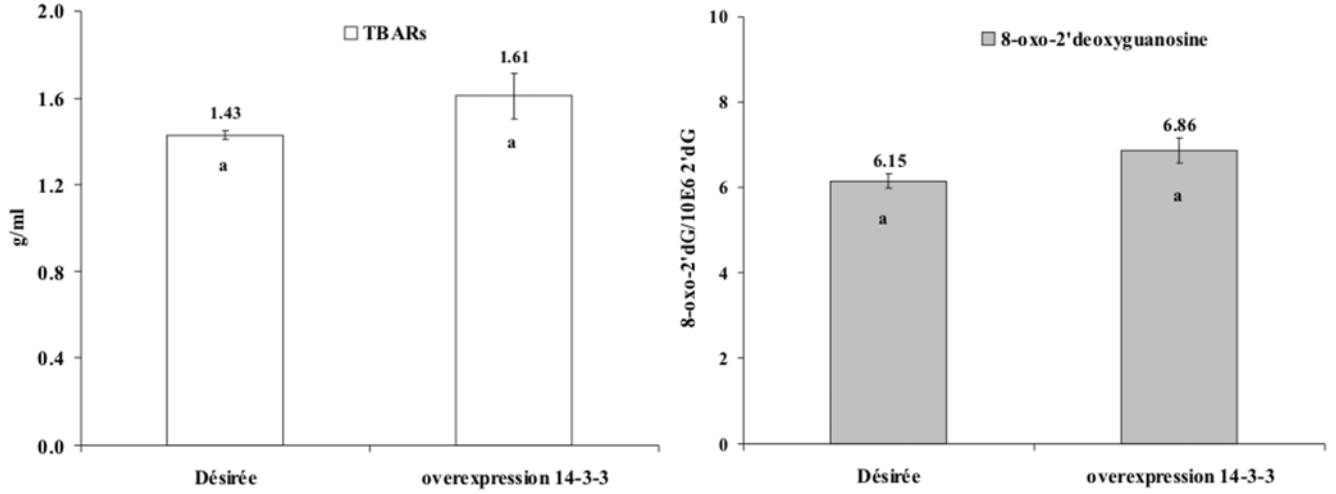

Fig. 9 Thiobarbituric acid-reactive substances (TBARs) and 8-oxo-2'deoxyguanosine in liver of rats fed diets with transgenic tubers with overexpression 14-3-3 protein. (modified from Kosieradzka *et al*. 2005b).

radzka *et al.* 2008a). Yet, the concentration of DNA adduct 8-oxo-2'-deoxyguanosine in the liver of rats receiving potatoes with the repression of α and χ isoforms was significantly higher than in the liver tissue of rats fed control potatoes of cv. 'Désirée', whereas the overexpression of 14-3-3 did not cause any increase in the concentration of DNA degradation product in brain tissue (**Figs. 8, 9**), (Kosieradzka *et al.* 2005b, 2008a). The administration of potatoes with overexpression of 14-3-3 protein with diets elicited an increase in the number of phagocytic neutrophiles and in the number of cells phagocytated by monocytes (Kosieradzka *et al.* 2003), which may indicate that the immune

system has been stimulated by components of the GMO-containing diet.

Nutritional and safety aspects of modification

The 14-3-3 protein influences the synthesis of anthocyanins and phenolic acids (Łukaszewicz and Szopa 2005). It has been demonstrated that overexpression of that protein in a plant resulted in almost two-fold increase of its antioxidative capacity (Łukaszewicz *et al.* 2001). Resulting from that, the enhancement of 14-3-3 protein synthesis may have a beneficial effect on agrotechnical properties of potato plants, may stimulate plants' resistance to stress and increase synthesis of anthocyanins and phenolic acids in tubers. Overexpression of 14-3-3 protein causes an increased content of total protein in tubers, whereas repression of its synthesis results in the intensification of starch synthesis and changes in the amino acid composition of protein. In the reported study, dry material obtained from transgenic potatoes was characterized by a higher content of total protein (by *ca.* 60%) as compared to potato tubers of the parental line 'Désirée'. It may point to more intensive synthesis of nitrogen compounds in those plants. The higher content of starch found in transgenic tubers (by over 30%) was probably a consequence of an enhanced activity of starch synthase (Żuk *et al.* 2005). The reported significant decrease in the concentration of NO_3 in tubers was likely to result from the effect of 14-3-3 protein on the activity of nitrate reductase (Szopa *et al.* 2001; Łukasiewicz and Szopa 2005).

Results obtained in our study (Kosieradzka *et al.* 2004a, Kosieradzka *et al*. 2005a,b; Kosieradzka *et al.* 2008b) indicate that a change in the chemical composition of potato tubers with modified synthesis of 14-3-3 protein does not diminish their nutritional properties, does not decrease the growth rate nor deteriorates the health status and does not disturb the metabolism of rats. Such an effect was accomplished by feeding rats with diets containing considerable amounts of dry material of tubers from plants with repression and overexpression of 14-3-3 protein (30 and 20% of the diet, respectively). The lack of significant differences in the morphological picture of blood and basic biochemical parameters of rats indicates the proper course of vital functions in the animals is effectively unaltered. Although some deviations were reported in selected physiological parameters, we did not demonstrate any explicit negative effect of the experimental diets which could be considered as the occurrence of an unexpected and unfavorable effect of transgenesis. The results obtained enable concluding that tubers of transgenic potatoes with repression and overexpression of 14-3-3 protein are a nutritionally equivalent dietary component, as compared to tubers of the initial conventional cultivar.

FUTURE RESEARCH

Successful advances in genetic manipulation of the genomes of plants and animals have aroused much controversy. This has also recently been evident also in Poland. For two years, the Polish Government has been trying to enact a more restrictive "Law of GMO" that would, for example, ban the release of GMOs to the environment and use GMO feeds in animal nutrition. The solutions put forward are contrary to the European Union law and the opinions of many animal feeding experts. Unfortunately, the number of *in vivo* experiments conducted in Poland was too low to weaken the opinions of these experts. For these reasons, future research is needed that focuses on the safety of using transgenic plans in feed and food production. In the presented investigations, the period of experimental feeding of rats was restricted to 3-5 weeks, mainly owing to the difficulty of accumulating sufficient transgenic materials to carry out experiments of longer duration. The contents of autoclaved and dried potato tubers in the diet were, however, relatively high (20-40%), which allowed us to make some first conclusion. The general conclusion is as follows: results of *in vivo* experiments indicate that tubers of GM potato are a substantial and nutritional equivalent to the non-transgenic cultivars, however some indices of the physiological response of animals point to the need for conducting longer investigations. The results obtained in the first experiments (Juśkiewicz *et al*. 2004; Juśkiewicz *et al*. 2005; Zduńczyk *et al*. 2005a, 2005b) are insufficient to explain the reason for the higher content of essential amino acids in tubers of line R2.P (truncated gene for PVY^N polymerase in antisense orientation), the increased bulk of cecal digesta and the higher production of short chain fatty acids in the cecum of rats fed a diet with tubers of line NTR2.27 (non-translated regions pf PVY^N genome in antisense orientation), the lower percentage of phagocytic cells in rats fed a diet with tubers of transgenic clone R1.F (truncated gene for PVY^N polymerase in sense orientation) or lower retention of nitrogen in rats fed a diet with tubers of line NTR1.16 (non-translated regions pf PVY^N genome in sense orientation). Similar uncertainties remain concerning deviations of some physiological indices in the experiment with tubers of potatoes with overexpression or repression of 14-3-3 protein synthesis, as well as in potatoes with modified of enzymes of the flavonoid synthesis pathway.

REFERENCES

Amin A, Hamza AA (2005) Hepatoprotective effects of *Hibiscus*, *Rosmarinus* and *Salvia* on azathioprine-induced toxicity in rats. *Life Science* **77**, 266-278

Augustyniak A, Waszkiewicz E, Skrzydlewska E (2005) Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol. *Nutrition* **21**, 925-932

Brown JE, Khodr H, Hider RC, Rice-Evans CA (1998) Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. *Biochemical Journal* **330**, 1173-1178

Catchpole GS, Beckmann M, Enot DP, Mondhe M, Żywicki B, Taylor J, Hardy N, Smith A, King RD, Kell DB, Fiehn O, Draper J (2005) Hierarchical metabolomics demonstrates substantial compositional similarity between genetically modified and conventional potato crops. *Proceedings of the National Academy of Sciences USA* **102**, 14458-14462

Central Statistical Office (2007) *Statistical Yearbook*, Statistical Publishing Establishment, Warsaw, 888 pp

Chachulska AM, Chrzanowska M, Flis B, Krzymowska M, Lipska-Dwużnik A, Robaglia C, Zagórski W (1997) Potato and tobacco cultivars transformation towards potato virus *Y* resistance. *Biotechnologia* **4**, 48-54

Chrzanowska M, Doroszewska T (1997) Comparison between PVY isolates obtained from potato and tobacco plants grown in Poland. *Phytopathologia Polonica* **8**, 15-20

Chye M-L, Zhao K-J, He Z-M, Ramalingam S, Fung K-L (2005) An agglutinating chitinase with two chitin-binding domains confers fungal protection in transgenic potato. *Planta* **220**, 717-730

Davidson MM, Butler RC, Wratten SD, Conner AJ (2006) Field evaluation of potato plants transgenic for a cry1Ac gene conferring resistance to potato tuber moth, *Phthorimaea operculella* (Zeller) (*Lepidoptera: Gelechiidae*). *Crop Protection* **25**, 216-224

Finotti E, Bertone A, Vivanti V (2006) Balance between nutrients and antinutrients in nine Italian potato cultivars. *Food Chemistry* **99**, 698-701

Flis B, Zimnoch-Guzowska E (2000) Field performance of transgenic clones obtained from potato cv. 'Irga'. *Journal of Applied Genetics* **41**, 81-90

Fridman M, McDonald GM (1997) Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. *Critical Review of Plant Sciences* **16**, 55-132

Gatehouse AMR, Davison GM, Newell CA, Merryweather A, Hamilton WDO, Burgess EPJ, Gilbert RJC, Gatehouse JA (1997) Transgenic potato plants with enhanced resistance to the tomato moth, *Lacanobia oleracea*: growth room trials. *Molecular Breeding* **3**, 49-63

Gebhardt C, Valkonen JPT (2001) Organization of genes controlling disease resistance in the potato genome. *Annual Review of Phytopathology* **39**, 79-102

Hashimoto W, Momma K, Katsube T, Ohkawa Y, Ishige T, Kito M, Utsumi S, Murata K (1999) Safety assessment of genetically engineered potatoes with designed soybean glycinin: compositional analyses of the potato tubers and digestibility of the newly expressed protein in transgenic potatoes. *Journal of the Science of Food and Agriculture* **79 (12)**, 1607-1612

Hollman PCH, Katan MB (1999) Dietary flavonoids: intake, health effects and bioavailability. *Food and Chemical Toxicology* **37**, 937-942

Jondedijk E, De Schutter AAJM, Sollte T, van den Elzen PJM, Cornelissen BJC (1992) Increased resistance to potato virus X and preservation of cultivar properties in transgenic potato under field condition. *Bio/Technology* **10**, 422-429

Juśkiewicz J, Zduńczyk Z, Frejnagel S, Fornal J (2004) Caecal fermentation in rats fed diets containing tubers of transgenic potato. *Journal of Animal and*

Feed Sciences **13 (Suppl. 2)**, 93-96

Juśkiewicz J, Zduńczyk Z, Fornal J (2005) Nutritional properties of conventionally bred and transgenic lines of potato resistant to necrotic strain of *Potato virus Y* (PVY^N). *Acta Biochimica Polonica* **52 (3)**, 725-729

Kaniewski W, Lawson C, Samson B, Haley L, Hart J, Delannay X, Tumer N (1990) Field resistance of transgenic russet Burbank potato to effects of infection by *Potato virus X* and *Potato virus Y*. *Nature Biotechnology* **8**, 750-754

Kosieradzka I, Winnicka W, Kluciński W, Sawosz E, Szopa J (2003) Effect of diets containing tubers of genetically modified potatoes on parameters of non-specific resistance in rats. *Annals of Animal Science* **(Suppl. 2)**, 281-285

Kosieradzka I, Sawosz E, Pastuszewska B, Żuk M, Szopa J, Bielecki W (2004a) Effect of feeding potato tubers modified by 14-3-3 protein overexpression on metabolism and health status of rats. *Journal of Animal and Feed Sciences* **13**, 329-339

Kosieradzka I, Borucki W, Matysiak–Kata I, Szopa J, Sawosz E (2004b) Transgenic potato tubers as a source of phenolic compounds. Localization of anthocyanins in the peridermis. *Journal of Animal and Feed Sciences* **13 (Suppl. 2)**, 85-90

Kosieradzka I, Sawosz E, Skomiał J, Szopa J (2005a) Transgenic potato tubers with overexpression of 14-3-3 protein in growing rat diets. 1. Selected hormone activities and liver function status. *Journal of Animal and Feed Sciences* **14 (Suppl. 1)**, 545-548

Kosieradzka I, Sawosz E, Skomiał J, Szopa J, Dudkowska I, Pastuszewska B (2005b) Transgenic potato tubers with overexpression of 14-3-3 protein in growing rat diets. 2. Redox indices in blood and brain. *Journal of Animal and Feed Sciences* **14 (Suppl. 1)**, 549-552

Kosieradzka I (2008) *Transgenic Potato with Overexpression of the Enzymes of the Flavonoid Synthesis Pathway as a Dietary Source of Bioactive Substances*, Wydawnictwo SGGW Rozprawy Naukowe i Monografie **330**, 176 pp (in Polish)

Kosieradzka I, Sawosz E, Szopa J, Bielecki W (2008a) Potato genetically modified by 14-3-3 protein repression in growing rat diets Part 2: Health status of experimental animals. *Polish Journal of Food and Nutrition Sciences* **58 (3)**, 377-382

Kosieradzka I, Sawosz E, Szopa J, Vasko V (2008b) Potato genetically modified by 14-3-3 protein repression in growing rat diets. Part 1: chemical composition and digestibility of nutritional compounds. *Polish Journal of Food and Nutrition Sciences* **58 (1)**, 125-129

Kulma A, Wilczyński G, Milcarz M, Prescha A, Szopa J (2000) Modulation of carbohydrate metabolism in transgenic potato through genetic engineering and analysis of rabbits fed on wild type and transgenic potato tubers. *Food Biotechnology* **17**, 19-23

Lin Y-L, Cheng Ch-Y, Lin Y-P, Lau Y-W, Juan I-M, Lin JK (1998) Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase and glutathione *S*-transferase in rats. *Journal of Agriculture and Food Chemistry* **46**, 1893-1899

Lorenc-Kukuła K, Jafra S, Oszmiański J, Szopa J (2005) Ectopic expression of anthocyanin 5-o-glucosyltransferase in potato tuber causes increased resistance to bacteria. *Journal of Agriculture and Food Chemistry* **53**, 272-81

Lorenc-Kukuła K, Korobczak A, Aksamit-Stachurska A, Kostyń K, Łukaszewicz M, Szopa J (2004) Glucosyltransferase: the gene arrangement and enzyme function. *Cellular and Molecular Biology Letters* **9**, 935-46

Łukaszewicz M, Matysiak-Kata I, Aksamit A, Oszmiański J, Szopa J (2001) 14-3-3 Protein regulation of the antioxidant capacity of transgenic potato tubers. *Plant Science* **163**, 125-130

Łukaszewicz M, Matysiak-Kata I, Skała J, Fecka I, Cisowski W, Szopa J (2004) Antioxidant capacity manipulation in transgenic potato tuber by changes in phenolic compounds content. *Journal of Agriculture and Food Chemistry* **52**, 1526-1533

Łukaszewicz M, Szopa J (2005) Pleiotropic effect of flavonoid biosynthesis manipulation in transgenic potato plants. *Acta Physiologiae Plantarum* **27 (2)**, 221-228

Machado RMD, Toledo MCF, Garcia LC (2007) Effect of light and temperature on the formation of glycoalkaloids in potato tubers. *Food Control* **18**, 503-508

Matthews D, Jones H, Gans P, Coates S, Smith LM (2005) Toxic secondary metabolite production in genetically modified potatoes in response to stress. *Journal of Agriculture and Food Chemistry* **53 (20)**, 7766-7776

Missiou A, Kalantidis K, Boutla A, Tzortzakaki S, Tabler M, Tsagris M (2004) Generation of transgenic potato plants highly resistant to *Potato virus Y* (PVY) through RNA silencing. *Molecular Breeding* **14**, 185-197

Mullins E, Milbourne D, Petti C, Doyle-Prestwich BM, Meade C (2006) Potato in the age of biotechnology. *Trends in Plant Science* **11 (5)**, 254-260

Pęksa A, Gołubowska G, Rytel E, Lisińska G, Aniołowski K (2002) Influence of harvest date on glycoalkaloid contents of three potato varieties. *Food Chemistry* **78**, 313-317

Prescha A, Świędrych A, Biernat J, Szopa J (2001) Increase in lipid content in potato tubers modified by 14-3-3 gene overexpression. *Journal of Agriculture and Food Chemistry* **49**, 3638-3643

Rogan GJ, Bookout JT, Duncan DR, Fuchs RL, Lavrik PB, Love SL, Mueth M, Olson T, Owens ED, Raymond PJ, Zalewski J (2000) Compositional analysis of tubers from insect and virus resistant potato plants. *Journal of Agriculture and Food Chemistry* **48**, 5936-5945

Sadowska J, Błaszczak W, Fornal J, Jeliński T, Budny J (2008) Technological usability of tubers of genetically modified potato. *Polish Journal of Food and Nutrition Sciences* **58 (2)**, 199-210

Sadowska J, Budny J, Fornal J (2007) Starch, protein, glycoalkaloids, and L-ascorbic acid content in tubers of genetically modified potato cv. Irga. *European Food Research and Technology* **227 (1)**, 232-241

Sarma AD, Sreelakshmi Y, Sharma R (1997) Antioxidant ability of anthocyanins against ascorbic acid oxidation. *Phytochemistry* **45**, 671-674

Schwall GP, Safford R, Westcott RJ, Jeffcoat R, Tayal A, Shi Y-C, Gidley MJ, Jobling SA (2000) Production of very-high-amylose potato starch by inhibition of SBE A and B. *Nature Biotechnology* **18**, 551-554

Shahin EA, Simpson RB (1986) Gene transfer system for potato. *HortScience* **21**, 1199-1201

Shahjahan M, Vani G, Shyamaladevi CS (2005) Effect of *Solanum trilobatum* on the antioxidant status during diethyl nitrosamine induced and phenobarbital promoted hepatocarcinogenesis in rat. *Chemico-Biological Interactions* **156**, 113-123

Stobiecki M, Matysiak-Kata I, Frański R, Skała J, Szopa J (2003) Monitoring changes in anthocyanin and steroid alkaloid glycoside content in lines of transgenic potato plants using liquid chromatography/mass spectrometry. *Phytochemistry* **62**, 959-969

Świędrych A, Prescha A, Matysiak-Kata I, Biernat J, Szopa J (2002) Repression of the 14-3-3 gene affects the amino acid and mineral composition of potato tubers. *Journal of Agriculture and Food Chemistry* **50**, 2137-2141

Szopa J, Müller-Röber B (1994) Cloning and expression analysis of an ADP-ribosylation factor from *Solanum tuberosum* L. *Plant Cell Reports* **14**, 180-183

Szopa J, Wróbel M, Matysiak-Kata I, Świędrych A (2001) The metabolic profile of the 14-3-3 repressed transgenic potato tubers. *Plant Physiology and Biochemistry* **41**, 417-423

Szopa J (2002) Transgenic 14-3-3 isoforms in plants: the metabolite profiling of repressed 14-3-3 protein synthesis in transgenic potato plants. *Biochemical Society Transactions* **30**, 405-410

Wilczyński G, Kulma A, Feiga I, Wenczel A, Szopa J (1998a) Manipulating of 14-3-3 protein expression result in the changes of catecholamine content in potato plants. *Cellular and Molecular Biology Letters* **3**, 75-91

Wilczyński G, Kulma A, Szopa J (1998b) The expression of 14-3-3 isoforms in potato is developmentally regulated. *Journal of Plant Physiology* **153**, 118-126

Zduńczyk Z, Frejnagel S, Fornal J, Flis M, Palacios MC, Flis B, Zagórski-Ostoja W (2005a) Biological response of rat fed diets with high tuber content of conventionally bred and transgenic potato resistant to necrotic strain of *Potato Virus Y* (PVY^N). Part. I. Chemical composition of tubers and nutritional value of diets. *Food Control* **16**, 761-766

Zduńczyk Z, Juśkiewicz J, Fornal J, Mazur-Gonkowska B, Koncicki A, Flis B, Zimnoch-Guzowska E, Zagórski-Ostoja W (2005b) Biological response of rat fed diets with high tuber content of conventionally bred and transgenic potato resistant to necrotic strain of *Potato Virus Y* (PVY^N). Part. II. Caecal metabolism, serum enzymes and indices of non-specific defense of rats. *Food Control* **16**, 767-772

Żuk M, Weber R, Szopa J (2005) 14-3-3 protein down-regulates key enzyme activities of nitrate and carbohydrate metabolism in potato plants. *Journal of Agriculture and Food Chemistry* **53**, 3454-3460