CHANGES OF POTENTIALLY ANTINUTRITIVE COMPONENTS IN HUNGARIAN POTATOES UNDER ORGANIC AND CONVENTIONAL FARMING

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Anti-nutritive components in multi resistant potato varieties were investigated in relation to conventional and organic farming for three years. Glycoalkaloids, nitrate, nitrite, asparagine and glutamine content of tubers were examined. Farming technology was found not to have

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an effect on the level of glycoalkaloids; which were influenced mostly by the genotype and season. Nitrogen fertilisation caused significant increase in nitrate, asparagine and glutamine content as compared to organic farming. Nitrite content was found to be more independent of farming technologies then nitrate. Tubers of Rioja had the lowest nitrate content irrespective of season or technology. In conclusion the absolute amount and changes of different antinutritive components of potato tubers were influenced differently by the technology, genotype and season in a complex manner. Organic farming could not effect on glycoalkaloid content, but it has other positive effect, like lower nitrate levels which found to be beneficial according health critics.

Keywords: organic farming; potato; glycoalkaloids; nitrate; asparagine; glutamine.

Organic farming extends rapidly because crop production without or limited use of fertilizers and pesticides is beneficial to the consumers and environment as well. However, what the researches have been conducted during the last decades to compare organic and conventional foods with respect to nutritional composition, the effects of organic farming on vegetable crop quality and nutrient content are still not consistent. Some studies concluded that vegetables originated from organic production are richer in nutrients, particularly organic acids, vitamins and poly-phenols than conventionally produces ones (WINTER & DAVIS, 2006). Other studies could not confirm significant differences in nutrients between organic and conventional production methods (WARMAN & HAVARD, 1998).

Two major hypotheses have been suggested to explain the possible increases in secondary metabolites in organic versus conventional foods. According to the first hypothesis in conventional agriculture synthetic fertilizers make N more available for the plants and may accelerate plant growth and development. This rapid growth results a decrease in the production of plant secondary metabolites. The second hypothesis considers the chemical defence mechanisms of plants to stressful environment (ASAMI et al., 2003). The same mechanisms may result in the elevation of metabolites having both of toxic and positive nutritional effects (WINTER & DAVIS, 2006).

Potato is one of the most important staple foods and it plays a significant role in human diet worldwide. Its intensive cultivation assumes the operation of effective plant nutrition and plant protection systems. In most cases this means the intensive use of chemicals leading to serious environmental and food safety concerns. Growing of potato under organic conditions to overcome such problems can be a solution. However organic potato producers face difficulties in terms of dealing with adequate plant nutrients, especially nitrogen application; weed, insect and disease control issues (HAJSLOVA et al., 2005; HERENCIA et al., 2011). The use of varieties having a wide range of adaptability to different factors is prerequisite for successful organic potato production.

From the human nutrition point of view occurrence of several types of glycoalkaloids threatens consumer's health (FRIEDMAN & LEVIN, 2009). The total glycoalkaloid (TGA) content in tubers is affected by the genotype, climate, production technology, storage time, sprouting and exposure to light and heat. Results of researchers did not prove a definite effect on the formation of glycoalkaloids in tubers. Some of them did not reveal any differences between organic and integrated farming methods, others found higher or lower the TGA content in organic plants (ABREU et al., 2007).

Acrylamide (AA) is a well known hazardous component of foods with neurotoxic, carcinogenic effects. The major route for AA formation is the thermal degradation of free asparagine in the presence of reducing sugars during the Maillard reaction. Potato tubers contain substantial amounts of the AA precursors which explain the high concentrations of acrylamide in potato products. Reducing sugars are regarded as limiting factors with respect to acrylamide formation, thus maintaining low sugar content in tubers is crucial. However, reducing sugars can accumulate rapidly in tubers stored at temperatures below 5 °C and increase to a point where reducing sugar content is no longer limiting and asparagine content becomes the critical driving factor (MATSURRA-ENDO et al., 2006). This was the reason to investigate also the glutamine and asparagine content in potato tubers.

Consumption of high levels of nitrate may cause health problems, especially in babies and some cancers. Several scientists concluded that the nitrate content of organically grown foodstuffs (including potato) is generally is lower than in conventionally grown products (RUTKOWSKA, 1999).

The objective of the present study was the comparison of internal components of three Hungarian potato varieties having complex resistance traits due to their wild species origin and grown under conventional and organic farming practices with special focus on the measurement of TGA, nitrate, nitrite, asparagine and glutamine content of tubers. The seasonal effect (3 years) on such antinutritive compounds was also aimed.

1. Materials and methods

The standards materials were purchased from Sigma-Aldrich Ltd. (St Louis, USA). Acetonitrile, acetic acid, potassium hexacyanoferrate (II), zinc acetate, sulfanilamide chloride and n-(1-naphtyl) ethylenediamine were purchased from Merck (Darmstadt, Germany). Acids used for sample digestion were of Hiperpur grade from Panreac (Darmstadt, Germany). Standard solutions for elemental analysis were purchased from Carlo Erba (Paris, France), Merck and Pancreac. All reagents were of analytical reagent grade. Ultrapure water generated by the Milli-Q System (Millipore, Darmstadt, Germany) was used. SPE cartridge (ENVI-18 6 ml) and PTFE sample filter (25 mm x 0,45 µm) were purchased from SUPELCO Co. (St Louis, USA)

1.2 Plant material and growth conditions

Potato varieties Rioja, Hópehely and White Lady originate from the commercial breeding programme of University of Pannonia, Potato Research Centre, Keszthely, Hungary. The varieties show complex resistance to potato virus Y, X A and leaf roll virus (PVY, PVX, PVA and PLRV) as to common scab (*Streptomyces scabies*). Cv. Hópehely and White Lady are resistant to potato golden cyst nematode (*Globodera rostochiensis*, pathotype Ro1 and Ro4) while White Lady has high field resistance to potato late blight (P*hytophthora infestans*). During their breeding exotic potato species *S. acaule, S. demissum, S. stoloniferum, S. vernei, S. tuberosum ssp. andigenum* were used as source of resistance genes. Varieties were cultivated under conventional and organic farming conditions at Keszthely and Rábcakapi, Hungary in four replications where 56 plants represented one replicate in 2007-2009. Organic farming conditions, applied plant nutrition and plant protection were certified by Biocontroll Hungária Nonprofit Ltd. Soil parameters were determined by official soil sampling and measurements. No irrigation was applied at any location and any of the years. Applied plant p1

2.3. Sampling

After harvest 20 kg of tubers from each experimental parcel representing the farming technologies were collected. For the 3 parallel measurements 3 x 3 tubers were selected and prepared to get homogenous samples.

1.4. Potato processing

Potatoes were washed, peeled (2 mm thickness) according to the food processing technology, crushed by chopper (Philips HR 1392). All of analyses were carried out with peeled raw materials but for the comparison of the farming systems the same sample preparation is adequate. All samples were then freeze dried and subjected to further analysis. The lyophilised samples were ground to powder (Bosch MKM6003) and were stored at room temperature. The investigated components are stable at room temperature and our preliminary experiments proved that under the applied circumstances the freeze drying method did not damage any of these compounds.

1.5. Chemical determinations

1.5.1. Glycoalkaloid analysis. The analysis of glycoalkaloids was carried out by methods of TÖMÖSKÖZI-FARKAS and co-workers (2006).

1.5.2. Analysis of glutamine and asparagine content. 50 mg of the liophilized potato tuber sample was extracted with 1 ml water for 30 minutes in an ultrasonic bath then centrifuged for 10 minutes at 2000 g. The supernatant was complemented to eluent composition with ammonium acetate and acetonitrile and was was centrifuged for 10 minutes at 30 000 g. 5 µl of the sample was injected into a Perkin Elmer Series 200 chromatograph. The components were separated on a 150x2.1 mm, 5 µm, ZIC-HILIC (Merck) column by gradient elution at a flow rate of 150 µl min^{-1}. Mobile phase component A was 20 mM ammonium acetate (pH 4.0), component B was acetonitrile. From 0 to 8 minutes mobile phase B was kept at 80%, from 8 to 20 minutes it was decreased to 40% then kept at this value for 3 minutes. The analytes were detected with a Perkin Elmer Sciex API 365 triple quadrupole mass spectrometer with an ESI ion source (Sciex, Toronto, Canada) in positive multiple reaction monitoring mode. Asparagine and glutamine were measured at the m/z 133.1→m/z 73.9 and m/z147.1 → m/z 84.1 transitions, respectively and quantitated using calibration standards prepared from an amino acid standard mix (Sigma-Aldrich).

1.5.3. Determination of nitrate and nitrite content. Measurements were carried out with the standard method of A.O.A.C. official method (2003)

1.6. Statistics

For statistical analysis of experimental data Mann-Whitney test, with Excel software was used.

2. Results and discussion

The results of statistical analysis of TGA indicated that no direct relationship between the farming technology and the amount of glycoalkaloids (Table 1.). ASAMI and co-workers (2003) found that secondary metabolite production was more intensive for organic farming technology because the biosynthesis of these components played a role in the plant defence system. On the basis of our three-year results this conception could not be proved. In the case of variety "Rioja" from organic production contained significantly higher amount of TGA in 2007 (Table 2), there was no difference in 2008 and the conventional circumstances resulted in a significantly higher TGA concentration in the third year (2009). In the case of "Hópehely" there were no significant differences between the technology and years (Table 2.). This variety had the lowest and most stable level of TGA in all cases. However, the conventionally farmed "White Lady" contained significantly higher amount of TGA in 2007 and 2009. Considering the average TGA content of the same genotypes and years the results of the statistical analysis proved that the effect of year was the limiting factor. Furthermore, statistical differences were found between the varieties in all years. In summary, the results showed that the season and the genotypes had a greater effect than the farming technology on the amount of alkaloids.

Out of the nine cases (3 years x 3 varieties) the conventionally grown tubers had higher values in six cases for asparagine and in six cases for glutamine (Table 1). Although the detected alterations were proven to be statistically significant only in 3 cases for asparagine and 2 cases for glutamine, the tendency is clear. Nitrogen fertilisation during conventional farming has a positive effect on the amount of the examined amino acids. The data also revealed the genotype effect on the measured differences. Rioja had the highest asparagine content (62.4-122.0 mmol kg^{-1}) under conventional farming in all the cases. On contrary, White Lady had higher amino acid content in 4 cases under organic farming (3 times significant). This phenomenon can reflect the existence of differences in nitrogen reaction of the varieties. The season itself had significant effect on asparagine and glutamine because the lowest values in all three varieties, under both farming technologies were detected in 2007. This seasonal effect can be explained by the beneficial environmental circumstances for mineralisation of nitrogen. Summarizing, the data of amino acids were affected primarily by the technology.

Probably because of the fertilizers used, the nitrate content of tubers grown under conventional practice were higher than that of organically grown ones but the differences were not significant (Table 3.). In case of the same variety and farming technology we could not detect significant effect of the season. However, nitrate content was primarily influenced by the genotype (Table 2.). The results of HERENCIA and co-workers (2011) showed high data variability because there were a large number of factors that could have effect on the nitrate content of crops. These results of high variability in nitrate content are in agreement with our data. The nitrite content of tubers was lower than 0.1 mg kg^{-1} except in a few cases and there were no significant differences under the various circumstances (farming technology, variety or year, data are not shown).

3. Conclusion

In our study the absolute amount and changes of examined antinutritive components of potato tubers were influenced differently by the technology, genotype and season in a complex manner. TGA content of tubers is under strong genetic control. Nitrate and consequently the asparagine and glutamine content can be increased by nitrogen fertilizers but its extent is very much dependent on the genotype and seasonal effects. Nitrite content is more independent of farming technologies then nitrate. The effect of organic farming on nitrate and amino acid levels is not significant in all cases but the tendency is clear and it possible health effects.

The Hungarian potato varieties bred by conventional breeding utilising resistance genes of wild species were proved to be appropriate for organic production without the risk of increased toxic TGA content. From the point of toxic nitrate content Rioja is the most advised variety due to its lowest nitrate level irrespective of used farming technology.

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Table 1. Comparison of conventional (C) and organic (O) farming technology on antinutritive components of potato tubers of cv. White Lady, Rioja and Hópehely in 2007- 2009 using Mann-Whitney probe (Data are significant at P<5 %.)

Components	2007			2008			2009		
	White Lady	Rioja	Hópehely	White Lady	Rioja	Hópehely	White Lady	Rioja	Hópehely
Asparagine (mmol kg^{-1})	C<O	C>O	C=O	C=O	C>O	C>O	C>O	C>O	C=O
Glutamine (mmol kg^{-1})	C<O	C>O	C=O	C=O	C=O	C>O	C>O	C=O	C=O
Nitrate (mg kg^{-1})	C>O	C>O	C>O	C>O	C>O	C>O	C>O	C>O	C=O
TGA (mg kg^{-1} raw potato)	C>O	C<O	C=O	C=O	C=O	C=O	C>O	C>O	C=O

C>O organically grown potato tubers contained higher amount of the investigated component.

C<O conventionally grown potato tubers contained higher amount of the investigated component.

C = O No difference in data.

Table 2. Effect of conventional and organic farming technology on the amount and ratio of asparagine and glutamine in potato tubers (mmol/kg liophilized potato) of cv. White Lady, Rioja and Hópehely in 2007- 2009.

Year	Variety	Farming technology	Asparagine ($mmol\ kg^{-1}$)		Glutamine ($mmol\ kg^{-1}$)		Asp+GLU	Asp/Glut
			average	SD	average	SD		
2007	White Lady	Conventional	35.4	1.6	16.8	1.4	51.5	2.1
	White Lady	Organic	47.3	1.6	33.9	2.6	77.5	1.4
	Rioja	Conventional	62.4	3.7	28.3	2.3	86.7	2.2
	Rioja	Organic	43.9	4.6	13.3	1.3	50.4	3.3
	Hópehely	Conventional	43.7	7.8	22.1	4.5	66.5	2.0
	Hópehely	Organic	45.8	1.4	22.7	0.9	70.2	2.0
2008	White Lady	Conventional	69.9	6.5	96.3	28.9	198.7	0.7
	White Lady	Organic	87.1	5.4	112.3	10.8	79.7	0.8
	Rioja	Conventional	122.0	25.9	110.9	39.1	256.7	1.1
	Rioja	Organic	85.1	9.0	60.4	7.6	127.0	1.4
	Hópehely	Conventional	74.6	9.7	144.4	29.9	191.0	0.5
	Hópehely	Organic	56.1	1.3	113.4	15.2	176.3	0.5
2009	White Lady	Conventional	90.9	11.3	74.6	9.8	186.4	1.2
	White Lady	Organic	54.0	4.2	40.1	1.4	92.3	1.3
	Rioja	Conventional	113.3	16.5	47.5	5.4	136.9	2.4
	Rioja	Organic	83.7	6.3	39.1	7.2	122.1	2.1
	Hópehely	Conventional	78.1	29.3	70.2	23.2	135.7	1.1
	Hópehely	Organic	52.2	13.4	46.0	11.0	123.9	1.1

Table 3. Effect of conventional and organic farming technology on nitrate content of potato tubers of cv. White Lady, Rioja and Hópehely in 2007-2009.

Nitrate content of studied potato cultivars (mg kg^{-1})

Year	Crop system	Variety					
		White Lady		Hópehely		Rioja	
		mean	SD	mean	SD	mean	SD
2007	organic	79.5	46.2	77.8	41.4	26.0	5.3
	conventinal	293.7	91.0	141.4	25.5	52.8	12.8
2008	organic	133.0	34.6	65.3	8.5	50.0	9.5
	conventinal	198.0	16.5	192.7	24.7	68.3	8.4
2009	organic	90.0	30.3	81.3	43.0	11.7	1.2
	conventinal	239.3	11.0	78.0	58.0	17.7	3.1

Table 4. Effect of conventional and organic farming technology on TGA content of potato tubers of cv. White Lady. Rioja and Hópehely in 2007-2009.

TGA content of studied potato cultivars (mg kg^{-1} raw potato)							
		Variety					
Year	Crop system	White Lady		Hópehely		Rioja	
		mean	SD	mean	SD	mean	SD
2007	organic	1.59	1.32	0.28	0.07	4.45	2.26
	conventional	3.86	0.43	0.24	0.77	0.53	0.14
2008	organic	28.09	17.25	0.00	0.00	0.47	0.51
	conventional	15.10	5.63	0.00	0.00	1.94	2.65
2009	organic	0.00	17.25	0.00	0.00	2.04	1.18
	conventional	8.85	5.63	1.04	1.81	8.39	2.19