TECHNOLOGICAL USABILITY OF TUBERS OF GENETICALLY-MODIFIED POTATO

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In the presented work the effects of 3-year own investigations relative to technological usability of potato tubers of parental cultivar and its genetically-modified clones were recapitulated.

Potato tubers of cultivar Irga were transformed with viral genome sequences in order to improve their resistance to a necrotic strain of potato virus Y (PVY^N) at the Institute of Biochemistry and Biophysics, Warsaw, and tubers of the transgenic clones were produced at the Plant Breeding and Acclimatisation Institute, Młochów, in 2000-2002.

At the first stage, the variability of parameters characterizing raw material quality was determined. The characteristics of physical properties (size, shape, and mechanical resistance of tubers), microstructure, and content of main chemical constituents (starch, protein, ash, ascorbic acid, glycoalkaloids: α-solanine and α-chaconine) of 15 genetically-modified clones were investigated during three successive years. Tubers of these GM clones were next subjected to culinary processing (microwaving, cooking, and frying) and physico-chemical changes, which are decisive for texture formation and texture properties of end-use product, have been investigated and technological usability of GM potatoes was established. In this work, due to a considerable number of collected data, the final results were presented as means for modification group or, in two cases, as results relative to single clones representative for modification groups. Analysis of variability of physical and chemical parameters studied for the raw and heat-treated potato tubers enabled classifying Irga and its genetically-modified clones as similar, and did not allow distinguishing a clone of special usability for heat processing.

INTRODUCTION

Potatoes are an excellent source of carbohydrates, proteins, and bioactive compounds, *i.e*. inhibitors of digestive enzymes, polyphenols, vitamins and others, but the tubers contain also toxic glycoalkaloids which content should be controlled carefully because of human health safety. The consumption of potatoes varies from country to country, but takes remarkable position in a human diet (150–400 g and more per capita per day). The major pests limiting the yield or quality of potato tubers are: Colorado potato beetle (CPB), potato loaf roll virus (PLRV), and potato Y (PVY) viruses. The latter are considered as damaging viruses which cause economically significant yield reduction, by as much as 80% [Błaszczak *et al*., 2005a]. While the effectiveness of insecticides used to prevent undesired changes of tuber quality and yield losses varies due to pesticidal characteristics limitations, genetical transformation seems to be the most effective method introducing the required resistance [Fornal *et al*., 2002; Mullins *et al*., 2006].

MATERIAL

Potato tubers of cultivar Irga were transformed with viral genome sequences in order to improve their resistance to a ne-crotic strain of potato virus Y (PVY^N). The transgenic clones were produced at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw. The first group (Group I) of constructs (R) introduced into the genome of cv. Irga contained a truncated gene coding PVY^N polymerase (Nib) (GenBank Acc. No. D00441). The second group (Group II) of clones (NTR) contained a fragment of cDNA corresponding to 184 nucleotides of the 5'-end of PVY of PVY^{N}Wi isolate (GenBank Acc. No.Z70238). These two groups of transgenic clones contained the following subgroups: R1 with a truncated gene coding PVY^N polymerase in sense orientation (subgroup Is - 4 clones), and R2 with the same viral gene in antisense orientation (subgroup Ias - 6 clones); as well as NTR1 with introduced fragment of cDNA of PVY of PVY^{N}Wi isolate in sense orientation (subgroup IIs - 3 clones), and NTR2 with the same sequence introduced in antisense orientation (subgroup IIas - 2 clones).

The transformation process, selection and identification of transformed plants from cv. Irga were described by Chachulska *et al*. [1997]. Non-transgenic tubers obtained from normally planted cv. Irga were also examined as a control sample. The potatoes were bred and collected at the Plant Breeding and Acclimatization Institute, Młochów Research Center. Modified clones were propagated by successive pas-

sages of *in vitro* obtained minitubers [Flis & Zimnoch-Gu-zowska, 2000] which were used in a field experiment.

The weight of potato samples ranged from 1000 to 1500 g while the number of tubers in a sample ranged from 18 to 63. Mean dry matter content of the modified potatoes ranged from 19.72 to 23.02%, from 19.63 to 23.23% and from 20.69 to 24.38% for tubers from 2000, 2001 and 2002. Samples of all the examined potatoes were stored under the same conditions and tested at the same time after harvest.

SIZE AND SHAPE OF TUBERS

Variation in potato tuber size is usually very high, even for a single plant, because of different development of particular tubers during harvesting. Additionally, tuber size of potato cultivars depends on many outer factors as climatic conditions and agricultural treatment [Cepl & Vokal, 1996; Wurr *et al.*, 1997]. For a long time the size of tuber has been recognised to strongly influence distribution of dry mass and other basic constituents, especially starch [Burton, 1966; Dale & Mackay, 1994; Rastovski & van Es, 1981]. Baritelle & Hyde [1999] noted that tuber size influenced resistance of potato tissue on failure and Cmunt [1997] found also that tuber resistance to mechanical damage during harvest was increasing with an increasing weight of tubers. On the other hand, the food industry demands potato cultivars for special uses, *i.e.* for chips or French fries of characteristic shape and size distribution. By genetical selection and use of special agricultural treatment, appropriate cultivars of desired geometrical parameters have been bred. Then, remembering the above-mentioned restrictions, changes of tuber geometry can be considered as the variation of characteristic feature of a cultivar.

The variability of tuber weight for all GM clones and parental Irga cultivar was very high but the results of variance analysis confirmed a statistically significant (at $p < 0.05$) effect of modification and cultivation year [Sadowska *et al.*, 2004a]. Such a high weight variability for particular clones suggested the examination of tuber weight and its distributions for modification groups (Table 1, Figure 1). Mean weight of tubers of modification groups correlated closely in 2000 and 2001 although GM tubers were lighter (in 2000) or heavier (in 2001) than tubers of Irga. In 2002, the mean weight of tuber of modification groups changed irregularly being always very low and significantly smaller than this of Irga tubers (Table 1). Tuber weight distributions of modification groups and parental cultivar Irga were statistically similar in 2000 whereas in 2001 and 2002 tuber weight distribution also changed irregularly (Figure 1). It is worthy attention that distributions of tuber weight from 2000 and 2001 were characterised by a lack of the smallest tuber range, while in 2002 these ranges predominated in all distributions. Though the results of Anova confirmed a statistically significant effect of modification type, still they pointed out various single clones and modification subgroups as different in the successive years of cultivation.

Most often used index of tuber shape is the length : width ratio, *i.e.* shape coefficient (Table 1). Only two clones from 2002 (R2P and R2Y) can be classified to the class longish/long, which corresponded to shape coefficient ranging from 1.40 to 1.69 (according to Winiger & Ludwig [1974]). All the
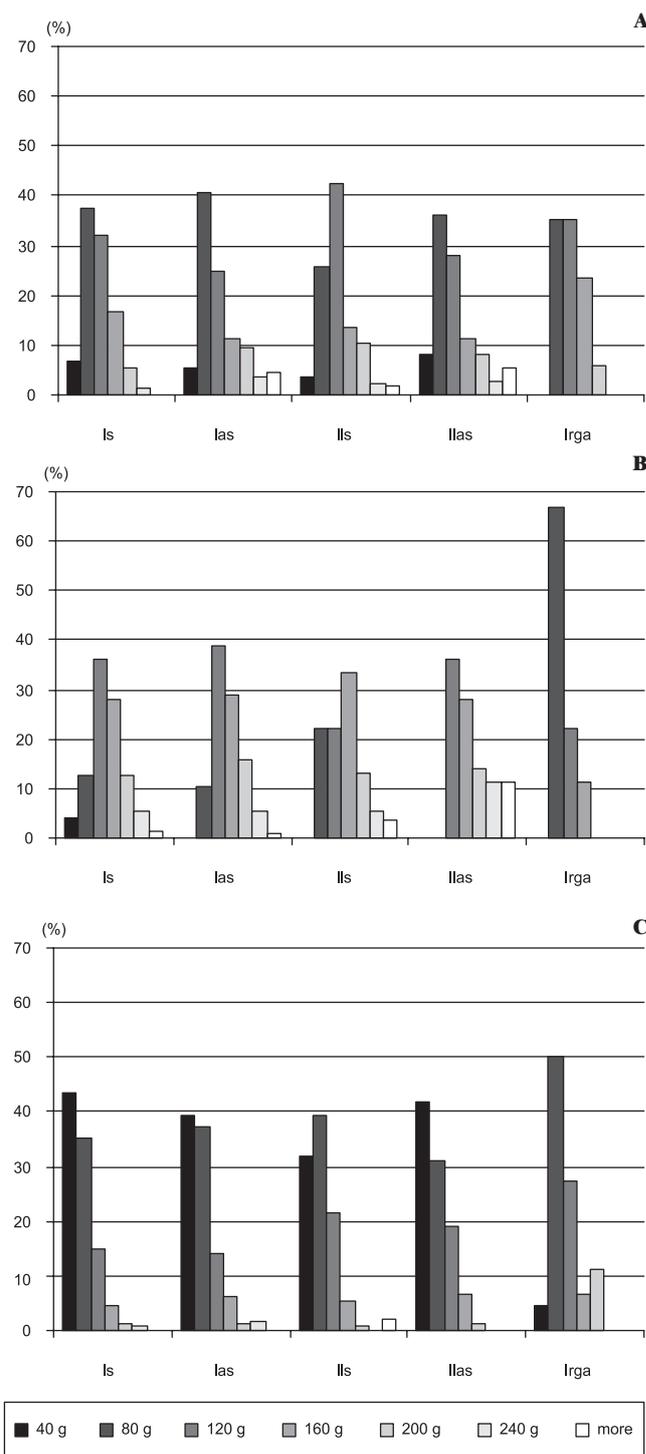

FIGURE 1. Tuber weight distribution for modification groups of GM clones and parental cultivar Irga (A – 2000, B – 2001, C – 2002).

remaining tubers were classified as roundish/longish with a respective range of shape coefficient from 1.10 to 1.39 [Sad-owska *et al.*, 2004a]. It is worthy of attention that tubers of all the examined groups collected in 2001 were characterised by higher shape coefficient values, *i.e.* more oblong shape. Actually, the shape was a more constant feature than weight of single tubers, which was confirmed by the range of variation coefficients clearly narrow for the shape coefficient (from 6 to 12%) than for the mean weight of tubers (even up to 81%).

TABLE 1. Physical properties of tubers of GM potatoes.

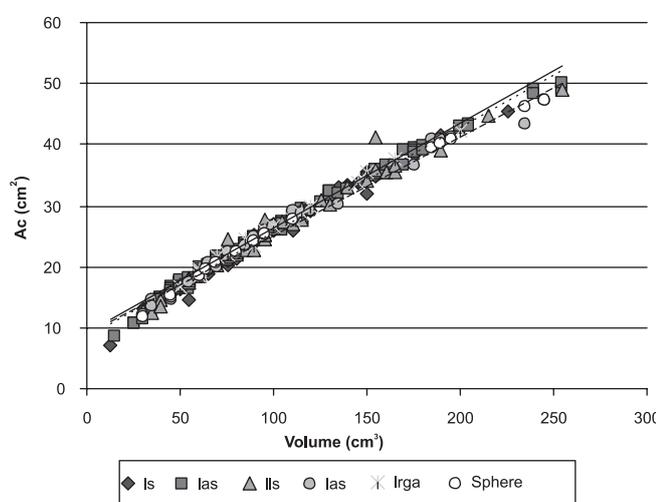|	Modification groups				Irga
	Is	Ias	IIs	IIas	
2000					
Tuber weight (g)	93.2	103.8	103.0	104.1	107.3
Shape coefficient (-)	1.16	1.17	1.17	1.13	1.10
2001					
Tuber weight (g)	124.2	128.4	125.9	155.9	77.9
Shape coefficient (-)	1.34	1.39	1.39	1.34	1.31
2002					
Tuber weight (g)	52.5	49.9	68.6	60.5	88.2
Shape coefficient (-)	1.19	1.19	1.21	1.25	1.21

FIGURE 2. Comparison of relationship between criterion area (Ac) and volume for tubers of all GM potato clones (solid line) and parental cultivar Irga (dotted line) and ideal sphere (broken line).

A similar opinion was presented by Wurr e*t al.* [2001] who found that time of harvesting the seed crop did not affect tuber shape nor the number of ground stem without interaction with tuber size. Additionally, for the description of volume shape of tubers collected in 2000 criterion area was determined. Based on the theory that the average projected area of convex body is 1/4 the surface area, Mohsenin [1970] presented the equation: $A = K.V^{2/3}$ where constant K is a measure of the degree of sphericity (for sphere K=1.21). Values of this constant ranged from 1.18 to 1.37 showing small differentiation of tubers shape. Classic relationship between criterion area and size of potato tubers allowed presenting visually deviation of the examined tubers shape from ideal sphere (Figure 2). It was confirmed that parental Irga tubers were rather roundish and the relationships calculated for tubers of modification subgroups were similar to that for Irga.

MECHANICAL PROPERTIES

During harvest and transport, potato tubers are prone to impact damage which ranges from internal black spot bruising through shatter bruising and finally tissue cracking [Mathew & Hyde, 1997]. The number of damages can be primarily attributed to physiological changes which affect structural components [Thybo *et al.*, 1998, Alvarez & Canet, 1998; Konstankiewicz *et al.,* 2001; Zdunek & Umeda, 2005], tissue turgor and temperature affecting failure properties of tubers [Alvarez & Canet, 2002; Bajema *et al.*, 1998]. Thus, the recognition of their mechanical characteristics allows to improve harvesting and handling equipment and operations for diminishing economic losses. Mechanical properties of tubers (as a measure of texture) are, beside size and shape, important factors of cultivar classification for such technological destinations as chips, French fry strips, and cooked potato [Dale & Mackay, 1994]. Mechanical/rheological parameters of tubers obtained using various instrumental methods have also been widely studied for elaboration of an algorithm of instrumental methods for prediction of sensory attributes [Laza *et al.*, 2001; Solomon & Jindal, 2003; Thybo & van den Berg, 2002; Truong *et al.*, 1997]. The mathematical modelling of the mechanical properties of turgid plant tissue could enable an understanding of why different fruits and vegetables have different mechanical properties and how these properties can be changed [Blahovec, 2001; Hepworth & Bruce, 2000].

The examination showed that the changes of mechanical parameters (expressed by failure stress, failure strain, end elasticity modulus) for particular clones were found irregular during three years of cultivation [Sadowska *et al.*, 2004b]. The mechanical properties of tubers of parental Irga cultivar did not remained at a constant level either. In 2000, the mechanical properties of potatoes of control group were lower than those of modified clones, but in 2001 and 2002 the mechanical characteristics of control tubers was similar to the characteristics of most clones. A typical correlation between fracture stress and elasticity modulus (correlation coefficients were 0.9283, 0.9276 and 0.9354 for 2000, 2001 and 2002 years, respectively) could be accepted as the confirmation of different mechanical characteristics of the examined clones despite significant variability of collected data. Yet, the expected correlation between fracture stress and strain was statistically significant only for tubers from the year 2001. Bajema *et al.* [1998] found that changes in strain rate did not affect failure stress but failure strain was reduced dramatically with an increasing strain rate. Then, they concluded that cell rupture pressure is not a function of the strain rate and reduction of fracture strain suggested different fracture mechanisms compared with the standard viscoelastic material. A high variability of mechanical parameters found within a clone was confirmed by Thybo & van den Berg [2002] who also pointed out that the samples of potatoes were characterised by large variation between replications for particular cultivar and even for tubers of the same cultivar. However, the obtained average results of fracture stress and strain were similar or slightly lower than data for tubers of other cultivars presented by Alvarez & Canet [1998, 2000].

Thus, tendencies in mechanical properties for groups of clones representing the same modification model were investigated. Mean values of mechanical parameters for groups were presented in Table 2. Mean values of failure stress and elasticity modulus for group II have always appeared higher

TABLE 2. Mechanical resistance of tubers of GM potatoes [Sadowska *et al*., 2004b with kind permission of International Agrophysics].

	Modification groups				Irga
	Is	Ias	IIs	IIas	
2000					
Fracture stress (MPa)	1.23	1.25	1.24	1.26	1.17
Elasticity modulus (MPa)	5.11	5.00	5.06	5.02	4.66
2001					
Fracture stress (MPa)	1.20	1.21	1.25	1.38	1.21
Elasticity modulus (MPa)	4.60	4.61	4.66	5.08	4.71
2002					
Fracture stress (MPa)	1.27	1.24	1.30	1.24	1.07
Elasticity modulus (MPa)	4.70	5.02	5.16	4.73	4.27

than those for groups I and cultivar Irga. It has been also observed that the tubers in both subgroups of antisense position *i.e*., Ias and IIas were more mechanically resistant than the tubers of sense position subgroups *i.e*., Is and IIs, respectively. The results of statistical analysis (discriminant analysis) did not allow distinguishing groups of statistically different mechanical properties (Figure 3). Wilks coefficients, *i.e.* the measure of the accuracy of group recognition, which ranges from 0 (ideal group discrimination) to 1 (lack of group discrimination), were 0.4318, 0.7698 and 0.8575 for 2000, 2001 and 2002, respectively, and confirmed unsharp separation of the group of different genetical modifications. The percentage of proper classification of cases in all the accepted groups in the successive years was also very low (68%, 35% and 42%, respectively). Thus, it was concluded that the fracture stress and other mechanical properties expressed by fracture strain and elasticity modulus of potato tubers of cultivar Irga have not been permanently affected by the type of genetical modification used.

CONTENT OF MAIN CHEMICAL CONSTITUENTS

Among various quality factors the level of chemical compounds (responsible for nutrient and biological activity) of clone tuber produced has to be carefully controlled because nutritional quality is a delicate balance between nutritional and anti-nutritional compounds [Finotti *et al*., 2006]. In the evaluation of potato cultivar quality, the major compounds determined are: starch (15-18% of fresh weight) and protein (about 2.1% of fresh weight), tuber solids (as the most important determinant of culinary appeal), ash (as a measure of mineral level) and two compounds of opposed nutritional effect, *i.e.* L-ascorbic acid and glycoalkaloids (α-solanine, and α-chaconine). Ascorbic acid plays an important role as an enzyme cofactor, radical scavenger, and donor/acceptor in the electron transport system [Davey *et al*., 2000]. Unfortunately, L-ascorbic acid is sensitive to air, heat, and water treatment [Haase & Weber, 2003; Han *et al*., 2004]. Although potatoes are always consumed after processing, they are still an excellent source of vitamin C, because of significant share of potatoes in daily diet, as they can provide over 30% of the intake of vitamin C from fruit
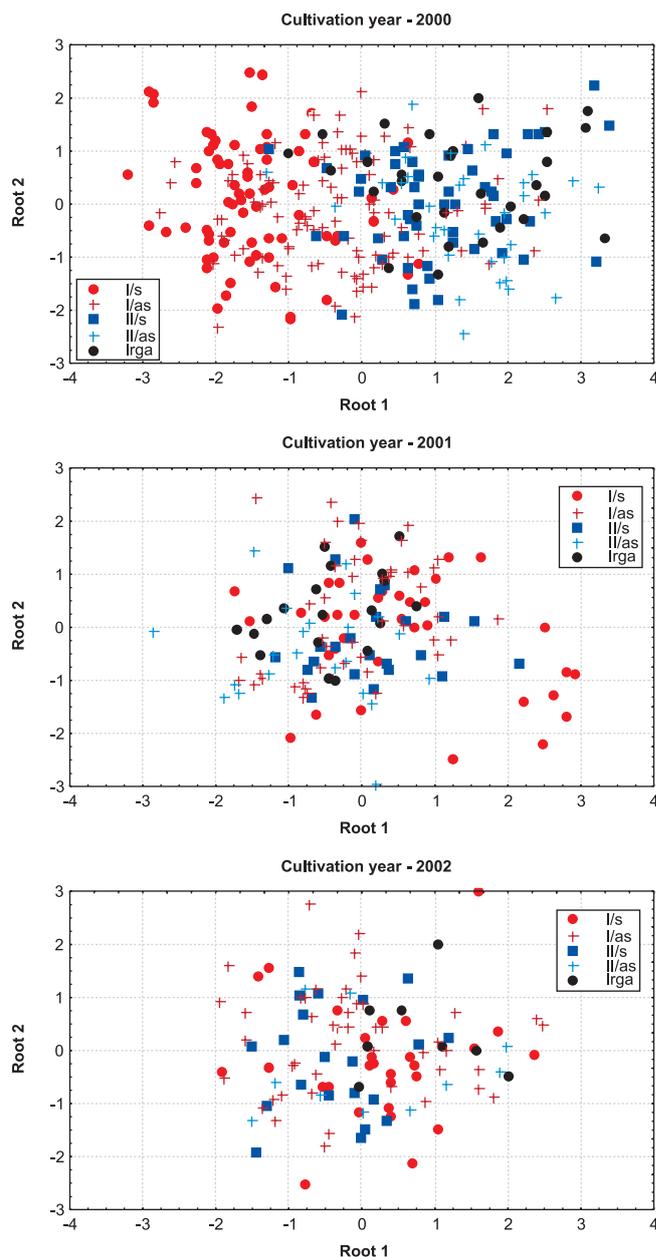
FIGURE 3. Classification of modification groups according to parameters of mechanical resistance [Sadowska *et al*., 2004b with kind permission of International Agrophysics].

and vegetables. In order to evaluate the nutritional quality of potatoes it is also needed to examine the content of anti-nutrients [Novak & Haslberger, 2000; El Sanhoty *et al*., 2004] the main representative of which are toxic glycoalkaloids – all the more they are resistant to typical heat treatment *i.e.* cooking, frying, baking or microwaving [Friedman & McDonald, 1997] and storage [Edwards & Cobb, 1997]. The concentration of glycoalkaloids in potatoes depends, among other things, on their variety [Finotti *et al*., 2006; Şengül *et al*., 2004], tubers maturity [Pęksa *et al*., 2002] and tuber part [Friedman & Da, 1990; Pęksa *et al*., 2006]. Thus, after consideration of the role of potato as a source of nutrients in human diet, chemical components of tubers of the modified clones mentioned above were selected for especially careful examination.

Tuber greening (caused by their exposure to light) is often used by the public as a measure of the health risk posed by potato. The mechanism of greening is known – this is due to the conversion of amyloplasts to chloroplasts and subsequent synthesis of chlorophyll and photosynthetic pigments [Edwards & Cobb, 1997]. However, explicit confirmation of a close relationship between exposure to light resulting in greening and accumulation of phenolic compounds, such as chlorogenic acid and glycoalkaloids is still the subject of numerous studies [Dao & Friedman, 1994; Edwards *et al*., 1998].

Content variability of compounds for particular clones was very low for ash, protein, and starch whereas for L-ascorbic acid (AA), α-solanine, and α-chaconine was distinctly high [Sadowska *et al*., 2007]. The content of glycoalkaloids (TGA) should be controlled carefully because of human health safety. Commonly recommended limit of glycoalkaloids in potato is 20 mg/100 g of fresh weight [Friedman & McDonald, 1997]. The results of statistical analysis did not indicate any stable tendencies for changes of particular chemical compounds (Table 3) and confirmed a random character of changes of compound contents of modification type groups over the 3-year period of cultivation. The results of statistical calculation implied that the effect of particular modification version on the chemical content of clones was not constant during years of cultivation. The recognition of a uniform group of the best chemical characteristics was impossible, like in the case of single clones.

The results of statistical analysis did not indicate any stable tendencies for changes of particular chemical compounds either but comparison of compounds content expressed as a percentage of clones differing from parent cultivar (Table 4)

TABLE 3. Chemical characteristics of tubers of GM potatoes [Sadowska *et al*., 2008 with kind permission of Springer Science and Business Media].

	Modification groups				Irga
	Is	Ias	IIs	IIas	
2000					
Ash (% dm)	4.09	4.20	4.16	4.12	4.21
Protein (% dm)	9.31	9.51	9.55	9.42	78.29
Starch (% dm)	76.80	77.01	78.73	79.14	71.90
α-Solanine (mg/100 g dm)	14.60	13.84	15.27	17.09	25.99
α-Chaconine (mg/100 g dm)	40.03	39.90	43.33	45.68	71.90
2001					
Ash (% dm)	4.48	4.29	4.26	4.31	4.67
Protein (% dm)	7.28	7.67	7.16	8.49	10.08
Starch (% dm)	80.19	77.83	79.59	80.11	69.17
L-ascorbic acid (mg/100 g dm)	34.91	33.00	36.07	36.27	42.35
α-Solanine (mg/100 g dm)	14.86	26.94	16.07	10.44	37.76
α-Chaconine (mg/100 g dm)	46.92	67.50	40.65	41.37	75.02
2002					
Ash (% dm)	4.28	4.16	4.39	4.28	4.66
Protein (% dm)	8.55	8.71	8.69	8.15	10.45
Starch (% dm)	83.72	83.89	84.03	83.25	80.33
L-ascorbic acid (mg/100 g dm)	18.06	20.25	20.04	19.22	28.78
α-Solanine (mg/100 g dm)	49.52	46.79	39.16	54.49	35.89
α-Chaconine (mg/100 g dm)	73.88	63.95	57.78	70.52	46.53

was extremely interesting. For vast majority of clones changes of the chemical compounds examined manifested desirable direction. High percentage of clones of an increased content of protein and starch (26.7-86.7% and 46.7–100%, respectively) and decreased content of α-solanine and α-chaconine (86.7-100% and 93.3-100%, respectively) was worthy of special attention. Similar results were also presented by Rogan *et al*. [2000] and Bianco *et al*. [2003] for modified, insect and virus resistant potato tubers.

The content of both glycoalkaloids was mostly higher in green tubers than in normal tubers of modified clones and parental cultivar Irga (Figure 4). A significant increase of TGA in tubers exposed to light under both experimental and natural conditions was often noted [Dao & Friedmann, 1994], but cultivars of stable TGA content were found as well [Edwards & Cobb, 1997]. The reason of such large differences in the greening response between cultivars is not completely recognized; it can be presumed rather as "varietal" influence, though the effect of uncertain factors cannot be precluded [Edwards & Cobb, 1999]. Yet it was concluded that some of modification versions (mainly from Ias group) eliminated or distinctly diminished the difference in TGA content between normal and green tubers of parental Irga through TGA decrease in green tubers of respective clones. L-ascorbic acid content in green tubers was found lower than in the normal ones, although a statistically significant difference was confirmed only for some clones and parental cultivar Irga (Figure 4).

Distribution of particular chemical compounds and even dry matter within the tuber was strongly diversified [Burton, 1989]. The concentration of glycoalkaloids was several times higher in peel than in flesh, which resulted in different contents of glycoalkaloids – higher in small than in big tubers [Friedman & McDonald, 1997; Pęksa *et al*., 2006]. L-ascorbic acid is situated mainly in flesh of tubers [Finlay *et al*., 2003]. Thus, the relationship between tuber size and glycoalkaloids content in green and normal tuber of representative clones was presented in Figures 5-6. Correlation coefficients for parental cultivar Irga for average data and those for normal tubers for both glycoalkaloids were statistically significant (at $p \leq 0.05$), whereas for green tubers, a statistically significant correlation between glycoalkaloids and tuber size was confirmed only for R1F clone and parental cultivar Irga. No relationships were either observed between L-ascorbic acid content and tuber size for both normal and green tubers of the modified clones.

TABLE 4. Percentage of clones differing from parental cultivar Irga in the content of particular compounds.

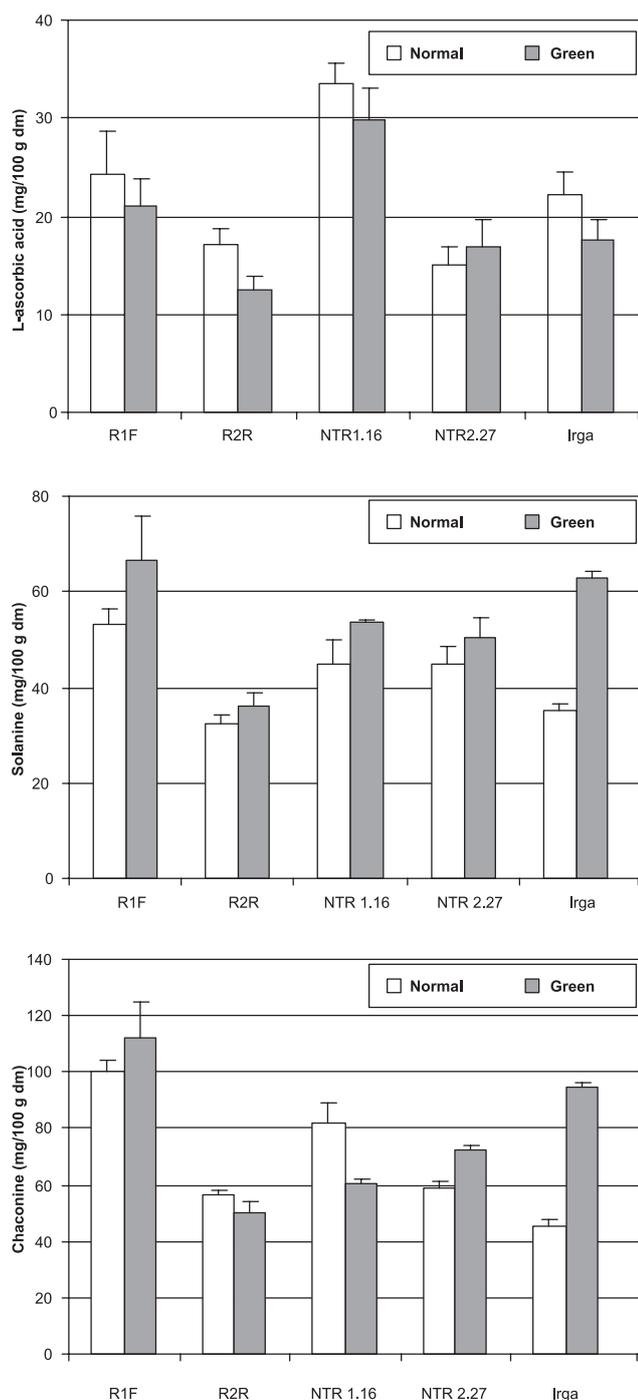| Compounds	Cultivation year		
	2000	2001	2002
Higher in clones than in Irga			
Ash	20.0	6.7	6.7
Protein	86.7	60.0	26.7
Starch	46.7	100.0	100.0
L-ascorbic acid	-	0.0	73.3
Lower in clones than in Irga			
α-Solanine	100.0	100.0	86.7
α-Chaconine	100.0	93.3	93.3

FIGURE 4. Comparison of L-ascorbic acid, α-solanine, and α-chaconine contents in normal and green tubers of GM clones and parental cultivar Irga.

FIGURE 5. Tuber size *vs*. α-solanine content for clones representing modification versions for normal (upper) and green tubers (below) [Sadowska *et al*., 2008 with kind permission of Springer Science and Business Media]. Ranges of tuber size: 1< 27.0 g; 27.1<2<46.0 g; 46.1<3<65.0 g; 65.1<4<84.0 g; 84.1<5<103.0 g; 6>103.0 g.

It could be assumed that some version of modification influenced the content of glycoalkaloids in normal and green tubers but the final conclusion requires further investigation.

TECHNOLOGICAL USABILITY

Plant tissue subjected to simple culinary processing (microwaving, cooking, and frying) undergoes physico-chemical changes which are decisive for texture formation and texture properties of end-use product. The texture is of critical impor-
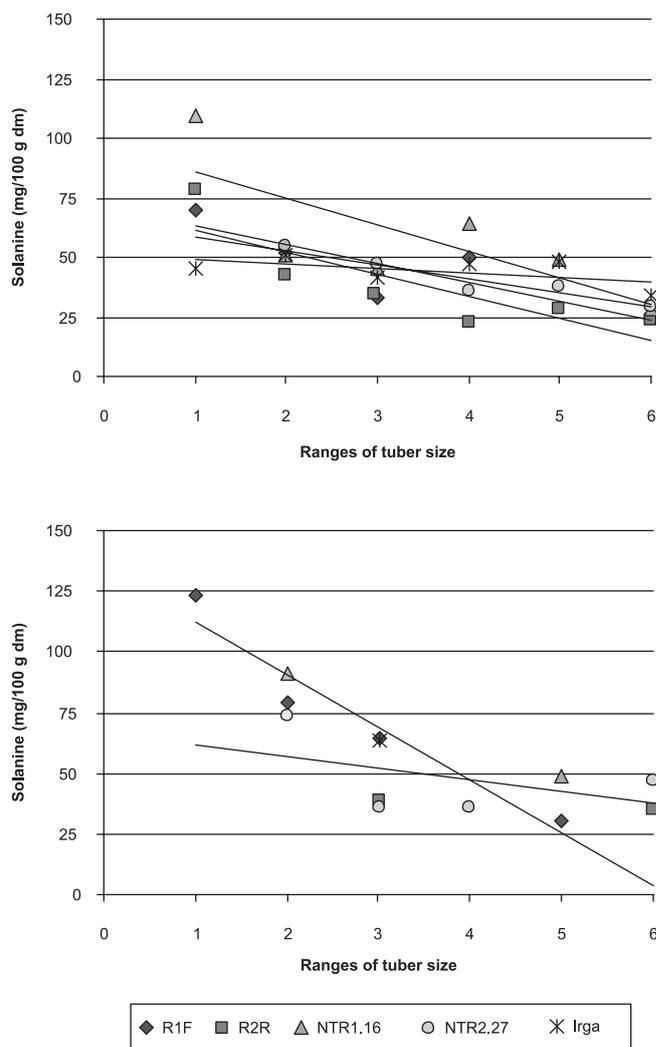
tance of the consumers choice and acceptability. Moreover, various processing indices are considered, *e.g.* cookability (expressed by ratio: hardness of raw/ hardness of microwaved or cooked potatoes), and losses of potato mass [Costa & Oliveira, 1999], for establishing technological usability of potatoes.

Błaszczak *et al*. [2004], who presented changes of texture of microwaved tuber of all GM clones, found an evident effect of microwave heating on their mechanical properties, independently of genetic modification. Although statistical analysis confirmed a high variability of tuber hardness (raw and microwaved) for all GM clones, average cookability of modification groups (except Is) was a little lower than this of parental cultivar Irga, and differences of mass losses were statistically not significant (Figure 7).

Cooking is most often applied process in the food processing, hence the mechanisms of thermal softening have been widely studied. Generally, starch swelling in tissue cells and pectin dissolvation in the middle lamella has been con-
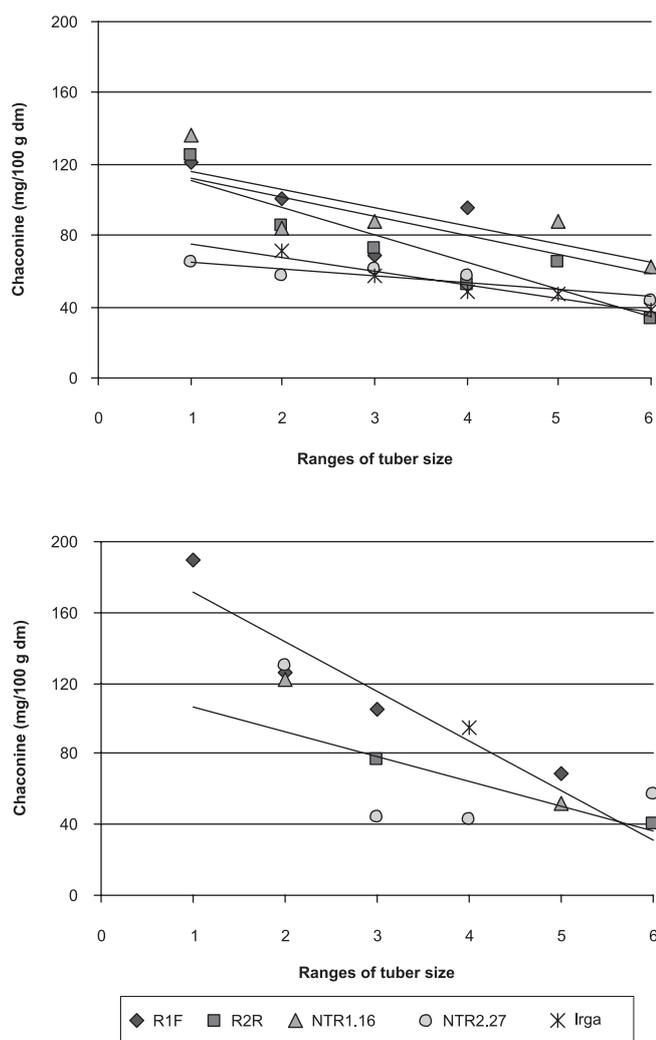
FIGURE 6. Tuber size *vs.* α-chaconine content for clones representing modification versions for normal (upper) and green tubers (below) [Sadowska *et al.*, 2008 with kind permission of Springer Science and Business Media].

Ranges of tuber size: 1< 27.0 g; 27.1<2<46.0 g; 46.1<3<65.0 g; 65.1<4<84.0 g; 84.1<5<103.0 g; 6>103.0 g.
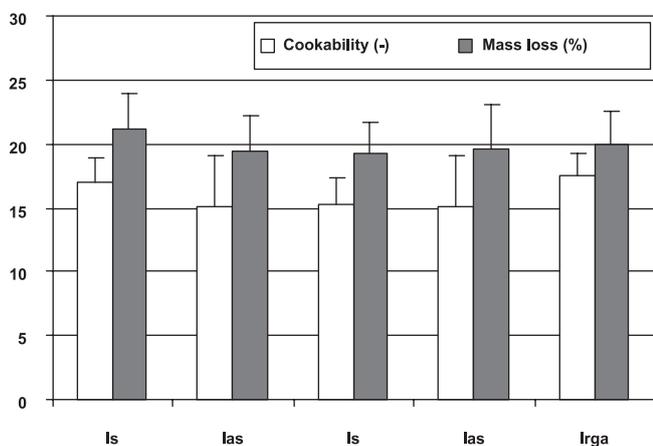

FIGURE 7. Cookability and mass losses of modification groups of GM clones and parental cultivar Irga during microwaving.

sidered to be responsible mainly for cooking results [Thybo *et al.*, 1998]. These phenomena resulted in changes of the mechanical properties and rheological behaviour of tubers, thus, various mechanical and rheological tests were used to determine texture softening [Solomon & Jindal, 2003], and to estimate both the influence of potato origin and conditions of processing. The effect of heat treatment of GM clones in water medium was studied in details by Sadowska *et al.* [2005]. They showed high variability of hardness values (expressed by fracture stress) for all raw and cooked GM clones (Table 5) at the similar rheological behaviour of their tissues. It is worthy of attention that in 2001 and 2002 the tubers stored and cooked under the same conditions showed softening (cookability and loss of mass) almost 1.5–2 times higher than in 2000 (Table 6). It implied the effect of cultivation conditions to be stronger than the influence of modification. Results of a statistical analysis showed irregularly changing relationships between modification groups in the successive years and impossibility of recognition of particular modification group. Such results of the analysis of differences between particular groups confirmed definitely a lack of constant tendencies.

As French fries are widely produced both domestically and commercially, food companies offer various kinds of semi-products – for example, blanched and frozen potato strips. Considering that deep-fat frying is a widely used method of potato processing, the usability of genetically--modified potatoes for frying should be examined above all. The effect of heat treatment of GM clones in oil medium was presented by Sadowska *et al.* [2006]. They did not observe any essential differences in the mechanical behaviour, especially in hardness, between Irga and its genetically--modified clones during heat treatment. Many authors have

TABLE 5. Variability of tuber hardness of GM clones before and after cooking.

Years	Tubers	Fracture stress (kPa)		Variance coefficient (%)
		max	min	
2000	raw	1403.5	1116.0	20.5
	cooked	132.0	68.0	48.5
2001	raw	1417.0	978.0	31.0
	cooked	70.0	39.0	44.3
2002	raw	1340.0	1100.0	17.9
	cooked	65.0	35.0	46.2

TABLE 6. Average cookability and losses of mass of modification groups of GM clones and parental cultivar Irga.

	Cookability (-)			Mass loss (%)		
	2000	2001	2002	2000	2001	2002
ls	13.3	23.8	24.8	3.84	5.03	4.43
las	12.2	22.0	21.9	4.34	5.05	4.15
lls	14.9	19.6	27.0	4.07	4.23	4.37
llas	13.8	21.5	23.2	3.92	4.49	4.07
Irga	17.2	24.2	18.1	3.61	4.41	4.64

suggested the effect of strip position on hardness of raw, blanched and French fried strips resulting from diversified microstructure of morphological parts of a tuber [Agblor & Scanlon 1998; Aguilera *et al.,* 2001; Anzaldua-Morales *et al.,* 1992]. They pointed out that the stem zone, especially in the cortex layer, is characterised by the highest hardness. Taking this into account, it was decided to show the variability of hardness measured at various sites of potato tuber. The determined values of puncture force (characterising the hardness of strips) were, however, not constant, even for this morphological zone (Figure 8).

There were not observed any essential differences in the mechanical behaviour between Irga and its particular genetically-modified clones or modification groups of clones during heat treatment. The mechanical and technological characteristics of raw and heat-treated tubers of modification groups or representative clones examined during successive years did not allow distinguishing a clone of special usability for microwaving, cooking, nor frying.
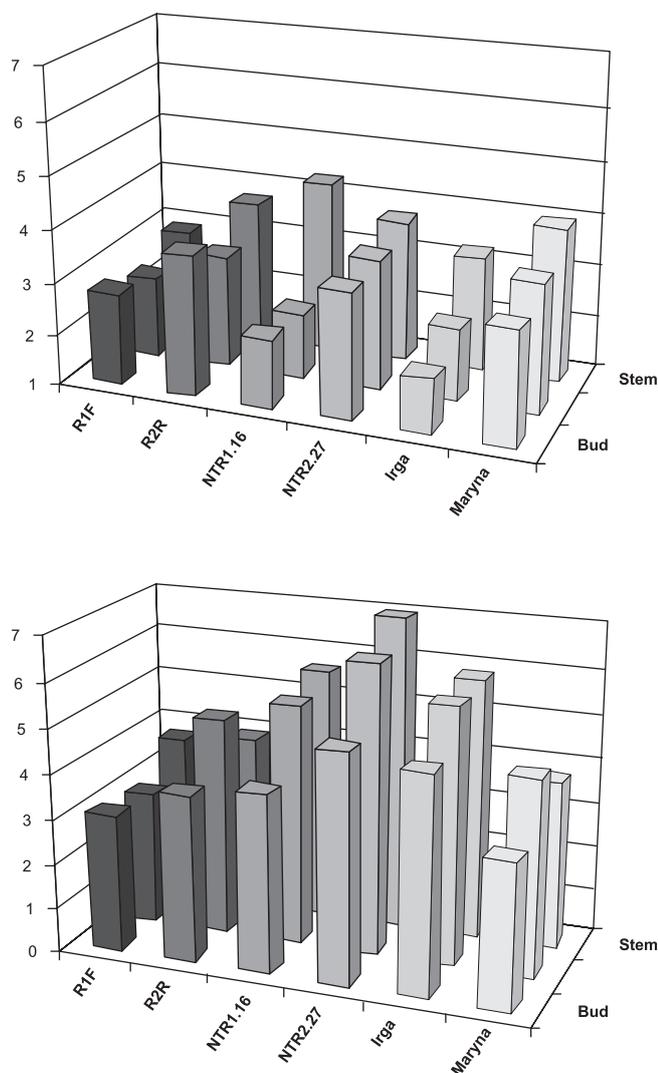

FIGURE 8. Variability of hardness changing along length of fried strips for representative GM clones, parental cultivar Irga, and cultivar Maryna applied for French fries production for fresh (upper) and stored (bottom) blanched potato strips.

MICROSTRUCTURE

The scanning electron microscopy analysis demonstrated differences in the microstructure of raw tubers between control potato and transgenic clones. The control sample of Irga potato tuber and transgenic clones of R1F one were presented as an example of differences in tuber microstructure evoked by the genetical modification of potato plant.

A cross section through the potato tuber of Irga (Figure 9A) showed the cells of storage parenchyma tissue filed with starch granules with a diameter of about 30–50 μm. The size of cells of parenchyma tissue in the Irga potato tuber did not exceed 200 μm. Bigger cells (up to 300 μm) formed the parenchyma tissue of the R1F clone (Figure 9B). The SEM observation of microstructure of R1F clone demonstrated a higher concentration of the storage material in the parenchyma cells compared to the control tuber of Irga. The starch granules appeared to be covered by some cellular material that, according to Nuss & Hadziyev [1980], may be assigned to the protein bodies. Using SEM, theses authors characterised protein material appearing in the parenchyma cells of tuber as cubical or globular crystalline bodies strongly adhered to starch granules. The SEM observation showed also differences in the walls character of the parenchyma cells between control tuber and the clone one. In opposite to control tuber, the cell walls of parenchyma in the R1F tuber appeared thicker and folded. That phenomenon probably resulted from a higher thickness of the middle lamella and also a higher concentration of the cell wall pectins compared to Irga potato.

Potato tubers subjected to microwave heating also demonstrated significant changes in storage material and cell walls microstructure (Figures 9C-D). However, these changes were of different character as compared to those of the cooked ones. The microwave treatment of potato tuber did not evoke a total solubilisation of the cell walls as in the case of tuber cooking. The microwaved potatoes manifested more collapsed and deformed cells of parenchyma tissue, and the cell walls were strongly adhered to the starch-protein matrix formed during treatment. Cells deformation resulted probably from the significant moisture loss in the tubers upon their microwave heating. The phenomenon of parenchyma cells collapsing after treatment was related by Huang *et al.* [1990] to a breakdown of hemicellulose and cellulose components in the cell walls. It is worth mentioning that the cellular water evaporation also limited gelatinization of starch granules inside the cells, which in turn resulted in the formation of a compact and closed structure of the gel.

The hydro-thermal treatment of potato tubers resulted in significant changes in microstructure of parenchyma tissue. The tubers subjected to cooking process manifested changes not only in the microstructure of storage material but also in the cell walls of parenchyma tissue. The deformation of parenchyma cells, observed under SEM, were mainly triggered by starch granules gelatinization. The gelatinization process of granules induced the swelling pressure inside the cells, which in turn affected breakdown of the cell walls [Agblor & Scanlon, 1998]. The gelatinised starch formed a continuous gel structure that completely filled the cell lumen (Figures 9E-F). The cooking process resulted also in the formation of
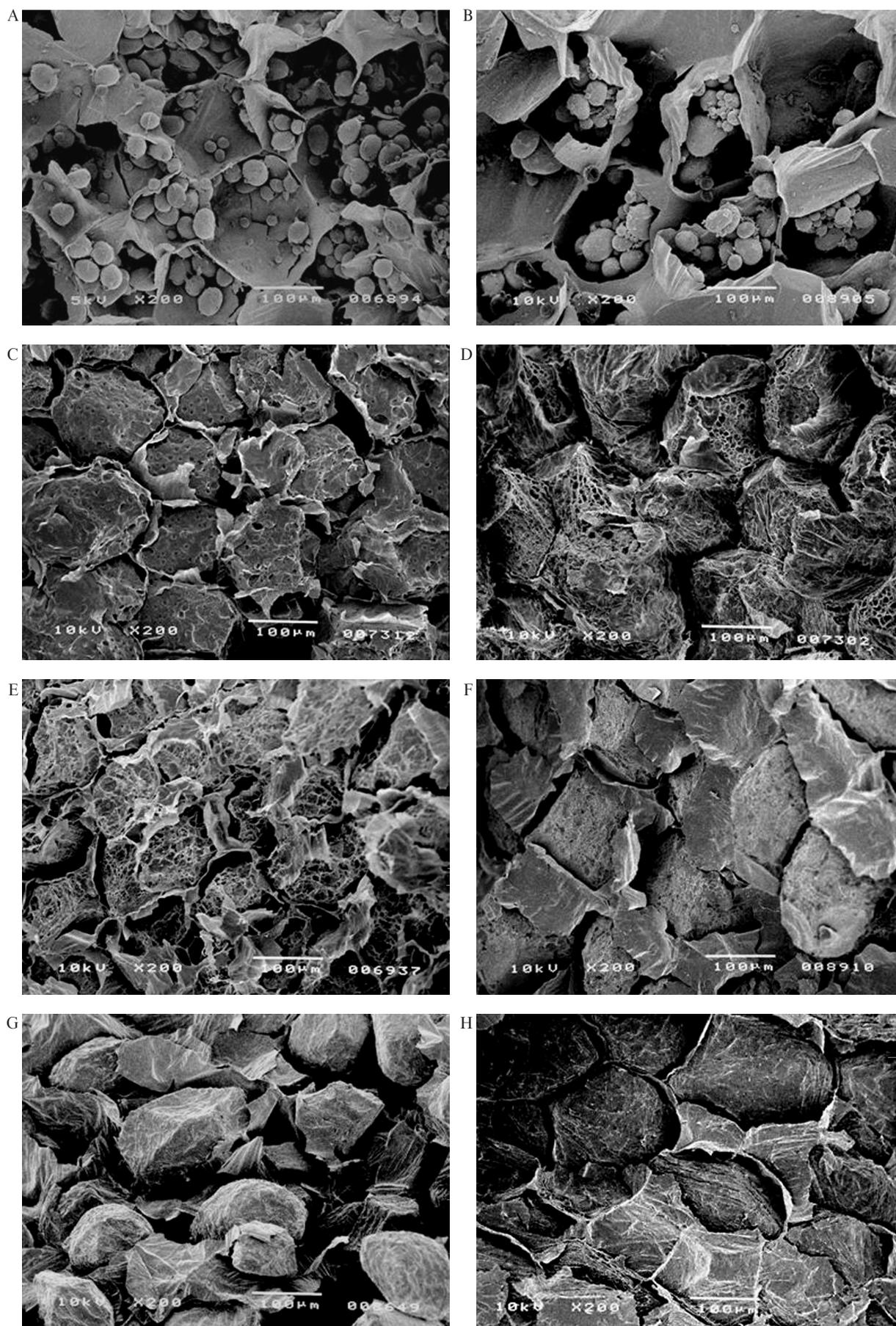
FIGURE 9. SEM micrographs of parenchyma tissue of parental cultivar Irga (A-raw, C-cooked, E-microwaved, G-fried) and GM clone R1F (B – raw, D – cooked, F – microwaved, H – fried).

large and numerous intercellular spaces in the parenchyma tissue of treated tubers (control and clones). That phenomenon appeared probably due to partial solubilisation of the cell wall pectic substances that led to easy separation of cells. It is worth mentioning that the degree of solubilisation of the pectic lamella responsible for intercellular cohesion significantly affects potato texture properties [Moledina *et al.,* 1978]. These authors pointed out that during cooking potato tissue some solubilisation of the cell binding material took place as well as intercellular binding material which might remain in the form of bridges interconnecting the cells. Also Thybo *et al.* [1998] reported that the pectin substances were the major component of the middle lamella and played a key role in the texture of potato.

The frying process following blanching led to dehydration of parenchyma tissue resulting in the formation of not only compact but also more fragile structure (Figures 9G-H). The cells of parenchyma tissue of R1F clone after frying appeared shrunken (Figure 9H) but the cell walls remained almost intact. Whereas, the microstructure of fried Irga was characterised by significantly broken and separated individual cells (Figure 9G), indicating the progressive degradation of pectins – first by blanching, and finally by frying in oil [Tajner-Czopek, 2003]. The thermal processes evoked significant changes not only in starch and cells structure but might as well result in proteins denaturation within cell structure. The denatured protein together with gelatinized starch formed a starch-protein matrix within cell structure [Nuss & Hadziyev, 1980].

CONCLUSIONS

The presented comprehensive characteristics of physical, chemical, microstructural, and technological parameters of tubers of clones genetically modified for improving their resistance to a necrotic strain of potato virus Y (PVY^N) enabled classifying parental cultivar Irga and its clones as similar. However, it should be strongly pointed out that the genetical modification of other type (especially that influencing the content of selected chemical constituents) may result in other, even negative, effects.

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