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Cell structural parameters of potato tuber tissue**

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A b s t r a c t. The present study reviews results of research on the quantitative determination of cell structural parameters such as: surface area, perimeter, Ferret's diameters, elongation, compaction, for the parenchyma of potato tuber, taking into consideration inner and outer core tissues. Tissue images were obtained for the samples in their natural state without any preparation using an optic confocal microscope. The quantitative analysis of the microscopic image of the cross-sections of the cell's structure, was carried out according to the method worked out by the present authors earlier. Four potato varieties were chosen for the present experiment (Danusia, Kuba, Mila, Triada), in three consecutive crop years in the conditions selected (1999, 2000, 2001) and with a controlled storage system. Our studies showed that the quantitative structure described by means of cell size and shape parameters is a characteristic feature of potato tuber tissues, outer and inner core, for a given variety and harvest date. The size of cells changes, whereas their shape is similar in each variety, type of core and harvest date.

K e y w o r d s: cell structural parameters, image analysis, potato-tuber tissue, inner and outer core of potato-tuber parenchyma

INTRODUCTION

One of the basic physical properties characterising study material is its structure which exerts a decisive influence on the remaining properties, i.e., physical, chemical and biological. In the case of materials with high water content, it is a cell structure with a high degree of complexity. It shows features of a discreet medium with a relatively high degree of discontinuity, stochastic and metamorphic (Fornal, 1998; Haman and Konstankiewicz, 2000; Jackman and Stanley, 1995; Khan and Vincent, 1993). Such materials are especially susceptible to various kinds of impacts, e.g., mechanical, thermal, etc., and changes in its structure can cause a series of processes that may result in inner damage and as a result in the lowering of its quality or rotting. The above is true for various agricultural materials (fruit, vegetables) as these are common phenomena well known and described in literature presenting empirical research (Brook, 1996; Dean, 1996; Hallett *et al*., 1992; Harker and Hallett, 1994; Sanz *et al*., 1998).

Physical processes such as cracking on mechanical impact or shrinking as a result of drying are all initiated at cell level. They require in-depth knowledge of the structure of plant tissue, i.e., location, shape and size of cells. Earlier studies showed that, among other things, cell size influenced tensile stress in their walls and cracking processes in the whole structure. Larger cells are the first to be damaged and the development of the cracking process depends on the probability distribution of their sizes (Haman *et al*., 2000; Konstankiewicz *et al*., 2001b; Konstankiewicz and Zdunek, 2000, 2001). A quantitative description of the cell structure parameters becomes indispensable for the interpretation of experimental data and also for working out theoretical models of destruction in such media (Czachor and Góźdź, 2001; Endy and Brent, 2001; Gao and Pitt, 1998; Góźdź and Pietrow, 1999; Haman and Konstankiewicz, 2000; Tomita, 2001).

The problem of a quantitative description of cell structure parameters is related to the choice of a suitable method of obtaining structure image, preferably in a natural state without preparation and registering and analysing the image adjusted to the object analysed (Petran *et al*., 1995; Konstankiewicz *et al.*, 2001a).

In the present studies, a potato (*Solanum tuberosum* L.) was selected as a master object as, on the one hand it has relatively big cells with weak membranes so it is susceptible to damage, and on the other hand, there was a need to solve the practical problem of losses caused by decreasing

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production. Due to losses resulting from outer and inner damage a considerable part of potato crops cannot be used for industrial processing. It should be stressed that Poland occupies one of the top positions in world potato production. The potato is a popular component of our diet widely used as fodder but it is not sufficiently utilised as a raw material for industry (~1%). Modern processing requires raw materials of high quality, homogeneity and a stable characteristics fulfilling specified conditions for directed usage (Brook, 1996; Dean, 1996; Peters, 1996; Zgórska 1989; Zgórska *et al*., 2000).

The aim of the present research was the description of the plant tissue cell structure on the basis of its parameters determined. In this study, results of research on the quantitative determination of cell structure parameters such as: surface area, perimeter, Ferret's diameters, elongation, compaction for the potato tuber parenchyma tissue taking into consideration outer and inner core tissues are present. Studies were carried out on samples in a natural state without any prior preparation using an optic confocal microscope. A quantitative analysis of microscopic images of the flat cross-sections of the cell structure was carried out according to the method worked out earlier (Konstankiewicz *et al*., 2001a). Four potato varieties in three consecutive years of breeding in pre-determined conditions and controlled storage systems were chosen for the present experiment. The present results are part of a wider programme on the mechanical properties of the parenchyma tissue of the potato tuber, and especially in conditions for the occurrence, detection and recording of its inner cracking.

MATERIALS AND METHODS

Potato tuber samples

The materials studied were potato tubers (*Solanum tuberosum* L.), of the Polish varieties selected: Danusia, Kuba, Mila, and Triada, from three consecutive harvest years: 1999, 2000, 2001 bred in the Department of Potato Proces- sing and Storage of the Institute of Plant Breeding and Acclimatisation in Jadwisin. All varieties with various designations were bred in the same soil with identical fertilisation and harvested at full crop maturity. On the basis of detailed examinations of the yield obtained, it was found that the highest content of dry mass and starch was observed in var. Kuba, and the lowest in the tubers of var. Danusia, whereas varieties Mila and Triada showed similar values of both components both in the outer and inner core. The varieties selected differed mainly in their susceptibility to damage determined on the basis of an impact test: Mila and Triada (edible) with a higher susceptibility to damage and Danusia (edible) and Kuba (starch) with lower susceptibility to damage, (Zgórska *et al.*, 2000; Czerko, 2001).

Immediately after harvesting, the material studied was stored in a controlled temperature (6°C) and a relative air humidity (90-95%). Each of the varieties was represented by tubers of a medium size without any visible outer damage. Laboratory studies were carried out at a stable room temperature (~ 20°C) and a relative air humidity (50-60%).

Samples for microscopic examination were collected from the middle part of the tuber according to the scheme shown in Fig. 1. Slices with a diameter of 7 mm and height of 1mm, were rinsed in distilled water in order to remove the starch remains and then were placed on glass plates to be observed under a microscope (Konstankiewicz *et al*., 2001a; Konstankiewicz *et al.*, 2001b; Konstankiewicz and Zdunek, 2000).

Samples taken from two locations, i.e., the inner and outer core, for each of the varieties and three harvest dates, were prepared for microscopic observation in the above way.

Microscopic observations

Microscopic observations were carried out by means of an optic confocal microscope (Tandem Scanning Light Microscope - TSRLM) which makes observations on biological samples in their natural state possible without any fixation or grinding (Petran *et al*., 1995). The Plan 10/0.25 and 20/0.4 lens were used. They allowed from a few to

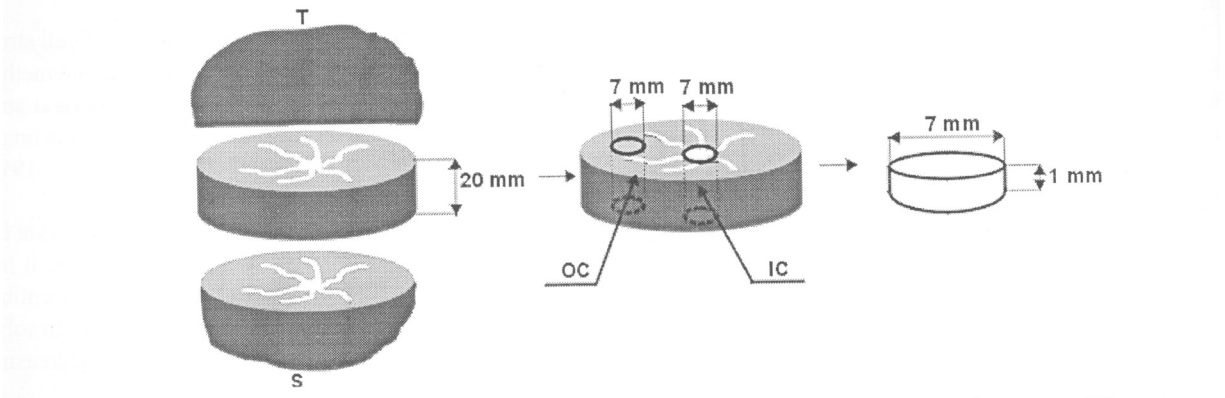

Fig. 1. Sample collection scheme of potato tubers for microscopic observations: T - top, S - stolon, IC - inner core, OC - outer core.

several cross-sections of full cells in one image to be obtained.

A precision continuous shift of the object in the x-y plane made possible a full observation (~20 images) of one sample in a few minutes, which in a stable ambient temperature (~20°C) and relative air humidity (50-60%) did not cause it to dry. In each measuring series, for each sample, a few non-overlapping images were chosen to obtain several tens of full cells in one repletion.

Examples of the images of the parenchyma cell structure of potato tubers subjected to analysis were shown in Figs 2 and 3, for the inner and outer core, respectively. The images show clear contours of cell walls. However, there are also regions visible as light spots coming from the surface of cell membranes or the remnants of liquids in the bottom of the cell. Hence, the images obtained cannot be subjected to direct automatic computer analysis.

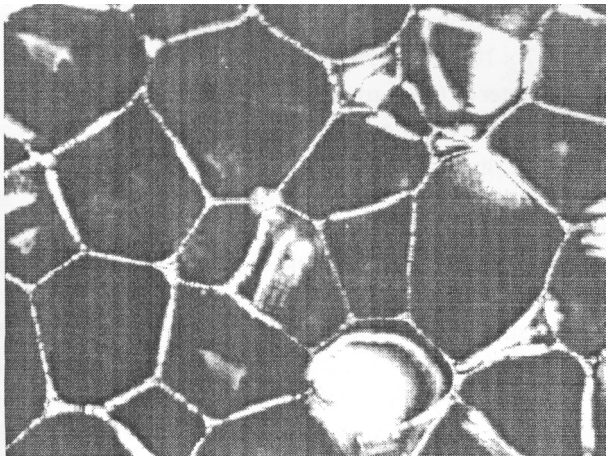

Fig. 2. Microscopic image of potato tuber tissue var. Kuba (2001) - outer core - lens Plan 20/0.4).

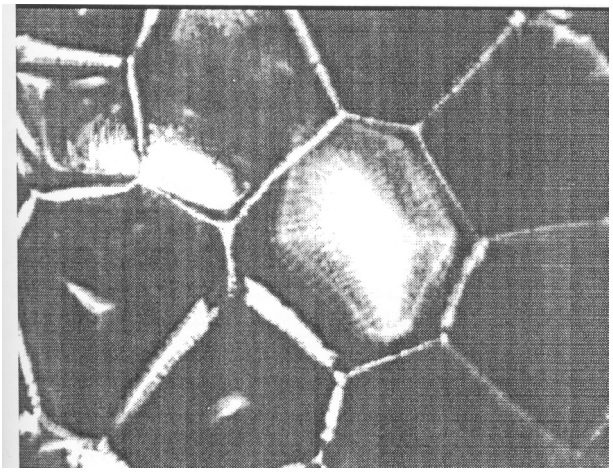

Fig. 3. Microscopic image of potato tuber tissue var. Kuba (2001) - inner core - lens Plan 20/0.4).

Image analysis

The quantitative analysis of the microscopic image of the cross-sections of the cell's structure, was carried out according to the method worked out by the present authors earlier (Konstankiewicz *et al*., 2001a). The analysis of microscopic images is time consuming but it allows the unique separation of the cell walls. Each image was manually prepared by the outlining of the visible cell walls. A few observers carried out independent parallel outlining which allowed the elimination of subjective mistakes and obtained repeatedly good results. A structural skeleton in the form of a net of polygons formed from the sections joining the visible knots of the cell walls was viewed on the background of a microscopic image. A knot was identified as the connecting point for at least three cell walls (Figs 4 and 5). The binary skeletons of the microscopic images thus obtained were then subjected to computer analysis in order to determine their structural parameters.

Image analysis determined the parameters related to the size of each cell, i.e., surface area - A, perimeter - P, Ferret's diameters, maximum - F_{max} and minimum - F_{min} , and cell shape, i.e., ratio of Ferret's diameters - F_{min} / F_{max}, elongation - E (ratio between the difference of the maximum and minimum ellipse diameter inscribed into a cell up to the sum of these diameters), compaction - C (ratio between 16 × the surface and the square of cell elongation), (Konstankiewicz *et al*., 2001) possible.

Calculation of results - surface areas, perimeter and diameters - obtained in pixels for the units of their lengths was immediately done on the basis of a scale obtained by the carrying out of an analogue analysis of an object with known flat dimensions. The statistical analysis and distribution of the measured values was carried out with Excel software.

RESULTS AND DISCUSSION

Microscopic observations and image analysis was carried out for the parenchyma tissue of the potato tuber: four varieties - Danusia, Kuba, Mila, and Triada, inner and out core and three consecutive years of crop: 1999, 2001, 2002. Five repetitions were made for each measuring series. In total, 1553 photos of microscopic images of cross-section of the potato tuber cell structure in a natural state were obtained. Examples of microscopic images of the structures studied were shown in Figs 2 and 3.

A skeleton of a cell structure on the background of a microscopic image prepared for the quantitative parameter analysis has been shown in Figs 4 and 5. The images are adjoining polygons most often hexagon and pentagon and various sizes. Structural parameters such as: surface area A, perimeter P, Ferret's diameters F_{max} and F_{min} , elongation E and compaction C were determined for each of the cells separated. The amount of cells in individual varieties, types of core and years has been shown in Table 1. In the first year of

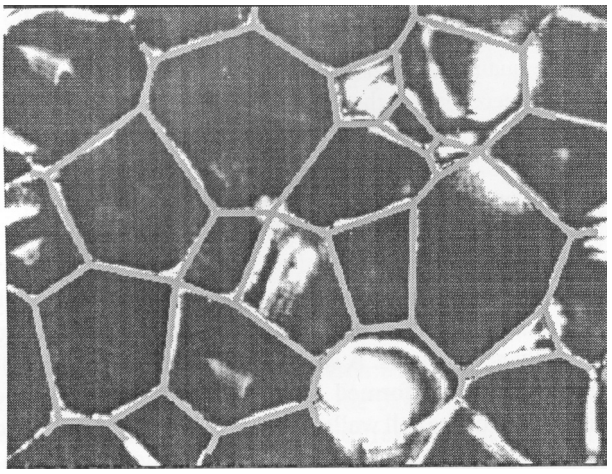

Fig. 4. Microscopic image of potato tuber tissue with the skeleton of the cell structure marked, var. Kuba (2001) - inner core - lens Plan 20/0.4).

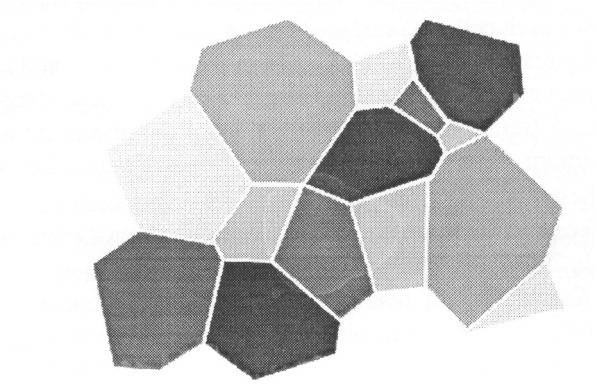

Fig. 5. Images with cells for the quantitative analysis of structure parameters separated.

study, the above numbers were the highest due to the confirmation of the repetitiveness of the results obtained by a few researchers. The repetitiveness obtained was high and hence, in the following years, the number of cells subjected to measurements decreased. At such a high number of trials it was possible to compare the mean values of parameters measured which together with standard deviation were given in Table 2. For all the results obtained, a λ compatibility Kołmogorow - Smirnow's test was carried out at the significance level $\alpha = 0.05$ and the cases for which the values determined are not differ significantly were marked in the table.

The results obtained showed that the cell structure of the parenchyma of potato varieties studied is not homogenous. There were differences in the structure parameters for the inner and outer core of each variety studied and for all three harvest years. Generally speaking the inner core is made of smaller cells than is the outer core. Cells with the highest surface area, i.e., 20.7×10^3 μm^2 (1999) and 19.2×10^3 μm^2 (2000), and the perimeter of 690.1 μm (1999) and 675.4 μm (2000) were found in the outer core of var. Danusia, whereas the lowest values of the surface area, i.e., 10.5×10^3 μm^2 (1999) and 5.5×10^3 μm^2 (2001), and perimeter of 488.3 μm (1999) and 447.6 μm (2001) were observed in the inner core of var. Kuba. Only for var. Mila were there cases in which their outer core was made of cells with smaller surface areas, i.e., 17.7×10^3 μm^2 (1999) and 11.2×10^3 μm^2 (2001), and the perimeter of 634.1 μm (1999) and 491.4 μm (2001) than the cells of the outer core, i.e.: 17.9×10^3 μm^2 (1999) and 13.3×10^3 μm^2 (2001) and 641.9 μm (1999) and 548.3 μm (2001), respectively. On the other hand in tubers of var. Danusia from the 2000 and 2001 harvests, no significant differences in the size distribution of the inner and outer core were observed.

The structure parameters: ratio between Ferret's diameters, elongation and compaction, showed that no matter what the size was, all cells were of a similar shape. Elongation parameters - E, were ranged from 0.30 to 0.35 for the outer core and from 0.27 to 0.33 for the inner core for all varieties and harvest years studied. On the other hand, the C content for all cell measured ranges from 0.62 to 0.66. All cells were of a similar shape intermediating between a circle and a flattened ellipse with a similar ratio of Ferret's diameters F_{min} / F_{max}, which changed from 0.74 to 0.80.

The greatest changes were observed in the structure parameters related to the cell size, i.e.: surface area - A and perimeter - P. Further images (Fig. 6) show surface area

T a b l e 1. Number of cells collected for quantitative analysis from individual varieties, type of core and years

Variety	Year					
	1999		2000		2001	
	N_{IC}	N_{OC}	N_{IC}	N_{OC}	N_{IC}	N_{OC}
Danusia	3718	4553	475	414	452	483
Kuba	4438	4911	707	602	1375	741
Mila	3676	3348	902	574	527	598
Triada	8069	5509	933	533	500	563

N_{OC} - number of cells of the outer core, N_{IC} - number of cells of the inner core.

T a b l e 2. Mean values of the structural parameters measured with standard deviation for all varieties, types of core and years studied

Variety	Parameter	1999			2000			2001		
		OC (s.d.)	IC (s.d.)	Δ=OC-IC	OC (s.d.)	IC (s.d.)	Δ=OC-IC	OC (s.d.)	IC (s.d.)	Δ=OC-IC
Danusia	*A*	20.7 (10.7)	16.9 (9.0)	3.7	19.2* (10.4)	18.6* (9.9)	0.6	13.1* (8.2)	13.8* (7.4)	-0.7
	P	690.1 (180.7)	616.8 (168.4)	73.3	675.4* (190.5)	648.0* (180.3)	27.4	559.3* (170.5)	552.1* (153.4)	7.2
	F_{max}	184.9 (51.1)	164.0 (45.1)	20.9	178.3* (52.9)	173.2* (48.8)	5.1	148.0 (47.3)	146.6 (40.8)	1.4
	F_{min}	143.1 (41.7)	130.3 (40.7)	12.8	137.0 (43.3)	135.7 (43.4)	1.3	112.0* (39.3)	117.5* (37.7)	-5.5
	F_{min}/F_{max}	0.78 (0.13)	0.79 (0.13)	-0.014	0.77* (0.14)	0.78* (0.14)	-0.008	0.76 (0.15)	0.79 (0.13)	-0.03
	E	0.30* (0.15)	0.29* (0.15)	0.01	0.32 (0.17)	0.30 (0.16)	0.02	0.33 (0.16)	0.28 (0.15)	0.05
	C	0.64 (0.07)	0.65 (0.07)	-0.01	0.62 (0.08)	0.65 (0.09)	-0.03	0.60 (0.08)	0.66 (0.08)	-0.06
Kuba	A	16.2 (8.0)	10.5 (5.2)	5.7	17.0 (10.8)	14.8 (7.6)	2.2	9.1 (6.4)	5.5 (3.3)	3.6
	P	609.7 (153.4)	488.3 (126.1)	121.4	624.5 (203.7)	579.9 (155.8)	44.6	447.6 (166.5)	356.1 (103.6)	91.5
	F_{max}	165.4 (45.2)	131.9 (36.1)	33.5	169.0 (59.1)	154.2 (42.1)	14.8	122.7 (47.0)	95.7 (29.2)	27.0
	F_{min}	126.5 (35.1)	102.4 (29.1)	24.1	126.1 (44.4)	121.8 (37.4)	4.3	90.1 (36.7)	73.4 (24.0)	16.7
	F_{min}/F_{max}	0.77 (0.14)	0.78 (0.13)	-0.01	0.76 (0.15)	0.79 (0.14)	-0.03	0.74 (0.16)	0.77 (0.15)	-0.03
	E	0.31 (0.15)	0.30 (0.15)	0.01	0.34 (17)	0.30 (0.16)	0.04	0.35 (0.18)	0.32 (0.16)	0.03
	C	0.65* (0.07)	0.66* (0.07)	-0.01	0.63* (0.08)	0.64* (0.08)	-0.01	0.62 (0.08)	0.63 (0.08)	-0.01
Mila	A	17.7 (10.0)	17.9 (8.1)	-0.2	16.0 (8.9)	10.8 (6.0)	5.2	11.2 (8.4)	13.3 (6.7)	-2.1
	P	634.1 (190.6)	641.9 (149.5)	-7.8	597.0 (174.0)	492.0 (141.8)	105	491.4 (194.7)	548.3 (149.3)	-56.9
	F_{max}	171.5 (55.5)	171.7 (42.2)	-0.2	162.6 (49.1)	132.7 (39.0)	29.9	133.7 (54.0)	145.0 (39.7)	-11.3
	F_{min}	130.1 (41.4)	134.9 (35.6)	4.8	124.9* (49.1)	103.3* (33.4)	21.6	99.7 (42.4)	115.1 (36.4)	-15.4
	F_{min}/F_{max}	0.77 (0.14)	0.79 (0.13)	-0.02	0.77* (0.15)	0.78* (0.14)	-0.01	0.75 (0.15)	0.79 (0.13)	-0.02
	E	0.32 (0.15)	0.29 (0.14)	0.03	0.33* (0.17)	0.31* (0.16)	0.02	0.33 (0.17)	0.29 (0.15)	0.04
	C	0.64 (0.07)	0.65 (0.07)	-0.01	0.65* (0.09)	0.65* (0.08)	0.00	0.63* (0.08)	0.64* (0.08)	-0.01

* marks values not differ significantly.

T a b l e 2. Continuation

Variety	Parameter	1999			2000			2001		
		OC (s.d.)	IC (s.d.)	Δ=OC-IC	OC (s.d.)	IC (s.d.)	Δ=OC-IC	OC (s.d.)	IC (s.d.)	Δ=OC-IC
Triada	*A*	15.1 (9.0)	12.8 (7.4)	2.5	15.7 (9.3)	12.0 (8.9)	3.7	11.9 (8.0)	11.5 (6.4)	0.4
	P	579.5 (174.1)	532.9 (154.1)	46.6	599.5 (187.0)	597.0 (174.0)	2.5	517.6 (169.8)	506.4 (143.0)	11.2
	F_{max}	155.8 (47.9)	141.9 (41.8)	13.9	164.0 (53.8)	162.6 (49.1)	1.4	138.8 (46.5)	134.3 (38.1)	4.5
	F_{min}	121.5 (40.4)	113.4 (36.6)	8.1	120.6 (41.3)	124.9 (41.1)	-4.3	105.6 (39.9)	107.3 (35.2)	-1.7
	F_{min}/F_{max}	0.78 (0.13)	0.80 (0.13)	-0.02	0.75 (0.15)	0.77 (0.15)	-0.02	0.76 (0.15)	0.79 (0.13)	-0.03
	E	0.30* (0.15)	0.28* (0.15)	0.02	0.35 (0.17)	0.33 (0.17)	0.02	0.32* (0.16)	0.27* (0.15)	0.05
	C	0.65* (0.07)	0.66* (0.07)	-0.01	0.63 (0.09)	0.65 (0.09)	-0.02	0.62 (0.09)	0.65 (0.07)	-0.03

A – area ($\times 10^3$ μm^2), *P* – perimeter (μm), F_{max} – Feret's maximum diameter (μm), F_{min} - Feret's minimum diameter (μm), *E* – elongation, *C* – compactness, s.d. – standard deviation, OC, IC – outer and inner core, respectively.

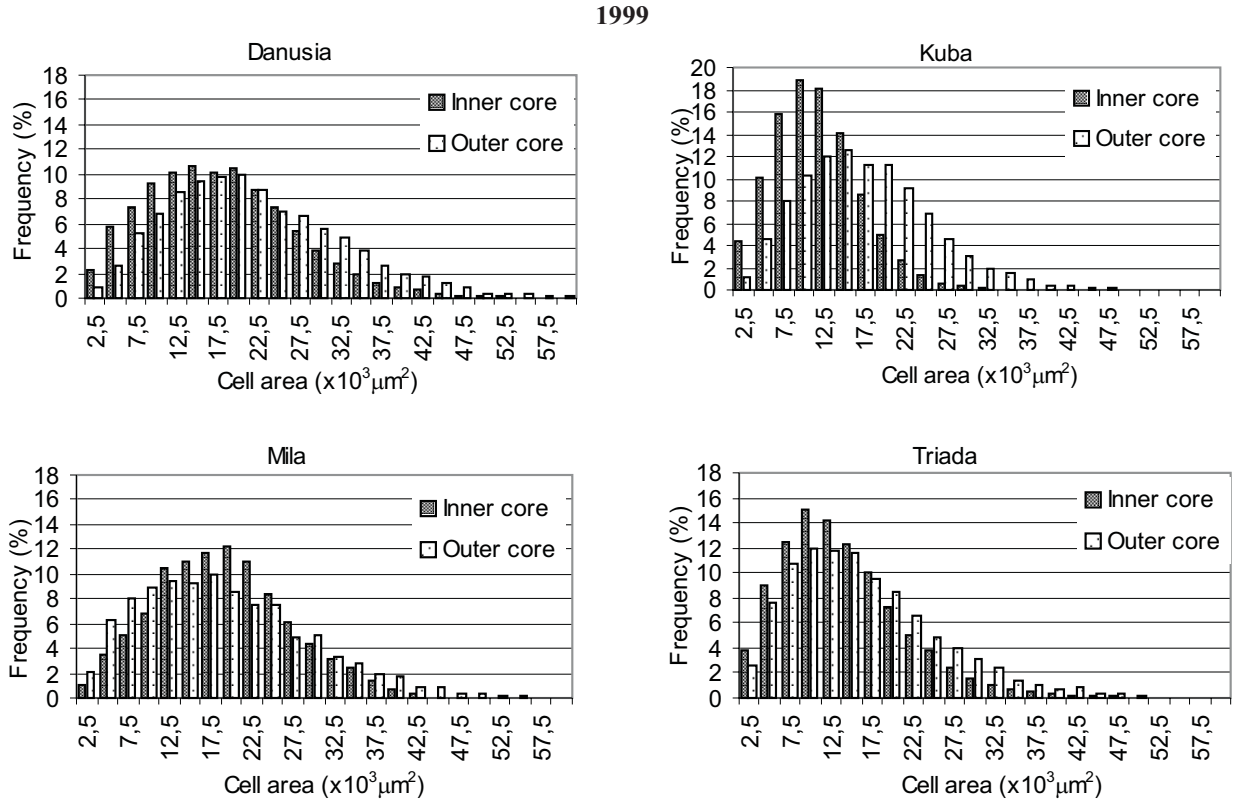

Fig. 6. Distribution of the cell's surface area of the four potato varieties studied taking into consideration the outer and inner core - Harvest of 1999, 2000, 2001.

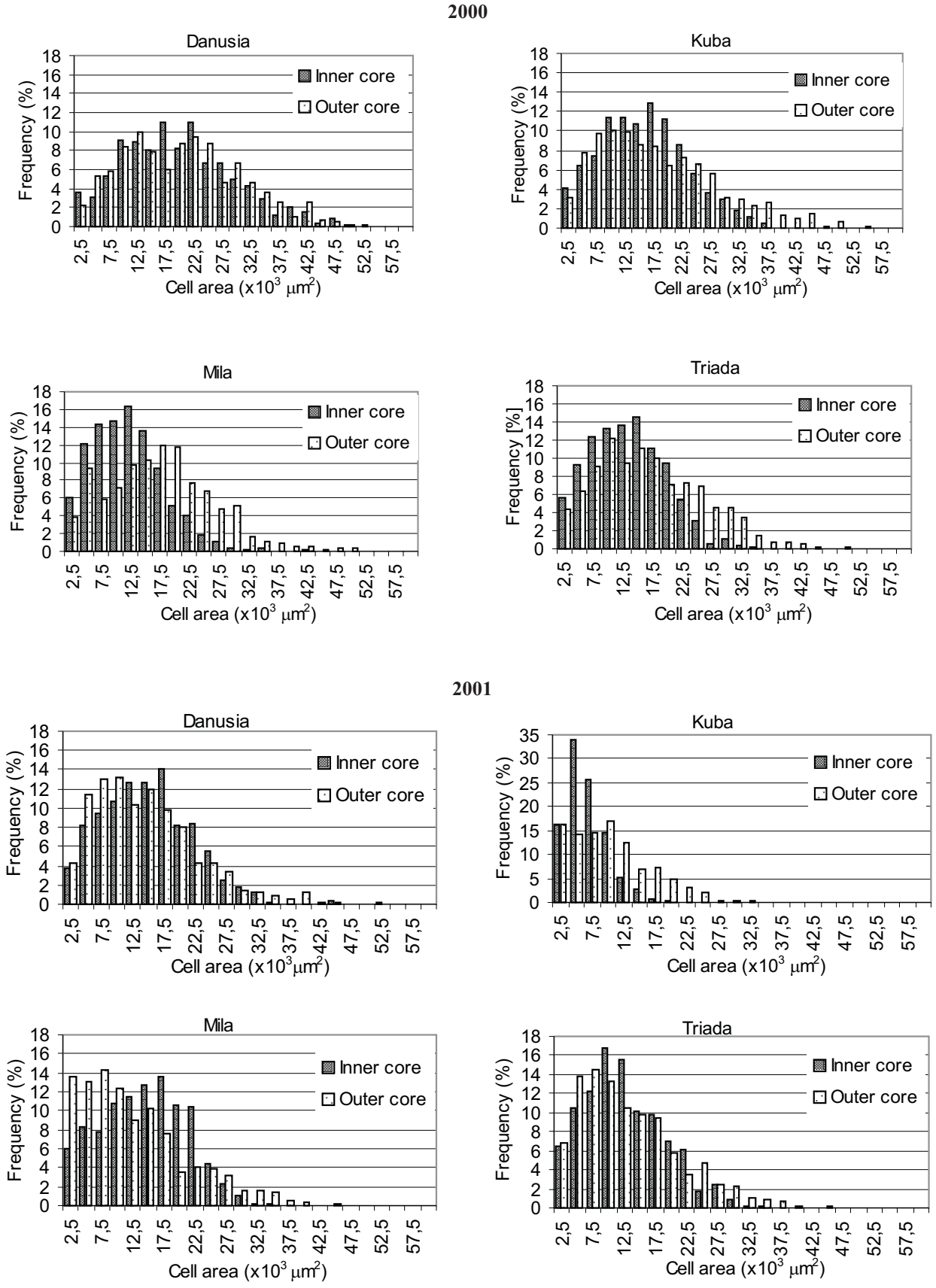

Fig. 6. Distribution of the cell's surface area of the four potato varieties studied taking into consideration the outer and inner core - Harvest of 1999, 2000, 2001.

distribution for all four varieties and three harvest years with values for the inner and outer core marked. The widest distribution range covering most intervals of size classes was observed for var. Danusia (1999), and var. Kuba (2001) showed the highest homogeneity of the structure even though for the small size intervals (5-7.5) $\times 10^3$ μm^2 and (7.5-10) $\times 10^3$ μm^2 a significant increase in the number of cells in the area of the inner core. All diagrams show a higher contribution of the outer core cells in intervals with higher values. Whereas for the inner core there were more cells in intervals with lower values. The numerical values of cell contributions in individual classes were not stable either for individual varieties or harvest years. The lack of structural homogeneity occurs in the area of both types of tissue studied and cells with similar sizes occurred in both areas, there was no sharp border between them.

CONCLUSIONS

The studies on the quantitative determination of structural parameters of the parenchyma tissues of potato tubers: four varieties, two types of core and three harvest years, showed that:

1. The quantitative structure described by means of size and shape parameters is a characteristic feature for inner and outer tissue of the potato tubers studied in a given variety and harvest date.

2. Structural parameters related to cell size change for each variety and type of parenchyma of potato tubers, i.e., outer or inner core, and harvest date.

3. Structural parameters related to the cell shape preserve similar and constant values for all varieties, types of core and harvest dates.

4. Size distribution of cell surface areas show individual features in the varieties studied, type of core and harvest dates considered.

The results obtained for potato tubers despite identical breeding and storage conditions, showed that the structure of parenchyma tissue, both in the outer and inner cores, is neither homogenous nor stable. To characterise it and use it correctly in other research programmes, requires the quantitative determination of its structural parameters, especially those related to cell size in each individual case.

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