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Cell orientation in potato tuber parenchyma tissue

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A b s t r a c t. The paper presents the results of the research of the size and shape of parenchyma tissue cells in potato tubers depending on the direction and site of sampling in tubers. An optical confocal microscope was used to observe samples in their natural state. The investigation was carried out for 1 mm thick samples cut from cylindrical samples (10x10 mm) taken in two mutually perpendicular directions of the inner and outer core of each variety. The analysis was done ten times.The methods developed for the composition and image analysis ensure obtaining a sufficient number of cells to determine tissue structure parameters (surface, shape, elongation and number of cells per 1 mm^2) and decays of these parameters were obtained. Statistical analysis was performed using the λ-Kolmogorov-Smirnov compliance test. A relationship between the direction of sampling and the size and shape of the inner core of cells was found. Greater surface area and elongation of the inner core cells for the longitudinal direction in the tuber (stolon – top) was demonstrated. There was no such a correlation for the outer core in the tubers of the cultivars examined.

K e y w o r d s: microstructure, cell parameters, image analysis, potato

INTRODUCTION

Consumers requirements concerning the quality of raw material and product as well as increasing competitiveness on the market encourages food producers to use better agricultural plant materials and improve their quality. The stability of morphological, chemical, empirical, and physical features decides about the quality of raw material and its usefulness for processing. Features such as colour, firmness, shape, size, and taste are critical for consumers choice. During the production process *ie* growth, harvest, transport, sorting, and storage, raw materials are exposed to stress, which can cause damage (Blahovec and Židová, 2004), visible as bruises, discoloration, or cracks that lead to a decline in the quality of raw material and end product as well as huge economic losses (Baranowski *et al*., 2012). If the damage is visible on the exterior side, it is easily detectable during the sorting stage. However, when the damage occurs inside the object, it is very difficult or in many cases impossible to detect (Baranowski *et al.*, 2009; Blahovec, 2006).

Studies conducted so far have confirmed the influence of the size and shape of fruits and vegetables and the site in the object on the formation of damage to their both external and internal tissues (Mohsenin, 1986).

The structure of raw material is a very important feature that affects other properties of fruits and vegetables (Aguilera, 2005). Quality losses of raw material are connected with damage to cellular structure. The features of structure have a major effect on appearance of damage (Baritelle and Hyde, 1999).

Many investigations show that, among others, the variety, tissue type, time of harvest, and storage exert an effect on the microstructure of plant tissue (Gancarz and Konstankiewicz, 2007) and that the microstructure undergoes changes during the impact (Chassagne-Berces *et al*., 2009; Sun and Li, 2002) and influences the mechanical strength of tissue (Zdunek *et al*., 2008). The size and shape of cells and their orientation have an influence on the mechanical resistance of tissue (Guillemin *et al*., 2004; Zdunek and Umeda, 2005).

Microscopic images obtained by various microscopic or macroscopic techniques are used to study the structure of plant tissues and their changes (Błaszczak and Fornal, 2008; Konstankiewicz and Zdunek, 2005).

The images are computer-analyzed by special programs for quantitative description of structural parameters. Computer image analysis is widely used in a variety of science disciplines (Asadi *et al*., 2012; Wojnar *et al*., 2002). It is applied for examinations of whole objects and plant tissue in the agriculture science as well (Zapotoczny, 2012).

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Quantitative analysis of structural parameters is possible for microscopic images with quality allowing detection of cellular wall boundaries of (Gancarz *et al*., 2003, 2007) and containing a sufficient number of structure elements – cells (Guillemin *et al*., 2004; Konstankiewicz *et al*., 2003).

The potato tuber was chosen as the study object because it is widely used in industry and it is a major part of our diet as well.

The puncture test was done by Sadowska *et al*. (2008) in three areas *ie* the inner core, outer core, and cortex. The test showed that the force values measured for the perpendicular direction to the stolon-top axis were lower than in the case of the parallel direction. This may be caused by heterogeneity of the cell size or shape in potato tubers tissue.

The aim of this study was to determine the influence of the direction and site of sample collection on the size of potato tuber parenchyma cells characterized by the surface of the cell cross-section (A) and their shape described by elongation of the cell cross-section (E).

MATERIALS AND METHODS

Eight potato varieties were used to be tested *ie* Danusia, Kuba, and Triada harvested in 2003 and Aster, Felka Bona, Krasa, Velox, and Triada harvested in 2004. The potatoes were received from the Plant Breeding and Acclimatization Institute, National Research Institute in Radzików, Department of Potato Storing and Processing in Jadwisin, Poland. All varieties were cultivated on the same soil with the same fertilization. The harvest was carried out in the full maturity phase. Tubers of the same size and shape without external damage were chosen for analysis.

The computer image analysis was used to choose the size and shape of tubers as described by Gancarz and Konstankiewicz (2007). This method involved taking photos of 30 potato tubers and next determining their size and shape. The size and shape of the tubers were determined by the surface S_b and elongation E_b of the cross-section image. Tubers with S_b of 12.56 to 28.26 cm^2 and E_b of 0 to 0.2 were chosen for the analyzes. This surface corresponds to the 4-6 cm fraction of tubers, which are the most desirable in the industry. The laboratory tests were conducted at constant room temperature 20°C and relative humidity 50-60%.

The samples were taken from the inner and outer core for each variety and each type of the core in two directions in the tuber *ie* transverse – X and longitudinal – Y (Fig. 1). Cylindrical samples for microscopic examination with a height of *ca*. 20 and 10 mm diameter were taken from the outer and inner core of the tubers. Two slices were cut off from those samples. The first slice was 1 mm and the other 10 mm thick (Fig. 1). The first slice was used for microscopic examinations for the transverse direction of tuber sampling. A cube with a thickness of 1 mm and the other two dimensions of 10 mm each was cut perpendicular to the previous one from the second slice and used for the same kind of examination. Homogeneous material was obtained from these areas (Fig. 1).

The method of composition of 25 single consecutive images in order to obtain a larger surface was used in the study. In this case, more whole cells were obtained than from images analyzed individually, and omission of very large cells that were not visible in a single image or multiple counting of the same cells in adjacent images was prevented (Gancarz *et al*., 2003).

Microscopic images of the structure of the potato tuber tissue were performed using an optical confocal microscope TSRLM equipped with a 10/0.24 lens and a table that allows shifting the object in the plane of the sample (Gancarz *et al*., 2003). The entire observation (25 images) for one sample lasted for about a minute.

The microscopic images obtained were analyzed by quantitative parameters of the cell structure in accordance with the methodology developed previously using Aphelion software, ADCIS, France (Konstankiewicz *et al*., 2001). Parameters and distributions of these parameters relating to

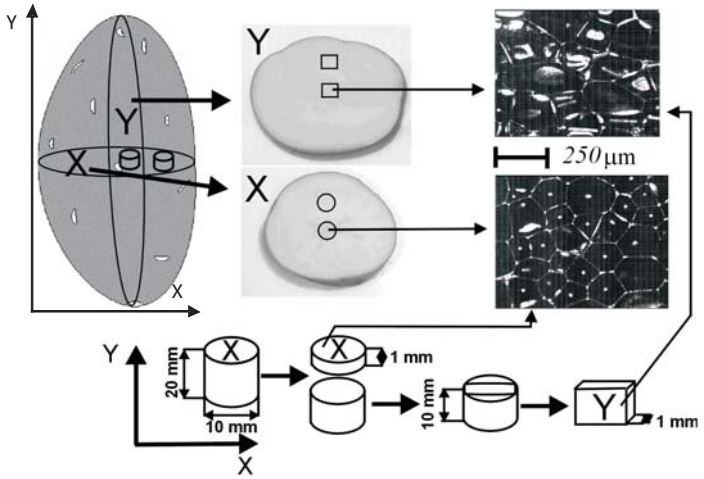

Fig. 1. The directions of the tuber sampling and sample sections of the tubers in these directions. Schematic images of tuber sampling and samples showing the microstructure of the internal core of tissue obtained from two sampling directions: X, Y transverse and longitudinal.

the size of cells: surface (A), the number of cells per unit surface (1 mm^2) (N_A), and the shape of the cell, elongation (E) (ratio of the difference between the maximum and minimum diameter of the ellipse inscribed in the cell to the sum of the diameters) were obtained from the structure of the flat cross-sections. The analyzes of the images were performed using Aphelion software.

8 000 microscopic images were analyzed, and the parameters and their distributions for approximately 128 000 cells were obtained. More than 300 cells were obtained from each location and direction, which is a sufficient number for statistical analysis (Konstankiewicz *et al*., 2003).

The statistical analysis of results using the λ Kolmogorov-Smirnov test of compliance was carried out with Excel 2003 software, Microsoft, USA.

RESULTS AND DISCUSSION

The images of tissue structure obtained vary between the tested areas in the tuber. The outer core cells are bigger than the inner core cells in all cases and the cells of the internal core exhibit differences in the shape depending on the direction of sampling (Fig. 2).

Images of the structure of the potato tuber tissue obtained without any preparation were of good quality and had visible cell walls.

A procedure of linking adjoining images was employed shifting the confocal microscope table in the sample observation plane and a bigger number of objects for the analysis of the structural parameters were obtained (Fig. 3, Gancarz *et al*., 2003).

Distributions of the parameters for the Danusia, Kuba, and Triada varieties tested are presented in Figs 4, 5.

The λ Kolmogorov-Smirnov test of compliance was performed for all parameters obtained with a significance level $\alpha = 0.05$ for its statistic $\lambda_{0.05}$=1.358. The λ_α value for the accepted level of significance α was taken from the table of the λ Kolmogorov-Smirnov test and compared with the calculated λ value. The relation $\lambda \geq \lambda_\alpha$ constitutes scientific evidence that there is a difference between the distributions of the tested values in both cases. If the $\lambda < \lambda_\alpha$ relation occurs, it cannot be excluded that both samples were taken from the same population or that the elements compared exhibit insignificantly different distributions of analyzed data.

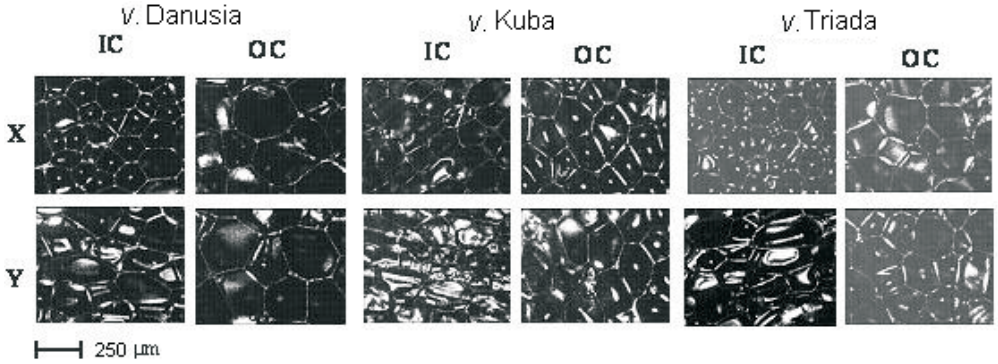

Fig. 2. Sample microstructure images of potato tuber parenchyma tissue for the outer (OC) and inner core (IC) in the tested varieties in two sampling directions.

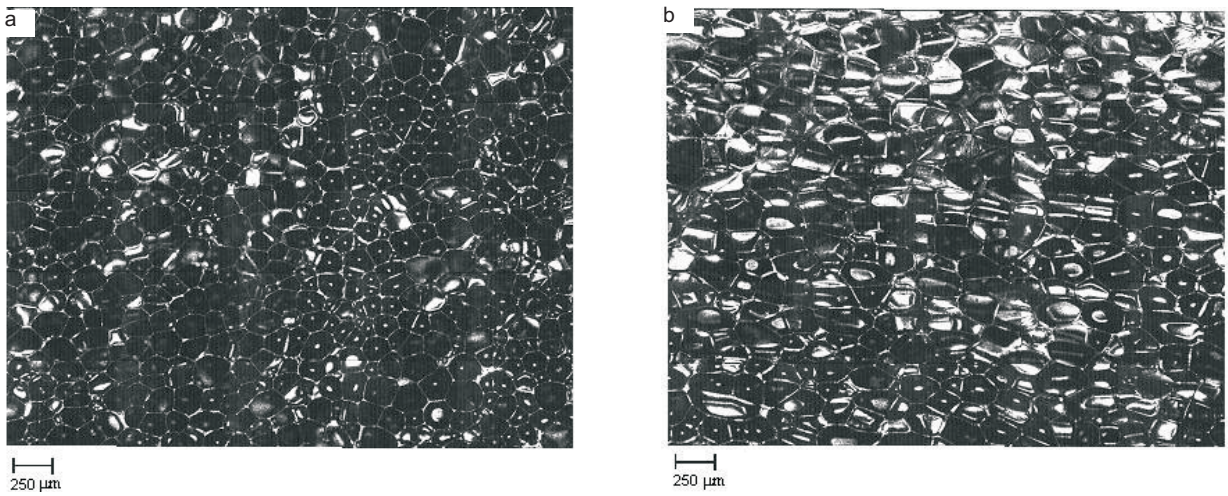

Fig. 3. Sample image of the flat cross-section cellular structure of the internal core of potato tuber parenchyma composed of 25 individual microscopic images: a – transverse section, b – longitudinal section.

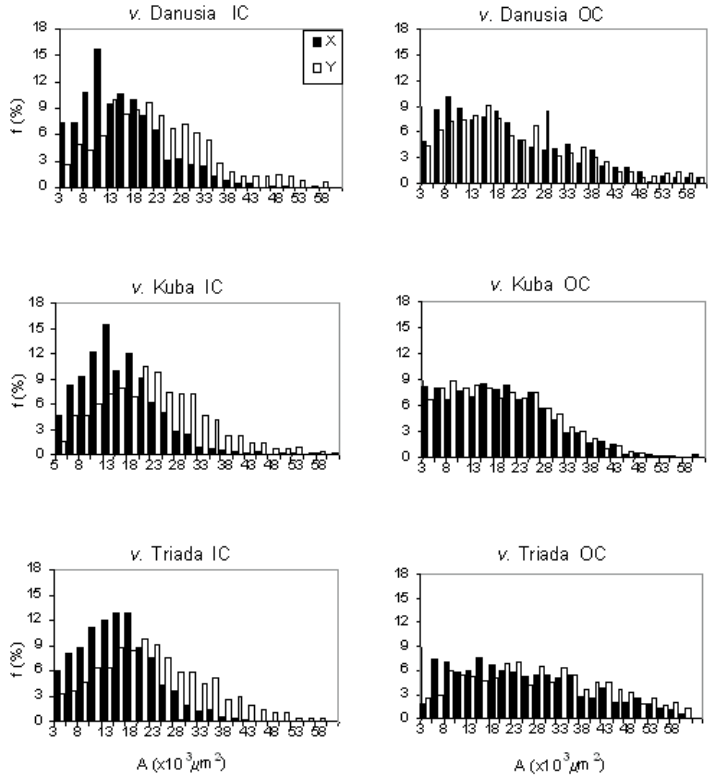

Fig. 4. Sample distributions of cell size - A for the tested cultivars, the type of core and tuber sampling direction. IC – inner core and OC – outer core, X and Y – sampling directions: transverse and longitudinal section, respectively.

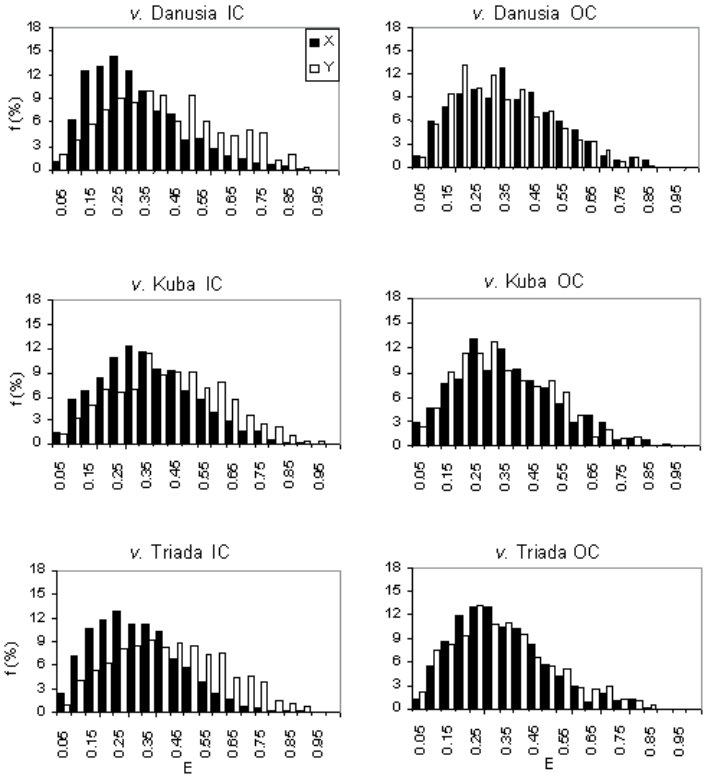

Fig. 5. Sample distributions of cell elongation – E for the cultivars studied, the type of core and tuber sampling direction. Explanations as in Fig. 4.

It allowed comparison of the distributions of the parameter values for all the locations studied, collecting directions in the tuber, and each variety.

Parameters of the mean size (the surface area of the cell, A, and the number of cells per unit surface (1 mm^2) N_A) and shape (elongation, E, ratio of the difference between the maximum and minimum diameter of the ellipse inscribed in the cell to the sum of the diameters) were determined for each tested variety depending on the location of sampling and the direction of observation. The results of standard deviation and the value of λ_{XY} were obtained after testing the λ Kolmogorov-Smirnov compatibility shown in Tables 1, 2.

These results indicate that the cellular structure of the potato tuber parenchyma tissue is specific to each variety and is not homogeneous. There are differences in the size and shape parameters of the structure between the direction of observation and location in the tuber. The biggest tissue cells were observed for the outer core in the Aster variety in both directions of sampling: AX = 27.3 10^3 μm^2 in the transverse direction and AY = 26.1 10^3 μm^2 in the longitudinal direction. The smallest cells (AX = 13.7 10^3 μm^2) were found in the inner core of the tissue sampled in the transverse direction (stolon-top) in the Danusia variety. The biggest difference in the cell size depending on the direction of sampling was obtained for the inner core tissue sampled in the longitudinal direction Y in the Aster variety and the smallest for the outer core tissue sampled in the transverse direction X in the Danusia variety.

The most elongated cells were observed for the longitudinal direction Y of the sampling from the internal core. The least elongated cells were found in these two varieties at the same sampling site but for the transverse direction X. The biggest difference between the cell shapes depending on the direction of sampling was obtained for inner core tissue sampled in the longitudinal direction Y in the Aster variety and the smallest for the outer core tissue collected in the transverse direction X in the Velox variety.

The largest number of cells per unit surface N_A = 73.0 was found in the inner core tissue in the Danusia variety sampled in the transverse direction X. The smallest number

Table 1. The mean values of the parameters obtained with the standard deviation for the tested varieties from the 2003 harvest, the types and direction of core sampling

Variety	Type of core	Parameter	A (10^3 μm^2)		E		N_A	
			X	Y	X	Y	X	Y
Danusia	IC	mean	13.7	21.3	0.29	0.40	73.0	46.9
		sd	4.7	4.4	0.02	0.06		
		λ_{XY}		1.739		2.742		
	OC	mean	22.3	22.4	0.34	0.32	44.8	44.6
		sd	7.6	7.8	0.03	0.02		
		λ_{XY}		0.853		0.624		
Kuba	IC	mean	14.6	22.1	0.34	0.40	68.5	45.2
		sd	3.7	4.4	0.02	0.04		
		λ_{XY}		2.356		1.865		
	OC	mean	17.7	17.0	0.33	0.32	56.5	58.8
		sd	3.7	2.9	0.02	0.02		
		λ_{XY}		0.952		0.646		
Triada	IC	mean	14.4	21.8	0.29	0.40	69.4	45.8
		sd	2.5	4.4	0.03	0.03		
		λ_{XY}		3.846		2.151		
	OC	mean	14.5	16.5	0.31	0.32	68.91	60.6
		sd	3.8	2.9	0.11	0.12		
		λ_{XY}		0.737		0.658		

A – flat cross-sectional area of cells, E – elongation of cells, N_A – number of cells per unit (1 mm^2). Other explanations as in Fig. 4.

T a b l e 2. The mean values of the parameters obtained with the standard deviation for the tested varieties from the 2004 harvest, the type and direction of core sampling

Variety	Type of core	Parameter	A (10^3 μm^2)		E		N_A	
			X	Y	X	Y	X	Y
Aster	IC	mean	15.6	24.5	0.30	0.42	64.1	40.8
		sd	8.4	13.1	0.15	0.21		
		λ_{XY}		3.012		2.360		
	OC	mean	27.3	26.1	0.33	0.32	36.6	38.3
		sd	18.6	15.9	0.17	0.17		
		λ_{XY}		0.956		0.758		
Felka Bona	IC	mean	13.8	20.5	0.33	0.43	72.5	48.8
		sd	7.5	11.2	0.16	0.20		
		λ_{XY}		1.961		2.689		
	OC	mean	17.5	18.6	0.33	0.34	57.2	53.8
		sd	9.5	10.1	0.17	0.17		
		λ_{XY}		1.124		0.965		
Krasa	IC	mean	14.8	21.3	0.29	0.35	67.6	46.9
		sd	9.2	11.5	0.16	0.17		
		λ_{XY}		2.583		1.834		
	OC	mean	22.3	21.2	0.31	0.30	44.8	47.2
		sd	13.5	12.3	0.16	0.15		
		λ_{XY}		1.036		0.928		
Triada	IC	mean	16.7	23.8	0.28	0.39	59.9	42
		sd	9.7	12.1	0.16	0.21		
		λ_{XY}		1.952		1.977		
	OC	mean	23.5	22.3	0.32	0.31	42.5	44.8
		sd	13.1	13.6	0.17	0.17		
		λ_{XY}		1.024		0.886		
Velox	IC	mean	15.7	19.5	0.29	0.38	63.7	51.3
		sd	8.6	10.7	0.15	0.18		
		λ_{XY}		2.836		1.957		
	OC	mean	18.4	18.1	0.32	0.32	54.3	55.2
		sd	10.1	10.4	0.17	0.17		
		λ_{XY}		0.826		0.624		

Explanations as in Table 1.

of cells per unit surface N_A = 36.6 was found in the outer core tissue in the Aster variety sampled in the transverse direction X.

The results obtained perfectly complement the research conducted by Sadowska *et al.* (2008), Zdunek and Umeda (2005).

The force values for the test in the perpendicular direction to the stolon – top axis were lower in each case than those obtained in the parallel direction test, as shown by Sadowska *et al*. (2008). This could largely result from the difference between the size and shape in the tissue of potato tubers.

Sadowska *et al*. (2008) conducted these investigations for tubers of various sizes and shapes. This may explain the fact that for each type of the core they obtained differences in the measured strength of the test depending on the direction in which the test was conducted.

In their experiment, Zdunek and Umeda (2005) proved a relationship between the size of the inner core tissue cells in potato tubers and failure work, failure stress, and failure strain. However, they did not take into consideration the size and shape of tubers in their experiment.

Homogenous tubers in terms of the size and shape *ie* 4-6 cm fractions, were selected in this research. This allowed elimination of a possible impact of the size and shape on the result of the investigations. Differences in the cell size and shape in relation to the direction of sample observation were observed only for the inner core.

CONCLUSIONS

1. The cellular structure of potato tuber tissue is a characteristic feature of each variety and is not homogeneous.

2. The image analysis of the cellular structure of the tuber tissue in the tested varieties has shown that the structure parameters (cell surface of the cross-section, number of cells per 1 mm^2, and cell elongation of the cross-section) differ depending on the site in the tuber where the sample was taken from. In all the cases, the outer core cells are bigger than the inner core cells.

3. The size and shape parameters of the cellular structure differ (cell surface of cross-section, number of cells per 1 mm^2, and cell elongation of cross-section) depending on the direction and site of sampling in the tuber. This relationship was observed for the inner and outer core.

4. The biggest cells were found in the outer core tissue in the Aster variety for all sampling directions and the lowest in the inner core tissue sampled in the transverse direction in the Danusia variety.

5. The cells of the inner core only exhibited differences in the shape and size depending on the sampling direction. The most elongated and large cells were observed for the longitudinal sampling direction in all sampling tested varieties.

6. The least elongated and smallest cells were obtained for the same sampling site (the inner core) for the transverse direction in all the tested varieties.

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