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DIFFERENCES IN THE FRUIT PEEL STRUCTURES BETWEEN TWO APPLE CULTIVARS DURING STORAGE

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Abstract. *Malus* fruits are covered with peel, which consists of the cuticle, epidermis and several layers of hypodermis. This peel, and especially the cuticle and epicuticular wax formed on the fruit surface, plays a crucial role in preserving the fruit life by preventing water evaporation and the penetration of pathogen, as well as maintaining fruit firmness. The protective function of these two layers is particularly important after harvest during storage. Using light and scanning electron microscopy, the present study examined the structure of the fruit peel in two apple cultivars, 'Lobo' and 'Boskoop'; their fruits had been stored for 2 months in a controlled-atmosphere storehouse. The fruit epidermis in cv. 'Lobo', with a smooth and slick surface, was characterized by the occurrence of unidirectional microcracks that were less numerous and had a smaller depth than in cv. 'Boskoop'. The fruit surface in 'Boskoop' was coarse and dry, its numerous microcracks ran in different directions along the walls of the epidermal cells. Mycelium hyphae were observed in these microcracks and inside the lenticels of the fruits of 'Boskoop', whereas no mycelium hyphae were found on the surface of the fruits in 'Lobo'. The apple cultivars differed in the thickness of the cuticle layer, the height of the epidermal cells as well as in the thickness and number of hypodermis layers.

Key words: *Malus*, fruit storage, epicuticular wax, cuticle, epidermis and hypodermis

INTRODUCTION

The fruit of many plant species, e.g. apples, pears, cherries, and plums, is covered by the peel consisting of the cuticle, epidermis, and several layers of hypodermis [Babos et al. 1984, Homutová and Blažek 2006, Zamorskyi 2007]. The most important function of the epidermis and cuticle is to protect the fruit surface against environmental stresses such as wind, temperature, drought, chemicals, insects, and microorganisms throughout the fruit life: on the tree and later, after harvest, during transportation and storage [Jenks

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et al. 1994, 1995, Markstädter et al. 2000]. The cuticle is mainly composed of two hydrophobic components: the cuticle proper containing cutin and intracuticular waxes. Epicuticular wax occurs on the surface of the cuticle proper [Jeffree 1986, Barthlott 1990]. The epicuticular waxes form an amorphous wax film on the fruit surface or/and crystalline structures of various shapes [Barthlott and Wollenweber 1981, Riederer and Schreiber 1995]. Holloway [1982] defined 6 types of cuticle structure. The fruit of *Malus* generally has the first type of cuticle: lamellate-reticulate [Kerstiens 1996] or the fourth – only reticulate [deVries 1968]. The protective role of the cuticle and in particular epicuticular waxes is the most important during apple storage.

Shelf life and quality (firmness, surface greasiness) of fruits taken from a storage room largely depend on the thickness of the cuticular epithelium and the presence of a waxy coating on the surface of apples [Amarante et al. 2001a, 2001b, Veraverbeke et al. 2001a, 2001b]. Apple cultivars differ in the thickness of the epidermis and cuticle, the amount and form of wax produced on the fruit surface as well as in the degree of fruit russeting [Gardingen et al. 1991, Roy et al. 1994, Rinallo and Mori 1996, Belding et al. 1998, Gordon et al. 1998, Veraverbeke et al. 2003b]. The values of these parameters depend on many factors: fruit exposure to sunlight or fruit shading (fruits growing in shade produce more wax and a thicker cuticle layer than those growing with full exposure to the sun) [Babos et al. 1984], fruit ripening period (late-ripening varieties have a thicker layer of cuticle than early varieties) [Kumachova 2003], or the place on the fruit (the stem and calyx parts have a thicker cutin and waxes than the equatorial region) [Homutová 2005]. Moreover, variations are observed in the values of the above mentioned parameters under different apple storage conditions and different climatic conditions [Glenn et al. 1990, Veraverbeke et al. 2001a, Homutová and Blažek 2006].

Evaporation of water from the fruit was found to be dependent primarily on the number of open lenticels present in their peel and number and depth of microcracks occurring in the epidermis of the fruits, but it no depended on the thickness of cuticle [Faust and Shear 1972, Maguire et al. 1999, Veraverbeke et al. 2003a, 2003b]. The cultivars with a dry rough surface of the fruit, a large number of microcracks as well as containing little wax lost more water during storage than those with a greasy skin, a small number of microcracks, and a large quantity of wax [Belding et al. 1998, Veraverbeke et al. 2001a].

In spite of the fact that Poland is one of the leading apple producers in Europe and in the world, in the literature there is no information on the structure of the layers covering the fruit of some apple varieties grown in the climatic conditions of Poland and on changes taking place in these tissues during apple storage. The present paper provides preliminary information on the differences between the structure of the fruits peel after the two-month storage of two apple cultivars: 'Lobo' and 'Boskoop', which are quite frequently encountered in commercial plantings in Polish orchards.

MATERIAL AND METHODS

The material comprised fruits of two autumn-ripening apple cultivars: 'Lobo' and 'Boskoop', sampled after a two-month storage period from a controlled-atmosphere

storehouse (O_2 – 2%; CO_2 – 3%; temperature +4°C; and relative humidity 90–95%). The fruits came from an orchard in the Lublin region in which conventional growing methods are used. Medium-sized and similarly coloured fruits were selected for investigation. Pieces of fruits with a peel and without russeting were sampled for examination with the equatorial, blushed part of the fruit.

Examination of the structure of the fruit peel was performed using light (LM) and scanning electron microscope (SEM).

SEM. Tissue block (fruit transverse sections with the epidermis of 25 mm^2 in area) were fixed with a 2% solution of glutaraldehyde with 2.5% paraformaldehyde in 0.75 M phosphate buffer (pH 6.8) at a temperature of 4°C for 12 h. Subsequently, the fragments were dehydrated in an ethanol series and dried at the critical point in liquid CO_2. Using the CS 100 Sputter Coater, they were coated with gold and examined by means of a Tesla BS-340 scanning electron microscope at an accelerating voltage of 30 kV.

LM. To prepare microscopic slides, hand-cut cross-sections were made from the 10 apples from each cultivar and they were mounted in glycerol-gelatin; the sections contained the epidermis with the cuticle, the hypodermis, and the flesh. In each slide, the thickness of the cuticle, the height of the epidermal cells, the number of layers of hypodermis and its overall thickness, the thickness of the tangential collenchyma cell walls were determined in 5 different locations. Hand-cut sections of fresh material were tested for the presence of lipids with alcoholic Sudan III. Semi-thin sections were prepared using the following methodology. Fragments of fruit with the size of epidermal face 16 mm^2 were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde buffered at pH 7.4 in 0.1 M cacodylate buffer. Fixation was performed at room temperature for two hours, followed by 12 h at 4°C. When fixed, the samples were rinsed with 0.1 M cacodylate buffer at 4°C for 24 h and then treated with 1% aqueous solution OsO_4. Subsequently, the samples were transferred to re-distilled water and stained with a 0.5 aqueous solution of uranyl acetate. After passage through increasing concentrations of propylene oxide in ethanol and finally through pure propylene oxide, the samples were embedded for 12 h in Spurr Low Viscosity resin at 70°C. The transverse sections were cut at 0.9 μm thick using a Reichert Ultracut-S ultramicrotome and a glass knife and were stained with 1% methylene blue with 1% azure II in a 1% aqueous solution of sodium tetraborate. Sections were observed under a Jenaval Contrast microscope at an accelerating voltage of 120 kV. Five replicates per each sample were analyzed.

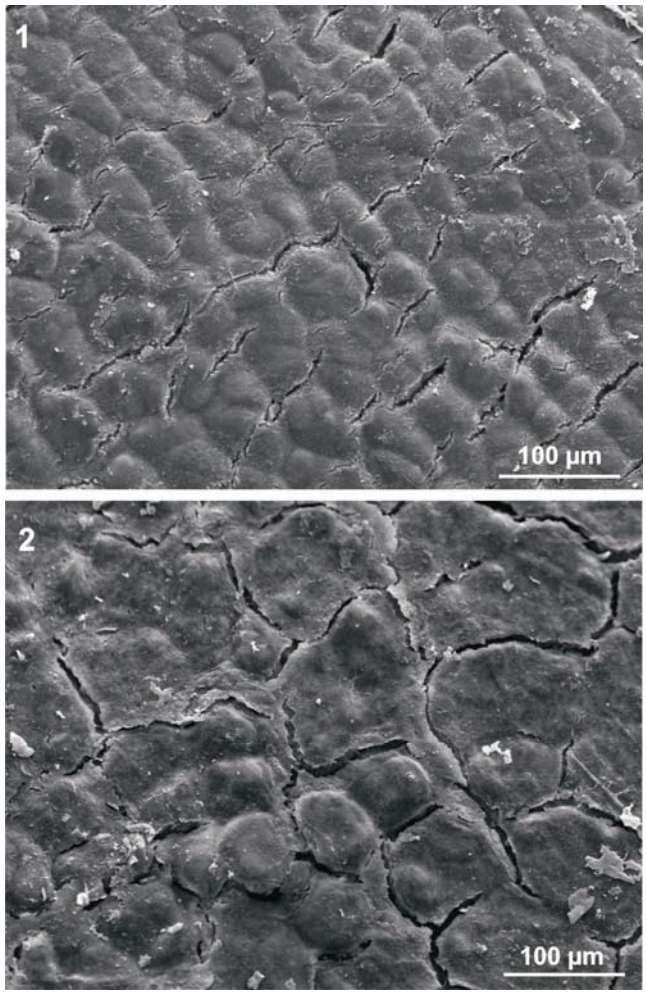
Statistical analyses. Data for each variety was analyzed as a standard deviation and the correlation coefficient at the five percent level.

RESULTS

The apples of the cultivar ‘Lobo’ were characterized by a smooth glossy peel with large green-yellow coloured lenticels, whereas the fruits of the cultivar ‘Boskoop’ were marked by a massive, rough green-coloured skin with numerous russets. The lenticels were large with light borders, also heavily russetted.

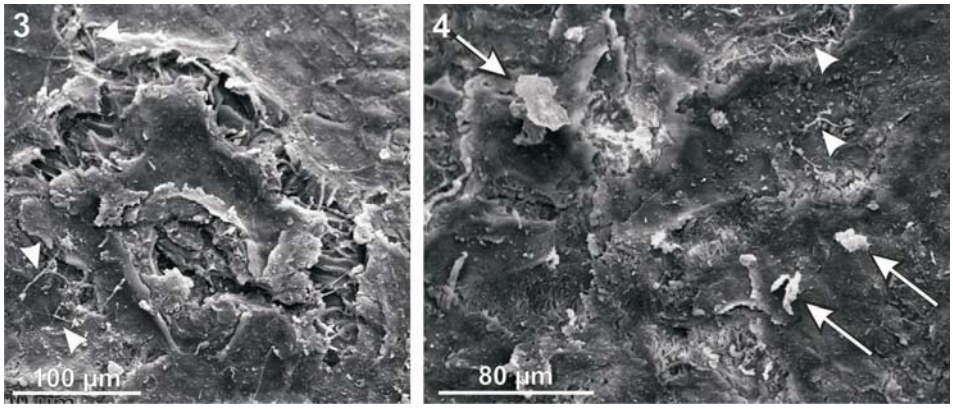
SEM. Based on scanning microscopy observations, the fruit epidermis in both cultivars was found to be covered with a cuticle characterized by the occurrence of micro-

cracks of varying depth and length, forming two different patterns (figs 1, 2). In the fruit epidermis of the cultivar 'Boskoop' these microcracks run perpendicular one to another, they parallel the cell walls, forming a reticulate network; they were also more numerous than in the cultivar 'Lobo' in which the microcracks had only an parallel arrangement (figs 1, 2). In 'Boskoop', the cracks in the cuticle were extensive (36–48 μm in width) (figs 3, 4), while those in 'Lobo' were characterized by a smaller width (6–10 μm) (figs 1, 5). Moreover, some patches of ruptured cuticle could be seen in cv. 'Boskoop', and mycelium hyphae were noticeable both in the cracks and inside the lenticels (figs 4, 5), while their presence was not observed in the fruits of cv. 'Lobo'.



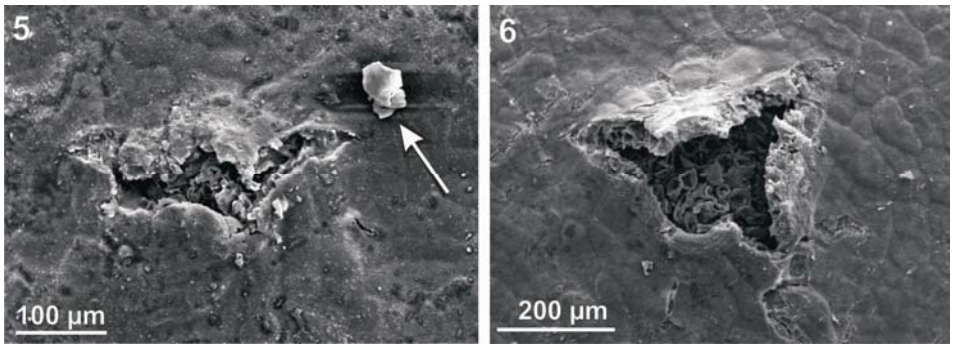
Figs 1, 2. Scanning electron micrographs of the apple epidermis surface with two different patterns of cracking; 1 – 'Lobo', 2 – 'Boskoop'

Fot. 1, 2. SEM. Powierzchnia epidermy jabłek z dwoma wzorami ułożenia mikroszczelin; 1 – 'Lobo', 2 – 'Boskoop'



Figs 3, 4. Scanning electron micrographs of the epidermis surface in 'Boskoop' apples. Cuticular cracks penetrating deep into the cuticular epithelium. Mycelium hyphae (arrowheads) and wax crystals (arrows) are visible on the cuticle surface

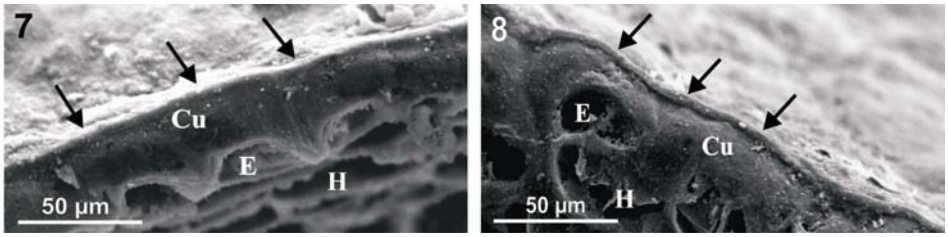
Fot. 3, 4. SEM. Powierzchnia epidermy jabłek 'odmiany 'Boskoop'. Widoczne głębokie spękania kutykuli oraz strzępki grzybni (groty strzałek) i kryształy wosku (strzałki)



Figs 5, 6. The cuticle surface of the 'Lobo' apple. Note a microcrack, wax crystal (arrow) (5) and a lenticel (6)

Fot. 5, 6. Powierzchnia kutykuli owoców odmiany Lobo. Widoczne mikrospękanie, kryształ wosku (strzałka) (5) i przetchlinka (6)

Few crystalline wax structures, in the form of platelets or flocculent deposit, could be seen on the surface of the cuticle in both cultivars (figs 4, 5). Lens- or star-shaped lenticels, being at different stages of maturity, were observed in the epidermis of the apples of both cultivars (fig. 6). In the cross-sectional view of the fruit epidermis and hypodermis, there was also observed a thin layer of amorphous wax, distinguished in SEM by the lighter colour than that of the cuticular epithelium (figs 7, 8); this layer formed a continuous covering with a thickness of 3.13 µm in the cultivar 'Lobo' and 2.08 µm in cv. 'Boskoop'.



Figs 7, 8. SEM. The cross-sections of the fruit peel in 'Lobo' (7) and 'Boskoop' (8). An amorphous wax film is visible on the cuticular layer (arrows). Cu – cuticle layer, E – epidermis

Fot. 7, 8. SEM. Przekroje poprzeczne przez skórkę jabłek 'Lobo' (7) i 'Boskoop'(8). Na powierzchni kutykuli widoczny pokład wosku amorficznego (strzałki). Cu – kutykula, E – epiderma

LM. The epidermis covering the fruit of the apple cultivars under investigation was composed of one layer, or more rarely two layers, of frequently flattened and crushed cells of different height (tab. 1). Protoplasts with a dark colour and dense contents were visible in the epidermal cells (figs 9–12).

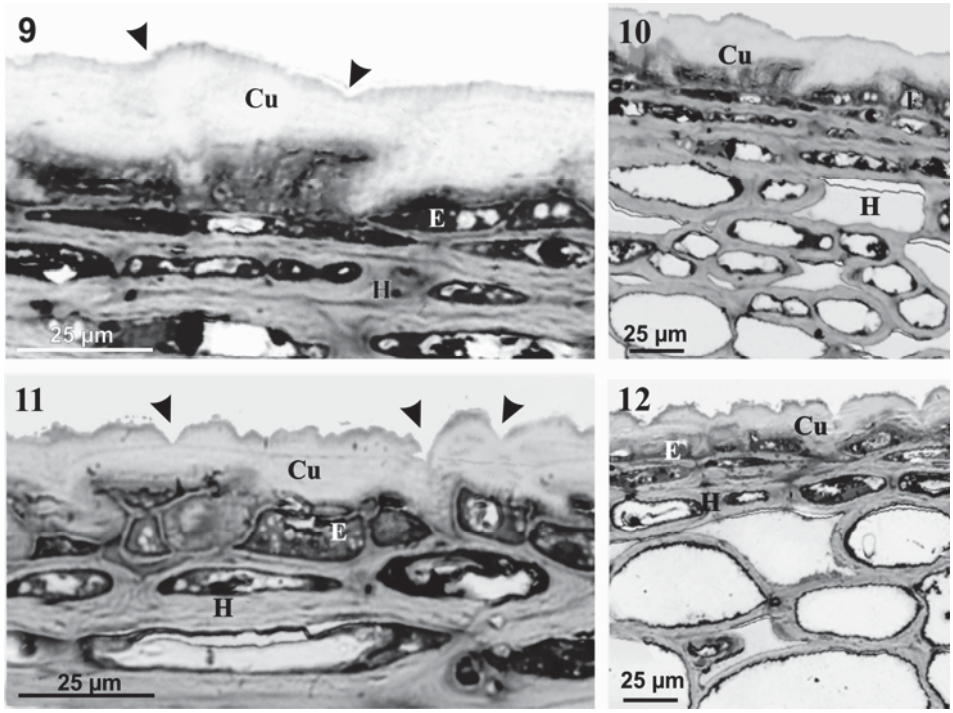
Table 1. Characteristics of 'Lobo' and 'Boskoop' peel
Tabela 1. Charakterystyka skórki jabłek odmiany Lobo i Boskoop

Investigated feature Badana cecha (µm)	'Lobo'	'Boskoop'	'Lobo'	'Boskoop'	Correlation coefficient Współczynnik korelacji p = 0.05
	average – ś rednia (min–max)		standard deviation odchylenie standardowe		
Thickness of cuticle layer Grubość kutykuli	19.32 (17.63–25.43)	13.64 (13.8–17.80)	2.16	1.34	0.53 b
Height of epidermis cells Wysokość komórek epidermy	9.04 (7.63–12.71)	13.06 (7.63–17.80)	1.97	3.26	0.90 a
Thickness of hypodermis layer Grubość pokładu hipodermy	161.24 (139.86–190.71)	156.99 (155.70–160.20)	15.96	6.94	0.17 c
Total thickness of a peel Całkowita grubość skórki	189.60 (155.12–228.85)	183.69 (170.96–195.80)	39.43	24.76	0.29 c
Number of hypodermis layers Liczba warstw hipodermy	8 (6–9)	4 (3–5)	1.51	0.95	0.89 a
Thickness of hypodermis cell wall Grubość ścian komórek hipodermy	5.09 (5.08–6.36)	6.11 (5.09–7.63)	0.68	0.94	0.25 c

a – very high correlation; b – high correlation; c – low correlation

In the cultivar 'Boskoop', the height of the epidermal cells was larger by 44.5% compared to cv. 'Lobo', and it was on average 13 µm (tab. 1). The epidermal cells in both cultivars were covered by a cuticle of varying thickness. In the cultivar 'Lobo', the massive cuticular epithelium formed a layer that was thicker by 42% relative to cv.

'Boskoop', and its average thickness was 19.3 µm (tab. 1, fig. 9). The fruit cuticle in the cultivar 'Lobo' had a slightly undulated surface with small depressions of varying thickness (from 7.63 µm to 25.43 µm). In turn, numerous slits with sharp edges and varying depth were observed in the cuticle layer covering the fruits of the cultivar 'Boskoop' (figs 10, 12). It is worth noting that the thickness of the cuticular epithelium in either cultivar was always greater than the height of the epidermal cells (tab. 1). Furthermore, the cuticle oftentimes penetrated also into the radial walls of the epidermal cells, increasing their thickness (figs 9, 10).



Figs 9–12. Light micrographs of the cross-sections of the apple fruit peel in 'Lobo' (9, 11) with shallow cuticular cracks and in 'Boskoop' (10, 12) with deep cuticular cracks (arrowheads). Protoplasts with a dark colour and dense contents were visible in the epidermal cells. Cu – cuticle, E – epidermis, H – hypodermis

Fot. 9–12. LM. Przekroje poprzeczne przez skórkę owoców 'Lobo' (9, 11) z płytkimi spękaniami kutykuli oraz 'Boskoop' (10, 12) z głębokimi spękaniami kutykuli (groty strzałek). W komórkach epidermy i hipodermy widoczne ciemno wybarwione protoplasty o gęstej zawartości. Cu – kutykula, E – epiderma, H – hipoderma

In both apple cultivars under study, the hypodermis consisted of a different number of layers of tangential collenchyma cells (figs 11, 12). In the fruit of cv. 'Lobo', the hypodermis was composed of 6 to 9 layers of collenchyma cells, whereas in cv. 'Boskoop' the hypodermis was made up of 3–5 collenchyma layers (tab. 1). These cells

had different diameters; the closer to the inside of the fruit the cells were, the larger the diameter was. The hypodermis in cv. 'Lobo' was composed of smaller cells compared to cv. 'Boskoop' (figs 11, 12). In addition, the sub-epidermal cells in cv. 'Lobo' made up a layer thicker by 2.5% than that in the other studied cultivar (tab. 1). The thickness of the tangential collenchyma cell walls was on average 5 µm in cv. 'Lobo', while in cv. 'Boskoop' it was nearly 20% larger (tab. 1). In the collenchyma cells lying directly beneath the epidermis, dark coloured protoplasts with small vacuoles were visible, while the cells of the deeper layers of this tissue showed a greater degree of vacuolization (figs 9, 10).

The total thickness of the fruit peel, which includes the aggregate thickness of the cuticle, epidermis and hypodermis, differed slightly between these two cultivars; in the case of the cultivar Lobo it was 190 µm, whereas in cv. 'Boskoop' 184 µm.

DISCUSSION

The term "peel" is commonly used to describe jointly the several surface layers of the tissues protecting the juicy, fleshy ground tissue containing an aqueous sugar solution with organic acids and micronutrients [Homutová i Blažek 2006, Zamorskyi 2007]. These layers perform a very significant role in the protection of fruits on the tree and, especially, in a storage room [Amarante et al. 2001a, 2001b, Veraverbeke et al. 2001a, 2001b, 2003a, 2003b]. The peel thickness determines the shelf life and quality of fruits collected from a storehouse. The cuticle, which is an epithelial covering with limited permeability that inhibits excessive water loss, plays a special role.

The fruit of the studied cultivars, characterized by a similar harvest maturity period, distinctly differed in the type of fruit surface. The cultivar 'Lobo' was distinguished by a smooth and slick epidermis, whereas the fruit of the cultivar 'Boskoop' had a rough and coarse surface with numerous russets. Reports about the varying structure of the epidermis in different apple cultivars can be found in numerous sources [Babos et al. 1984, Veraverbake et al. 2001b, 2003b, Zamorskyi 2007]. According to some researchers, the rough epidermis covering different organs in many plant species is an effect of the irregularity of the cuticle structure or of the epidermal cells with a varying thickness of the cuticular epithelium [Glenn and Poovaiah 1985, Glenn et al. 1990]. In the case of apples, the fruit surface roughness is caused by numerous microcracks that form on the epidermis surface and by the production of a small quantity of epicuticular wax [Glenn et al. 1990, Jenks et al. 1995, Veraverbake et al. 2001a, 2001b, 2003a, 2003b]. The present study results confirm the observations of the above mentioned authors, since the cultivar 'Boskoop' was characterized by more numerous microcracks on the fruit surface than the cultivar 'Lobo'. As reported by Roy et al. [1994], Maguire et al. [2000] as well as Knoche and Grimm [2008], microcracks develop on the tree, as a result of fruit enlargement and development when fruit surfaces were exposed to water or high humidity, as well as during fruit storage.

During the present study, the cultivars 'Lobo' and 'Boskoop' were also found to differ in the depth of the microcracks and their direction. Glenn et al. [1990] and Maguire et al. [1999] also observed in other apple cultivars a reticulate arrangement of micro-

cracks that was similar to that of the cultivar 'Boskoop' and a similar depth of them relative the thickness of the cuticle. These authors found that the total area of cracking could occupy even up to 40% of the apple surface area, increasing cuticle permeability even 15 times. Observing the fruit of the cultivar 'Jonagold', Veraverbake et al. [2003a] found in turn that microcracks occupied ca. 10% of the fruit surface area and had a depth ranging from 20 to 100 μm. The microcracks found on the surface of 'Lobo' and 'Boskoop' apples could have a decisive influence on the quantity of water lost during storage and on the quality of the fruits taken from the storehouse.

The presence of a wax coating on fruits is another important feature determining their good and long storage [Belding et al. 1998, Veraverbake et al. 2001a, 2003a]. After 2-month storage, the fruit of both cultivars was characterized by two forms of wax found on the fruit surface: crystalline wax in flocculent and/or platelet form as well as amorphous wax forming a continuous layer on the surface of the cuticle. Wax crystals in the form of platelets on the fruit surface have also been described in various apple cultivars by other researchers. They were found in particular abundance on apples being at the harvest maturity stage [Glenn et al. 1990, Belding et al. 1998, Veraverbake 2001b]. According to Babos et al. [1984], apple cultivars differ in the structure of crystalline wax produced on the surface of apples. After 2-month storage of the fruits of the studied cultivars, continuous amorphous wax layer in the form of a wax film was well visible. The amorphous wax layer with few platelet structures of crystalline wax was also observed in the cultivar 'Golden Delicious' by Faust and Shear [1972]. It should be remembered that the climatic conditions and fruit storage conditions have a major effect on the quantity of wax as well as on the variability of the structure of wax found on the apple fruit.

Moreover, the present study found that the cultivar 'Lobo', with a smooth and slick skin, had a layer of cuticle thicker by more than 40% compared to cv. 'Boskoop' with a rough and dry skin. Similar differences in the thickness of this layer in different apple cultivars have been observed by Veraverbake et al. [2001a, 2003a] and Ghafir et al. [2009]. Furthermore, Hull et al. [1975] noted that the cuticle could have a different thickness even in the same fruit, which is associated with micro-environmental conditions: light intensity, moisture stress, and temperature. In the present study, the thickness of the cuticle was measured in the equatorial, blushed part of the fruit. Babos et al. [1984] and Homutová [2005] think that the thickness of the cuticle is greatest near the stem and calyx and on the part of the fruit growing in shade (on the opposite side of the blush).

The studied cultivars differed only slightly in the thickness of the entire layer of the so-called peel that includes all the cuticle, epidermis and hypodermis (respectively, 190 μm in 'Lobo' and 184 μm in 'Boskoop'). But Zamorskyi [2007] reports that there are great variations in the thickness of this layer in different apple cultivars; he found that the peel thickness in 'Golden Delicious' was 110 μm and as much as 350 μm in the cultivar 'Mantuanske'. The above differences in the peel thickness suggest that the thickness of particular rows and layers of cells are determined by the climatic conditions, but it is also associated with the rate of fruit development and ripening, fruit storage conditions and time as well as the place of particular layers on the fruit [Glenn et al. 1990, Veraverbeke et al. 2001a, 2001b, Kumachova 2003, Homutová 2005].

The observed presence of mycelium hyphae on the surface of the fruit of the cultivar 'Boskoop' (with a thinner wax layer and a smaller thickness of the cuticular epithelium) may suggest the susceptibility of this cultivar to fungal diseases, while on the other hand it may indicate the resistance of the fungus to the conditions prevailing in the storehouse. Markstädter et al. [2000] and Marcell and Beattie [2002] report about the role of wax in the protection against insects as well as bacterial and fungal infections.

CONCLUSIONS

1. The fruit of the apple cultivars under study differed in the type of surface; the fruit of 'Lobo' had a smooth and slick surface, while the fruit of 'Boskoop' was characterized by a dry and rough surface.

2. The epidermis of cv. 'Lobo' was marked by the presence of shallower and less numerous microcracks compared to the fruit epidermis in cv. 'Boskoop'.

3. Mycelium hyphae were found on the surface of the fruit of 'Boskoop', but they were not observed on the surface of the fruit of 'Lobo'.

4. The fruit of the cultivars 'Lobo' and 'Boskoop' differed in the thickness of the cuticle layer, the height of the epidermal cells as well as in the number of hypodermis layers.

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RÓŻNICE W STRUKTURZE SKÓRKI OWOCÓW DWU UPRAWNYCH ODMIAN JABŁONI W CZASIE PRZECHOWYWANIA

Streszczenie. Struktura powierzchniowych warstw owoców, budujących tzw. „skórkę" dwu odmian jabłoni uprawnych: 'Lobo' i 'Boskoop', przechowywanych przez 2 miesiące w przechowalni o kontrolowanej atmosferze, była analizowana w mikroskopach: świetlnym oraz elektronowym skaningowym. Epiderma owoców odmiany Lobo o gładkiej i śliskiej powierzchni odznaczała się występowaniem mikrospękań o jednokierunkowym przebiegu, które były mniej liczne i miały mniejszą głębokość niż u odmiany Boskoop. Powierzchnia owoców odmiany Boskoop była szorstka i sucha, a licznie występujące mikrospękania przebiegały różnokierunkowo wzdłuż ścian komórek epidermy. W mikroszczelinach, a także we wnętrzu przetchlinek owoców odmiany Boskoop zaobserwowano strzępki grzybni, których nie stwierdzono na powierzchni owoców odmiany Lobo. Badane odmiany jabłoni różniły się ponadto grubością pokładu kutykuli, wysokością komórek epidermy oraz liczbą warstw hipodermy.

Słowa kluczowe: *Malus*, przechowywanie owoców, wosk epikutykularny, kutykula, epiderma, hipoderma

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