

DIFFERENCES IN THE STRUCTURE OF FRUIT BUDS IN TWO APPLE CULTIVARS WITH PARTICULAR EMPHASIS ON FEATURES RESPONSIBLE FOR FRUIT STORABILITY AND QUALITY

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Abstract. ‘Jonagold’ and ‘Szampion’ are winter apple cultivars, whose fruits are suitable for long-term storage. However, fruits of these cultivars differ markedly in the type of the surface and the rate and volume of water transpiration, which is manifested in fruit quality after storage and the length of apple shelf life. A majority of factors responsible for fruit quality and storability are genetically conditioned traits that are mainly developed before fruits reach harvest maturity or still develop during the storage period. The micromorphology, anatomy, and ultrastructure of 21-day-old fruit buds of the ‘Jonagold’ and ‘Szampion’ were examined using light microscopy as well as scanning and transmission electron microscopy. The analyses were particularly focused on the traits that determine fruit firmness and storability, which contribute to long-term storage capacity. It was found that the fruit buds in both cultivars differed significantly in the number of trichome scars and stomata on the fruit surface, the thickness of the hypodermis layer and the hypodermis cell walls, and in the content of phenolic compound deposits. At the fruit bud stage, the following features related to increased or decreased fruit firmness and storability were observed: platelet crystalline wax, cuticle microcracks, stomata and trichome scars, and presence of phenolic compounds.

Key words: apple fruit buds, fruit peel, micromorphology, histology and ultrastructure, cuticle and epicuticular wax, microcracks, phenol compounds

INTRODUCTION

Apple cultivars are hybrids of *Malus domestica* (Borkh). They differ in many traits, e.g. the timing of reaching harvest and consumption maturity and long-term shelf life of fruits, which are largely dependent on the genetic background as well as on climatic and storage conditions [Rejman 1994, Kruczyńska 2008]. Apple fruits develop from the ovary of the flower and from the floral tube (receptacle). The development proceeds in

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two distinct stages, and the fruit growth curve has a form of a sigmoid curve [Miller et al. 1987, Westwood 1995]. During the development, the fruit size and weight increase systematically, while firmness and acidity decline [Atay et al. 2010]. The first stage of fruit development, i.e. the so-called fruit bud stage, is characterised by very intensive cell divisions (up to 8–10 weeks after anthesis). During this period, fruits are firm and contain a lot of starch, tannins and acids; therefore, they are tart and sour. In the second stage, between mid-June and harvest time, the fruit volume increases through enlargement of the intercellular spaces and cell volume. Additionally, accumulation of food reserves occurs, large vacuoles emerge in the cells, and the water content in fruits increases in this stage. Physical changes in fruits are accompanied by chemical processes leading to reduction of the content of tannins and intensive accumulation of pigments, sugars, organic acids, fatty substances and pectins, volatile substances, vitamins, and minerals [Pieniżek 2000, Atay et al. 2010].

Fruits of different varieties of *Malus* vary with the type of the fruit surface and peel structure and exhibit variation in the intensity of transpiration [Belding et al. 1998, Veraverbake et al. 2001a]. These traits are primarily associated with the amounts of cuticular (intra- and epicuticular) waxes as well as the number and depth of epidermal microcracks [Gordon et al. 1998, Maguire et al. 2000, Veraverbake et al. 2003b, Konarska 2012]. A great impact on fruit transpiration is also exerted by the number of “opened” (active) stomata and lenticels per unit surface area and, to a small extent, by cuticle thickness and the number of hypodermis layers [Veraverbake 2001b, 2003b, Homutová and Blažek 2006]. These features prevent or promote fruit water loss, which is reflected in their firmness, post-storage quality (attractiveness), and the length of shelf life [Riederer and Schreiber 1995, Czernyszewicz 2007]. The peel morphology play also an important role in determining the distributions of water, carbohydrates, and nutrients inside the fruit [Cieslak et al. 2013]. According to Khanal and Knoche [2014] the epidermal and hypodermal cell layers represent the structural backbone of an apple peel during pre- and postharvest development, whereas cutical membrane microcracking has limited relevance to the overall mechanical properties of the peel. Recent research shows that, similar to the cuticle layer, the outer layer of the cuticle (cuticle proper) contains polysaccharides (cellulose and pectin), which can affect the rheological properties of cuticles and may actively contribute to the bi-directional transport of water and solutes [Guzmán et al. 2014]. Moreover, optical coherence tomography which is a new non-destructive technique to visualize subsurface structures of materials, can be used to demonstrate peel structural differences between apples, as well as to measure structural changes that occur during storage [Verboven et al. 2013].

In previous studies, the author of the paper found that, the peels of two apple cultivars ‘Jonagold’ and ‘Szampion’ differed in many quantitative and qualitative traits of their micromorphology, anatomy, and ultrastructure at the harvest and consumption maturity stage [Konarska 2013]. The most substantial differences between the cultivars involved the quantity and forms of epicuticular wax and the total wax weight; the depth of cuticular microcracks; the number of stomata and lenticels; cuticle thickness and the size of epidermal cells; the thickness of the hypodermis layer; and the presence of phenolic compounds. The extensive literature concerning apple development and structure does not provide detailed information about the morphology, anatomy, and ultrastruc-

ture of apple fruit buds or the mechanism and time of development of traits responsible for fruit firmness and shelf-life. Determination of the duration of development of the major qualitative fruit traits may be important for growers, who could control and modify the trait development through application of appropriate fertilisation and/or irrigation and other agricultural treatments. Therefore, the aim of the present study was to analyse the structure of 21-day-old 'Jonagold' and 'Szampion' fruit buds at the intensive cell division stage and to present differences in the structure of the covering layer in these cultivars at the micromorphological, tissue, and cellular level. Particular attention was paid to the occurrence of traits that have an impact on subsequent quality and firmness of fruit.

MATERIAL AND METHODS

21-day-old (21 days after anthesis) fruit buds (ca. 1-cm diameter) of 'Jonagold' and 'Szampion' apples were collected on May 15–20, 2012 in a commercial, conventionally managed orchard near Lublin. Trees of the analysed cultivars grew in close proximity and identical climate and soil conditions. 20 fruit buds were sampled from the crowns of 5 randomly chosen trees of both cultivars. Further analyses were carried out on peel-comprising fragments of fruits sampled from their equatorial part.

Scanning electron microscopy (SEM). The fruit buds were carefully transported to the laboratory to avoid damage or destruction of their surface wax layer. Next, $4 \times 4 \times 1$ -mm peel fragments were sampled from 5 fruits of each cultivar. Since fixation of the material for SEM induces changes in the structure and destruction of the epicuticular wax layer [Reed 1982], freshly sampled material was carefully mounted onto stubs, sputter-coated with gold, and examined "live" under a TESCAN/VEGA LMU (Tescan, USA) scanning electron microscopy at an accelerating voltage of 30 kV. Quantity of wax platelets was roughly determined, while the length and the number of stomata and trichome scars within an area of 1 mm^2 of the epidermis were assessed using the morphology software combined with SEM.

Light microscopy (LM). Using a razor blade, hand-cut cross-sections perpendicular to the fruit axis were made through fresh peel of 10 fruit buds of 'Jonagold' and 'Szampion'. Further, the samples were stained with Sudan III (a saturated ethanol solution of Sudan III) to visualize lipophilic substances in cuticle, with Lugol's iodine in order to detect starch, and with FeCl_3 to detect phenolic substances. Later, the samples were embedded in glycerol gelatine on a glass slide and observed under the Nikon SE 102 light microscope where the thickness of the cuticle (at the mid-width of a randomly chosen epidermal cell), the height of the epidermal cells, the number of layers of hypodermis and its overall thickness, the thickness of hypodermis cell walls, and the thickness of 3 layers of the parenchyma located under the hypodermis were determined in five places under a Nikon SE 102 light microscopy. Hand-cut samples obtained from fresh material were also observed with a stereoscopic Nikon Eclipse 90i microscope combined with an UV filter set comprising the wavelength of EX 330–380 nm stimulating autofluorescence of cuticle and chlorophyll in order to analyse the distribution of that substances. Images were obtained by using a digital camera (Nikon Fi1) and

NIS-Elements Br 2 software, respectively a Zeiss AxioImager Z1 fluorescence microscope equipped with an AxioCam MR digital camera.

0.7- μm semi-thin sections were also prepared from fragments of the fruit buds, which were stained with 1% methylene blue with 1% azur II in a 1% aqueous solution of sodium tetraborate. The material was fixed and embedded in synthetic resin with the standard method used in transmission electron microscopy (see below). Sections were observed by means of a Nikon Eclipse 90i microscopy.

Transmission electron microscopy (TEM). Small samples ($2 \times 2 \times 2$ mm) of 5 fruit buds of ‘Jonagold’ and ‘Szampion’ 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 hr at 4°C. When fixed, the samples were rinsed with 0.1 M cacodylate buffer at 4°C for 24 hr and then treated with 1% OsO_4 . After passage through increasing concentrations of propylene oxide in ethanol and finally through pure propylene oxide, the samples were embedded for 12 hr in Spurr Low Viscosity resin at 70°C [Spurr 1969]. Subsequently, ultrathin sections (70 nm thick) obtained using the Reichert Ultracut-S ultramicrotome (Vienna, Austria) and a glass knife were transferred to re-distilled water and stained with a 0.5 M aqueous solution of uranyl acetate and lead citrate [Reynolds 1963]. Images were observed and recorded using the FEI Technai G2 Spirit Bio TWIN transmission electron microscopy at an accelerating voltage of 120 kV. Images were captured using a Megaview G2 Olympus Soft Imaging Solutions camera.

Statistical analyses. Means ($\pm\text{SD}$) were calculated for all the parameters measured. Data were analysed by one-way analysis of variance (ANOVA) and Tukey’s multiple range test for comparison of means, using software Statistica 7. The difference was considered statistically significant at the level of $P < 0.05$.

RESULTS

SEM. The ‘Jonagold’ and ‘Szampion’ fruit buds were covered by dense, easily broken off, unicellular, ca. 1-mm-long non-glandular trichomes (figs 1A, C). The epidermis among the trichomes exhibited few trichome scars, i.e. traces left by broken off non-glandular trichomes (figs 1D–E), and stomata in various developmental stages showing different degrees of stomatal opening (figs 1F, G). In both cultivars, the sizes of stomata and trichome scars were comparable, but their number per unit surface area was by ca. 30% greater in ‘Jonagold’ (tab. 1). The cuticle of both cultivars exhibited microcracks of varied depths: superficial, which were more abundant, and deeper microcracks, which occurred sporadically and resembled a fastened zipper (fig. 1H). Moreover, the cuticle surface displayed vertically and horizontally oriented crystalline wax platelets (figs 2A–D). In ‘Jonagold’, the wax platelets were more ordered and more numerous than in the ‘Szampion’ cultivar. Additionally, a majority of the platelets were vertically or obliquely arranged (fig. 2A, B).

LM. The surface layer of the 21-day-old ‘Jonagold’ and ‘Szampion’ fruit buds was composed of a single-layered epidermis covered by a cuticle layer and several hypodermis layers (figs 3A–F).

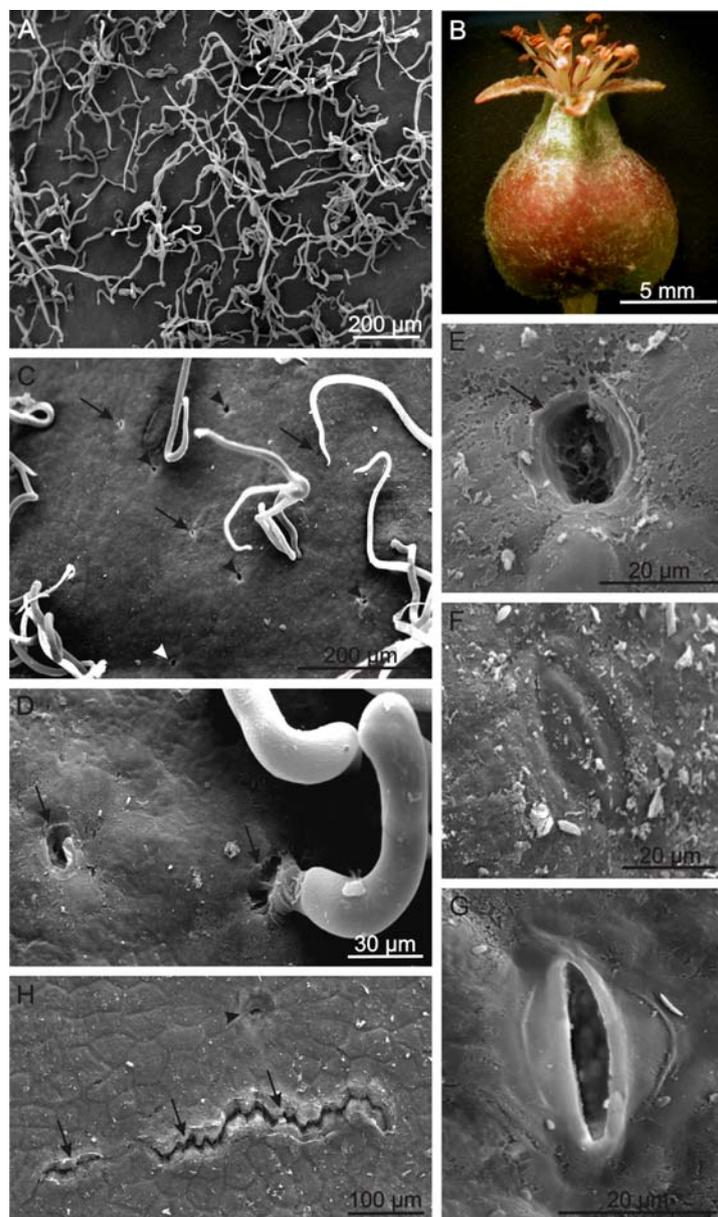


Fig. 1. Epidermis surface of the fruit buds in the 'Jonagold' (A, C, D, F, G) and 'Szampion' cultivars (B, E, H); A, C – fragments of the epidermis surface with numerous subulate non-glandular trichomes; B – a fruit bud of 'Szampion' in stereoscopic microscopy. C – visible trichomes, stomata (arrows) and trichome scars (arrowheads); D, E – trichome scars (arrows) visible on the fruit bud surface; D – note a breaking off non-glandular trichome; F, G – stomata in different stages of development; H – microcrack (arrows) and trichome scar (arrowhead)

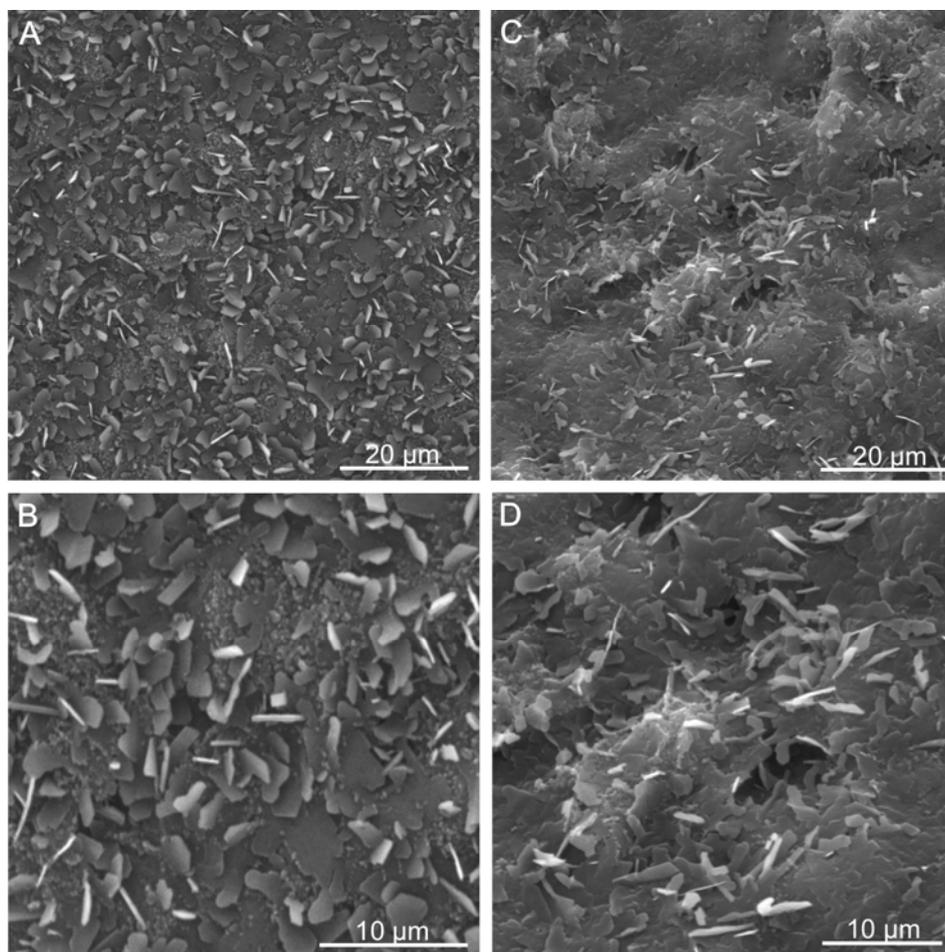


Fig. 2. SEM. Fragments of the epidermis surface of the fruit buds 'Jonagold' (A, B) and 'Szampion' cultivars (C, D) with vertical and horizontal crystalline wax platelets. Note arrangement and a greater number of vertical and inclined wax plates in 'Jonagold'

Epidermis covering the fruit buds in both cultivars had a nature of a meristematic tissue. Cells produced through anti- and periclinal divisions were visible among anticlinal elongated cells, particularly in the 'Szampion' cultivar (figs 3C, D). The height of epidermal cells was by 8% greater in 'Jonagold' (tab. 1). The epidermis was covered by a different-thickness cuticle layer which was stained orange-red by Sudan 3 (not shown) and exhibiting light blue fluorescence under UV light (fig. 3E). The mean cuticle thickness was by 11% greater in the 'Szampion' cultivar (tab. 1). The epidermis of both cultivars had stomata with large air chambers underneath (fig. 3F). In the 'Jonagold' cultivar, the hypodermis layer was by 11% thicker than in 'Szampion' (tab. 1) and consisted of 5–6 layers of the rectangular outline collenchyma cells with distinctly thick-

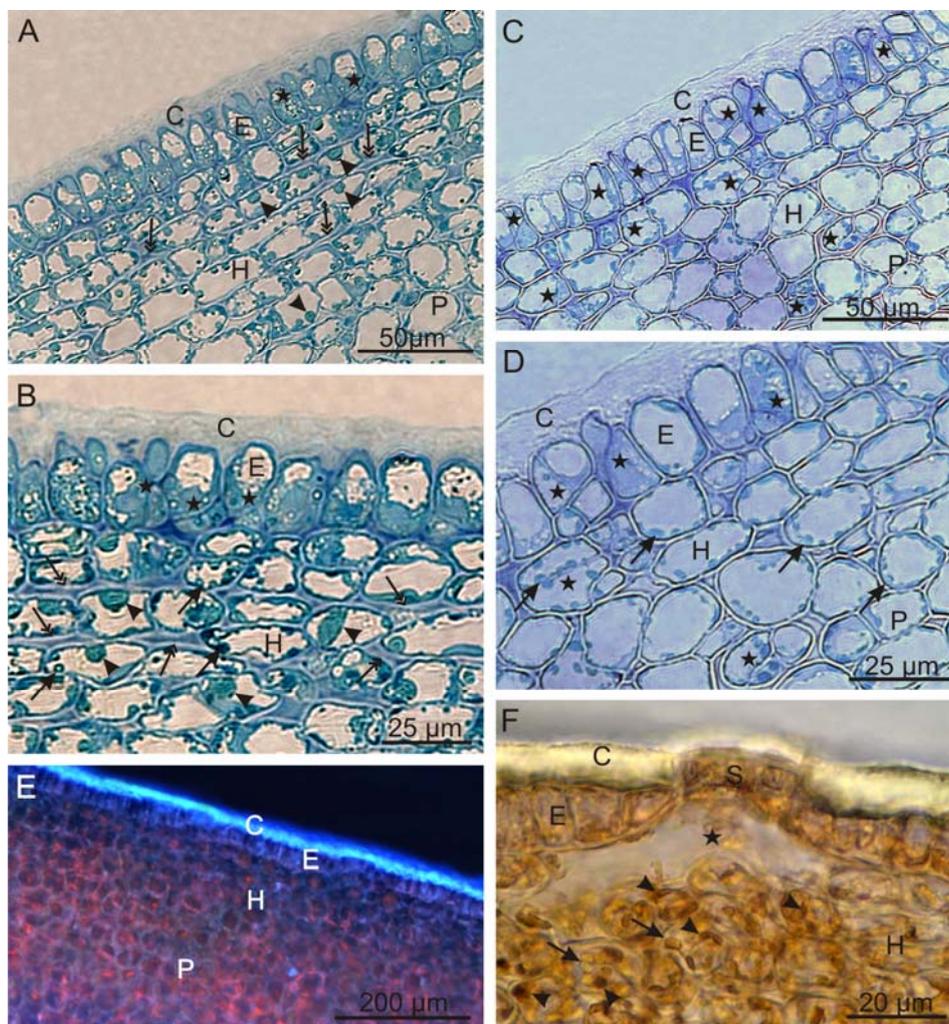


Fig. 3. LM. Fragments of the cross-sections through the 'Jonagold' (A, B, E, F) and 'Szampion'(C–D) surface layer of the fruit bud. A, B – in hypodermis and parenchymatic cells visible large deposits of phenolic substances (arrowheads) and thickened tangential cell walls in the hypodermis cells (arrows with two heads). Note epidermis cells after mitotic divisions (asterisks); B – visible chloroplasts (arrows) in the hypodermis; C, D – visible chloroplasts (arrows) (D) and cells after mitotic divisions in the epidermis, hypodermis and parenchyma layer (asterisks); E – visible blue-fluorescent cuticle and red – fluorescent chloroplasts (fluorescence microscopy); F – visible stoma (S) with the air space (asterisk). Note chloroplasts (arrows) and deposits of phenolic substances (arrowheads) in the hypodermis cells; C – cuticle, E – epidermis, H – hypodermis, P – parenchyma

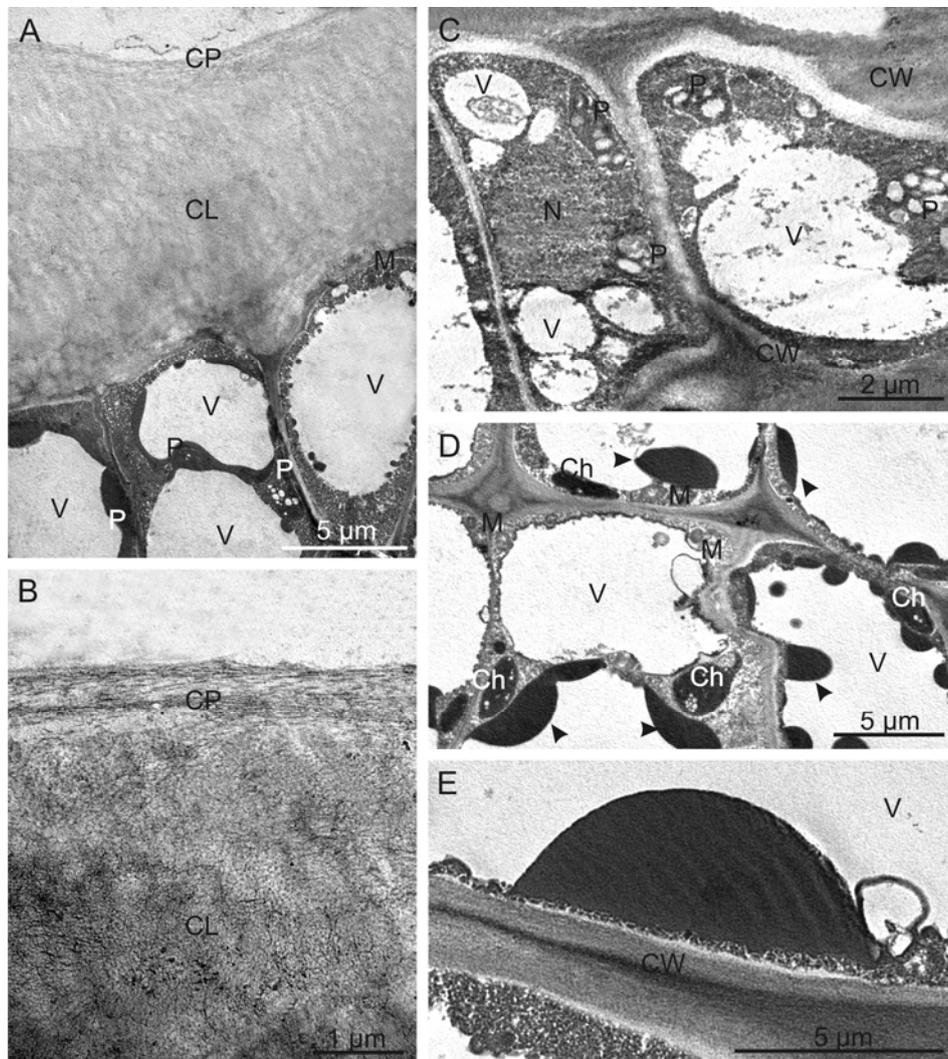


Fig. 4. TEM. Ultrastructure of 'Jonagold' (A, D, E) and 'Szampion' (B, C) fruit peel at the fruit bud stage; A, B – visible cuticle composed of lamellar cuticle proper (CP) and a reticulate cuticular layer (CL); A, C – plastids (P) with starch grains visible in the epidermis and hypodermis cells; D – chloroplasts (Ch) with starch grains and electron-dense deposits of phenolic substances (arrowheads) visible in the hypodermis cells; E – deposit of phenolic substances visible in the vacuole; N – nucleus, M – mitochondrion, V – vacuoles, CW – cell wall

ened tangential walls (tab. 1, figs 3A, B). In turn, the hypodermis cells in the ‘Szampion’ fruit buds exhibited local and only slight cell wall thickenings or they were still at the differentiation stage and had an oval outline characteristic of parenchymatic cells (tab. 1, figs 3C, D). The diameters of parenchymal cells located below the hypodermis were comparable in both cultivars (tab. 1, figs 3A, C). Likewise in the epidermis, cells after mitotic divisions were also observed in hypodermal and parenchymal layers, particularly in the ‘Szampion’ cultivar (figs 3C, D). Numerous chloroplasts that produced red fluorescence under the fluorescence microscopy (fig. 3E) and contained starch (reaction with IKI) were visible in the cytoplasm of these tissues in both cultivars (figs 3B, D, F). Additionally, the hypodermis and parenchymal cells in the ‘Jonagold’ fruit buds contained oval deposits of phenolic compounds (figs 3A, B, F) characterised by dark brown staining in FeCl_3 .

Table 1. Characteristics of fruit buds of ‘Jonagold’ and ‘Szampion’ cultivars

| Parameters (n = 10) | ‘Jonagold’ | ‘Szampion’ |
|--|--------------------|--------------------|
| Number of stomata and trichome scars (per mm^2) | 23 \pm 5.0 a | 18 \pm 4.0 b |
| Length of stomata pores (μm) | 27.8 \pm 3.3 a | 30.1 \pm 2.9 a |
| Length of trichome scars (μm) | 23.32 \pm 4.6 a | 22.56 \pm 4.2 a |
| Thickness of cuticle (μm) | 8.64 \pm 0.7 a | 9.77 \pm 0.9 a |
| Height of the epidermis cells (μm) | 22.93 \pm 1.7 a | 21.1 \pm 1.2 a |
| Number of the hypodermis layers | 6 \pm 1.0 a | 4 \pm 1.0 a |
| Thickness of the hypodermis layer (μm) | 85.51 \pm 9.7 a | 76.18 \pm 5.8 b |
| Thickness of the hypodermis cell walls | 3.65 \pm 1.0 a | 0.09 \pm 0.4 b |
| Thickness of the three parenchyma layers (μm) | 65.71 \pm 11.1 a | 67.98 \pm 6.0 a |
| Total thickness of peel (μm) | 114.0 \pm 23.6 a | 106.3 \pm 18.4 a |

Values are mean \pm SD (standard deviation). The same letters within a row mean no statistically differences ($P < 0.05$)

TEM. Only slight differences in the ultrastructure of epidermal, hypodermal, and parenchymal cells were found between the analysed cultivars. The cuticle on the surface of the fruit buds was composed of two layers: a substantially larger internal reticulate layer, the so-called cuticular layer and an external lamellate layer, the so-called cuticle proper, accounting for ca. 8–10% of the total cuticle thickness (figs 4A, B). The epidermal cells exhibited a thin layer of parietal cytoplasm with visible mitochondria, and plastids containing starch grains (figs 4A, C). Similarly, the cytoplasm of hypodermal and parenchymal cells had plastids containing starch grains, whereas the vacuoles in the ‘Jonagold’ cultivar exhibited numerous, large electron-dense deposits of phenolic compounds (figs 4D, E). They usually adhered to the tonoplast and had different sizes.

DISCUSSION

Most traits related to fruit quality and firmness are genetically conditioned [Faust and Shear 1972a]. In the fruits of 'Jonagold' and 'Szampion', these traits were fully developed at harvest and consumption maturity [Konarska 2013]. 3-week-old fruit buds of the 'Jonagold' and 'Szampion' cultivars exhibited most features associated with the protective function of the surface covering layer. Differences in the structure of the fruit buds between the cultivars were visible primarily at the level of anatomy and micro-morphology and were less evident than in the stage of harvest and consumption maturity.

Babos et al. [1984] and Zamorsky [2007] report that protection of the fruit interior is ensured by fruit peel composed of an epidermis covered by a cuticle and a multi-layered hypodermis. In the early stage of 'Jonagold' and 'Szampion' fruit development, the protective role of this layer is additionally strengthened by abundant non-glandular trichomes densely distributed in the fruit bud epidermis. Similar non-glandular trichomes with a similar function were found to cover fruits of other plant species in different developmental stages [Bain 1961, Miller 1984, Bednorz and Wojciechowicz 2009, Celano et al. 2009]. In 21 day-old 'Jonagold' and 'Szampion' fruit buds only few trichome scars remaining after broken off trichomes were visible. Maguire et al. [1999] and Veraverbake et al. [2003b] report that trichome scars, stomata and lenticels, present in the fruit epidermis at all developmental stages facilitate gas exchange and promote fruit transpiration, thereby contributing to wilting, softening and quality deterioration during storage and shelf life. However, the total number of trichome scars and stomata in the 'Szampion' fruit buds was lower than that in 'Jonagold'. As reported by Konarska [2013], this relationship persisted in the consumption maturity stage, although 'Szampion' fruits exhibit worse and shorter storability. According to Veraverbake et al. [2003a], at the consumption maturity stage, approximately 60% of 'Jonagold' lenticels are "closed" (non-transpiring), while only "opened" lenticels promote transpiration. A vast majority of the 'Szampion' lenticels may have been "opened"; yet, no such investigations have been conducted. The author of the present study considers that the intensity of fruit transpiration is dependent on a set of several water-loss promoting traits rather than on a single feature.

The surface of the fruit buds in both cultivars examined showed few microcracks present mostly on the surface cuticle layers. The presence of the microcracks indicated the onset of the cell expansion process, although cells after mitotic divisions were still found by the author in all the layers of the fruit bud tissues, i.e. the epidermis, hypodermis, and parenchyma. Microcracks appearing in the cuticle may indicate more rapid expansion of the volume and turgor of epidermal and internal fruit cells, while the amount of cuticle per fruit is constant [Faust and Shear 1972a, Roy et al. 1994, Knoche et al. 2004]. Similar results were obtained by Harker and Ferguson [1988], who found that the fruit bud stage in apples is a period of dynamic cell divisions, particularly in the epidermis, and the beginning of intensive cell growth. According to many authors, microcracks enhance water loss and reduce fruit firmness and weight [Höhn 1990, Lau and Lane 1998, Maguire et al. 1999, De Bellie 2000, Link et al. 2004].

The surface of the fruit buds in the cultivars examined was covered by a layer of crystalline epicuticular waxes arranged in vertical and horizontal platelets, which were more abundant and ordered in the 'Jonagold' cultivar. Unlike the trichome scars, stomata as well as microcracks, cuticular waxes are an efficient protective barrier against excessive transpiration [Faust and Shear 1972b, Babos et al. 1984, Roy et al. 1994, Belding et al. 1998, Veraverbeke et al. 2001a, b]. The number of wax platelets increases together with fruit maturation, reaching a maximum value during the storage period, particularly in varieties with a greasy and smooth peel, which was observed by Konarska [2013] in 'Jonagold' fruits stored in a controlled-atmosphere storehouse for 6 months. Koch et al. [2004] and Curry [2009] suggest that production of the highest possible numbers of vertically oriented platelets is especially important for reduction of transpiration, since this form of wax promotes healing and "repair" of microcracks. According to Curry [2001, 2005] and Müller [2005], apple trees employ a 'Tear and Repair' mechanism involving continuous synthesis of epicuticular waxes and closure of microcracks appearing along with fruit growth.

The surface of the fruit buds of the cultivars examined had a characteristic reticulate-lamellate cuticle, whose layer was thicker in the 'Szampion' cultivar. As shown by Konarska [2013], 'Szampion' fruits were characterised by greater weight loss and more intensive transpiration during the storage period. A thicker cuticle does not restrict the decline in fruit firmness and their better quality after storage. Similar results concerning the role of the cuticle in fruits of other apple varieties were obtained by Riederer and Schreiber [1995] and Knoche et al. [2000].

Numerous deposits of phenolic compounds were found in the hypodermis and parenchymal cells in the 'Jonagold' fruit buds; these, however, were not observed in 'Szampion'. Similarly, the deposits were visible, although in smaller numbers, only in the harvest and consumption maturity stage in 'Jonagold' [Konarska 2013]. The results obtained by the author correspond to the findings of Mehrabani and Hassanpouraghdam [2012], who reported that the content of phenolic compounds was higher in younger fruits than in the harvest maturity stage. Additionally, a varied content of polyphenols in different apple and pear cultivars has been described by other researchers [Solovchenko and Schmitz-Elberger 2003, Drogoudi et al. 2008, Łata et al. 2009]. Absence of polyphenols in the 'Szampion' cultivar may be one of the causes of the poorer quality and storability of its fruits, as evidenced by literature data indicating that presence of polyphenols improves and extends fruit storage by increasing resistance to pathogens [Garry et al. 1995, Lattanzio et al. 2001, Cheynier 2005].

CONCLUSIONS

1. The fruit buds in the 'Jonagold' and 'Szampion' cultivars differed significantly in the number of trichome scars and stomata, thickness of hypodermis layer and hypodermis cell walls, as well as the quantities of deposits of phenolic compounds.

2. The following factors exert an impact on the fruit quality and storability in the fruit bud stage: trichome scars and stomata, microcracks, crystalline wax platelets and phenols compounds.

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REFERENCES

- Atay E., Pirlak L., Atay A.N., 2010. Determination of fruit growth in some apple varieties. *J. Agric. Sci.*, 16, 1–8.
- Babos K., Sass P., Mohácsy P., 1984. Relationship between the peel structure and storability of apples. *Acta Agron. Acad. Sci. Hung.*, 33, 41–50.
- Bain J.M., 1961. Some morphological, anatomical, and physiological changes in the pear fruit (*Pyrus communis* var. Williams Bon Chrétien) during development and following harvest. *Aust. J. Bot.*, 9, 99–123.
- Bednorz L., Wojciechowicz M.K., 2009. Development of the multilayered epidermis covering fruit of *Sorbus torminalis* (Rosaceae). *Dendrobiology*, 62, 11–16.
- Belding R.D., Blankenship S.M., Young E., Leidy R.B., 1998. Composition and variability of epicuticular waxes in apple cultivars. *J. Amer. Soc. Hort. Sci.*, 123, 348–356.
- Celano G., Minnocci A., Sebastiani L., D’Auria M., Xiloyannis C., 2009. Changes in the structure of the skin of kiwifruit in a relation to water. *J. Hortic. Sci. Biotech.*, 84, 41–46.
- Cieslak M., Génard M., Boudon F., Baldazzi V., Godin C., Bertin N., 2013. Integrating architecture and physiological perspectives in fruit development. Proceedings of the 7th International Conference on Functional-Structural Plant Models. 9–14 June 2013, Saariselkä, Finland, 127–130.
- Cheyrier V., 2005. Polyphenols in foods are more complex than often thought. *Am. J. Clin. Nutr.*, 81, 2235–2295.
- Curry E.A., 2001. Lenticel and cuticle disorders: a survey. Proceedings of the Washington Tree Fruit Postharvest Conference. 13–14 March 2001, Wenatchee, WA, USA.
- Curry E.A., 2005. Ultrastructure of epicuticular wax aggregates during fruit development in apple (*Malus domestica* Borkh). *J. Hortic. Sci. Biotech.*, 80, 668–676.
- Curry E.A., 2009. Growth-induced microcracking and repair mechanisms of fruit cuticles. *Plant Physiology*. Proceedings of the SEM Annual Conference. 1–4 June 2009, Albuquerque New Mexico, USA.
- Czernyszewicz E., 2007. A consumer’s look at the apple quality. *Annales UMCS, sec. EEE, Horticultura*, 17, 70–82.
- De Bellie N., Schotte S., Soucke P., De Baerdemaeker J., 2000. Development of an automated monitoring device to quantify changes in firmness of apples during storage. *Postharv. Biol. Technol.*, 18, 1–8.
- Drogoudi P.D., Michailidis Z., Pantelidis G., 2008. Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. *Sci. Hort.*, 115, 149–153.
- Faust M., Shear C.B., 1972a. Russeting of apples, an interpretive review. *HortSci.*, 7, 233–235.
- Faust M., Shear C.B., 1972b. Fine structure of the fruit surface of three apple cultivars. *J. Am. Soc. Hortic. Sci.*, 97, 351–355.
- Garry L.L., Suttill N.H., Wall K.M., Beveridge T.H., 1995. Localization of condensed tannins in apple fruit peel, pulp, and seeds. *Can. J. Bot.*, 73, 1897–1904.

- Gordon D.C., Percy K.E., Riding R.T., 1998. effects of uv-radiation on epicuticular wax production and chemical composition of four *Picea* species. *New Phytol.*, 138, 441–449.
- Guzmán P., Fernández V., García M.L., Khayet M., Fernández A., Gil L., 2014. Localization of polysaccharides in isolated and intact cuticles of eucalypt, poplar and pear leaves by enzyme-gold labelling. *Plant Physiol. Biochem.*, 76, 1–6.
- Harker F.R., Ferguson I.B., 1988. Transport of calcium across cuticles isolated from apple fruit. *Sci. Hortic.*, 36, 205–217.
- Homutová I., Blažek J., 2006. Differences In fruit skin thickness between selected apple (*Malus domestica* Borkh.) cultivars assessed by histological and sensory methods. *Hort. Sci. (Prague)*, 33, 108–113.
- Höhn E., 1990. Quality of apples. *Acta Hort.*, 285, 111–118.
- Khanal B.P., Knoche M., 2014. Mechanical properties of apple skin are determined by epidermis and hypodermis. *J. Am. Soc. Hortic. Sci.*, 139(2), 139–147.
- Knoche M., Peschel S., Hinz M., Bukovac M.J., 2000. Studies on water transport through the sweet cherry fruit surface: Characterizing conductance of the cuticular membrane using pericarp segments. *Planta*, 212, 127–135.
- Knoche M., Beyer M., Peschel S., Oparlakov B., Bukovac M.J., 2004. Changes in strain and deposition of cuticle in developing sweet cherry fruit. *Physiol. Plant.*, 120, 667–677.
- Koch K., Neinhuis C., Ensikat H.J., Barthlott W., 2004. Self assembly of epicuticular waxes on living plant surfaces imaged by atomic force microscopy (AFM). *J. Exp. Bot.*, 55, 711–718.
- Konarska A., 2012. Differences in the fruit peel structures between two apple cultivars during storage. *Acta Sci. Pol., Hort. Cult.*, 11(2), 105–116.
- Konarska A., 2013. The structure of the fruit peel in two varieties of *Malus domestica* Borkh. (Rosaceae) before and after storage. *Protoplasma*, 250, 701–714.
- Kruczyńska D., 2008. Jabłonie: nowe odmiany. Hortpress Warszawa.
- Lattanzio V., Di-Venere D., Linsalata V., Bertolini P., Ippolito A., Salerno M., 2001. Low temperature metabolism of apple phenolics and quiescence of *Phlyctaena vagabunda*. *J. Agric. Food Chem.*, 49, 5817–5821.
- Lau O.L., Lane W.D., 1998. Harvest indices, storability, and poststorage refrigeration requirements of ‘Sunrise’ apple. *HortSci.*, 33, 302–304.
- Link S.O., Drake S.R., Thiede M.E., 2004. Prediction of apple firmness from mass loss and shrinkage. *J. Food Quality*, 27, 13–26.
- Łata B., Trampczyńska A., Paczesna J., 2009. Cultivar variation in apple peel and whole fruit phenolic composition. *Sci. Hortic.*, 121, 176–181.
- Maguire K.M., Lang A., Banks N.H., Hall A., Hopcroft D., Benneti R., 1999. Relationship between water vapour permeance of apples and micro-cracking of the cuticle, *Postharv. Biol. Technol.*, 17, 89–96.
- Maguire K.M., Banks N.H., Lang A., Gordon I.L., 2000. Harvest date, cultivar, orchard and tree effects on water vapour permeance in apples. *J. Am. Soc. Hort. Sci.*, 125, 100–104.
- Mehrabani L.V., Hassanpouraghdam M.B., 2012. Developmental variation of phenolic compounds in fruit tissue of two apple cultivars. *Acta Sci. Pol., Technol. Aliment.*, 11(3), 259–264.
- Miller A.N., Walsh C.S., Cohen C.D., 1987. Measurement of indole-3-acetic acid in peach fruits (*Prunus persica* L. Batsch cv Redhaven) during development. *Plant Physiol.*, 84, 491–494.
- Miller R.H., 1984. The multiple epidermis-cuticle complex of Medlar fruit *Mespilus germanica* L. (Rosaceae). *Ann. Bot.*, 53, 779–792.
- Müller I., 2005. Relationship between preharvest soybean oil application and postharvest behavior apples. Dissertation, Washington State University.
- Pieniążek S.A., 2000. Sadownictwo. PWRiL Warszawa.

- Reed D.W., 1982. Wax alteration and extraction Turing electron microscopy preparation of leaf cuticles. In: The plant cuticle. Cutler, D.F., Alvin K.L., Price C.E., eds. Academic Press, London.
- Rejman A., 1994. Pomologia. PWRiL Warszawa.
- Reynolds E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain for electron microscopy. *J. Cell Biol.*, 17, 208–212.
- Riederer M., Schreiber L., 1995. Waxes – the transport barriers of plant cuticles. In: Waxes: Chemistry, molecular biology and functions, Hamilton R.J (ed.). The Oily Press, Scotland, 131–156.
- Roy S., Conway W.S., Watada A.E., Sams C.E., Erbe E.F., Wergin W.P., 1994. Heat treatment affects epicuticular wax structure and postharvest calcium uptake in ‘Golden Delicious’ apples. *Hort. Sci.*, 29, 1056–1058.
- Solovchenko A., Schmitz-Eiberger M.J., 2003. Significance of skin flavonoids for UV-B-protection in apple fruits. *Exp. Bot.*, 54, 1977–1984.
- Spurr A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultra. Res.*, 26, 31–43.
- Veraverbake E.A., van Bruaene N., van Oostveldt P., Nicolaï B.M., 2001a. Non destructive analysis of the wax layer off apple (*Malus domestica* Borkh.) by means of confocal laser scanning microscopy. *Planta*, 213, 525–533.
- Veraverbake E.A., Lammertyn J., Saevels S., Nicolaï B.M., 2001b. Changes in chemical wax composition of three different apple (*Malus domestica* Borkh.) cultivars during storage. *Postharv. Biol. Technol.*, 23, 197–208.
- Veraverbake E.A., Verboven P., van Oostveldt P., Nicolaï B.M., 2003a. Prediction of moisture loss across the cuticle of apple (*Malus sylvestris* subsp. *Mitis* (Wallr.)) during storage: part 1. Model development and determination of diffusion coefficients, *Postharv. Biol. Technol.*, 30, 75–88.
- Veraverbeke E.A., Verboven P., van Oostveldt P., Nicolaï B.M., 2003b. Prediction of moisture loss across the cuticle of apple (*Malus sylvestris* subsp. *Mitis* (Wallr.)) during storage: part 2. Model simulations and practical applications. *Postharv. Biol. Technol.*, 30, 89–97.
- Verboven P., Nemeth A., Abera M.K., Bongaers E., Daelemans D., Estrade P., Herremans E., Hertog M., Saeys W., Vanstreels E., Verlinden B., Leitner M., Nicolaï B., 2013. Optical coherence tomography visualizes microstructure of apple peel. *Postharv. Biol. Technol.*, 78, 123–132.
- Westwood M.N., 1995. Temperate zone pomology: Physiology and culture. (Third Edition). Timber Press, Oregon.
- Zamorskyi V., 2007. The role of the anatomical structure of apple fruits as fresh cut produce. *Acta Hort.*, 746, 509–512.

RÓŻNICE W STRUKTURZE ZAWIĄZKÓW OWOCÓW DWÓCH UPRAWNYCH ODMIAN JABŁONI ZE SZCZEGÓLNYM UWZGLĘDNIENIEM CECH DECYDUJĄCYCH O ICH TRWAŁOŚCI I JAKOŚCI

Streszczenie. ‘Jonagold’ i ‘Szampion’ należą do zimowych odmian jabłoni, których owoce są przystosowane do długotrwałego przechowywania. Jednak owoce różnią się wyraźnie rodzajem powierzchni oraz tempem i ilością transpirowanej wody, co przekłada się na

jakość owoców po wyjęciu z przechowalni oraz na długość życia jabłek na półce sklepowej. Większość cech odpowiedzialnych za jakość i trwałość owoców to cechy uwarunkowane genetycznie, rozwijające się w różnym czasie. Mikromorfologię, anatomię oraz ultrastrukturę 21-dniowych zawiązków owoców odmian 'Jonagold' i 'Szampion' badano za pomocą mikroskopii świetlnej oraz elektronowej: skaningowej i transmisyjnej. Szczególną uwagę zwrócono na cechy mające wpływ na jędrność oraz trwałość owoców. Stwierdzono, że zawiązki badanych odmian różniły się istotnie liczbą szparek i blizn włoskowych obecnych na jednostce powierzchni owocu, grubością pokładu hipodermy oraz jej ścian komórkowych, a także zawartością depozytów związków fenolowych. Na etapie zawiązka u obydwu odmian zaobserwowano następujące cechy mające związek ze wzrostem lub obniżeniem jędrności i trwałości owoców: wosk krystaliczny w postaci płytek, mikrospękania w kutykuli, szparki i blizny włoskowe oraz obecność związków fenolowych.

Słowa kluczowe: zawiązki jabłek, skórka owoców, mikromorfologia, anatomia i ultrastruktura, kutykula, wosk epikutykularny, mikrospękania, zawiązki fenolowe

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