INTRODUCTION

The microstructure changes of plant tissue maybe cause changes of its mechanical properties. Materials and products of plant origin are using in studies on material structure. The image analysis of plant tissues is methods which enables analysis and evaluation their material structure, texture and geometrical properties of tissues.

Piggott et all. (2006) have described utilization of image analysis tools to study the deformation of cells in biological plant tissue. The tissue was taken from upper onion epidermal layers, chosen because they form a single structural layer and are relatively large and easy to work with. The authors have described image analysis techniques to investigate the structure of deforming notched and un-notched onion epidermal cellular structures and have compared the influence of vertically and horizontally oriented cells. They used image processing to track and quantify dimensions on a cell-by-cell basis. Changes in the cell size were described quantitatively as a function of time for tissues stretched parallel and perpendicular to the cell axis. Tissue orientation had a marked effect on cell deformation and failure for an edge-notched sample. Wang et all. (2001) have presented a segmentation-free tree-structure image representation. In order to learn the structure representation, a back-propagation through structure (BPTS) algorithm was adopted. Experiments on plant image classification and retrieval refining using only six visual features were conducted on a plant image database and a natural scene image database, respectively.

The material of plant tissue is also especially susceptible to a variety of effects, e.g. electrical (Hlaváčová, Kertész, 2008), thermal (Božíková, 2007), (Hlaváč, 2007) which often result in structure changes that may subsequently cause processes lowering the quality of the product. The continually perfected methods of microscopy permit the observation of structure under magnification and digital techniques of image recording allow the analysis of information contained in the images obtained. The microscope methods with abundance of accessories and software enable obtaining of good results on plant tissues (Konstankiewicz and Zdunek, 2005). Chien and Lin (2005) used a non-destructive measurement method of plant features image processing technique provides a means to analyze the continuous growth process of a plant. They developed a non-destructive measurement method by using elliptical Hough transform to search for seedling leaves from a top-view image. To improve the accuracy of the leaf number estimation and leaf area measurement, this study further incorporates two side-view images with a top-view image to extract and reconstruct the three-dimensional structure of selected vegetable seedlings, and thus to measure the features related to plant growth. Ganczarz and Konstankiewicz (2007) studied cellular structure of potato tuber parenchyma tissue. As the results of analysis they obtained mean values parameters of cells size and shape: surface area of plane section of the cell and elongation of cells.

METHODS

Samples and storage properties

The study of varieties Picasso, Laura and Red Anna was realized. Experimental measurements were realized after the potato storage which was performed from 4th October 2007 to 3rd March 2008. One group of potato samples was stored in the standard conditions and the second was stored in the nonstandard conditions. The standard storage temperature was (4 – 7) °C and the environment humidity was (85 – 95) %. The nonstandard storage temperature was (8 – 14) °C and the air humidity was (40 – 60) %.

Image analysis

Image analysis starts with an acquisition of real objects by means of digital equipment as a digital camera or scanner. The digital images are obtained in original form in color or gray scale. The processing and analysis of grayscale image follows next steps (Aguilera and Stanley, 1999). The color image is transformed to the binary image. Than follows binary image editing, segmentation, object selection, measure analysis and statistical analysis.

The image can be additionally processed by thresholding to create a binary image that can be further processed by binary image editing. Segmentation divides the image into regions of structures intended for analysis. Object selection is followed by measurement and analysis and the collection of quantitative or qualitative data. The data are finally subjected to statistical analysis.

Binarization

Image processing might proceed by binarization. In binarization, the original gray level image is changed from a continuum
of colors or gray levels into a black-and-white image by assigning to each pixel a value of black or white. The binary image, once created, can then be manually edited by selecting objects for removal or inclusion or by using a spectrum of well-documented and extraordinarily powerful binary image-editing techniques. Binary image editing permits the selective removal of artifacts and noise, edge discrimination, skeletonizing, hole filling, application of Boolean operators using selected overlay or time-sequenced images, and other operations.

**Segmentation**

Images must then be segmented into measurable structures on the basis of color, brightness, edge discontinuities, elemental composition, temperature, or some other property that can be used to distinguish a feature from background. Segmentation refers to the process of extracting the desired object of interest from the image background. In image analysis, segmentation may be done by manual or automated methods and may be applied to an original image, to an image following filter transformation, or to a binary image. When segmentation is complete, every pixel in the image is included as an object or as “background.” Pixels contained in an object form a connected region in the image and have values similar to those of other pixels in that category but dissimilar to those of adjacent pixels in different categories.

Segmentation can proceed by thresholding, edge-based methods, and region-based methods. In binarization, thresholding is the dynamic process of taking the original gray level image from a continuum of colors or gray levels and assigning to each pixel a value of white or black, but in this context it can mean a wider range of categories with more than one cutoff point. Thresholding, in other words, involves limiting the intensity values within an image to a certain bounded range. Each pixel in an 8-bit gray scale image has a value between 0 (black) and 255 (white), and it may be decided that all pixels below a certain value do not contribute significantly to the object of interest and can be eliminated. This can be done by scanning the image one pixel at a time and keeping a pixel if it is at or above the selected intensity value or setting it to 0 (black) if it is below that value. This can be done either by manually tracing around the regions of interest with the mouse or by using an automated routine. Thresholding is the simplest and most commonly employed segmentation technique. Edge-based segmentation separates pixels into those that are on an edge of a region and those that are not. Non-edge pixels that form connected regions are then allocated to the same category. Region-based methods use algorithms to group adjacent pixels having similar values and to divide groups of pixels that have dissimilar values.

The processing of images was realized in the software ImageJ ver. 1.39s and we used next procedures (Rasband, 1997 – 2008): Sharpener, binarization (Make binary, thresholding), segmentation (Find Maxima), erosion, dilation, opening and analyzing of particles.

**Sharpen**

Increases contrast and accentuates detail in the image or selection, but may also accentuate noise. This filter uses the following weighting factors to replace each pixel with a weighted average of the 3x3 neighborhood:

-1 -1 -1
-1 12 -1
-1 -1 -1

**Make Binary**

Procedure converts an image to black and white. The threshold level is determined by analyzing the histogram of the current selection, or of the entire image if there is no selection. The algorithm used to calculate the threshold is

\[
\text{Threshold} = \frac{\text{average background} + \text{average objects}}{2}
\]

**Find Maxima**

Procedure determines the local maxima in an image and creates a binary (mask-like) image of the same size with the maxima, or one segmented particle per maximum. Output type of procedure was: "Display Point selection" - Displays a multi-point selection with a point at each maximum and the procedure "Exclude Edge Maxima" - Excludes maxima if the area within the noise tolerance surrounding a maximum touches the edge of the image (edge of the selection does not matter).

**Erosion**

The procedure removes pixels from the edges of black objects.

**Dilation**

The procedure adds pixels to the edges of black objects.

**Opening**

The procedure performs an erosion operation, followed by dilation. This smoothes objects and removes isolated pixels.

**Measurement analysis**

Techniques by which numerical measurements are extracted from images vary considerably in technological complexity. At the simpler end of the scale are linear measurements performed in the microscope or taken from a photographic image. If the determination is to be made in a light microscopy (LM), the microscope must be provided with a measuring eyepiece and micrometer and a stage micrometer. Measurements made in an LM can provide accurate data, but they are limited by the resolution of the micrometer and the subjectivity of the operator. In lieu of any digital-imaging capability, conventional photographic prints can be scored or measured by combining a variety of digitizing tablets with any of the analytical software packages; measurements are then acquired using computer-based analytical software. The extraction of quantitative data from images is often the main goal of the researcher, and what has gone before are attempts to convert the image to a form in which measurements can be made easily and accurately.

We are interested in the measurement of the area, perimeter, circularity and Feret’s diameter of the cells by software ImageJ ver. 1.39s (Rasband, 1997 – 2008). The measured object definitions follow:

**Area of cross section of cells**

Area of selection is measured in square pixels. Area is in calibrated units, such as square micrometers.

**Perimeter of cross section of cells**

This is defined as length of the outside boundary of the selection.

**Circularity of cross section of cells**

This is defined:

\[
\text{Circularity} = \frac{\pi \times \text{area}}{\text{perimeter}^2}
\]

A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated shape.

**Feret's diameter of cross section of cells**

Feret's diameter is the distance between pairs of parallel tangents to the projected outline of the particle in some fixed direction. There is longest distance between any two points along the selection boundary, also known as maximum caliper.
Experimental measurement

Potato tubers were always cut on two half parts and the cylindrical samples of the height of 30 mm and diameter of 5 mm were taken from the internal parenchyma – on periphery of tuber tissue (Fig 2). Than the samples of diameter of 5mm and depth of 0.1 mm were prepared by means of the razor blade. Digital imaged of the sections were scanned by video microscope combined with colour digital CCD camera GKB CS-8606S with the array of size 768x576 pixels and trinocular microscope MI XSZ 107. The experimental equipment is shown in the Fig. 3.

The images were digitized by the frame grabber KAPA PLUS which provided the collaboration with PC. The control software IMPOR’99 was used for a camera to provide a preprocessing of the snapshots. The software ImageJ ver. 1.39s was used for processing of images and the analysis. The digitized samples were adjusted on the size 768x576 pixels with the resolution 2.84 pixels/mm. The 200 times magnification was obtained. The real area of scanned surface had size 1.36x1.02 mm for each digital image. The one pixel of image was 0,0001mm on original sample.

The image of a cross section of internal parenchyma of the potato tuber tissue is presented in Figure 4A as the original 24-bit color RGB image. This image was then processed to minimize noise, contrasted and brightened. The image was transformed to a gray level (8 bit) and the filter of sharpening was selected (Figure 4B). The foregrounding of cells skeleton was realized manually by means of the paintbrush tool (Figure 4C). The gray level image was then thresholded. The image was then converted to a binary image (Figure 4D) which by definition comprises white or black pixels. The binary image was edited using opening, for removal and elimination noise in the form of one- or two-pixel specks. This step was followed by the inverting of image (Figure 4E). After binary image editing, all remaining structures were analyzed. This particular structure enabled itself to automated analysis. The analysis of cellular skeleton was realized by means of methods Find maxima and Analysis of particles (Figure 4F).

RESULTS AND DISCUSSION

The one cross section of cellular skeleton was evaluated for each variety of the standard and the nonstandard conditions of the storage. The statistical processing of data was realized in software Microsoft Excel. The arithmetical averages and the standard deviations were calculated. The obtained values are presented in the Tab 1. and Tab. 2.

Sl. 4. Digitalne slike tkiva gomolja krompira (internal parenchyma) varijeteta Picasso. a) originalna struktura (RGB boje, 24bit), b) 8 bitna transformacija, primenjen filter za izoštravanje (Greyscale, 8 bit), c) cellularni skeleton u prvom planu, d) binarizacija, granica osjetljivosti (Binary, 2 bit), e) invertovano, f) analiza geometrije i distribucija čelija. Originalna veličina slike: 768x596 pixels (27.1x20.3cm), 72 DPI, uvećanje 200 puta
and the Feret’s diameter were bigger for the standard conditions than the nonstandard conditions. The standard deviation of the parameters of the variety Red Anna for standard conditions were bigger than the standard deviations for the nonstandard conditions and bigger than the deviations of the all parameters of the varieties. It is results that the cell parameters of the variety Red Anna were smaller significant than the parameters of other varieties.

Table 1. Means values of cells of potato varieties: Picasso, Red Anna and Laura. Area of cells. Perimeter of cells and their standard deviations

<table>
<thead>
<tr>
<th>Varieties Varietet</th>
<th>Area Površina (μm²)</th>
<th>Stdev (μm²)</th>
<th>Stdev (%)</th>
<th>Perimeter Perimetar (μm)</th>
<th>Stdev (μm)</th>
<th>Stdev (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picasso nonstandard (61 cells)</td>
<td>6720,16</td>
<td>550,57</td>
<td>8,19</td>
<td>3347,90</td>
<td>13,44</td>
<td>3,86</td>
</tr>
<tr>
<td>Picasso standard (72 cells)</td>
<td>5573,39</td>
<td>416,92</td>
<td>7,48</td>
<td>3360,40</td>
<td>11,59</td>
<td>3,44</td>
</tr>
<tr>
<td>RedAnna nonstandard (80 cells)</td>
<td>5039,80</td>
<td>255,93</td>
<td>5,08</td>
<td>2959,79</td>
<td>7,91</td>
<td>2,67</td>
</tr>
<tr>
<td>RedAnna standard (80 cells)</td>
<td>5020,81</td>
<td>561,34</td>
<td>11,18</td>
<td>3030,04</td>
<td>33,88</td>
<td>11,18</td>
</tr>
<tr>
<td>Laura nonstandard (51 cells)</td>
<td>8066,31</td>
<td>757,82</td>
<td>9,39</td>
<td>370,98</td>
<td>17,85</td>
<td>4,81</td>
</tr>
<tr>
<td>Laura standard (60 cells)</td>
<td>6757,90</td>
<td>529,50</td>
<td>7,84</td>
<td>348,98</td>
<td>15,78</td>
<td>4,52</td>
</tr>
</tbody>
</table>

Table 2. Means values of cells of potato varieties: Picasso, Red Anna and Laura. Circlearity of the cells. Feret’s diameter and their standard deviations

<table>
<thead>
<tr>
<th>Varieties Varietet</th>
<th>Circularity Zaokrženost (%)</th>
<th>Stdev (-)</th>
<th>Stdev (%)</th>
<th>Feret’s diameter Feretov prečnik (μm)</th>
<th>Stdev (μm)</th>
<th>Stdev (%)</th>
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</thead>
<tbody>
<tr>
<td>Picasso nonstandard (61 cells)</td>
<td>0,63</td>
<td>0,01</td>
<td>2,26</td>
<td>126,28</td>
<td>4,84</td>
<td>3,83</td>
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<tr>
<td>Picasso standard (72 cells)</td>
<td>0,57</td>
<td>0,02</td>
<td>3,54</td>
<td>122,40</td>
<td>4,01</td>
<td>3,28</td>
</tr>
<tr>
<td>RedAnna nonstandard (80 cells)</td>
<td>0,68</td>
<td>0,01</td>
<td>1,70</td>
<td>105,03</td>
<td>2,61</td>
<td>2,49</td>
</tr>
<tr>
<td>RedAnna standard (80 cells)</td>
<td>0,65</td>
<td>0,07</td>
<td>11,18</td>
<td>110,25</td>
<td>12,33</td>
<td>11,18</td>
</tr>
<tr>
<td>Laura nonstandard (51 cells)</td>
<td>0,64</td>
<td>0,02</td>
<td>3,12</td>
<td>131,25</td>
<td>5,80</td>
<td>4,42</td>
</tr>
<tr>
<td>Laura standard (60 cells)</td>
<td>0,63</td>
<td>0,02</td>
<td>2,97</td>
<td>121,53</td>
<td>4,51</td>
<td>3,71</td>
</tr>
</tbody>
</table>

We also study the problem of the small resolution of the taken cross section images. The background of the cross section and the border of the cells merged together and the borders of cells we had to draw manually. The problems were created by sample preparation and by the nonconfocal light microscope utilization.

**CONCLUSION**

The method of image analysis of the potato of the varieties Picasso, Red Anna and Laura was used at the study of the potato tissue microstructure and its modification in the period of the storage in the standard and nonstandard conditions. The image processing of cross sections of the varieties were realized and the structural parameters characterized the shape of the cells were determined. The area of the cells, the perimeter of the cells, the circularity of the cells and Feret’s diameter were evaluated. The influence of the variety and the storage conditions were observed and determined.

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**REFERENCES**


