The aim of our work was the non-invasive maturity determination of Golden Delicious apples during 35 days long cold (2°C) and shelf-life storage (20°C). Significant difference was found between cold and at 20°C stored samples in case of mass loss, acoustic stiffness coefficient, RGB color parameters and chlorophyll content related parameters (IAD, F0, FM, FV/FM). The DA-value and the chlorophyll fluorescence values did not show significant differences between the blushed and shaded sides of the samples.

All the applied non-invasive measuring methods were found to be suitable for the detection of postharvest maturation related quality changes of apple.

**Keywords**

DA-index®, chlorophyll fluorescence, acoustic stiffness, computer/machine vision system.

**1. Introduction**

Nowadays, the consumers’ demand for fresh apples in excellent quality became more and more important. Apple is one of the main fruits available during all the year in Hungary for fresh consumption and postharvest storage. Concerning this, the objective quality determination of fresh apple samples is also an important task to solve. In order to store the apples for months it is essential to know the internal processes. Internal and external quality features such as stiffness, taste and color are the most valuable parameters for the consumers.

The new non-destructive quality evaluating methods offer the possibility to supply precise information about the quality and its changes during the postharvest period. Abbott et al. [1] found the relationship between the resonance frequency of the apples and their firmness. Since then, more and more research groups carried out experiments using the acoustic method. More fruits’ and vegetables’ stiffness, such as pear [2], peach [3], tomato [4], carrot [5], were measured successfully by this technique.

The outlook is a very important parameter during quality evaluation and purchase. Computer aided machine vision system offers the possibility for size, shape and color analysis [6, 7].

The maturity related surface color change can be characterized by the logistic curve related color changes in case of many fruits and vegetables [8, 9]. Photosynthetically active chlorophyll content and postharvest quality related maturity changes can be determined by the use of chlorophyll fluorescence analysis [10, 11] and the measurement of DA-value (index of absorbance difference, IAD) by a DA-meter [3, 12, 13, 14, 15].

The aim of our work was the non-invasive maturity and quality determination of freshly harvested Golden Delicious apple samples during simulated postharvest cold and shelf-life storage.
2. Materials and methods

Mature green and fresh Golden delicious apple samples (Malus domestica cv. Golden delicious L.) were bought from an experienced grower in uniform maturity. According to uniform size, mass, shape and freedom from defects, 50 pieces were randomly divided into two groups. They were stored in temperature-controlled refrigerator at 2 ± 0.5 °C and at ambient temperature of 20 ± 1 °C for 35 days wrapped in thin LDPE (low-density polyethylene) bags.

Two measuring points on the blushed and shaded side were selected for the nondestructive measurements as surface color measurements carried out by a machine vision system, chlorophyll fluorescence analysis and DA-index® evaluation. In case of the optical methods the parameters were calculated as the average on the two sides measured values. Mass loss (% of fresh weight) was calculated based upon the measured weight data of each sample on every measuring day.

Surface color changes were determined by a specific self-developed machine vision system consisting homogeneous illumination provided by halogen lamps and a high-performance camera. Average red, green and blue color characteristics (and additionally the normalized values too) of the different sides of each sample were evaluated during the 35 day long cold and shelf-life storage period. The normalized R, B, and G values were calculated upon the intensity of R or B or G value divided by the sum of the R, B and G values.

Maturity related changes of photosynthetic activity, integrity and efficiency of photosystem II (PSII) were characterized by the measurement of chlorophyll fluorescence parameters. F0 (dark fluorescence signal), Fm (maximum dark fluorescence signal) and Fv (variable fluorescence Fv = Fm-F0) parameters at the above mentioned two points of each apple side were measured by a PAM WinControl-3 controlled MONI-PAM multi-channel chlorophyll fluorometer (Heinz Walz GmbH, Germany). The calculated index of Fv/Fm reflects the potential maximum photon yield of photochemistry, i.e. the maximum photochemical efficiency. It is a valuable tool to determine both photosynthetic capacity and stability in connection with chlorophyll degradation related postharvest maturation.

In order to characterize the change in fruit surface color or tissue related chlorophyll content, the DA index® was measured by a FRM01-F type Vis/NIR DA-meter® (Sinteleia s.r.l., Italy) on every two measurement points per side of each apple. Index of absorbance difference (IAD = A670nm-A720nm) is calculated upon the absorbance difference between 670 nm (near the Chl-a absorption peak) and 720 nm (background of the spectrum). The DA index® (or IAD) is proportional to the amount of chlorophyll present in the fruit and varies from 0 to 5 (Ziosi et al. [15]). For the calculation of DA index®, back light luminosity value (BK), Red light value (RED) and IR light value (IR) were also measured automatically by the device.

Textural changes were measured by a purpose-built laboratory device using the acoustic impulse response technique. Samples were tapped lightly on the equator with a wooden stick. The apples’ acoustic response was collected by microphone located under the cushioning sample holder. The microphone’s output was recorded by a sound card in a PC-compatible computer. Custom Fast Fourier Transform software was used to analyze the recorded acoustic response. From the resulting frequency spectrum, the characteristic frequency was selected. The characteristic frequency and the sample mass were used to calculate the acoustic stiffness coefficient [5, 16] by the equation below (Equation 1), where f is the characteristic frequency of the sample (Hz), m is the sample mass (g) measured with a high precision balance:

\[
S = f^2 m \times 10^{-6} \text{[N/mm]} \quad (1)
\]

Data were converted by means of routines in MS-Excel and were analyzed using the SPSS for Windows ver. 14. Statistical analysis was performed at 95 % significance level (in figures marked with 95 % CI).

3. Results and discussion

During the postharvest storage the firmness, the mass and the color changes of the apple samples were observed. Figure 1 shows the mass loss changes of the samples in the two different storage temperatures. It can be seen, that the mass loss of the 2°C stored apples was only 3% during the 35 days long storage period. Compared to that, the 20°C stored apple’s mass loss was more than 10% with significant difference from the 4th day.

During the storage the mass loss and the firmness changed together. Figure 2 represents the changes of the acoustic stiffness coefficient. The trend is similar to that of mass loss changes versus time. Here also significant difference was found between the two groups from the 4th day.

In contrast to that, in case of the 20°C stored samples, the degree of the decrease in the acoustic stiffness coefficient after the 25th day was not as...
significant compared to the mass loss changes. It is worth evaluating the relationship between the mass loss and the relative acoustic stiffness coefficient (Figure 3). Based on the observed changes, it can be concluded, that the acoustic stiffness coefficient is much more sensitive parameter compared to the mass loss (note the change of the relative acoustic stiffness: it was more than 80 %, and the mass loss changes was about 15 % during the 35 days).

The acoustic stiffness coefficient’s change also clearly and objectively showed the significant difference between the cold and room temperature stored samples quality decrease (tissue softening) during postharvest. Until having reached about the 4 % of mass loss, the stiffness change intensity was almost the same as the mass loss change suggesting a linear relationship (Figure 3). After this point, the stiffness change is less intensive compared to the mass change.

Figures 4-6 show the changes of the normalized R, G, B color parameter of the samples, respectively. The dominant color component was found to be the red. During maturation, the red color parameter increased, while the green and the blue parameters decreased continuously, following a logistic like trend.

In case of all three parameters, significant difference was found between the two storage conditions. Regarding the normalized color parameters, the speed of the color changes was approximately two times higher and more intensive in case of the 20 °C stored apples compared to the 2 °C stored samples. The large-scale changes were observed on the same day (after the 11th day) in case of the two groups. During the storage, the initially green samples became yellow due to the postharvest maturation process.

Figure 1. Mass loss changes of the apples stored at 2°C and 20°C.

Figure 2. Acoustic stiffness changes of the apples stored at 2°C and 20°C.

Figure 3. Relationship between mass loss and relative acoustic stiffness coefficient

Figure 4. The changes of the normalized red color parameter of the samples

Figure 5. The changes of the normalized green color
In Figure 7 the change in chlorophyll content related DA index® (IDA) values can be seen. It was about 1 at the beginning of the storage and decreased continuously during the measurement period. From the 4th day of storage, significant difference was observed between the two groups. The decrease was only about 0.2 DA value in case of 2°C sample and about 0.7 in case of 20°C stored apples. In case of 20°C stored apple it means that almost all the chlorophyll content decayed during this 35 days long storage period. It confirms that the initial green color of the samples changed to yellow. That change was also shown in case of the normalized color parameters (Figure 4-6).

Compared to the initial values, the average IR value increased during the storage (Figure 8). From the 4th day of storage, in case of this parameter significant difference was found between the two groups stored at different storage conditions too. The IR parameter of the 20°C stored samples increased to almost twice as the initial.

The changes of the chlorophyll content related Fv parameter are shown in Figure 9. During the storage, the content of the photosynthetically active chlorophyll content decreased as it was already shown earlier by the DA index® values (Figure 7).

From the 4th storage day, significant difference was observed between the two groups. The Fv value decreased from 2500 to about 2000 relative value in case of 2°C stored apples and to about 250 in case of 20°C stored samples clearly representing the negative effect of higher storage temperature on postharvest maturity changes. After the 21st day, the rate of the decrease was almost negligible in case of the 20°C stored group.
between the groups, the way of decrease showed a similar trend. In contrast to that, after the 7th day significant difference occurred in the way of the two groups behavior. The change of the Fv/Fm value of 2°C samples was only 0.1, compared to the higher change of 0.3 in case of 20°C stored samples revealing a fast and rapid maturity change at improper storage conditions.

4. Conclusions

Non-destructive measurements such as chlorophyll fluorescence analysis, DA index® measurement, acoustic stiffness measurement and computer aided machine vision system-based surface color measurement were carried out during the test period of 35 days on the Golden delicious apple samples stored at 2°C and 20°C.

According to our results shown above, all the applied non-invasive quality measurement methods were found to be suitable for the detection and follow-up of postharvest maturation related quality changes of apples stored at different storage temperatures proving the positive effect of reduced temperature on apple quality.

As a main conclusion, it can be declared that by the use of the applied methods, significant differences were determined between at cold and ambient temperature stored samples quality from the 4th day of storage in case of textural changes such as mass loss, acoustic stiffness, surface color related DA index values, and additionally from the 7th-11th day of storage in case of chlorophyll fluorescence related parameters.

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