

# NONDESTRUCTIVE DETERMINATION OF 'GOLDEN DELICIOUS' APPLE QUALITY AND HARVEST MATURITY

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## A B S T R A C T

The usefulness of nondestructive methods for measuring chlorophyll content in skin of 'Golden Delicious' apples and for evaluating quality parameters and fruit maturity using optical reflectance spectrometry was studied. The fruit weight, firmness, total soluble solids content and titratable acidity during vegetation period and short-term storage at 10°C of 'Golden Delicious' apples were measured. Additionally, internal ethylene concentration and chlorophyll *a* and chlorophyll *b* contents were analyzed as indicators of fruit maturation. The quality parameters were measured using standard destructive methods and non-destructively using Chlorophyll Content Meter CCM-200 and CP Pigment Analyzer PA1101. The results of measurement using CCM-200 were expressed as Chlorophyll Index. Spectra collected from CP PA1101 were used for calculating two indices: normalized difference vegetation index  $NDVI = (I780 - I660) / (I780 + I660)$  and normalized anthocyanin index  $NAI = (I780 - I570) / (I780 + I570)$ .

Chlorophyll content, fruit firmness, and acidity decreased during vegetative and postharvest period. The indices obtained from nondestructive measurements also decreased. Good correlations between NAI and fruit firmness, chlorophyll content and titratable acidity were noticed. The results of the experiment indicate a big potential of using NAI index for sorting 'Golden Delicious' apples according to their ripening stage (based on ethylene evolution).

**Key words:** 'Golden Delicious', chlorophyll, firmness, acidity, ripening, IEC, storage, optical reflectance, NAI, NDVI

## INTRODUCTION

Nowadays, the consumers demand high quality products, including fresh fruit and vegetables. For long-term storage of apples with good retention of their quality it is essential that fruits should be harvested at the optimal maturity (Streif, 1996). The prediction of the proper ripening stage at harvest is also essential for postharvest treatment of apples with 1-methylcyclopropen (1-MCP). The effectiveness of the treatment depends strongly on fruit maturity stage at harvest. Only apples harvested at the optimum maturity stage and treated with 1-MCP retain high quality for a long time (Lafer, 2006).

Fruit quality indices like flesh firmness, total soluble solids content and titratable acidity (all measured destructively) are commonly used as a standard procedure to determine the optimal harvest date. A number of methods for predicting the optimum harvest date for apples were listed by Blanpied (1960). There are several mathematical formulas, based on meteorological or destructive measurement's data, which are used for this purpose (Streif, 1996; de Jager and Roelofs, 1996). Sensory attributes and consumer expectations are taken into consideration as well in making decision on fruit harvest time (Molina et al., 2006; Casals et al., 2006). It has been also demonstrated that apple fruit maturation can be followed by changes of the background skin colour and the chlorophyll content in apple skin (Blanke and Notton, 1992; Faragher et al., 1984). However, the

chlorophyll content is commonly measured by destructive methods, after sample homogenization and extraction. All these destructive methods are time consuming and require a new batch of fruits for each analysis. The fruit industry needs non-destructive techniques for on-line sorting and certifying high quality fruit. Several non-destructive methods were developed to overcome this problem (Abbott, 1999; Nicolai et al. 2005). However, the precision of these methods is still not satisfactory, especially in comparison with standard destructive methods of determining quality parameters. In some cases, such as in methods based on near infrared spectroscopy, the properties measured are not well defined and the data obtained can be converted into conventionally measured parameters using calibration models (Eccher Zerbini, 2006). Zude et al. (2002) reported that the spectral optical chlorophyll determination is a promising tool for indicating the optimum harvest date of apples.

The aim of the study was to determine the usefulness of nondestructive measurement of chlorophyll content in the skin of 'Golden Delicious' by optical reflectance spectrometry as a tool for evaluating fruit quality parameters and estimating fruit maturity.

## MATERIAL AND METHODS

The experiment was done on 'Golden Delicious' apples. Samples of fruit were weekly collected from Experimental Orchard of Research Institute of Pomology and Floricul-

ture, starting on 23<sup>rd</sup> of July 2007 till 16<sup>th</sup> of October 2007. Apples harvested on 16<sup>th</sup> October were stored at +10°C till 6<sup>th</sup> of November 2007. Fruit weight, background skin colour, internal ethylene concentration (IEC), starch index, flesh firmness (FF), total soluble solids content (TSS), titratable acidity (TA) and chlorophyll *a* and chlorophyll *b* content were measured.

Weight of fruit was measured using WPS 2100/C/2 balance (Radwag, Poland). Background skin colour was measured using MiniScan XE Plus (Hunter Lab, USA). The colour parameters were expressed as an 'a\*' (green to red), 'b\*' (blue to yellow), and 'L' (black-white) indices. Starch degradation was determined using the standard iodine test and comparison with the colour chart proposed by Ctifl (France) for apples.

Flesh firmness was measured by penetrometer type EPT-1R (Kelowna, BC Canada) equipped with 11 mm probe. The results were expressed in kG.

The total soluble solids content (TSS) and titratable acidity (TA) were measured in freshly prepared juice. TSS was determined using a digital refractometer ATAGO 101 (Japan) and expressed in %. TA was determined using automatic titrator DL 50 Graphix (Mettler Toledo, Switzerland) by titration of juice with 0.1N NaOH to the end point at pH = 8.1. The results were expressed as a percentage of malic acid.

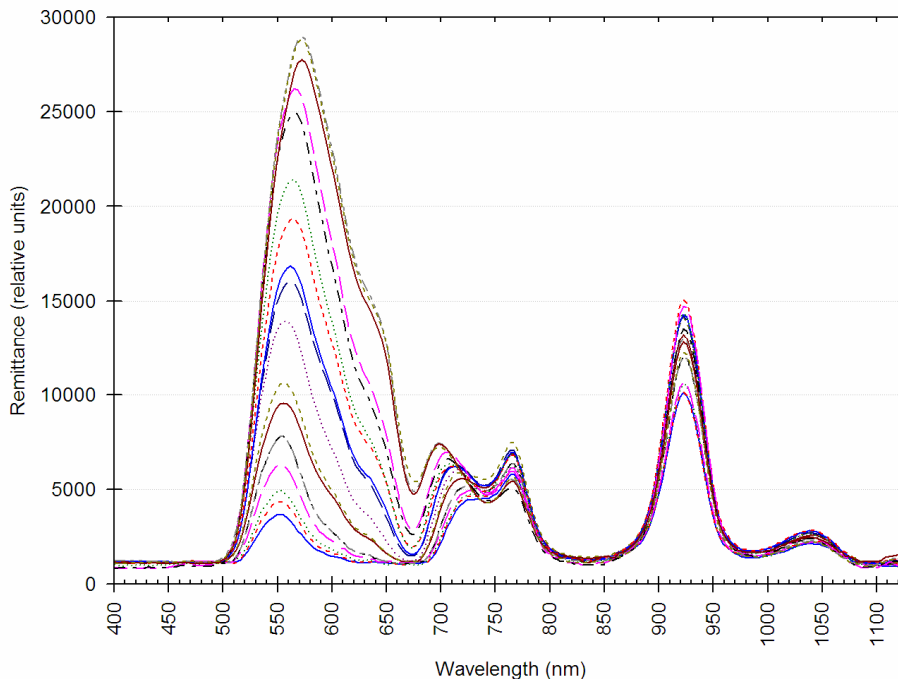
The chlorophyll *a* and *b* content in the fruit peel with app. 1 mm of the subepidermal cell layers attached (discs of 32 mm in diameter) were

measured by extracting chlorophyll pigments with methanol and spectrophotometric determination of their concentration in the solution, as described by Bruisma (1963). The results are presented in µg per gram of fresh weight. The Chlorophyll Index was measured using Chlorophyll Content Meter CCM-200 (OPTI-SCIENCE, USA). The measurements were semi non-destructive, because before measurement the discs of peel were cut and then the Chlorophyll Index was measured non-destructively.

CP Pigment Analyzer PA1101 (Produced by Control in Applied Physiology GbR., Germany) was used for non-destructive measurement of quality parameters of 'Golden Delicious' apples. According to the information given in the manual, during measurement process "radiation from the light source enters the sample matter and interacts with the texture and composition of the sample. Some radiation goes through the sample and reaches the fiber cross-section that is placed in the middle of the light cup". The raw spectra obtained from the instrument are presented on Figure 1. Using spectral data from CP, two standard indices: normalized difference vegetation index  $NDVI = (I780 - I660) / (I780 + I660)$  and normalized anthocyanin index  $NAI = (I780 - I570) / (I780 + I570)$  were calculated.

## RESULTS AND DISCUSSION

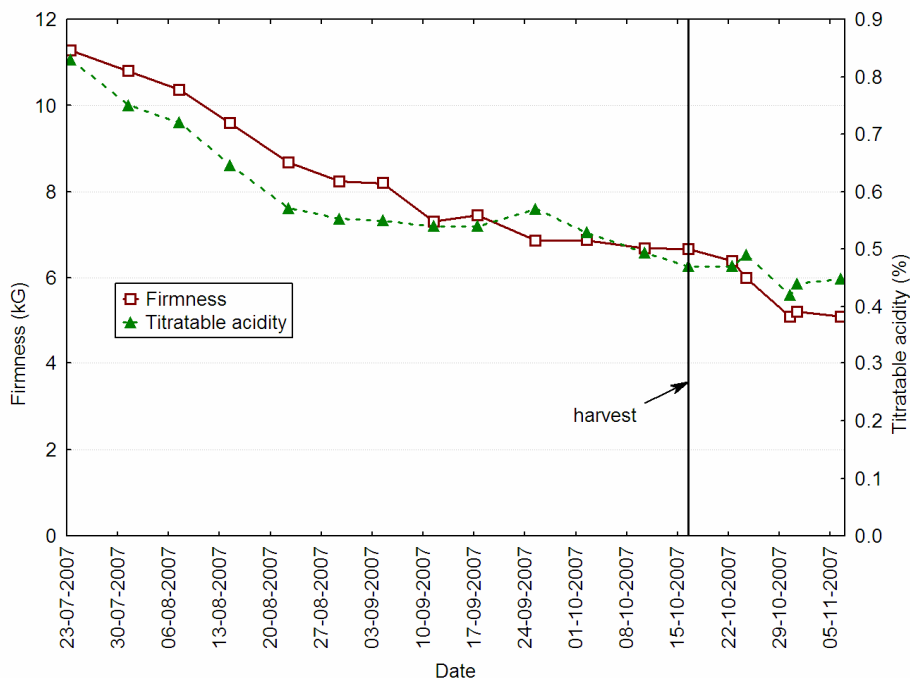
The measurements of quality parameters of 'Golden Delicious' apples during last 12 weeks before harvest showed enhanced values of



**Figure 1.** Spectra obtained from CP Pigment Analyzer PA1101 (each line represents subsequent measurement of 'Golden Delicious' apples)

fruit weight and TSS content. Fruit weight increased from an average of 60 g in July to 160 g in October, and TSS grew during this time from below 9% to above 12%. The rate of changes of both quality parameters was lower during the last month before the harvest than during the earlier period (data not presented). During the vegetative period (from 23<sup>rd</sup> of July to 16<sup>th</sup> of October) and during the ripening at 10°C both the flesh firmness and titratable acidity decreased gradually at comparable rates (Fig. 2). Fruit firmness diminished from 11.3 kG in July to 5.1 kG in November, and TA decreased from 0.83% to 0.45%. It

confirms the earlier findings that total organic acid content declines gradually during fruit maturation, ripening and storage (Olsen and Martin, 1980). However, the titratable acidity varies more between seasons and orchards than between harvest dates (Knee and Smith, 1989). For this reason, it is very difficult to set a specific value of TA as an indicator of the optimum harvest date. Similarly, absolute values of the fruit firmness cannot be used as a precise indicator of apple maturity. Although decrease of the fruit firmness during fruit maturation and ripening is well documented (Knee and Smith, 1989; Sams, 1999;

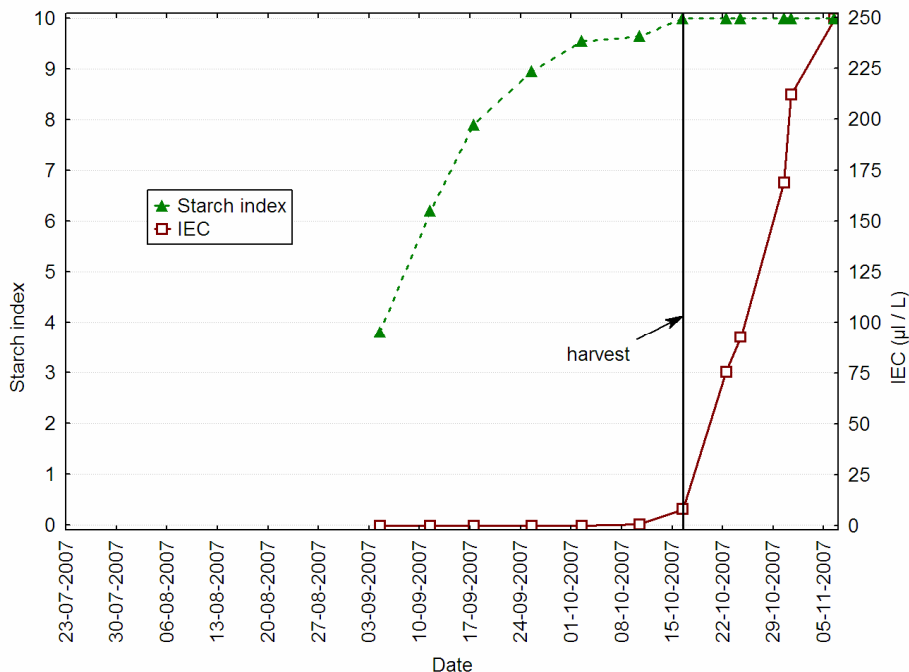


**Figure 2.** The changes of fruit firmness and titrateable acidity of 'Golden Delicious' apples during maturation and ripening

DeEll et al., 2001; Johnston et al., 2002), the high variability between orchards and seasons is noticed (Blanpied and Blak, 1977). Anyway, the firmness and titrateable acidity are very important for eating quality, and should be controlled during storage. Hoehn (2001) reported that at the minimal acceptable eating quality, 'Golden Delicious' fruit should attain 12% TSS, 3.5 g L<sup>-1</sup> of titrateable acidity and firmness of at least 4.5 kG.

Fruit destined for short-term storage were harvested on 16<sup>th</sup> of October when a significant increase of IEC was noticed (Fig. 3). Starch index at this time reached value 10,

which is higher than recommended for the optimum harvest maturity for 'Golden Delicious' apples. Alegre et al. (2006) recommended starch index in the range from 5 to 7 as an optimal value for the fruit destined for storage. Also Kupferman (2003) recommended the following parameters for a long-term CA storage of 'Golden Delicious' apples: firmness 7.3 kG, starch rating 2.7 (at 1 to 6 scale), TSS at 11.5% and acidity of 0.7%. Our results are not in contrast with the above mentioned recommendations because fruit harvested after onset of the ethylene climacteric are not suitable for the long term storage (Streif, 1996).

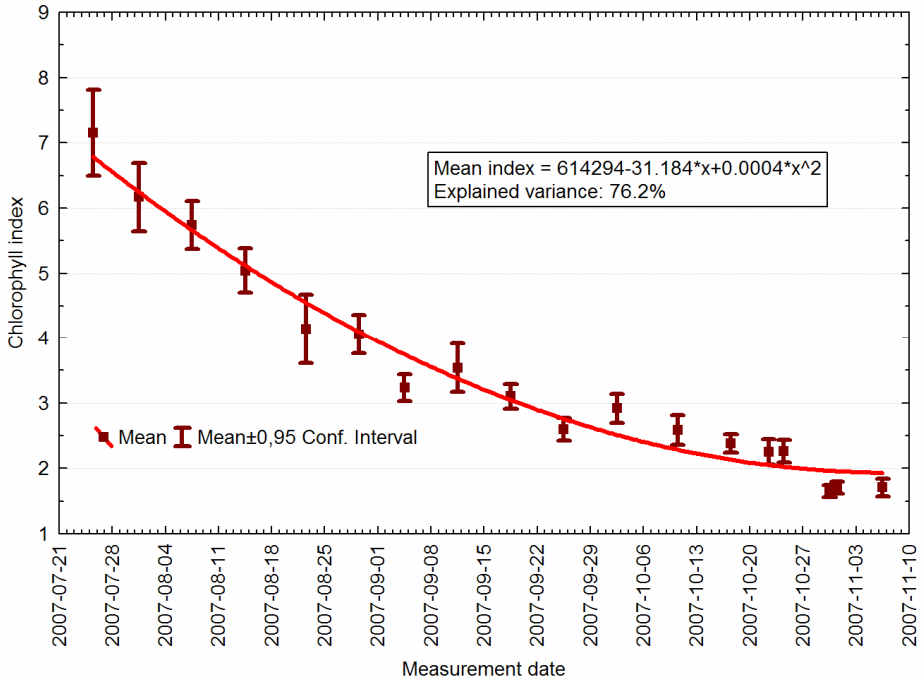


**Figure 3.** Starch index and internal ethylene concentration (IEC) in ‘Golden Delicious’ apples during maturation and ripening

During the maturation and ripening a steady decrease of chlorophyll content in the apple peel was observed. The chlorophyll *a* content decreased from ca.  $25 \mu\text{g g}^{-1}$  F.W. in July to  $3.5 \mu\text{g g}^{-1}$  F.W. in November and chlorophyll *b* content decreased from ca.  $10 \mu\text{g g}^{-1}$  F.W. to  $1.5 \mu\text{g g}^{-1}$  F.W. It well corresponds with results presented by Kuckenberget al. (2008) who found that after harvest and during four weeks of the shelf-life study the chlorophyll content in the skin of ‘Golden Delicious’ apples decreased from  $5.2$  to  $1.1 \mu\text{g cm}^{-2}$  on the shaded side of the fruit. The values of chlorophyll index (CI),

measured semi non-destructively (the discs of peel were cut off before measurement) also showed steady decrease (Fig. 4). Despite the fact that originally the instrument was developed for measuring chlorophyll content in leaves, the results of our study indicate that the Chlorophyll Content Meter CCM-200 can be used for a fast but still destructive estimation of chlorophyll status in the fruit peel as well.

Data of colour measurements show an increase of all measured parameters (L, *a*\* and *b*\*). The L value changed from 59.5 in July to 66.7 at harvest and finally to 70.5 after

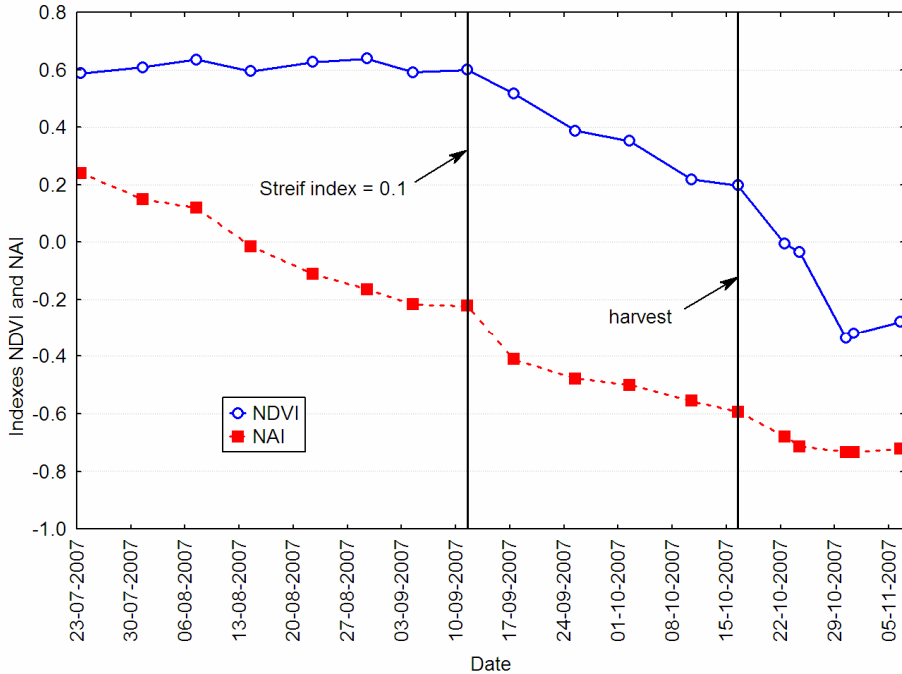


**Figure 4.** The changes of Chlorophyll Index in 'Golden Delicious' apple skin

storage. The changes of  $a^*$  value were from -10.5 through -7.6 to -2.4 and  $b^*$  value from 38.2 through 46.6 to 56.5, respectively. The changes of skin colour (yellowing) are caused by chlorophyll degradation. During fruit development and maturation both chlorophyll breakdown and synthesis is observed, but following maturation and ripening processes the decrease of the chlorophyll content is noticed, which results in decreasing intensity of green coloration (Kingston, 1992). The changes of chlorophyll content may be used as an indicator of harvest date (Knee et al., 1989). However, a decrease of green colour intensity due to the chlorophyll

degradation would be masked by the blush colour in red cultivars, thus the resulting colour would not provide dependable data for estimating the background chlorophyll decrease.

During vegetative period and after harvest the indexes NAI and NDVI, calculated from spectra gathered with the use of CP Pigment Analyzer PA1101, decreased (Fig. 5). The value of NDVI index was stable till 11<sup>th</sup> of September, and later gradually decreased. Streif's index at 0.1, which was calculated for fruits harvested on 11<sup>th</sup> of September, is considered to be optimal for 'Golden Delicious' fruit destined for long-term storage (Streif, 1996; Skrzynski,

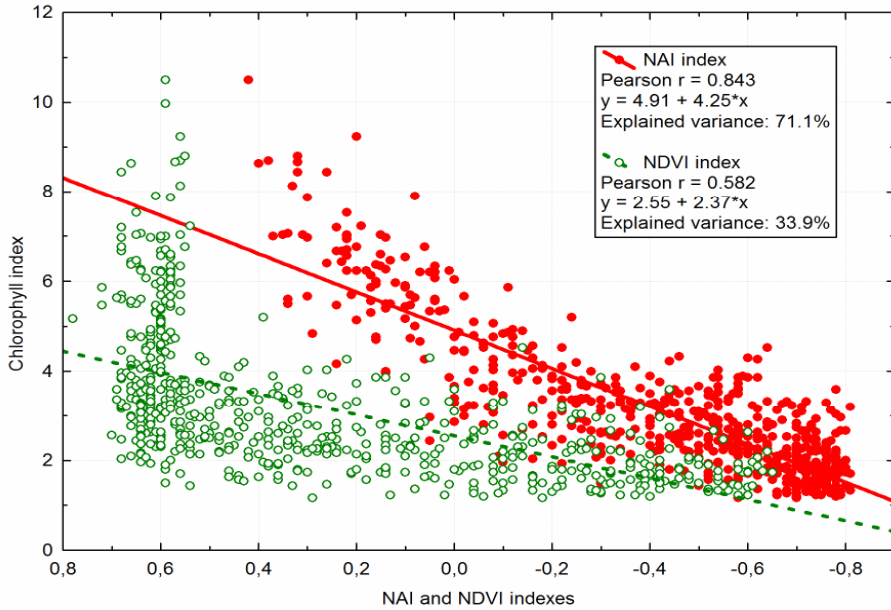


**Figure 5.** The changes of NDVI and NAI indexes during ‘Golden Delicious’ fruit maturation and ripening

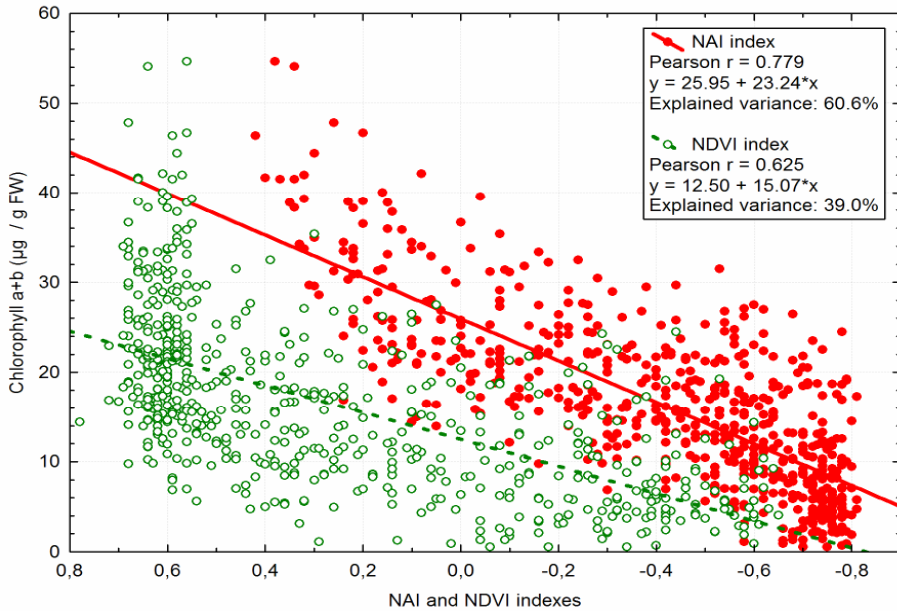
1996). In our study, the mean value of Streif’s Index in fruit harvested on 16<sup>th</sup> October reached 0.06. The values of NAI index gradually decreased during the whole observation period. However, before the Streif’s Index reached value 0.1, the plateau was observed (Fig. 5). There was a low correlation between NAI and NDVI indexes and starch index in the harvested apples (data not shown). Data presented on Fig. 6 show a better correlation between NAI index than NDVI index and Chlorophyll Index gathered with the use of CCM-200 instrument. Also, the data presented on Fig. 7 indicate a good correlation

between NAI index and the chlorophyll content measured destructively. It confirms data presented by Kuckenberg et al. (2008) who reported that, in ‘Golden Delicious’ apples, NAI index showed a close correlation with chlorophyll content independently of fruit side (sunny and shaded). Kuckenberg et al. (2008) also reported a good correlation between NDVI index and chlorophyll content in skin of ‘Golden Delicious’ apples. Our study indicated that NDVI index is less useful than NAI for estimating chlorophyll content. Anyway, our results confirm earlier findings that the changes in the

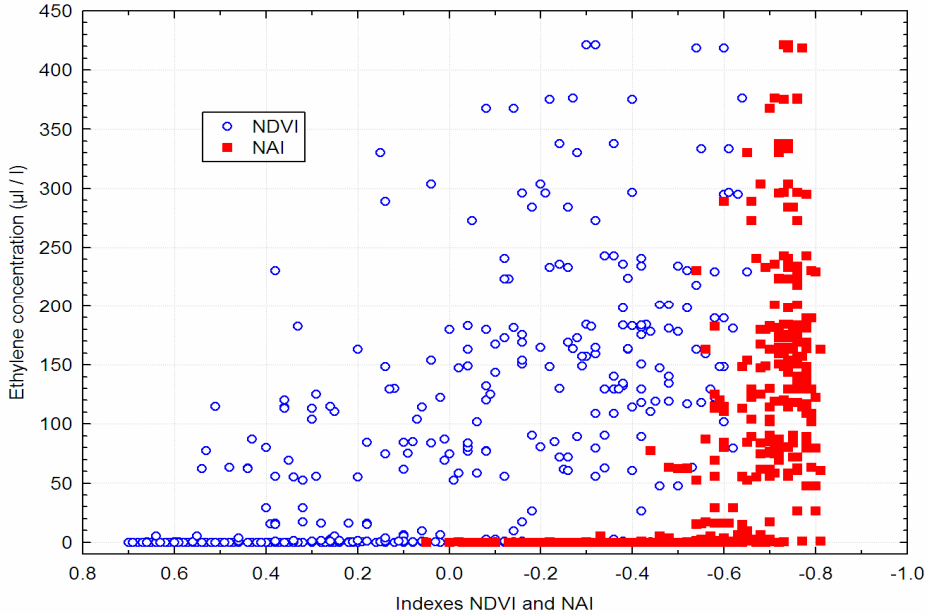




**Figure 6.** The relationship between NAI and NDVI indexes and Chlorophyll Index in 'Golden Delicious' apples



**Figure 7.** The relationship between NAI and NDVI indexes and chlorophyll  $a+b$  content in 'Golden Delicious' apple skin



**Figure 8.** The relationship between NAI and NDVI indexes and IEC in ‘Golden Delicious’ apples

chlorophyll content can be measured using spectral analysis (Merzlyak et al., 1999; Zude and Herold, 2002; Zude, 2003).

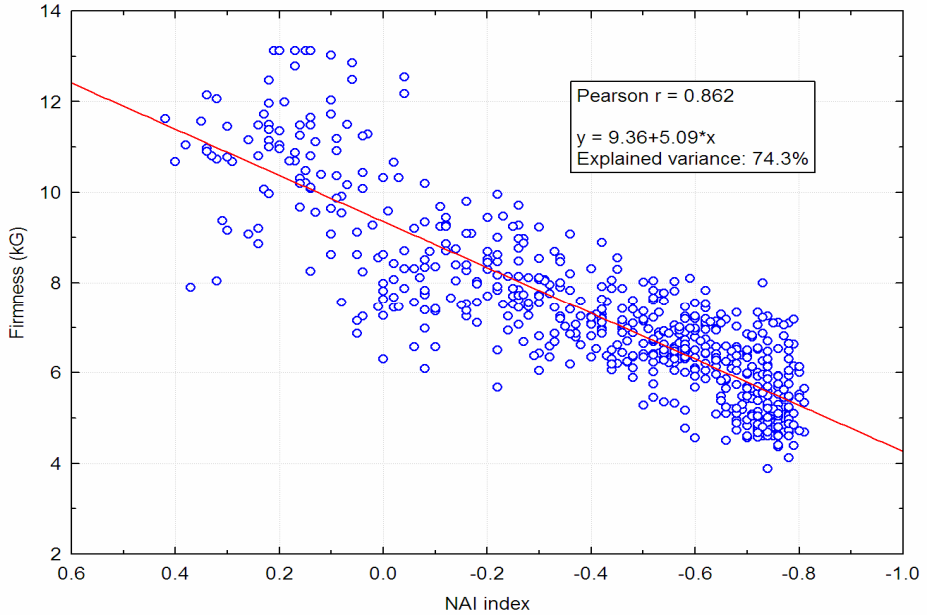
Data presented on Fig. 8 indicate that NDVI index is not useful for predicting IEC in the apple core. On the contrary, NAI index could be used for fruit grading according to the IEC level. Index higher than -0.4 indicate immature fruit (pre-climacteric), index between -0.4 and -0.6 characterise fruits with the onset of ethylene production while index below -0.8 is representative for fruits with more advanced ripening stage (ripe fruits). The determination of IEC seems to be not reliable enough for the selection of proper harvest time for commercial purpose (Streif,

1996). However, Luton (1996) reported that IEC is well correlated with the estimated harvest date for ‘Cox’s Orange Pippin’ apples.

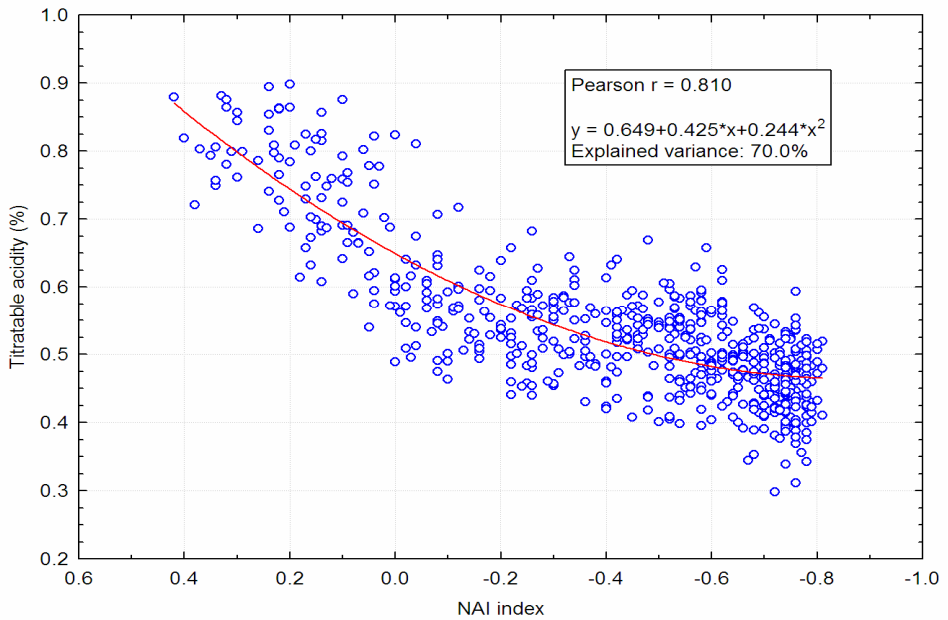
Our results indicate a good correlation between NAI and fruit firmness (Fig. 9) as well as between NAI and titratable acidity (Fig. 10). Also Kuckenberget al. (2008) reported that NAI index is strongly correlated with fruit firmness (Pearson’s correlation coefficient 0.7), but these authors concluded that it seemed to be insufficient for a reliable, non-destructive estimation of fruit firmness.

In general, contrary to Kuckenberget al. (2008) findings, in our study NDVI index was less useful than NAI index for estimating apple maturity and quality parameters.

## Nondestructive determination of 'Golden Delicious' apple quality



**Figure 9.** The relationship between NAI index and fruit firmness of 'Golden Delicious' apples



**Figure 10.** The relationship between NAI index and titratable acidity of 'Golden Delicious' apples

## CONCLUSION

The quality parameters of 'Golden Delicious' apples could be estimated using NAI index calculated from the spectra obtained with the use of CP Pigment Analyzer. Further experiments are needed to build and validate the model for predicting TSS, acidity and fruit firmness. The results of the presented experiment indicate a big potential of using NAI index for sorting of 'Golden Delicious' apples according to their ripening stage, based on ethylene evolution. It could be a good tool for fruit separation for different storage technologies (regular, CA, and ULO conditions) and 1-MCP treatment.

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## NIEDESTRUKCYJNE OKREŚLANIE JAKOŚCI I DOJRZAŁOŚCI JABŁEK 'GOLDEN DELICIOUS'

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### S T R E S Z C Z E N I E

W doświadczeniu oceniano przydatność dwóch urządzeń (miernik zawartości chlorofilu CCM-200 oraz analizator barwników-spektrofotometr CP Pigment Analyzer PA1101) do niedestrukcyjnego pomiaru zawartości chlorofilu w skórce owoców, określania cech jakościowych i dojrzałości jabłek 'Golden Delicious'. Podczas sezonu wegetacyjnego i krótkotrwałego przechowywania w +10°C, wykorzystując standardowe metody, mierzono masę owoców, stężenie etylenu w komorach nasiennych, jędrność, zawartość ekstraktu, kwasowość oraz zawartość chlorofilu a i b w skórce owoców. Na podstawie wartości sygnału odebranego przez CPPA1101 obliczono dwa znormalizowane indeksy NDVI (wegetacyjny) i NAI (antocyjanowy), wykorzystując odpowiednio wzory  $NDVI = (I_{780} - I_{660}) / (I_{780} + I_{660})$   $NAI = (I_{780} - I_{570}) / (I_{780} + I_{570})$ , gdzie  $I$  – wartość sygnału dla długości fal 780 nm, 660 nm i 570 nm.

Zawartość chlorofilu, jędrność owoców i ich kwasowość, a także wartości indeksów wyznaczonych na podstawie pomiarów niedestrukcyjnych ulegają spadkowi, zarówno w czasie sezonu wegetacyjnego, jak i w czasie przechowywania. Zanotowano dobrą korelację pomiędzy indeksem NAI a jędrnością owoców, kwasowością oraz zawartością chlorofilu. Wyniki przeprowadzonych analiz wskazują na potencjalne możliwości wykorzystania indeksu NAI do sortowania jabłek 'Golden Delicious' pod kątem ich dojrzałości podczas zbioru (biorąc pod uwagę zawartość etylenu w komorach nasiennych).

**Słowa kluczowe:** 'Golden Delicious', chlorofil, jędrność, kwasowość, dojrzałość, IEC, przechowywanie, NAI i NDVI