Spectral evaluation of apple fruit ripening and pigment content alteration

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Abstract

The aim of this study was to study spectral-based investigation methods of apple fruit quality parameters, for the evaluation of ripeness, water content, and quality monitoring of certain species by rapid, non-invasive way.

The research site is an intensive apple orchard with microirrigation, which located in North East part of Hungary. Considering ripening characteristics, two antocyanin containing (Gala Galaxy, Gala Must) and two antocianin free (Early Gold, Golden Reinders) apple species were studied. Based on the ripening process of the fruits of the four apple species, spectral, chlorophyll, carotenoid and moisture changes were investigated measuring both the properties of the skin and the pulp of the fruits.

Conforming to the results, carotenoid-chlorophyll ratio is appropriate for the characterization of ripening process in antocianin free species. Based on the spectral data, 678 nm wavelength is sensitive for low chlorophyll content, therefore RED interval (678nm) is suitable for examining the ripeness and ripening process. Though reflectances at 678nm ±30 nm wavelength showed greater variability for high chlorophyll content, therefore reflectance, measured on 700 nm can be applicable for monitoring the early stage of ripening and the pigment content changes. New kind of ripening monitoring spectral index were set up, which are feasible for surveying pigment changes within ripening process of antocianin free species.

The measurement of water-content and the WBI cannot be suitable for monitoring the ripening, but it can be applied for surveying the moisture content changes during the storage.

KEYWORDS: apple, chlorophyll, carotenoid, moisture content, spectral properties of fruit

1. Introduction

The fruit quality can be evaluated with many parameters, such as organoleptic properties (colour, shape, texture, flavor and aroma), nutritive value, as well as various chemical and physical characteristics (Lurie 2008). Typically, a large amount of pigment accumulates in the thick layer of the apple fruit parenchyma which strongly scatters the light. As a result, the physiological status and the quality of the fruit can be assessed based on the species specific reflection spectrum. The content of chlorophylls, carotenoids and anthocyanins as well as their proportions determine fruit color and appearance (Saure, 1990; Abbott, 1999; Solovchenko et al., 2005) and serve as markers of quality. Pigment changes occur during ripening, storage and as a result of various stresses (Knee, 1972, 1988; Merzlyak et al., 1997, 1999; Abbott, 1999; Merzlyak and Chivkunova, 2000; Merzlyak et al., 2003). The level of carotenoids, flavonoids and anthocyanins are further of importance because these compounds possess antioxidant properties hence they have beneficial effects on human health (Russo et al.. 2000).

Color is traditionally measured by destructive techniques, such as isolation and quantitation of pigments, but in recent decades, spectral based methods have been applied successfully for testing fruit quality parameters (Knee, 1980; Zude et al., 2006). The spectral -based, non-destructive technology has several advantages over conventional, destructive and alternate non-destructive methods. Simplicity, sensitivity, reliability of the method and its high performance are the most important advantages. Significant technological development has been started in apple fruit quality (ripeness, deterioration) monitoring tests, especially after the portable optical radiometer was placed on the market with which reliable spectral data can be supplied from a small fruit surface. Information can be obtained with the help of optical methods from the fruits on the basis of their reflective properties in the visible and near-infrared (NIR) wavelength ranges. The fruit reflectance depends on the pigment content (composition, localization), the cuticle, and the internal optical properties of the fruit (tissue structure, water content, and other factors) (Gitelson et al., 2003). Pigment sensitivity of the different spectral bands are often examined. Evaluation of fruit quality with modern equipment is relatively simple and it does not require expensive and complicated settings (Geyer et al., 2007). In recent years highly reliable spectral methods are used in fruit sorting and grading, and in the field of "precision agriculture "(Gitelson et al., 2003).

2. Materials and Methods

The aim of this research is the spectral evaluation of apple fruit quality parameters, with which the water content, quality monitoring and fruit ripeness of certain species can be assessed in rapid, non-invasive way.

In order to achieve this goal, not only the spectral profiles of the fruit skin and flesh samples were measured, but also conventional gravimetric method for water content and destructive chlorophyll and carotenoid measurements were carried out as a calibration for the spectral features. The principal component analysis and bivariate correlation statistical methods were used to select that wavelength which relates to water or chlorophyll content. Tukey variance analysis was used to determine the differences between different water and chlorophyll content and detect spectral differences.

Field sampling and field studies were carried out an intensive apple orchards with micro-irrigation system at Farm and Regional Research Institute of the University of Debrecen, located on the Northen part of Hungary. The spectral sampling and data processing were made at the Institute of Water and Environmental Management, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. Taking into account the ripening characteristics of the many available apple varieties, two anthocyanin -free, i.e., yellow fruit types (Early Gold and Golden Reinders) and two red fruit types containing anthocyanins (Gala Galaxy and Gala Must) were examined (Figure 1).

Figure 1. Golden Reinders and Gala Must apple at the end of July

Two individuals by each varieties, individually 4 pieces of healthy apple fruit were taken so the number of fruit sample was 8 pieces of each apple variety in a certain sampling period. Samplings were made four times during the ripening process, in 20 to 25 day steps from the beginning of July 2013 until complete ripeness. During the fruit ripening process, moisture carotenoid and chlorophyll content and spectral changes were examined in the the skin and the pulp four apple varieties.

In order to spectrally identify the maturity status of the fruits, moisture and pigment sensitive wavelength ranges were identified based on the selection spectral channels with high reflectanve variability. Based on their chlorophyll content fruits were separated into 4 maturity groups for each apple variety. The first and second groups represent the mean spectral properties of unripe fruits, the third group refers to the mean reflectance of medium ripe fruits, and the fourth group shows the mean spectral characteristics of ripe fruits.

Standard deviation curves of spectral profiles were also calculated to evaluate the effect of the pigment content on reflectance. First, reflectance curves were grouped based on increasing chlorophyll content, then the standard deviation curves of each group were calculated. 0-7 μg/g; 0-25 μg/g; 0-30 μg/g chlorophyll content groups were set for red fruits, and 0-10 μg/g; 0-15 μg/g; 0-20 μg/g; 0-25 μg/g; 0-40 μg/g for yellow fruit varieties.

The spectral profiles (reflectance) were measured by laboratory scale AvaSpec 2048 spectrometer at 400 – 1000 nm wavelength interval with 0.6 nm spectral resolution. The AvaSpec 2048 system consists of one spectrometer, AvaLight-HAL halogen light source which are joined by a fibre optic with 8 μm diameter and a self-innovated special sampling box in order to provide dark for measurements.

The halogen light source provides constant intensity of light emission on 400-1000 nm. The special sampling box is used to isolate samples from the permanently changing irradiation of other light sources (e.g. light bulb or neon or fluorescent lamp).

After spectral sampling, the clorophyll, carotenoid content of fruit skin and moisture content of fruit was determined. The moisture content of the collected fresh fruit samples were measured gravimetrically.

To determine the total chlorophyll and carotenoid content of the fruits fresh skin samples were taken. The weight of fruit skin samples were about 100 mg, samples were destructed by 10 ml acetone for extraction and 1 g quartz sand for homogeneity. After extraction the suspensions were centrifuged at 3000 rev/min for 3 min in Hettich ROTOFIX 32A, and the clean solution was placed to 2.5 ml quartz cuvette. The absorbance of the solution was measured by SECOMAN Anthelie Light II. UV-VIS spectrophotometer at 470, 644 and 663 nm wavelength. Based on the absorbances chlorophyll content was calculated by the followings (Droppa et al., 2003):

Chlorophyll (a+b) μg/g fresh weight=(20,2*A644+8,02*A663)* V/w, where: V = volume of tissue extract (ml), w = fresh weight of tissue (g), A = absorbance.

The carotenoid content was calculated by the following equotation (Lichenthaler and Wellburn, 1983)

Carotenoid μg/g fresh weight =

$(1000*A_{470nm}–3.27\ (12.21*A_{663nm}–2.81*A_{644nm})–104*(20.13A_{644nm}–5.03A_{663nm}))/229$

According to Merzlyak et al. (1999) two kinds of Plant Senescence Reflectance Index (PSRI) and Browning Reflectance Index (BRI) were calculated for better comparison and identification of the spectral changes during the ripening period:

$$PSRI = \frac{\rho_{678} - \rho_{500}}{\rho_{750}}$$

$$PSRI_{480} = \frac{\rho_{678} - \rho_{480}}{\rho_{750}}$$

$$BRI = \frac{1/\rho_{550} - 1/\rho_{700}}{\rho_{750}}$$

The Water Band Index (WBI) is also calculated. WBI is a reflectance measurement that is sensitive to changes in canopy water status. As the water content of vegetation canopies increase, the strength of the absorption around 970 nm increases relative to that of 900 nm. WBI is defined by the following equitation (Champagne et al. 2001):

$$WBI = \rho_{900} / \rho_{970}.$$

The principal component analysis and bivariate correlation statistical methods were used to select that wavelength which relates to water or pigment content. Based on the selected wavelength ranges, new ripening monitoring indices were set up. Linear regression were performed between vegetation indices and pigment content as well as between the Water Band Idices and fruit water content in order to identify spectral based pigment and water content estimation possibilities. Tukey variance analysis and Student's t-test was used to determine the differences between different water and pigment content and detect spectral differences in ripening process (Based on Kolmogorov-Smirnov test variables had normal distribution.). Statistical evaluation was performed by SPSS 17.0 software.

3. Results and discussions

3.1. Skin pigment content

During ripening, the chlorophyll concentration is decreasing. The chlorophyll concentration of anthocyanin-free variaties (Early Gold and Golden Reinders) were 31,1±7,07 μg/g in green, un-ripe state (02 July),while there was 7,73±2,81 μg/g in ripe state (Sept. 10),and it continually decreased during the ripenning. The decrease in red skinned Gála varieties could have been detected as well. Chlorophyll concentration was 19.91 ± 10.54 μg/g in unripe state and 4.99 ± 1.65 μg/g in ripe state. The chlorophyll concentration for red fruit apple varieties were smaller than the green-yellow apple fruit varieties in each measurement time. During fruit ripening, the chlorophyll concentration reduction was significantly ($p = 0.032$) detectable only between the ripe and unripe fruits, and among the ripeness stages there were no significant differences. This was probably due to the heterogenity of the samples and the differences among the varieties. However, these differences in chlorophyll concentration were declined among samples at the time of the ripening, resulting homogenous pigment concentration in ripe fruit skin samples. Paralell to the chlorophyll concentration, the carotenoid concentration of fruit are continuously decreasing (Figure 2.).

Figure 2. Correlation between carotenoid and chlorophyll content of apple's skin in the concerned sampling dates

The carotinoid concentration of the anthocyanin-free varieties were 870 ± 358 μg/g in the case of unripe fruits, and 340 ± 39.5 μg/g in the case of ripe fruits, in the case Gala varieties the decrease in carotinoid was also detectable (unripe: 517 ± 206 μg/g; ripe: 358 ± 70 6 μg/g). However, the carotenoid concentration remains relatively high even in the case of low chlorophyll concentration.

During the ripening process, the remaining high carotenoid level is well characterized by the relationship between carotenoid-chlorophyll ratio (car/chl) and the chlorophyll content (Figure 3). As it was described in more detailed in the research of Merzlyak et al. (2003) such a relationship between pigment contents reflects the phenomenon of carotenoid retention and/or accumulation in the progress of apple fruit ripening. The findings showed the time of fruit ripeness is determined by physiological state, which fruit has attined by date of harvest, but not the harvest date per se. At the same time Car/chl ratio should be used for characterization the ripening process in apple rater that the content of each of the pigment alone (Solovchenko et al., 2005)

Figure 3. Correlation between carotenoid and chlorophyll ratio and chlorophyll content

3.2. Spectral properties of the apple skin

Changes in fruit coloration, thus the differences in the spectral properties of apple skin are demonstrating well the significant changes in pigment (chlorophyll and carotenoid) content and their compounds in fruits during ripening process (Figure 4).

Figure 4. Changes of the fruit skin reflectance properties of Golden Reinders and Gala Galaxy within the period of fruit ripening (Concentration related to content of chlorophyll)

Low chlorophyll-containing fruits (both in the case of yelow and red fruit varieties) had high reflectance (65-80%) in the range of chlorophyll absorption, between 600 nm and 700 nm, whereas considerable spectral characteristics were not observed in the NIR range with high reflectance values. Decrease in reflectance (also for unripe fruits) was observed in the range of 900 to 970 nm, which is realized not the pigment content but it relates to the moisture content. It also reveals that the low pigment concentration, which is difficult to define analytically, it can be separated as a depression in the reflectance spectrum of the chlorophyll and carotenoid absorption zones. Due to the fruit ripening, the pigment concentration is decreasing, thus reflectance spectrum is successively flattening (Figure 4).

Due to the high carotenoids and chlorophyll content, the unripe fruits resulted a high absorption and low reflectance in the red and blue color range for all investigated varieties. Both the absorption site of the chlorophyll a (on the 678 nm visible reflectance minimum) and chlorophyll b (like a shoulder shape figure close to 650 nm ranges) can be well recognized. As shown in the diagram, the ripe fruit with low chlorophyll content indicated a high reflectance at 678 nm. With the increase in chlorophyll content, the reflectance underwent a marked decline over 7-10 μg/g chlorophyll concentration, and beside higher chlorophyll concentration, its values has not changed significantly for the effect of further increase in concentration. This was explained with the effect of the cuticle and the epidermis on reflectance by numerous researchers (BATT and MARTIN 1960; SOLOVCHENKO and MERZLYAK 2003). Both the combined absorption of chlorophyll b and carotenoids explain the storng absorption appearing in the blue range (MERZLYAK and CHIVKUNOVA 2000). Among the high chlorophyll concentrations reflectance data (at 678 nm) was not provable statistical difference in the cases of anthocyanin-free fruits. This indicates that 678 nm wavelength is less sensitive to the high chlorophyll values but it can be well characterized the ripeness. On the other hand, significant variability were showed in the reflectance wavelenght ranges located from 678 nm to ± 30 nm, which can be applied for monitoring the early stage of fruit ripening. During ripening for low chlorophyll concentration (kl <10 μg/g), the curve peak flattens and it tops among 540-600 nm. However, according to Merzlyak et al. (2003), during the ripening, the anthocyanin levels are growing, which are responsible for the red coloured pigment in the case of red skinned apple varieties. These are greatly affected the reflectance of ripen Gala varieties group. The ripen fruit is peculiarity to have high reflectance (75-80%), in the red range whilst it is low (below 15%) in the green and blue range (previous Figure). For the group of Gala varieties, significant changes were not observed among the red and the NIR reflectance values either. This could be explained by the effect of anthocyanin pigment which has a dominant role in the reflectance features (Merzlyak, 2003). For further investigation of the spectral characteristics of the fruits, the reflectance data were divided into several groups by the chlorophyll content. From the yellowing apple varieties, three- and from the reddening apple varieties five groups were prepared (Figure 5).

Figure 5. Changes of the standard deviation of fruit skin reflectance of Golden Reinders and Gala Galaxy within the period of fruit ripening in case of different chlorophyll concentrations

The first group showed standard deviation of the low chlorophyll containing reflectance data and the last one contained the reflectance data of all fruits for the yellowing varieties between 0-40 μg/g, and for the Gala groups between 0-35 μg/g chlorophyll-content. Independently from the pigment concent, a very low standard deviation were observed for both fruit types in the blue range. Due to the chlorophyll absorption characteristics of the red color range, a peak was realized at 680 nm. Standard deviation of reflectance values of certain sample groups with midle and high chlorophyll content at 680 nm were confirmed the previously assumed that this wavelenght-range for the low chlorophyll content was sensitive. As the chlorophyll content is growing, proportionally extening the measured standard deviation refelectance at 680 nm, especially in the direction of the yellow color range. The maximum reflectance NIR range shows low variability, so the ripenning in itself cannot be applied for monitoring the pigment content changes. Based on the standard deviation changes between the range of 550-600 nm and the 700-nm wavelength range of the yellowing apple varieties data were particularly pigment sensitive. Nevertheles, in case of low chlorophyll concentration, a lower peak can be observed at 510 nm, which may be sensitive to change of carotenoid content. This sensitivity decreases with the increaseing of chlorophyll content, and the peak becomes smooth. The reason is that the absorption enhances parallel with the increasing chlorophyll content. So, as the chlorophyll content increases, the spectral properties disappear. This is determined by ZUR et al. (2000) who had similar results given by examining the pigment content changes in tree leaves. This range can be useful to determine the ripening stage as well. It cannot be observed the peak at the afore-mentioned, measured 510 nm for the Gala varieties group. Significant difference can be measured at the 550 nm range but the spectral characteristics will be disappeared with the appearance of anthocyanin (parallel with the decrease in chlorophyll content). Correlation tests were performed between the reflectance, and the chlorophyll and carotenoid concentration. According to the results of the correlation tests, the maximum reflectance NIR range with low variability are not useful for monitoring the changes in pigment content (Figure 6).

Figure 6. Correlation between chlorophyll content and reflectance measured at RED NIR wavelengths

In the case of examined apple varieties, the total chlorophyll and carotenoid levels are decreasing in the full ripening stage. This decrease can be well followed spectrally for the anthocyanin-free apple fruit both the sensitive ranges of chlorophyll and carotenoids in the 500 to 530 nm range (Figure 7).

Figure 7. Correlation between carotenoid content and reflectance measured at BLUE and GREEN wavelengths

Specially, the range at 510 nm is sensitive to the carotenoid content. This occurrence is confirmed by the results of the correlation test between the chlorophyll content and the normalized reflectance spectra (Figure 8).

Figure 8. Correlation between chlorophyll content and reflectance data of Golden Reinders and Gala Galaxy species

Regardless of the chlorophyll content, the spectral characteristics of each fruits of the yellowing and redding apple varieties were used in the study. In case of both the yellowing and the reddening varieties, very low correlation can be measured in the NIR range (from 720 nm to 1000 nm). However, significant difference are detected in the visible range. Maximum correlation (r^2 = 0.7-0.9) was measured for the anthocyanin-free varieties in the orange and red range (590 to 700 nm). In the case of yellowing apple varieties, two peaks can be observed in the 430-440 nm and 460-470 nm blue range, which were indicated the absorption properties of the chlorophyll a and b. However, these two peaks represent smaller r^2 value than the 600-700 nm range. Below 430 nm, the correlation is strongly reduced. In the case of anthocyanins containing varieties, maximum (r^2=0.5-0.65) can have been detected in the wavelength range between 640-690 nm. The rapid decline of the r^2 can be observed below 640 nm. The measured low correlation between the reflectance and chlorophyll content shows that this wavelength range does not contain spectral information for the reddening varieties in the range of 400 and 600 nm. Based on the correlation test, the near infrared (750 nm) and the red color range (678 nm) can be suitable for examining the chlorophyll content. However, the aforementioned reflectance measurement at 680 nm has low variability so rather the 700 nm reflectance is suggested. But the 678 nm is suitable for monitoring the ripeness because it is sensitive to the continually decreasing low chlorophyll content during the ripening. The reflectances of both the chlorophyll sensitive red range and the chlorophyll and carotinoid sensitive green range are increasing with the ripening of the Gala Must and Gala Galaxy, meanwhile the pigment content decreasing. Whereas, in the case of Gala varieties group, the effects of chlorophyll and carotenoid reflectances are greatly changing with the increasing of anthocyanin level, and the reflectance of the red range is increasing while in the green range a minimizing can be observed. This is beacaouse than the green range is the common absorption zone of the anthocyanin and chlorophyll while the red range is specifically sensitive to the amount of chlorophyll. This is proved well on the correlation with the measured reflectance between 550 and 700 nm wavelengths (Figure 9).

Figure 9. Correlation between the reflectances of antocianin containing and antocianin free apple species at 550 and 770 nm

Independently of the chlorophyll content and ripening stages, strong correlation (r^2=0.929) was measured for the yellowing apple varieties (p=0.000), but this is not be clearly detected for the red skinned fruit varieties. Confirmed this results, Merzlak et al. (2003) were made similar conclusions with examining other apple varieties.

3.3. Vegetation indices and pigment content

In order to monitoring the ripening, the applicability of the spectral indices, which have been already written in the literature review, were examined for the yellowing apple varieties. Despite being used the Browning Reflectance Index (BRI) for monitoring the browning, so deformations and injuries and other stress effects were examined well. The BRI was, among the studied three indices, which the most reliable was for describe the pigment content changes during the ripneing (Table 1).

Table 1. Correlation between carotene, chlorophyll content and spectral indices

		carotene	chlorophyll
PSRI	Pearson correlation	-0.529	-0.546
	Significance level	0.002	0.001
PSRI 480	Pearson correlation	-0.560	-0.619
	Significance level	0.001	0.000
BRI	Pearson correlation	0.710	0.716

	Significance level	0.000	0.000
NCI	Pearson correlation	-0.638	0.650
	Significance level	0.000	0.000
CRMI	Pearson correlation	-0.634	0.633
	Significance level	0.000	0.000
PRMI	Pearson correlation	0.711	0.693
	Significance level	0.000	0.000

Besides, three new vegetation indices were calculated, which are suitable for monitoring the ripening:

1. *Modifing the NDVI, Normalized Chlorophyll Index was established, which is a specified index for the chlorophyll content changes during the ripening:*

$$NCI = \frac{\lambda_{750} - \lambda_{678}}{\lambda_{750} + \lambda_{678}}$$

This index is only calculated by the measured maximum absorption of chlorophyll content in the red range.

2. Chlorophyll sensitive Ripening Monitoring Index (CRMI).

$$CRMI = \frac{\lambda_{750} - \lambda_{678}}{\lambda_{480}}$$

This index calculates with the maximum absorption of chlorophyll content measured in the blue and red ranges.

3. Pigment sensitive Ripening Monitoring Index (PRMI)

$$PRMI = \frac{\lambda_{750} - \lambda_{678}}{\lambda_{550}}$$

This index calculates with the maximum absorption of the chlorophyll content measured in the red range and with the increasingly important effect of reflectance during the carotenoid ripening.

The new indices were not resulted a breakthrough against the BRI, but especially the PRMI index can be an alternative choice of PSRI indices, which can be recommended to monitor the ripening and the related quality parameters.

3.4. Fruit moisture content

The pigment content of the pulp was not measured, only the moisture content of it. Significant differences were not detected among the moisture contents neither within the varieties nor during the ripening, nor the anthocyanins containing and nor the yellowing varieties (*Table 2.*).

Table 2. Statistical differences between water contents of fruit

Species	mean±standard deviation	date of sampling	mean±standard deviation ’	colour of the fruit	mean±standard deviation*

Golden Reinders	83.8±4.48^{a}	July 02	81.9±2.59^{a}	yellow	82,1±4,23^{a}
Early Gold	80.6±3.41^{a}	July 20	82.6±3.13^{a}	red	80,6±3,19^{a}
Gala Galaxy	80.8±3.71^{a}	Aug. 15	80.3±6.47^{a}		
Gala Must	80.5±2.84^{a}	Sept. 10	82.3±2.64^{a}		

There was no significant difference between the same numeric indices

* based on Student t-test ($p<0.05$)

’ based on analysis of variance ($p<0.05$)

Accordingly, 81.56% (± 3.9%) moisture content was typify to the studied fruits. According to results, the moisture content of the apple fruit are not influenced by the varieties, the anthocyanin content and the degree of ripeness.

3.5. Reflectance characteristics of the pulp (sarcocarp)

The pulp of the yellowing varieties shows such spectral properties as the spectral characteristics of the skin at the entire studied wavelength range (400-1000 nm) in the different stages of ripening. The spectral curves of the pulp of the reddening Gala varieties showed only the early stages of ripening were similar to those observed on the skin. The reason is that, the redness and the anthocyanin contents of the fruit appears in the skin, but not in the pulp. Therefore, the dominant effect of the anthocyanin was not observed to the spectral characteristics. As a result, the spectral characteristics of the Gala apple are similar to the pulp characteristics of the yellowing skin apple varieties (Figure 10).

Figure 10. Effect of fruit ripening of Golden Reinders and Gala Galaxy on pulp reflectance

It represented well the Pearson's correlation between the spectral curves of the ripe Gala and the yellowing varieties, which shows a strong correlation (r=0.89 at p=0.002).

The water content of the pulp was measured as a quality parameter. Therefore the NIR range, which correlates with the moisture content, was examined in detail. The spectral properties of the fruit, against the foliage, allow the water band index (WBI) calculation. The theoretical background of the WBI calculation that increasing in moisture content, greater absorbance is measured at 970 nm, then at 900 nm (Figure 11).

Figure 11. Water content of orchards of Golden Reinders and Gala Galaxy (percentage means water content of apple)

Low correlation can be observed between the moisture content and the reflectance (Figure 12), which is particularly true in the 400-690 nm range, as well as in a narrow wavelength range, about the 930 nm.

Figure 12. Correlation between water content and reflectance

The average r^2 value is 0.15, between 700 and 810 nm wavelenght in the RED-NIR range, then the deterministic coefficient reaches its minimum at 935 nm. Then there is a sharp increase in r^2, and has reached its maximum at 970 nm (r^2=0.4) and then falls sharply. Based on the foregoing, it can be recommended the reflectance at 970 nm for measuring the water content of the fruits. Apparently, the 930 to 935 nm seems more suitable then the used 900 nm to calculate the WBI index, although the sharp decrease in the reflectance does not occur at 935 nm but it begins at 900 nm. Therefore, the calculation method of the WBI will suitable for analyzing the moisture content of fruit as well. Based on the significant ($p=0.021$) linear correlation between the moisture content and the WBI that the water content of the apple fruit can be well estimated with this index (Figure 13).

Figure 13. Correlation between water content and WBI index

The WBI is not applicabe for monitoring the ripeness but it can be used well to track changes in the moisture content. Monitoring the loss of water during the storage and sorting by the fruit nutritive values with the spectral methods can be useful for estimating the water content of apple fruit.

4. Conclusions

The fruit carotenoid content continuously decreases together with the chlorophyll content during the ripening. However, the carotenoid content remains relatively high even for low chlorophyll concentrations. So the carotenoid/chlorophyll ratio can serve for characterization of the ripening process. The reflectance of the skin is increasing during the ripening. It increases dramatically in the yellow color range, due to the increase of carotenoid levels, while pronounced valley (at 678 nm), which can be seen in the red range, become more shallow, parallel with the decrease in chlorophyll content during the ripening. The 678-nm wavelength is sensitive to the low chlorophyll values, so the red range (at 678 nm) may be suitable for testing the ripeness. The reflectances, which measured from the 678 nm to ±30 nm wavelength range, shows greater variability to the high chlorophyll content. Thus the reflectance measured at 700 nm can be suitable for monitoring the early stages of ripening and the pigment content. The standard deviation results of the reflectance also support the above findings, adding that the 500 to 530 nm range is sensitive to the carotenoid, which intensifies with the decrease of the chlorophyll content. Thus it may take part in monitoring the ripening. This phenomenon cannot be detected to the dominant character of the red apple varieties on anthocyanin reflectance. Based on this, there is close correlation between the measured reflectance 550 and 700 nm for the yellowing apple varieties, which also proves clearly the importance of this two ranges to monitor the ripening. Based on the results, using infrared, red and yellow color ranges, new ripening monitoring spectral indices have been created, which can be traced reliably the pigment content changes during the ripening for the yellowing apple varieties. Based on significant linear correlation between the moisture content and WBI, it could be established that that the water content of the apple fruit can be well estimated with the help of WBI. The WBI is not convenient for monitoring the ripeness, but it can be useful for tracking the changes in moisture content. The water loss of fruits can be monitored simply during the professional/unprofessional fruit storages in the 900-970 nm ranges, but further researches need for examining the effect of the storage on the moisture and pigment content.

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