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Research Article

Non-Destructive Detection Of Some Quality Characteristics Of Strawberry Fruit In The Ripening Stage Using Near Infrared Spectroscopy[a]

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ABSTRACT

The products' internal and external quality characteristics were predicted using Fourier transform (FT-NIR) near-infrared spectroscopy technique in Albion cultivar *(Fragaria ananassa)* strawberry samples. Since the shelf life of strawberry fruits is short after harvest, their quality characteristics are an essential criterion, especially for exported products. Determining the quality characteristics of products using non-destructive measurement systems such as FT-NIR is less time-consuming and less costly than chemical or physical methods. Quality features are significant for exported products. Non-destructive spectroscopic measurements of strawberries were made using reflectance (780-2500 nm) and transmittance (800-1725 nm) techniques. Generally, high calibration and validation results were obtained for both measurement methods (Reflectance and Transmittance) in color properties. Hue prediction values on transmittance were predicted to have the best result in the measurement at R^2=84.81 (RMSECV= 0.347) for validation, while R^2=91.77 (RMSEE= 0.268) for calibration. In reflectance mode, it showed high predictive performance of *a** value with the red color variable R^2=82.19 (RMSECV= 5.81) for validation and R^2=89.42 (RMSEE= 4.73) for calibration during the ripening period of the strawberry. On the other hand, the intrinsic properties' prediction performance remained lower than the color properties. The most successful prediction performance was found for soluble solids content (SSC) (R^2=50.66; RMSECV= 0.951) in reflectance mode, while pH (R^2=58.21; RMSECV= 0.0472) for transmittance mode. As can be seen from the results, using FT-NIR spectroscopy to predict color properties without damage during the ripening period of strawberry products was highly successful, while more restrictive results were obtained in predicting internal properties.

Key words: Strawberry, Near Infrared, Color, Non-destructive technologies.

Yakın Kızılötesi Spektroskopisi Kullanılarak Olgunlaşma Aşamasındaki Çilek Meyvesinin Bazı Kalite Özelliklerinin Hasarsız Tespiti[a]

ÖZ

Yapılan çalışmada, Albion çeşidi (Fragaria ananassa) çilek örneklerinde Fourier dönüşümü (FT-NIR) yakın kızılötesi spektroskopi tekniği kullanılarak ürünlerin iç ve dış kalite özellikleri tahmin edilmesi amaçlanmaktadır. Çilek meyvelerinin raf ömürleri hasattan sonra kısa olmasından dolayı özellikle ihraç edilmekte olan ürünler için kalite özellikleri önemli bir kriterdir. FT-NIR gibi hasarsız ölçüm sistemleri kullanılarak ürünlerin kalite özelliklerinin belirlenmesi kimyasal ya da fiziksel metotlara göre daha az zaman alıcı ve daha az maliyetlidir. Özellikle ihracatı gerçekleştirilen ürünler için kalite özellikleri önem arz etmektedir. Çilek örneklerinin hasarsız spektroskopik ölçümleri yansıma (780-2500 nm) ve geçirgenlik (800-1725 nm) teknikleri kullanılarak yapılmıştır. Genel olarak renk özellikleri açısından her iki ölçüm yönteminde (Yansıma ve Geçirgenlik) yüksek kalibrasyon ve doğrulama sonuçları elde edilmiştir. Geçirgenliğe ilişkin renk tonu tahmin değerlerinin doğrulama için R^2=84.81 (RMSECV= 0.347) ve kalibrasyon için R^2=91.77 (RMSEE= 0.268) ile en iyi sonucu vereceği tahmin edilmiştir. Yansıma modunda, olgunlaşma sırasında doğrulama için kırmızı renk değişkeni R^2=82.19 (RMSECV= 5.81) ve kalibrasyon için R^2=89.42 (RMSEE= 4.73) ile a* değerinin yüksek tahmin performansı göstermiştir. Diğer taraftan, içsel özelliklerin tahmin performansı, renk özelliklerine göre daha düşük kalmıştır. En başarılı tahmin performansı yansıma modunda çözülebilir kuru madde oranı (R^2=50.66; RMSECV= 0.951) için, geçirgenlik modunda ise pH (R^2=58.21; RMSECV= 0.0472) için bulunmuştur. Sonuçlardan da anlaşılacağı üzere çilek ürünlerinin olgunlaşma döneminde renk özelliklerinin zarar görmeden tahmin edilmesinde FT-NIR spektroskopisi kullanılması oldukça başarılı olurken, iç özelliklerin tahmininde daha kısıtlayıcı sonuçlar elde edilmiştir.

Anahtar kelimeler: Çilek, Yakın Kızıl Ötesi, Renk, Hasarsız teknolojiler

INTRODUCTION

Strawberry has a large production capacity in Turkey where the world's fifth most extensive production amount (FAO, 2022). Strawberries have rich nutrient content, vitamins, minerals, and polyphenols (Skrovankova et. al., 2015). Therefore, strawberries should be harvested at full maturity and presented to the end consumer as fresh. Strawberry is a tropical fruit that has a short shelf life. The product's shelf life and quality depend entirely on the maturity stages (Rahman et. al., 2016). Depending on the usage area, strawberry fruit can be harvested in several stages as red color breaking, semi-red color, quarter red color and full red color (Saad et al., 2022). Changes in properties such as color and taste in strawberries during ripening are related and directly affect consumer preference. Since strawberry fruit can not continue the ripening process after harvest, it should be harvested at full maturity in terms of flavor(Lewers et. al., 2020; Li et. al., 2022). After the products are harvested maturely, factory classification processes are time-consuming and costly. Detection of the internal and physical quality characteristics of strawberry fruits without damage provides convenience in the classification processes(Kumar et. al., 2021). Today, spectroscopic methods can be used to measure the quality of food products. The most significant advantages of these methods are that they can make non-destructive and rapid measurements. Near infrared (NIR) spectroscopy is generally used for food analysis. NIR can perform spectral measurements in the 780-2500 nm range. It consists of combination bands of fundamental vibrations of chemical bonds such as N-H, C-H and O-H. These bonds form the main structure of organic components (Włodarska et. al., 2019). Devices that can measure with the NIR spectroscopy technique can automatically predict soluble solid content (SSC), acidity, pH and color without damage (Weng et al., 2020). There are studies on determining the quality characteristics of fruit and vegetable products by spectroscopic techniques. Near-infrared spectroscopy is widely used for determined the internal quality characteristics of vegetables and fruits such as apricot (Berardinelli et al., 2010; Carlini et al., 2000; Özdemir et al., 2019), mango (Nagle et al., 2010; Schmilovitch et al., 2000), avocado (Olarewaju et al., 2016), pears (Guo et al., 2015; Li et al., 2013), tomatoes (Huang et al., 2018; Torres et al., 2015). This study aimed to determine the quality characteristic features of the strawberries non-destructively during the maturity stage between green to reddish color by using near infrared spectroscopy methods. The experiment used a single cultivar and looked for differences between the reflectance and transmittance methods of the NIR spectroscopy. Comparing the NIR methods gave us the best method to determine the quality parameters of the strawberries non-destructively.

MATERIAL AND METHODS

Materials

The Albion (*Fragaria ananassa*) strawberry variety was used for the experiments in this study. Strawberry samples were collected from a commercial strawberry open field in Lapseki, Çanakkale (approx. 40°19 N; 26°42 E) between 23 May and 08 June 2022. 50 strawberry samples were collected during each harvest period which was the early harvest at 23 May (Day 0), middle harvest at 31 May (Day 17) and late harvest at 08 June (Day 14). The samples were harvested by hand and brought to the laboratory on the same day. The fruits were collected at four maturity stages green, pink-red and red. Totally 150 samples were harvested for analysis. Non-destructive measurements, FT-NIR and physical features such as color, weight and dimensions were performed the same day after harvest.

Physical measurements of the strawberries

Color, size, weight, pH, and titratable acidity (TA) and SSC measurements were performed. Changes in the strawberry fruits were measured using a colorimeter (Model CR-400, Minolta, Japan). The color measurements were taken in the middle of the equatorial surface of the strawberries, and results were obtained at *L** a* b *(CIE *L** a* b*) color space format. Hue [arctan (b^*/a^*)] and Chroma ($\sqrt{a*^2 + b*^2}$) results were acquired from Lab Color Space. Size measurements were measured with a digital calliper, and weights were scaled with digital scales where accuracy was ±0.01 g. Strawberries were separated into five groups (6 fruits in each group) and a homogeneous juice was obtained by crushing them in a hand mortar. Extracted strawberry juice was filtered through cheesecloth and purified, and pH, SSC and TA measurements were performed. The pH values were measured using a Mi150 pH meter (Mi150 Bench Top pH Meter, Milwaukee Instruments, North Carolina, USA). TA was measured on the 10 mL of strawberry fruit juice and adding 40 mL of distilled water was used to complete the 50 mL diluted juice. The diluted juice was titrated with 0.1 mL NaOH until the pH of 8.1 was stable (Gündoğdu et. al., 2021). The SSC of the squeezed strawberry juice was measured using a digital refractometer (RF-104 BP, 32-10, Atago Honcho, Itabashi-ku, Tokyo, Japan).

Spectral Features

Bruker MPA (Multi-Purpose Analyzer) FT-NIR spectrometer (Bruker Optik, GmbH, Ettlingen Germany) was used for FT-NIR spectral measurements to perform in reflectance and transmittance. The FT-NIR spectrometer has a 20 W Near infrared light source (tungsten-halogen) and an InGaAs detector for reflectance and RT-InGaAs detectors for transmittance modes. In reflectance measurements, the light source and detector fibers are located at the tip of the fiber optic probe. The tip of the fiber optic probe has a detection area of approximately 11.7 mm^2. In this area, light is sent by touching the light sources and the detector sensor tip to the sample at 90 degrees, and the detector tips (TE-InGaAs) can detect the light reflected from the sample. Transmittance measurements were made using the transmittance probe. Measurements were taken so that the rays emanating from the light source in the probe system pass through the center of the strawberry samples placed in this system. The rays coming out through the product were collected by a RTInGaAs detector (Kavdir et. al., 2009). Reflectance and transmittance spectra were taken from each sample, reference material (spectral) and dark environment (reference and dark environment, once in every 10 samples). OPUS software (Bruker Optik, GmbH, Ettlingen Germany) was used for the experiment's spectral measurements and instrument control. Reflection measurements were taken at 780-2500 nm wavelength ranges using a fiber optic probe.

Relative spectra of the samples were obtained using the equation below, using the OPUS program:

$$Relative\ Spectra = \frac{Sample}{Referance} \quad (1)$$

Calibration and validation models were improved during the spectral analyses using Partial Least Squares (PLS). To predict SSC, colors, pH and TA results were used, leaving one out cross-validation technique which includes the PLS. This technique removed a sample from the other samples for calibration and then used it in validation. This process is continued until all the samples are used to create the validation model. The calibration and validation repeated technique averages determined the calibration model's performance.

Different processing techniques on strawberry spectra were used to predict the performance of the calibration models in PLS analysis. For this purpose, three different spectral processing techniques were applied to the strawberry samples. Validation and calibration models were established by using the OPUS Software (Bruker Optic, GmbH, Ettlingen, Germany). To predict the performance of the calibration models, the coefficient of determination, the root mean squared error of estimation (RMSEE) and the root mean squared error of cross-validation (RMSECV) methods were used. Also, comparison of the results was calculated with general linear model (ANOVA). Differences between groups were indicated by Tukey's multiple comparison test, at the $p<0.05$ significance level and Minitab 20.4 software was used for all analyses.(Buyukcan & Kavdir, 2017).

RESULTS AND DISCUSSIONS

The mean, standard deviation and maximum-minimum values of the physical attributes of strawberry samples are reported in Table 1.

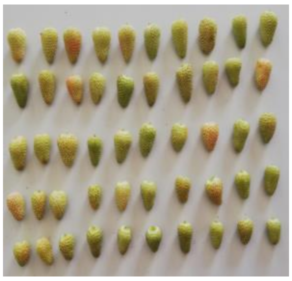
Table 1. The statistics for the physical attributes of the strawberry samples.

Physical Attributes	Days	Mean	StDev	Minimum	Maximum
	0	3.5975	0.0128	3.5800	3.6100
ph	7	3.6925	0.0492	3.6300	3.7700
	14	3.7025	0.0868	3.6300	3.8800
	0	1.4528	0.1442	1.2800	1.6640
TA	7	1.3408	0.0447	1.2928	1.4208
	14	1.4336	0.1160	1.2992	1.6064
	0	9.688	0.426	9.300	10.400
SSC(%)	7	10.712	1.027	9.400	12.500
	14	12.100	1.254	10.200	14.200
	0	54.325	3.592	47.110	61.900
L*	7	39.191	6.553	3.450	49.710
	14	31.459	3.667	26.470	43.340
	0	-5.729	3.166	-10.520	4.030
*a**	7	18.697	4.688	7.620	30.240
	14	23.197	3.781	11.380	30.210
	0	21.692	1.590	16.970	24.690
*b**	7	16.969	1.751	13.540	20.910
	14	11.641	2.568	6.520	16.980
	0	22.627	1.942	17.277	25.932
Chroma	7	25.499	3.479	17.930	35.398
	14	26.109	3.556	18.135	33.308
	0	-1.070	0.762	-1.550	1.562
Hue	7	0.7519	0.1469	0.5174	1.1563
	14	0.4689	0.1171	0.2984	0.8924

pH and SSC values of strawberries reduced with the ripening period. While there is a decrease in the *L** values, which show the brightness of the products, during the ripening period, there is an increase in the *a** parameters depending on time. Similar situations are observed for chroma and Hue results during the maturation period. The high standard deviation in some parameters was observed due to the extensive maturity range of the strawberry fruits.

Color Prediction Results

Color is a significant quality characteristic for determining fruit and vegetables. The amount of anthocyanin effectively transforms the strawberry from green to red during its development (Rodrigo et. al., 2007). Color prediction results are shown in Table 2 for both measurement methods and color changes during the harvest period were shown in figure 1.


23.05.2022


31.05.2022


08.06.2022

Figure 1. The color changes of strawberries during the harvest period.

The highest validation results were obtained for *a** prediction for reflection (R^2=82.19; RMSECV= 5.81) and Hue for transmittance (R^2=84.81; RMSECV= 0.347) method. The lowest prediction results were achieved in chroma, where the transmittance method was R^2=2.057 (RMSECV= 2.93) and the reflectance method was R^2=46.73 (RMSECV= 2.21) (Table 2).

Table 2. The calibration and validation results are summarized for color prediction values in reflectance and transmittance methods.

Color Features	Measurement Methods	Factors	Calibration		Validation	
			R^2	RMSEE	R^2	RMSECV
L	Reflectance	9	85.87	4.03	77.16	4.85
	Transmittance	7	77.63	4.97	65.5	5.91
*a**	Reflectance	9	89.42	4.73	82.19	5.81
	Transmittance	8	86.67	5.13	79.15	6.1
*b**	Reflectance	9	80.37	2.1	68.81	2.51
	Transmittance	6	68.73	2.62	58.3	2.91
Chroma	Reflectance	9	68.32	1.8	46.73	2.21
	Transmittance	1	5.395	2.91	2.057	2.93
Hue	Reflectance	2	59.82	0.598	57.59	0.605
	Transmittance	8	91.77	0.268	84.81	0.347

*L**, *a** and *b** prediction results were so close in both methods. *a** values describe the red color in *L a*b** color space. The transformation of strawberries from green to red during the ripening period is due to the change in the content of the anthocyanins. *a** values were the second highest validation results in reflectance (R^2=82.19; RMSECV= 5.81) and transmittance methods (R^2=79.15; RMSECV= 6.1).

800-1000 nm were the effective wavelength ranges for *L*, a*, b** and Hue to prediction in the transmittance method, respectively. Chroma in transmittance and L* value for the reflection method (999-1333 nm) was the same wavelength ranges. *a** values, which 1332-1886 nm and 2260-2355 nm, were nearly similar to the hue effective wavelength ranges in reflectance mode in 1332-1545 nm and 2173-235. The corresponding peaks were 975, 1187, 1450, and 1924 nm in the reflectance mode and 815, 1067, and 1280 nm in the transmittance method. (Włodarska et. al., 2019) pointed out that O-H and C-H groups included leading absorption bands in 980, 1192, 1451, 1778 and 1928 nm, which are similar results in this work. Water, sugars, and polyphenolic compounds such as anthocyanins, which gave color to the strawberry, may contribute to this absorption. Changes in color values for the maturity stages are shown in Table 3. During maturity, stages were statistically significant for the color parameters. Statistically, significance was found between the harvest times for the *L** values. The results of *L** values indicated that effective during the harvest period for the brightness of the strawberries. *L** and *b** values decreased rapidly while *a** values increased during the ripening period at the field. Nearly same results were reported by Ménager et al. (2004). Also, Chroma and Hue showed a similar increasing reaction for the strawberry fruits. Nunes et. al. (2006) reported that the strawberry fruits ripen from green to reddish in nearly 12-13 days, depending on air temperature.

Table 3. Determination of color characteristics of strawberries during the ripening period.

	Day 0	Day 7	Day 14
L	54.325±3.592 a	39.191±6.553 b	31.459±3.667 c
*a**	-5.729±3.166 c	18.697±4.688 b	23.97±3.781 a
*b**	21.692±1.590 a	16.969±1.751 b	11.641±2.568 c
Chroma	22.627±1.942 b	25.499±3.479 a	26.109±3.556 a
Hue	-1.070±0.762 c	0.751±0.147 a	0.469±0.117 b

[a-c]Means in the same row with different letters are significantly different($P<0.05$)

Physical Measurements Prediction Results

TA, pH and SSC prediction results are shown in Table 4. TA and pH values were obtained closely in reflectance modes of R^2= 35.99 (RMSECV=0.094) and R^2=26.16 (RMSECV=0.0627). Compared to reflectance, the transmittance mode seemed lower prediction results in TA (R^2=1.984; RMSECV=0.116) and SSC (R^2=33.2; RMSECV=1.11). Also, calibration predictions obtained similar TA and SSC measurements in both modes. Besides that, good pH prediction results (R^2=58.21; RMSECV=0.0472) were achieved in transmittance according to the reflectance (R^2=26.16; RMSECV=0.0627).

Table 4. The summary of the calibration and validation results for TA, SSC and pH prediction values in reflectance and transmittance methods.

Features	Measurement Methods	Factors	Calibration		Validation	
			R^2	RMSEE	R^2	RMSECV
TA	Reflectance	10	99.62	0.0121	35.99	0.094
	Transmittance	2	33.08	0.106	1.984	0.116
SSC	Reflectance	10	99.55	0.152	50.66	0.951
	Transmittance	6	92.01	0.499	33.2	1.11
pH	Reflectance	9	97.96	0.0162	26.16	0.0627
	Transmittance	10	99.57	0.008	58.21	0.0472

The most effective wavelength ranges detected for TA were 1731-1836 nm and 2173-2355, nearly identical wavelengths for SSC in reflection.800-1334 nm and 1639-1725 nm were the effective wavelength ranges for predicting the pH in transmission mode. During the maturity stage, 974, 1181, 1445, and 1922 nm were the main absorption peaks for the reflection, respectively. The wavelength at 974, 1181 and 1445 were effective peaks for determining the TA, pH and SSC prediction, similar to the reflection and transmittance measurement methods. These absorption wavelengths were essential for the organic molecules related to the C-H, O-H and N-H chemical bonds (Liu et. al., 2019). Carbohydrates, such as sugars, corresponded to C-H-related sugar at 1165 nm. Also, peaks at 974, 1445 and 1922 were described as water content, including O-H-related water content in strawberries (Seki et al., 2023). The peaks at 1443-1416 for reflectance and transmission are defined as O-H and C-H combinations described as SSC or TA (Mancini et. al., 2023).

Changes in the initial quality of strawberries samples were shown in Table 5 during the maturity stage. Acidity is an essential parameter in determining strawberries' taste and consumer attributes. Glucose, fructose and sucrose; sugar contents, constitute 99% of the sugar in strawberry fruit. In addition, this sugar content plays an essential role in customer satisfaction (Moing et. al., 2001; Park et. al., 2006). pH and SSC values increased during the maturity stage with statistically significant. Day 14 results for SSC values were significantly higher on day seven and day 0 while pH values were lower at day 0 compared to day seven and day 14. Nearly same results were decelerated for different strawberry cultivars in the maturity stage (Kafkas et. al., 2007; Karlidag et. al., 2009). During the ripening period invertase enzyme activity decreased so which caused the rise of the SSC levels for strawberries. Besides that after the respiration period, which continues throughout the ripening period in strawberries, the breakdown of organic acids into sugar may cause an increase in pH levels in the products. During the maturation period, the pH and SSC values enter an upward trend due to the decrease in malic and citric acids, which are essential organic acids in the respiratory function of the products (Basak et. al., 2022).

Table 5. Determination of TA, SSC and pH properties of strawberries during the ripening period.

	Day 0	Day 7	Day 14
TA	1.452±0.144 ns	1.340±0.044 ns	1.433±0.116 ns
SSC	9.688±0.426 b	10.712±1.027 b	12.100±1.254 a
pH	3.597±0.012 b	3.692±0.049 a	3.702±0.086 a

[a-c]Means in the same row with different letters are significantly different(P<0.05)
ns: There is no statistical difference (P>0.05).

Transmittance and Reflectance spectral measurements

The average spectrums of the strawberries can be seen in Fig. 2, where showing the differences in early, middle and late harvests, in reflectance mode. 972, 1179, 1443, 1784, 1923 and 2316 nm peaks were the significant peaks in reflection. Related absorption peaks are shown in Fig. 3 for the transmission mode which was 852, 970, 1161 and 1416 nm. 970-972, 1161-1179 and 1443-1416 nm peaks were described as water absorption peaks that contain O-H bonds(Williams & Norris, 1987). Similar wavelength results were described with C-H and O-H absorption in carbohydrates and water for 950-1000 nm, 1180-1200 nm and 1390-1610 nm wavelengths(Shen et. al., 2018). At around 1800 nm sugar overtones and around 1900-1970 water peaks were obtained for plums (Sánchez et. al., 2012) which were nearly same peaks for the strawberries in reflection mode. For transmittance spectra as shown in Fig 3. the average spectra for transmittance at day 0 are low when compared the day 14 between 1150-1450 nm wavelengths, since it is a strong water absorption band.

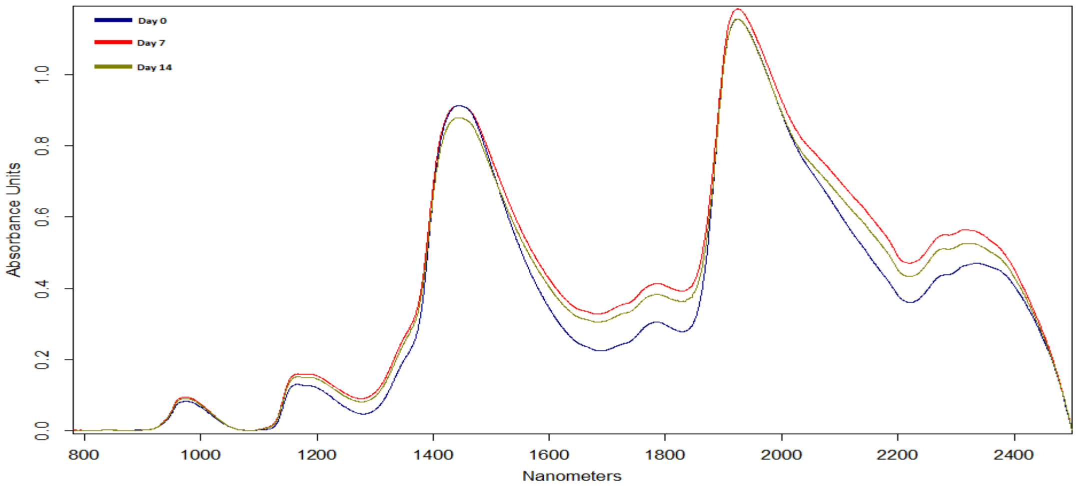


Figure 2. The average spectra of strawberries in reflectance mode during the ripening stage.

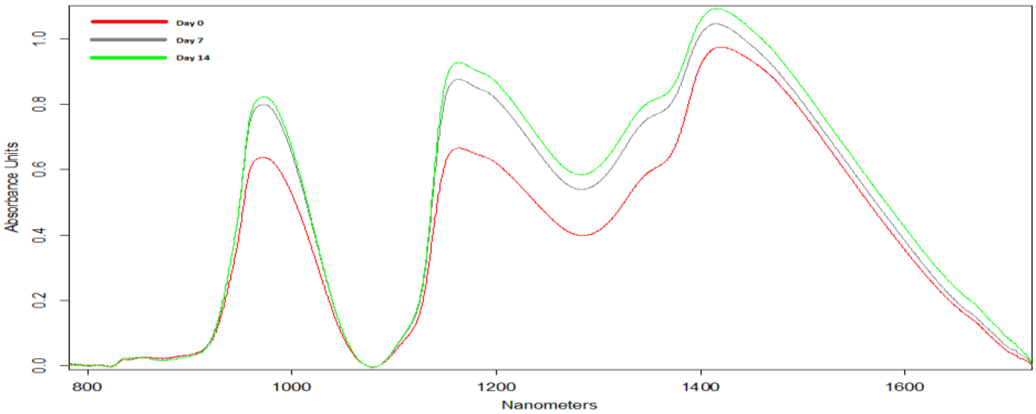


Figure 3. The average spectra of strawberries in transmittance mode during the ripening stage.

CONCLUSION

Our aim in this study is to predict some inner and outer quality characteristics of strawberry fruits during ripening by using FT-NIR spectroscopy. According to the results obtained in this direction, regarding color properties, the *a** value (R^2=82.19) showed the best prediction success in reflectance mode. In contrast, the lowest success rate was obtained in Chroma (R^2=46.73). Hue (R^2=84.81) values got the best prediction performance in transmittance mode. Transmittance and reflection modes successfully predicted strawberry fruits' color changes (from green to red) during the ripening period, as expected.

It was seen that the prediction success in TA, SSC and pH measurements remained at average levels. With the best prediction results during the analyzes, its performance was determined to be pH (R^2=58.21) in transmittance mode, while this performance was determined to be at SSC (R^2=50.66) in reflectance mode. So that pH and SSC prediction method can be improved to obtain higher results. As can be seen from the results, the prediction performance of color parameters in strawberry fruits with FT-NIR was higher during the ripening period. When both measurement methods were compared, it was concluded that the prediction success of the measurements made in transmittance mode was high. However, weaker results were obtained in predicting strawberries' internal properties (pH, SSC and TA) during the ripening period compared to the color properties.

Such new methods can be supported industrially and economically, as chemical analyses applied to agricultural products, especially in export, are reinforced and time-consuming. At the same time, they cause damage to the products. The manufacturer can determine the quality of the product in a shorter time and with traceability. At the same time, if he is cheating, he can have more precise and healthier information about the product purchased with the development of such new non-destructive methods. As a result, the FT-NIR spectroscopy technique may have the potential to be used in the non-destructive determination of quality characteristics during the ripening stages of sensitive products such as strawberries. More comprehensive studies will contribute to the literature when the results are evaluated.

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