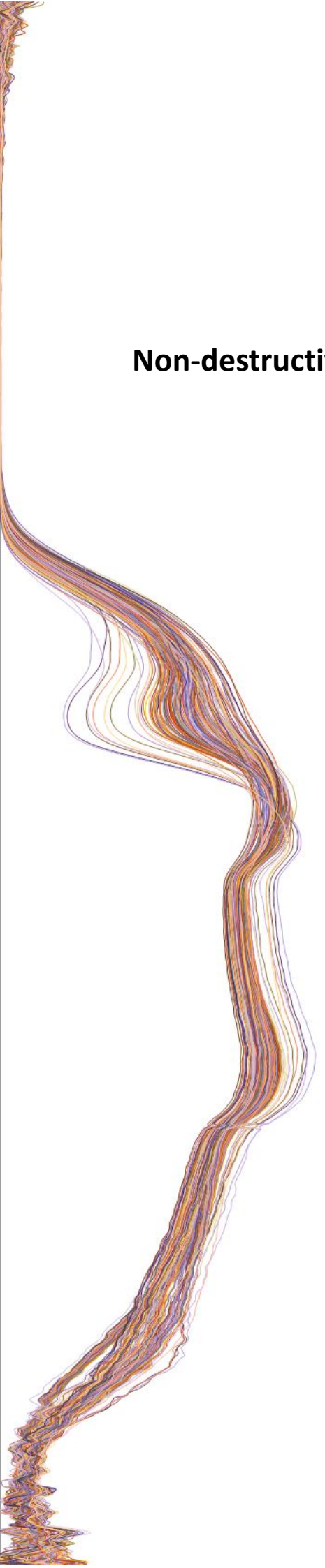
Non-destructive Phenotyping of Postharvest Quality Traits of Tomatoes and Strawberries



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Abstract

The development of non-invasive methods to efficiently evaluate fruit quality has become increasingly essential for plant breeding. In this study, visible and near infrared spectroscopies under different modes were investigated to non-destructively predict texture and taste quality of tomatoes and strawberries. A large number of tomato and strawberry genotypes was employed and the values of quality traits quantified by invasive methods were used as references. Invasive methods for firmness and juiciness were designed in our study. Fruit firmness was better evaluated by using fruit disks rather than whole fruits. OmicsFusion, a multivariate regression analysis, was performed on invasive and log transformed non-invasive data to select characteristic wavelengths as predictors. Low correlations were obtained between invasive and non-invasive data of each fruit for both tomatoes and strawberries, which was due to small variations in tested quality attributes and large genotypic variations in fruit size, shape, internal composition and etc. Correlations were dramatically improved by pooling genotypes with similar texture and taste quality values, separately, because of averaged variations in other physical parameters. Wavelengths obtained by NIR interactance were the best predictors for SSC, acidity, juiciness and disk firmness of tomatoes and that obtained from VIS-NIR transmittance was the best predictor for whole tomato firmness. Wavelength obtained by VIS-NIR transmittance was the best predictor for juiciness and disk firmness of strawberries, additionally NIR interactance could provide almost equal predictions considering the regression coefficient. NIR reflectance could not give useful predictions as it only had access to fruit surface rather than fruit tissues.

Acknowledgement

This MSc thesis is part of my study program in Plant Sciences at Wageningen University. The project was provided by Plant Breeding Group. First of all, I would like to extend my sincere gratitude to my supervisors Dr. AG (Arnaud) Bovy and Dr. YM (Yury) Tikunov, for their patient advising and critical supervision on the experimental design, equipment arrangement and data analysis. Next, I would like to thank Raquel Rosales Lopez and Jos Molthoff with my high gratitude for giving me instructive advices and helping me with my lab work. For sure, I could not finish my experiment without their supports. Furthermore, I want to thank Enza Zaden B.V. and Fresh Forward Breeding & Marketing for providing experiment materials and all the colleagues from PBR group for providing convenience during the whole process. Last but not least, special thanks to my parents, my husband and my friends for always being with me and encourage me in the completion of my thesis.

Content

1. Introduction

Nowadays, food security is challenged by a huge global population. In order to fulfil the population necessity, it is not only essential to increase the crop yield but also to reduce the food discarded (Kader, 2003). Postharvest shelf-life is an important determinant for the loss of fresh and processed fruit and vegetable products (Kader, 2002). Besides resistance to fungal and bacterial diseases, shelf-life is also determined by quality traits. From consumers' perspective, fruit quality is mostly defined by sensory attributes such as appearance, flavour and texture (ElMasry, Wang, ElSayed, & Ngadi, 2007). Currently, fruits are sorted manually or automatically based on their external qualities, for example, colour and firmness. However, their internal qualities, especially sweetness and sourness, play a key role in contributing the unique taste of fruits, which in turn specifically influences consumers' repurchase (Dong & Guo, 2015; Lu & Ariana, 2002). The quality traits are traditionally measured by destructive methods, which are inefficient and lack the ability to study the dynamics of postharvest physiological processes; therefore, the non-destructive methods, especially those based on optical properties, are needed and have been investigated in the past years in order to be employed in practice (Butz, Hofmann, & Tauscher, 2005). Implementation of non-invasive phenotyping technologies will increase the phenotyping efficiency, allow monitoring the kinetic parameters, assure grading the individual fruit quality before sale, facilitate developing models that imitating postharvest processes and accelerating breeding progresses (Nicolaï et al., 2014).

Tomato (*Solanum lycopersicum*) and strawberry (*Fragaria x ananassa* Duch) are economically important horticultural crops worldwide. Sweetness, sourness and juiciness are essential internal quality traits and firmness is a key external quality trait for tomatoes and strawberries. The destructive methods to measure these parameters have been mentioned in many studies. Sweetness is widely determined by soluble solids content (SSC) that is normally expressed in °Brix (Azodanlou, Darbellay, Luisier, Villettaz, & Amadò, 2004; Sirisomboon, Tanaka, Kojima, & Williams, 2012). Sourness is normally determined by titratable acidity (TA) (Aguayo, Jansasithorn, & Kader, 2006; Clément, Dorais, & Vernon, 2008). Firmness is a complex parameter that involves cell turgor, cell anatomy, intercellular spaces, chemical composition as well as spatial arrangement of the cell wall and middle lamella (Clément et al., 2008). Most of the firmness tests for tomatoes are based on compression or acoustic vibration and for strawberries are based on puncture (Azodanlou et al., 2004; Butz et al., 2005; Clément et al., 2008; De Ketelaere & De Baerdemaeker, 2001; Tallada, Nagata, & Kobayashi, 2006). Both whole fruits and pericarp disk have been used to determine tomato firmness in previous studies and they all worked well; however, the comparison of these two methods were employed on the same genotypes (Campbell, Huysamer, Stotz, Greve, & Labavitch, 1990). Thus, the accuracy of these two methods to compare a large amount of genotypes is unclear. In addition, the accuracy of puncture measurements were influenced by the variation of strawberry cores in our previous

firmness studies (Gudenschwager, 2016). Juiciness can be valued by sensory panel or quantified by measuring the amount of juice released and absorbed on the filter paper during mechanical crashing (Rocha, Deliza, Corrêa, do Carmo, & Abboud, 2013; SZCZESNIAK & SMITH, 1969). Due to the soft tissue and high water content, tomatoes and especially strawberries are quite perishable, therefore more effective and invulnerable methods are required.

Recently optical technologies that are flexible and versatile have been used as non-destructive methods for agro-product phenotyping, which require few sample preparations, enable large-scale individual assessments and real-time analyses (Flores, Sánchez, Pérez-Marín, Guerrero, & Garrido-Varo, 2009). The spectroscopy, hyperspectral imaging and multispectral imaging have been successfully used to measure SSC, TA and firmness (Clément et al., 2008; Nicolaï et al., 2014; Sanchez et al., 2012). Applications of these technologies to quantify fruit quality attributes are usually implemented in the visible and near infrared regions since spectra in this range incorporate abundant information concerning chemical compounds such as water, sugar and acids with OH-, CH- and NH- groups due to their particular overtone bands (Pissard et al., 2013). Visible near infrared (VIS-NIR; 400-1000 nm) and near infrared (NIR; 780-2500 nm) spectra are the interacted result of radiation with samples, allowing quantitative analysis of physical and chemical properties (Alves de Oliveira, Bureau, Renard, Pereira-Netto, & de Castilhos, 2014). Due to high water content of tomatoes and strawberries (90%-95%), the spectra are basically dominated by water absorption bands at 760, 970,1170, 1450 and 1930 nm caused by OH- stretching and bending absorbance (Clément et al., 2008). Visible wavelength carries more information about pigments such as chlorophyll, carotenoids and anthocyanins and NIR provides more information about sweetness and sourness of fruits (Butz et al., 2005).

The VIS-NIR and NIR spectra can be obtained by three different measurement setups, namely reflectance, transmittance and interactance (Figure 1) (Nicolai et al., 2007). In reflectance mode, the external diffuse reflection and scattering can only provide information about fruit surface. It is the easiest mode as there is no requirement to touch the fruits and the relative light level is high. However, it is susceptible to surface properties. Light source and light detector are placed under an angle to avoid specular reflection. In transmittance mode, the transmitted light carries both external and internal information about the fruit. The transmitted spectra are less sensitive to the fruit surface and measurements can also be done without contacting with fruit surface. As the amount of light that can penetrate the fruit is really small, light intensity should be high enough to penetrate the fruit surface and change its spectral properties during transmittance. However, the high light intensity might damage fruits by overheating, especially for thin-skinned fruits (Long & Walsh, 2006). The light source and the detector are normally positioned opposite to each other. In interactance mode, the interactance spectra contain external and part of the internal information of fruits,

which can be regarded as a compromise between reflectance and transmittance modes. The light source and detector are positioned parallel to each other (Schaare & Fraser, 2000).

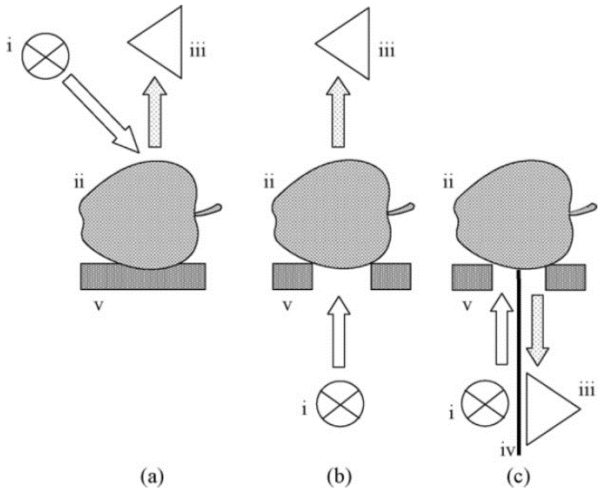

Figure 1. Measurement setup for (a) reflectance, (b) transmittance and (c) interactance spectra, with (i) a light source, (ii) a fruit, (iii) a light detector, (iv) a light barrier and (v) a fruit support (Nicolai et al., 2007).

A hyperspectral imaging system is used to obtain hyperspectral reflectance images in VIS-NIR and NIR regions. Generally, hyperspectral imaging analysis collects a substantial number of hyperspectral images which is represented by a 3-D spectral data cube with two spatial dimensions and one spectral dimension. The spectral information is extracted from hyperspectral images in order to predict the relevant quality attributes (Liu, Sun, & Zeng, 2014). The hyperspectral imaging combines NIR spectroscopy and machine vision and can obtain spatially distributed spectral information at each pixel of the image. However, the NIR spectroscopy can only acquire spectral information rather than spatial information (Fan, Huang, Guo, Zhang, & Zhao, 2015).

The VIS-NIR reflectance has been reported to have a good prediction for SSC and acidity of a single tomato genotype while a poor prediction for tomatoes from various genotypes. Its prediction for fruit firmness is good for either single genotype or multi genotypes (Clément et al., 2008; Ecarnot, Bączyk, Tessarotto, & Chervin, 2013; Shao et al., 2007). Clément et al. (2008) also find that absorptions at 680 and 722 nm are related to chlorophyll and lycopene content, respectively. In addition, NIR spectroscopy has been found useful to estimate SSC in both tomatoes (transmittance) and strawberries (at 908 nm) (Ito, 2000; Khuriyati & Matsuoka, 2004). According to Alves de Oliveira et al. (2014), the NIR spectroscopy is more accurate for fruits with homogeneous pulp and thin skin. Moreover, VIS-NIR and NIR hyperspectral reflectance have been suggested sufficient to estimate strawberry firmness at 528 nm and at 685, 865 and 985 nm, respectively (Nagata, Tallada, Kobayashi, Cui, & Gejima, 2004; Tallada et al., 2006).

According to previous studies in our group, the best non-destructive methods to assess fruit quality of tomatoes are hyperspectral imaging and VIS-NIR transmission and of strawberries is NIR reflectance. Especially for strawberry, the SSC, acidity and some volatiles were well predicted. However, their destructive method for measuring fruit firmness is not reliable (Gudenschwager, 2016). Therefore, in our study, firstly we designed new destructive methods to evaluate fruit firmness and juiciness. Afterwards, multivariate regressions were performed on all spectral information and quality attributes. Finally, the best non-destructive predictors were verified in order to further facilitate the high through-put phenotyping model development.

2. Research Aim

The aim of this project is to develop proper destructive methods to quantify fruit firmness and juiciness and to find the best non-destructive optical phenotyping methods based on multivariate regression to facilitate the development of robust non-invasive phenotyping methods for postharvest quality trait of tomatoes and strawberries.

3. Materials & Methods

Tomatoes were obtained from Enza Zaden B.V. and strawberries were obtained from Fresh Forward Breeding & Marketing. Tomato and strawberry crops were grown in a greenhouse with standard cultivation procedures and harvested at commercial harvest stage. There were 87 tomato genotypes and 98 strawberry genotypes used in the experiment. Upon arrival, tomatoes were stored at 15°C and 65% RH and strawberries were stored at 4°C and 60% RH. One night before strawberry measurements, selected fruits were removed from storage in order to reach room temperature. Fruit quality was monitored by invasive methods. Tomatoes and strawberries were firstly used for non-invasive measurements (NIR hyperspectral imaging, VIS-NIR hyperspectral imaging, VIS-NIR transmittance, NIR interactance and NIR reflectance) and then for invasive measurements (SSC, acidity, firmness and juiciness). The correlation between invasive methods and non-invasive methods was tested by Omicsfusion. The measurements were conducted in several days. After texture measurements, samples were frozen, ground and stored for brix and acidity measurements.

3.1 Non-invasive Phenotyping

All light sources were switched on in advance due to the change of spectral characteristics during warming period (Nicolai et al., 2007).

3.1.1 NIR Hyperspectral Imaging

The NIR hyperspectral imaging system contained a CCD camera (N17E), a spectrograph and a halogen illumination system. The tomatoes and strawberries were placed with the green calyxes down (for tomatoes the green calyxes were removed) and strawberries were fixed on custom made racks. Reflection image of tomatoes and strawberries were taken with in wavelength 900-1700 nm. The exposure time for tomatoes was 10 ms and for strawberries was 20 ms. Black and white calibrations were required before scanning. The detailed protocol was summarized in Appendix I. Three-dimensional hyperspectral data cubes were obtained from scanning and their values were corrected with dark and white references. Data analyses will be performed by Gerrit Polder.

3.1.2 VIS-NIR Hyperspectral Imaging

The VIS-NIR hyperspectral imaging system contained a CCD camera (V10E), a spectrograph and two DC regulated illuminators (Fiber-Lite® PL-900). Fruits were placed as described in 3.1.1. Reflection image of tomatoes and strawberries were taken within wavelength 400-1000 nm. The exposure time for tomatoes and strawberries were 50 ms. Black and white calibrations were required before scanning. The detailed protocol was summarized in Appendix II. Three-dimensional hyperspectral data cubes were obtained from

scanning and their values were corrected with dark and white references. Data analyses will be performed by Gerrit Polder.

3.1.3 VIS-NIR Transmittance

The VIS-NIR transmittance system contained a DC regulated illuminator (Fiber-Lite® DC-950) and a fiber optic spectrometer with a sensor attached on it (Ocean Optics, SD2000). Tomatoes and strawberries were posited to cover the light source and the transmitted light through fruits was detected by the fiber optic sensor at four different points on the equator. The wavelength of VIS-NIR transmittance ranged from 400 nm to 1000 nm. The intergration time for both tomatoes and strawberries was 20 ms. Black and white reference spectra were measured before measuring fruits. The output of transmittance signals has been already corrected by the dark and white references. The detailed protocol was summarized in Appendix III.

3.1.4 NIR Interactance

The NIR interactance system was consisted by a dual fibre light source (Schott, KL1500), a light sensor, a VIS-NIR spectrophotometer (Zeiss MCS 521 VIS NIR-E, Carl Zeiss, Jena, Germany; 305-962 nm) and a NIR spectrophotometer (Zeiss MCS 511 NIR 1, 7, Carl Zeiss, Jena, Germany; 962-1713 nm). Both spectrophotometers were connected to the light sensor. The intergration time of tomatoes is 200 ms and of strawberries is 100 ms. Dark and light calibrations were needed before measurements. During measurement, the most important thing was to prevent light leakage to the sensor. The detailed protocol was summarized in Appendix IV. The output for each scan contained four .csv files, namely corrected.csv, dark.csv, reference.csv and spectrum.csv. Only the corrected.csv files were used for analysis.

3.1.5 NIR Reflectance

The NIR reflectance spectra of tomatoes and strawberries were measured by a Perkin-Elmer Spectrum One NTS spectrophotometer (Perkin-Elmer, Beaconsfield Bucks, UK). The wavelength of NIR reflectance ranged from 700 nm to 2500 nm. Three spots of tomatoes and two spots of strawberries on the fruit equator were randomly selected for scan. Fruits were fixed on the lens and a white cap was used to cover the fruits, creating a dark environment. The spectrum obtained from each spot was the average of five scans. Before measurement, a white Spectralon®(Labsphere, Inc., North Sutton, NH) was used as reference to initiate the software. The detailed protocol was summarized in Appendix V.

3.2 Invasive Phenotyping

3.2.1 Firmness and Juiciness

Tomato and strawberry firmness were determined by a Texturemeter (TA. HD. Plus, Texture Analysis, Stable micro systems). Firmness was determined by the force (N) required to break the cell. Whole fruit firmness and disk firmness of tomatoes and disk firmness of strawberries were measured. The fruits or tissues were compressed by a cylindrical metal plate (D = 75 mm). The whole fruit firmness of tomatoes was measured at two points on the equator and the fruits were compressed by 10%. From each tomato or strawberry, two 10 mm diameter disks were cut using a cork borer and weighed (Verkerke, Janse, & Kersten, 1997). The disks of tomatoes and strawberries were compressed to 0.3 mm. Firness was expressed as N.

Tomato and strawberry juiciness were measured simultaneously with firmness. By crashing the tissue and quantifying the amount of juice released after crashing, juiciness was determined by the amount of juice per fresh weight. One disk was enclosed in a sheet of miracloth (Agratex scherm cloth) and placed with peel down between two pre-weighed filter papers (Whatman grade 3, catolog nn.1003-917). The filter papers were used to absorb fruit juice from compressed disks and the miraclothes were used to isolate loss cells or patches of fruit flesh from filter papers. After compression, the two filter papers were re-weighed (Verkerke et al., 1997). The amount of juice was determined by the weight differences of two filter papers before and after compression. The detailed protocol was summarized in Appendix VI.

3.2.2 SSC and Acidity

SSC and acidity of tomatoes were measured with a pocket Brix-Acidity Meter (Cat. No. 4703 PAL-BX|ACID3 ATAGO). Grinded tomato fruit powder was filled into a 1.5 ml Eppendorf tube and thaw at room temperature. Tubes were centrifuged at 13.3 g for two minutes and the supernatant was extracted to a new 1.5 ml Eppendorf tube for another two-minute centrifuge. Supernatant (200 µl) was used for brix measurement and after adding 9.8 ml Milli-Q water the acidity was measured. The measurements were done in duplicate and performed at room temperature. Milli-Q water was used as the zero reference. Acidity calibration was done using 0.04% Citric Acid. SSC and acidity were expressed as °Brix and citric acid (mg/L), respectively. The method to measure the SSC and acidity of strawberries are as the same as tomatoes and the measurements will be performed in the future.

3.3 Statistical Analysis

Phenotypic data per fruit obtained by both invasive and non-invasive methods were related together through OmicsFusion web application (http://www.plantbreeding.wur.nl/omicsFusion2/), a multivariate regression analysis tool developed by WUR (Acharjee, Finkers, Visser, & Maliepaard, 2013). The invasive data were submitted as responses and the non-invasive data were submitted as predictors. All the non-invasive values were log transformed before submission. The multivariate regression techniques included random forest regression (RF), lasso regression, elastic net regression (EN), partial least squares (PLS), sparse PLS regression (SPLS), ridge regression, principal component regression (PCR) and univariate regression. The OmicsFusion is done by a tenfold cross-validation procedure. The data set was split into two parts, 90% of the samples were the training data and 10% were the test data. After the analysis was finished, a summary table was provided. The overall rank was calculated for each predictor variable and the summary table was ordered accordingly. The best prediction variable was ranked on top of the table. The quality traits with a good correlation between invasive and non-invasive methods were selected.

4. Results

4.1 Research on Firmness and Juiciness Measurement Methods

In this section, the invasive methods to measure disk firmness and juiciness were studied and developed. The best methods were employed in our study as the reference invasive methods. The detailed procedure of final selected methods was described in Materials and Methods 3.2.1 and Appendix VI.

Different texturemeter settings for tomato disk firmness measurements were showed in Table 1. Compression was chosen in the test mode because the fruit disk would be compressed. Test speed was set according to Verkerke (1997). As the probe was commanded to go a certain distance, distance was selected in target mode. Fruit disks need to be completely compressed in order to imitate the bite behaviour, therefore, a distance between the probe and the bottom before compression and a distance the probe went during compression were adjusted to find the breaking peak for tomato tissues (Figure 2). When these two distances were the same, even though the tomato disk was completely broken, there was always an overload which was harmful to the machine, so overload should be avoided as much as possible. Height calibration with filter papers and miraclothes could avoid the errors cause by the thickness of these materials. By comparing different settings, the fifth was the best among all the tomato trials. The processes to develop a method for strawberry disk firmness measurements were as the same as tomatoes and the best setting was displayed in Table 2.

Table 1. Example for texturemeter settings for tomato disk firmness measurement.

Setting	Test mode	Test speed (mm/sec)	Post speed (mm/sec)	Target mode	Distance[1] (mm)	Trigger type	Probe setting[2] (mm)	Calibration	Description
1	Compression	1	5	Distance	10	Bottom	11	Bottom	No breaking peak
2	Compression	1	5	Distance	10	Bottom	10	Bottom	A breaking peak Overload
3	Compression	1	5	Distance	10	Bottom	10	Filter papers Miracloth	A breaking peak Overload
4	Compression	1	5	Distance	9	Bottom	10	Filter papers Miracloth	No breaking peak
5	Compression	1	5	Distance	9.7	Bottom	10	Filter papers Miracloth	A breaking peak

[1]. A distance that the probe went during compression.

[2]. A distance between the probe and the bottom before compression.

Table 2. Best texturemeter settings for strawberry disk firmness measurement.

Setting	Test mode	Test speed (mm/sec)	Post speed (mm/sec)	Target mode	Distance[1] (mm)	Trigger type	Probe setting[2] (mm)	Calibration	Description
1	Compression	1	5	Distance	11.7	Bottom	12	Filter papers Miracloth	A breaking peak

[1]. A distance that the probe went during compression.

[2]. A distance between the probe and the bottom before compression.

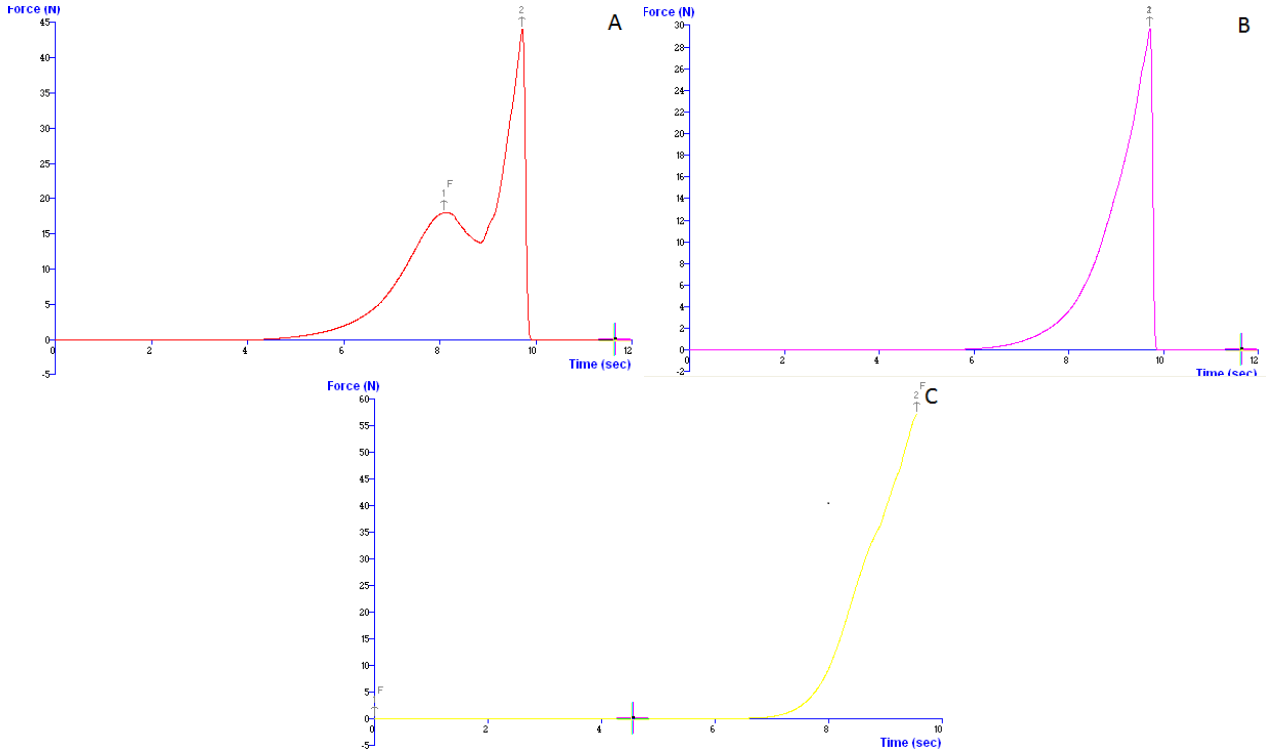

Figure 2. Line chart of disk firmness obtained from Texturemeter: (A) breaking point, (B) no breaking point and (C) overload.

Juiciness measurements were also set up based on Verkerke (1997). Miracloth was used to isolate mealy tomato cells from filter papers. As there was no mealy cell in strawberries, whether miracloth should also be used for strawberries was tested by compressing fruit disks with or without miracloth. After compression, some fleshes were hard to separate from filter papers (Figure 3), therefore, miracloth was determined to be used for both tomato and strawberry measurements.

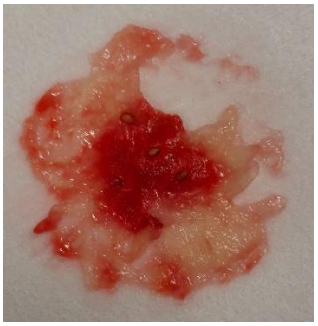

Figure 3. Example of strawberry flesh stick on the filter papers after compression.

4.2 Tomato Phenotyping

4.2.1 Invasive Measurements

Firmness of whole fruit and fruit disks of each tomato was measured and the results were displayed in Figure 4. Fruit firmness determined by whole fruits ranged from 7.26 N to 40.48 N and by fruit disks ranged from 1.58 N to 36.84 N. The disk measurement showed more sensitivity and variations than the whole fruit measurement. For some genotypes, two methods gave the similar firmness values or at least similar trends while for other genotypes, two methods gave significantly different values or even opposite trends. For example, the firmness of fruit 4269_2 measured by both methods were relatively similar while that of 4908_1 were significantly varied.

The correlation between whole fruit firmness and disk firmness was tested. Figure 5 showed a poor correlation between these two methods with a R^2 equalled to 0.03. As there were variations in fruit size and shape of different genotypes, the whole fruit firmness of 40 fruits was corrected based on diameter of two vertical equators. Figure 6A and 6B showed correlations between disk firmness and whole fruit firmness before and after size correction. The R^2 improved from 0.06 to 0.21 after correction. Therefore, the inconsistency of fruit size seemed to be a factor that partially influencing the precision of whole fruit firmness method.

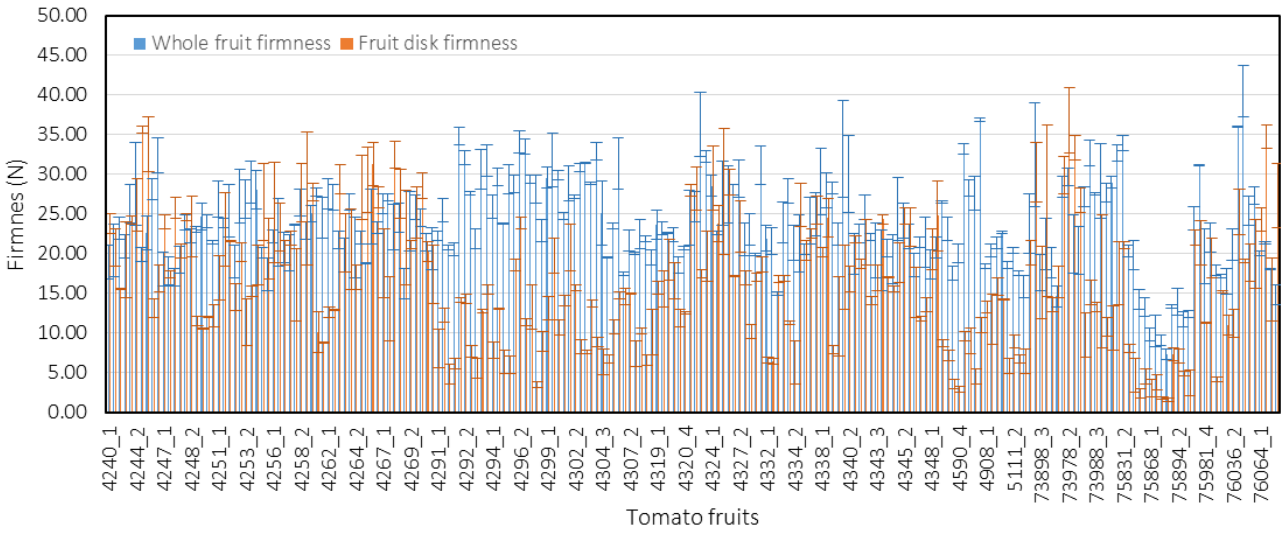

Figure 4. Tomato firmness quantified by whole fruits and fruit disks. Error bars represent SEM (n = 2). Y-axis represents fruit firmness (N) and x-axis represents the name of tomatoes. There are 214 tomato firmness values displayed in this figure, but due to area limitation, only a partial of tomato names was showed.

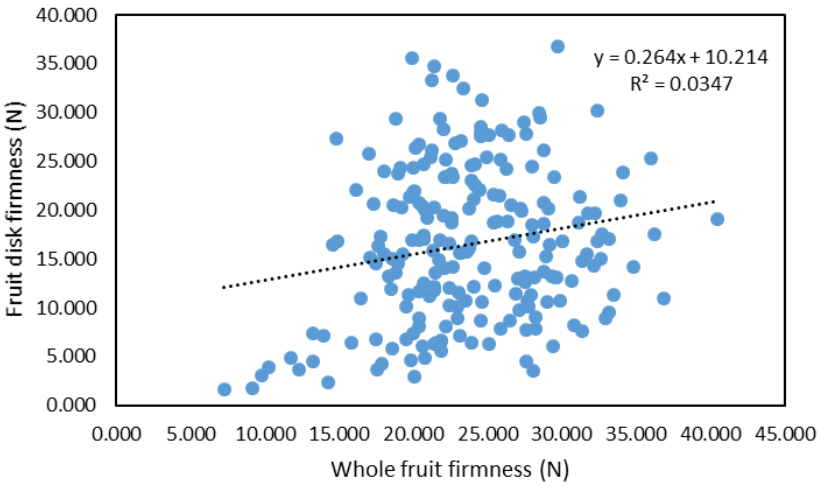

Figure 5. The correlation of whole fruit firmness (x-axis) and disk firmness (y-axis) of tomatoes. Single linear regression was performed on 218 fruits from 87 tomato genotypes.

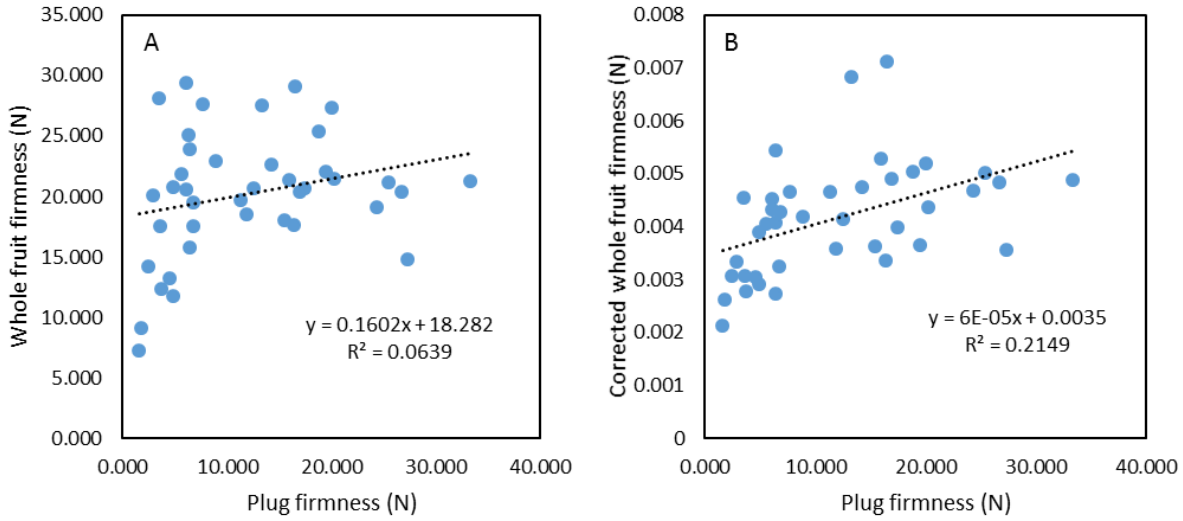

Figure 6. Correlations between disk firmness and whole fruit firmness (A) before and (B) after size correction. Single linear regression was performed on 40 fruits from 20 tomato genotypes.

Fruit juiciness was illustrated in Figure 7. Juiciness ranged from 8.64% to 47.50%, with more than 70% of fruits within 20% - 40%. Correlations between tomato juiciness and tomato firmness were also tested (Figure 8). Juiciness showed a better correlation with fruit disk firmness (R^2 = 0.403) rather than with whole fruit firmness (R^2 = 0.0026).

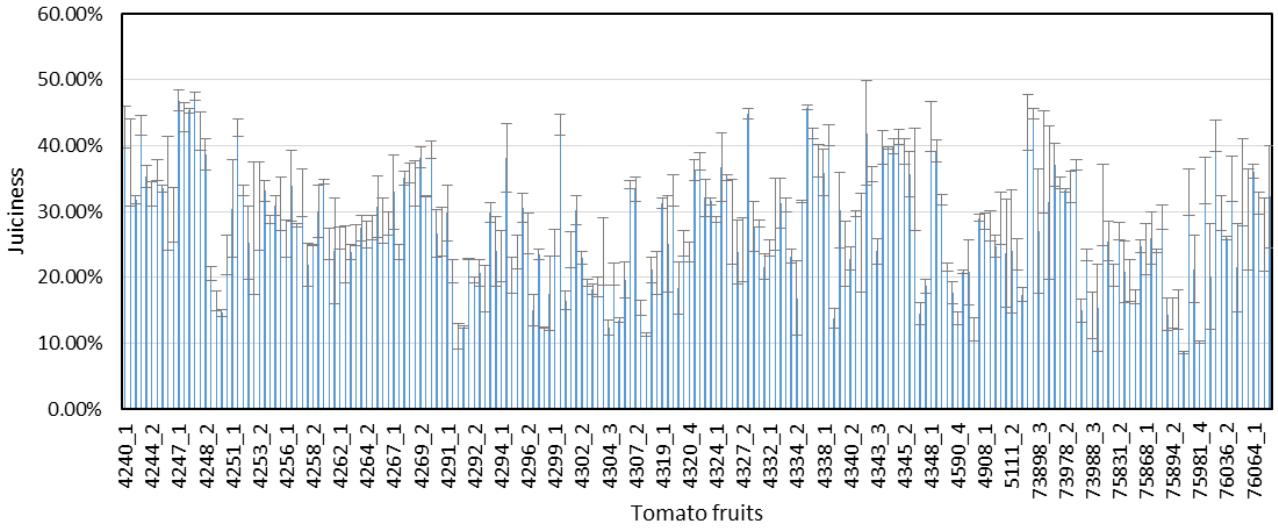

Figure 7. Tomato juiciness quantified by fruit disks. Error bars represent SEM (n = 2). Y-axis represents fruit juiciness (%) and x-axis represents the name of tomatoes. There are 214 tomato juiciness values displayed in this figure, but due to area limitation, only a partial of tomato names was showed.

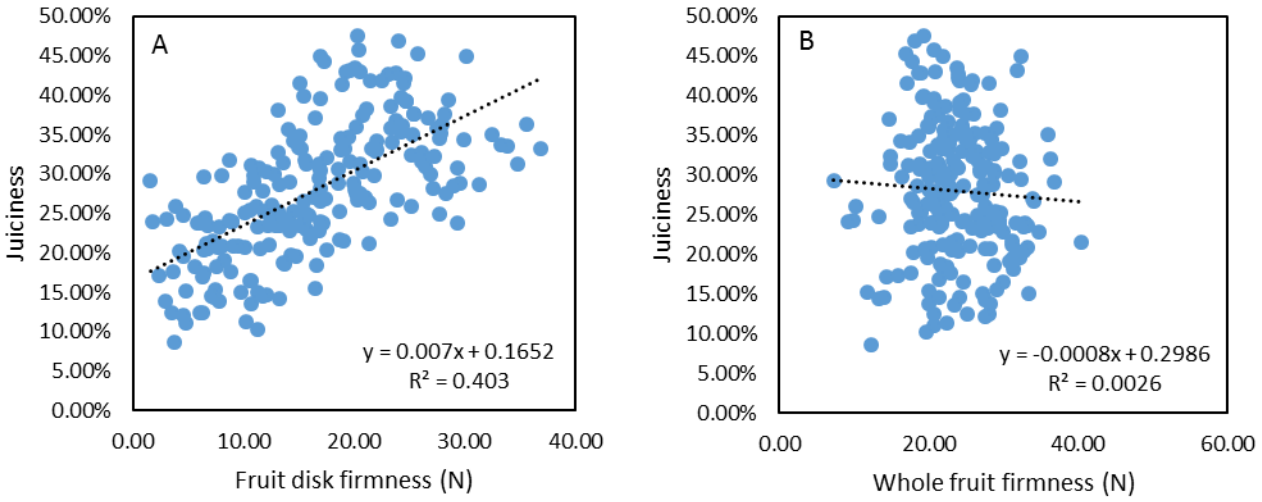

Figure 8. Correlations of (A) juiciness with fruit disk firmness and (B) juiciness with whole fruit firmness. Single linear regression was performed on 218 fruits from 87 tomato genotypes.

SSC of tomatoes were showed in Figure 9, ranging from 3.2 °Brix to 5.4 °Brix. The SSC of most fruits was within a narrow range between 3.5 °Brix and 4.5 °Brix, but variations could still be noticed due to small SEM.

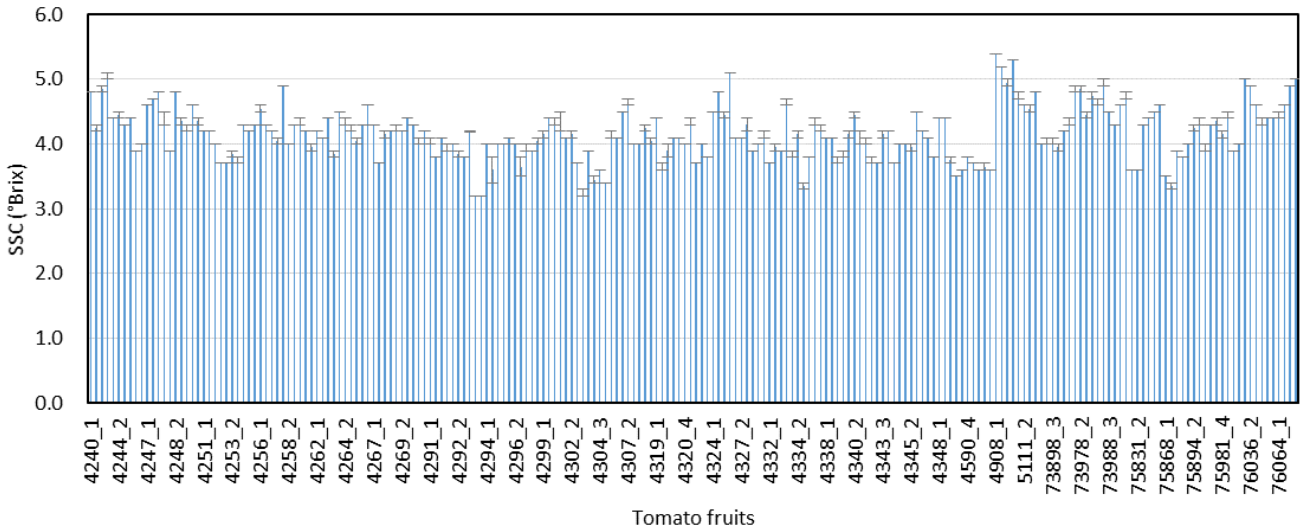

Figure 9. SSC of tomato fruits. Error bars represent SEM (n = 2). Y-axis represents fruit SSC (°Brix) and x-axis represents the name of tomatoes. There are 214 tomato SSC values displayed in this figure, but due to area limitation, only a partial of tomato names was showed.

Acidity of tomatoes was displayed in Figure 10. It was expressed as citric acid equivalents (mg L^{-1}). Acidity distributed from 3.6 to 7.0 mg L^{-1} with a majority portion around 5.0 mg L^{-1}. Most of the data were similar and significant differences could only be seen between polarization values.

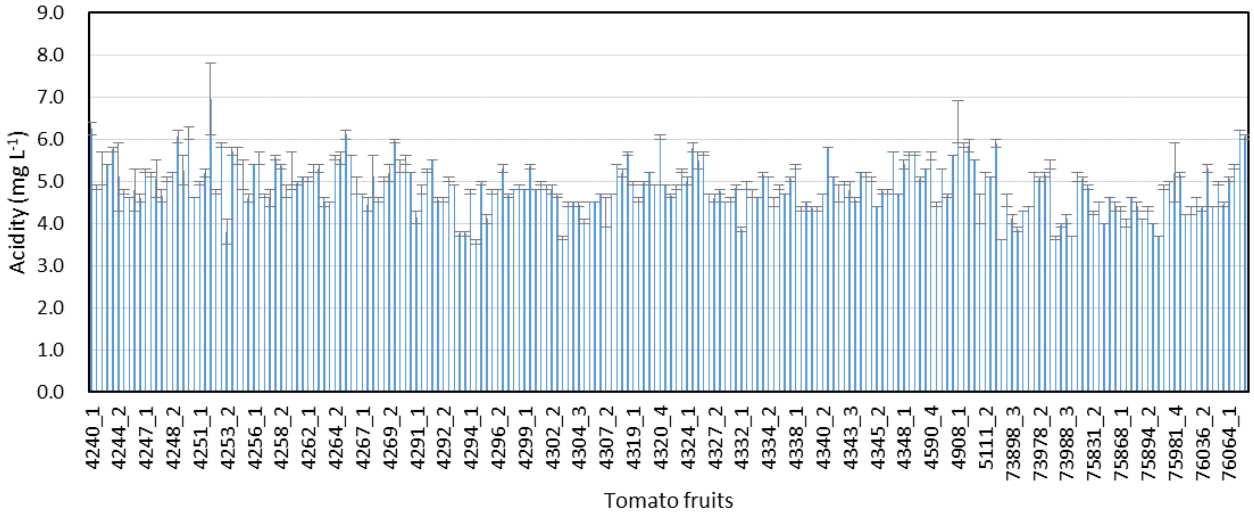

Figure 10. Acidity of tomato fruits. Error bars represent SEM (n = 2). Y-axis represents fruit acidity and x-axis represents the name of tomatoes. There are 214 tomato acidity values displayed in this figure, but due to area limitation, only a partial of tomato names was showed.

4.2.2 Non-invasive Measurements

Tomato fruits were measured by five non-invasive methods, VIS-NIR hyperspectral imaging, NIR hyperspectral imaging, VIS-NIR transmittance, NIR interactance and NIR reflectance. The data of two hyperspectral imaging methods have not been available yet, therefore, only the result of VIS-NIR transmittance, NIR interactance and NIR reflectance was presented. The general shape of the spectra of 87 tomato genotypes obtained by three modes was similar, respectively.

Spectra generated from raw VIS-NIR transmittance data were presented (Figure 11). The peaks at 680 nm were the absorption band of chlorophyll and at 722 nm were that of lycopene (Clément et al., 2008; McGlone, Fraser, Jordan, & Kunnemeyer, 2003). The peaks around 760 nm were one of the absorption band of water.

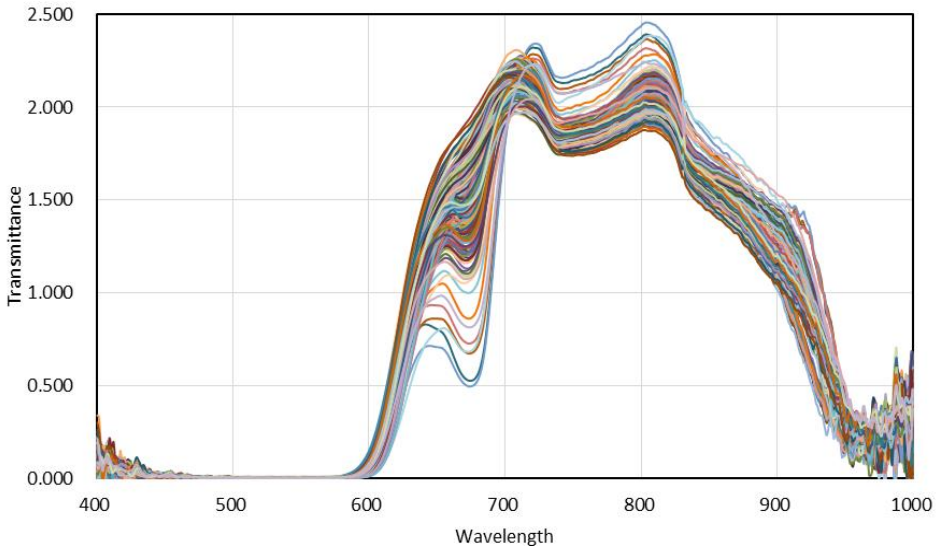

Figure 11. Transmitted spectra ranging from 400-1000 nm of 214 tomato fruits from 87 genotypes.

Raw data of NIR interactance was normalized by log transformation in order to obtain clear absorption bands. The NIR interactance spectra (Figure 12) were dominated by water absorption bands at 760 nm, 970 nm and 1170 nm and reflectance spectra (Figure 13) were dominated by that at 970 nm, 1170 nm, 1450 nm and 1930 nm due to the high water content in tomatoes. The broader bands at 1170 nm and 1450 nm could be due to overlapping with peaks at 1200 nm that corresponded to sugar related overtones and 1500 nm that corresponded to organic acid related overtones (Flores et al., 2009; Roberts, Stuth, & Flinn, 2004).

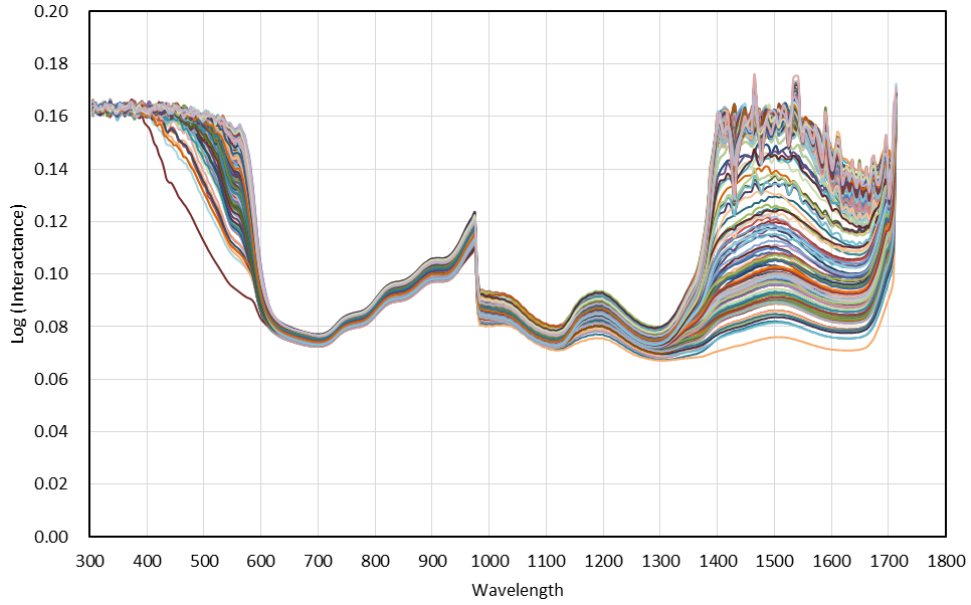

Figure 12. Interactance spectra ranging from 300-1750 nm of 214 tomato fruits from 87 genotypes.

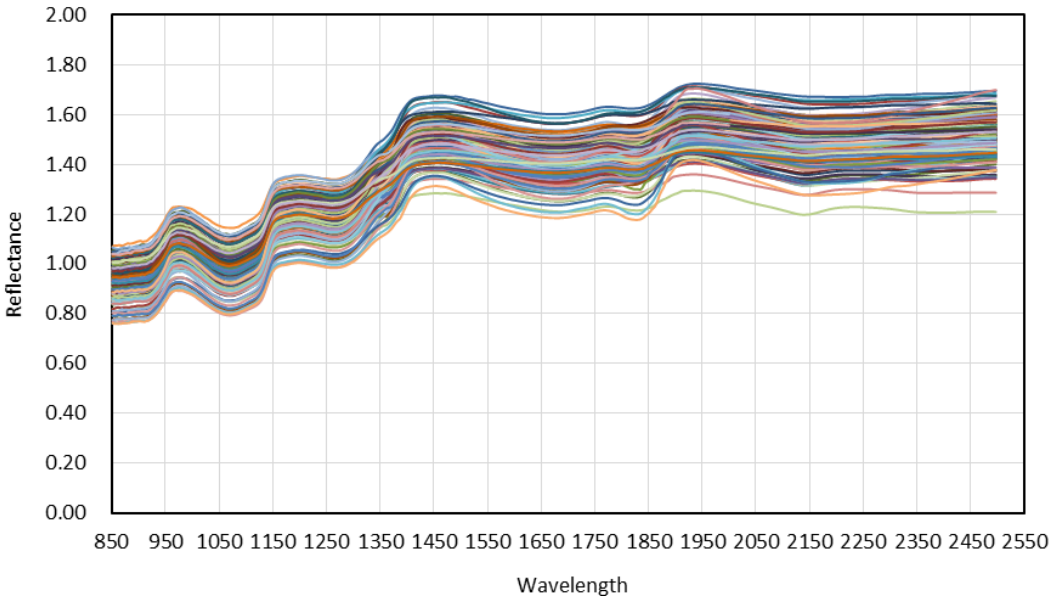

Figure 13. Reflected spectra ranging from 850-2500 nm of 214 tomato fruits from 87 genotypes.

4.2.3 OmicsFusion Analysis

The data obtained from invasive and non-invasive measurements were analysed by multivariate regression via OmicsFusion web tool. SSC, acidity, firmness and juiciness data were submitted as responses and VIS-NIR transmittance, NIR interactance and NIR reflectance data were submitted as predictors. The best three predictors from OmicsFusion analysis were summarized in Table 3. The regression coefficients (R^2) were calculated based on simple linear regression of raw invasive data and log transformed non-invasive data.

Poor correlations ($R^{2\,1}$) were observed between invasive and non-invasive data based on each fruit. $R^{2\,1}$ were slightly improved by making correlations based on each genotype ($R^{2\,2}$) and strongly improved by pooling

genotypes together ($R^{2\,3}$). Genotypes were ranked according to values of each invasive measurement, respectively, and every 11 genotypes were pooled together. In general, the quality traits could be mostly explained by predictors after pooling ($R^{2\,3} > 0.8$). Interactance was the best mode to predict SSC, acidity, juiciness and disk firmness and transmittance was the best mode to predict whole fruit firmness. Interactance spectroscopy at 588.71 nm, 593.00 nm and 597.29 nm were the best three predictors for SSC. These three wavelength were the successive scans performed by spectroscopy, therefore, this region might be a key factor to predict SSC. The top two predictors for acidity, 361.20 nm and 322.56 nm were also from a similar wavelength region, which indicates certain association of this region with acidity. Interactance spectroscopy at 678.55 nm and 674.56 nm (two continuous scans) were the best two predictors for juiciness, which suggested an important feature of this area to juiciness. Disk firmness was better predicted than whole fruit firmness. The adjacent wavelengths, 335.44 nm and 322.56 nm, detected by interactance mode and, 454.51 nm and 452.49 nm, detected by transmittance mode, were the top two predictors of tomato disk firmness and whole fruit firmness, respectively. Except for the third predictor of juiciness, all the listed predictors were fell in the visible range.

Table 3. The best three predictors (non-invasive) for quality traits (invasive) determined by OmicsFusion platform for tomatoes.

Quality trait	1st Predictor				2nd Predictor				3rd Predictor			
	Method	$R^{2\,1}$	$R^{2\,2}$	$R^{2\,3}$	Method	R^2	R^2	R^2	Method	R^2	R^2	R^2
SSC	Inter [4] λ = 588.71	0.25	0.31	0.82	Inter λ = 593.00	0.26	0.32	0.82	Inter λ = 597.29	0.26	0.33	0.82
Acidity	Inter λ = 361.20	0.08	0.12	0.70	Inter λ = 322.56	0.08	0.12	0.62	Trans [5] λ = 424.51	0.06	0.10	0.85
Juiciness	Inter λ = 678.85	0.23	0.30	0.87	Inter λ = 674.56	0.23	0.30	0.87	Inter λ = 919.24	0.19	0.25	0.81
Disk firmness	Inter λ = 335.44	0.22	0.28	0.82	Inter λ = 322.56	0.17	0.22	0.75	Trans λ = 440.55	0.13	0.17	0.68
Whole fruit firmness	Trans λ = 454.51	0.12	0.13	0.81	Trans λ = 704.35	0.09	0.10	0.37	Trans λ = 452.49	0.10	0.11	0.64

[1.] R^2 per fruit.

[2.] R^2 per genotype.

[3.] R^2 per 11 pooled genotypes according to ranked values of invasive measurement.

[4.] Interactance.

[5.] Transmittance.

4.3 Strawberry Phenotyping

4.3.1 Invasive Measurements

Firmness of strawberries was evaluated by compressing fruit disks. Strawberry firmness was ranged from 0.59 N to 7.34 N (Figure 14). The firmness of most fruits were similar around equators; however, due to partial decay some were quite different, which caused the big standard errors. Strawberry juiciness was ranged from 33.82% to 58.32%, with more than 63% of fruits within 40% - 50% (Figure 15). Brix and acidity data were not available to present.

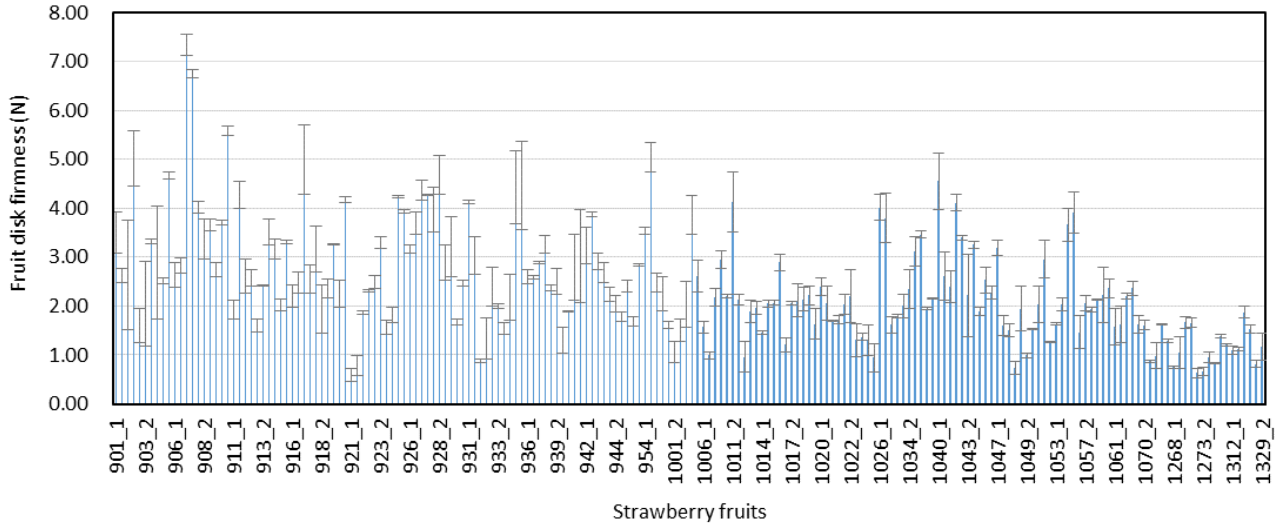

Figure 14. Strawberry firmness quantified by fruit disks. Error bars represent SEM (n = 2). Y-axis represents fruit firmness (N) and x-axis represents the name of strawberries. There are 196 strawberry firmness values displayed in this figure, but due to area limitation, only a partial of strawberry names was showed.

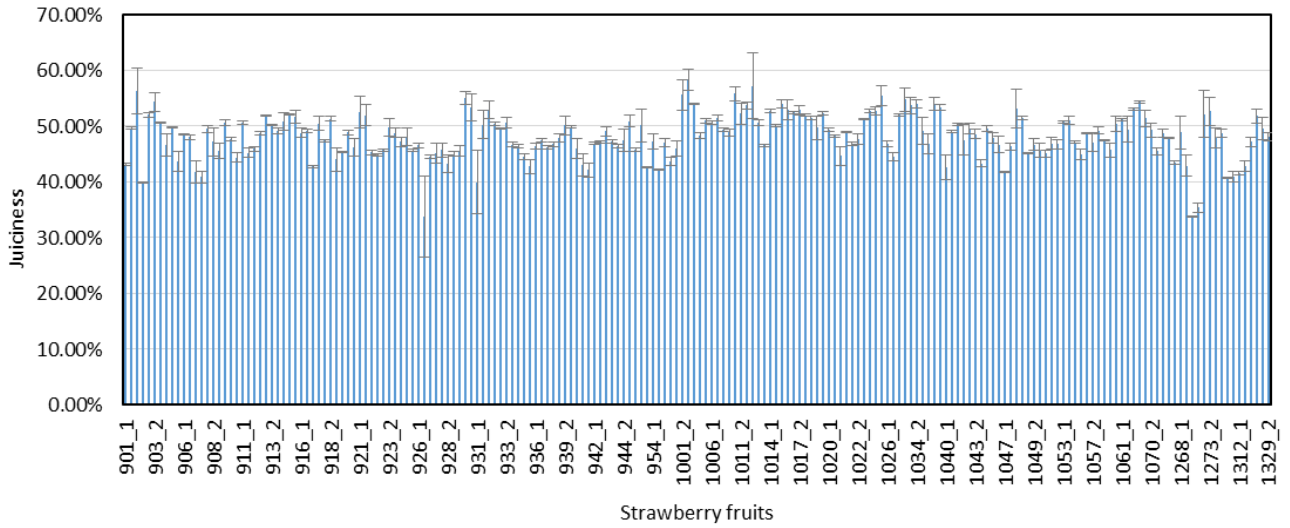

Figure 15. Strawberry juiciness quantified by fruit disks. Error bars represent SEM (n = 2). Y-axis represents fruit juiciness (%) and x-axis represents the name of strawberries. There are 196 strawberry juiciness values displayed in this figure, but due to area limitation, only a partial of strawberry names was showed.

4.3.2 Non-invasive Measurements

Strawberry fruits were also measured by five non-invasive methods. The data of two hyperspectral imaging methods have not been available yet, therefore, only the result of VIS-NIR transmittance, NIR interactance and NIR reflectance was presented. The general shape of the spectra of 98 strawberry genotypes obtained by three modes was similar, respectively.

Spectra generated from raw VIS-NIR transmittance data were presented (Figure 16). Similar to tomato, the peaks at 680 nm relating to chlorophyll absorption were observed.

Raw data of NIR interactance was normalized by log transformation in order to obtain clear absorption bands and that of NIR reflectance were directly used. Similar to tomatoes, the water content of strawberries was about 90%, thus the NIR spectra were dominated by water absorption bands. Overtones of water O-H bonds were absorbed at 760 nm, 970 nm and 1170 nm in NIR interactance spectra (Figure 17) and at 970 nm, 1170 nm, 1450 nm and 1930 nm in NIR reflectance spectra (Figure 18), respectively. The broader bands at 1170 nm and 1450 nm could be due to overlapping with peaks at 1200 nm, corresponding to sugar related overtones, and 1500 nm, corresponding to organic acid related overtone (Flores et al., 2009; Roberts et al., 2004).

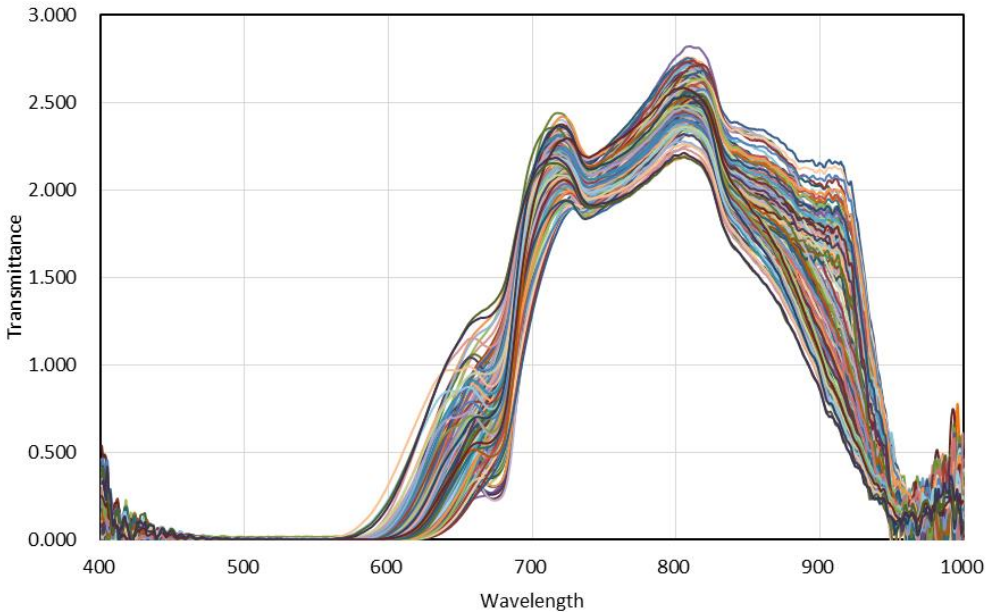

Figure 16. Spectra obtained from transmittance mode ranging from 400-1000 nm of 196 strawberry fruits from 98 genotypes.

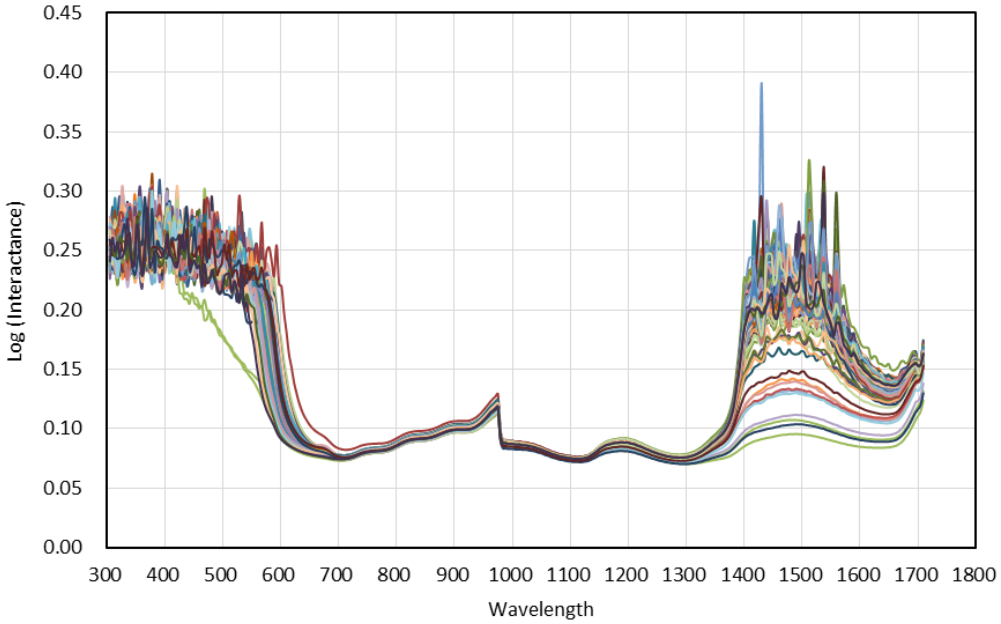

Figure 17. Spectra obtained from interactance mode ranging from 300-1750 nm of 198 strawberry fruits from 98 genotypes.

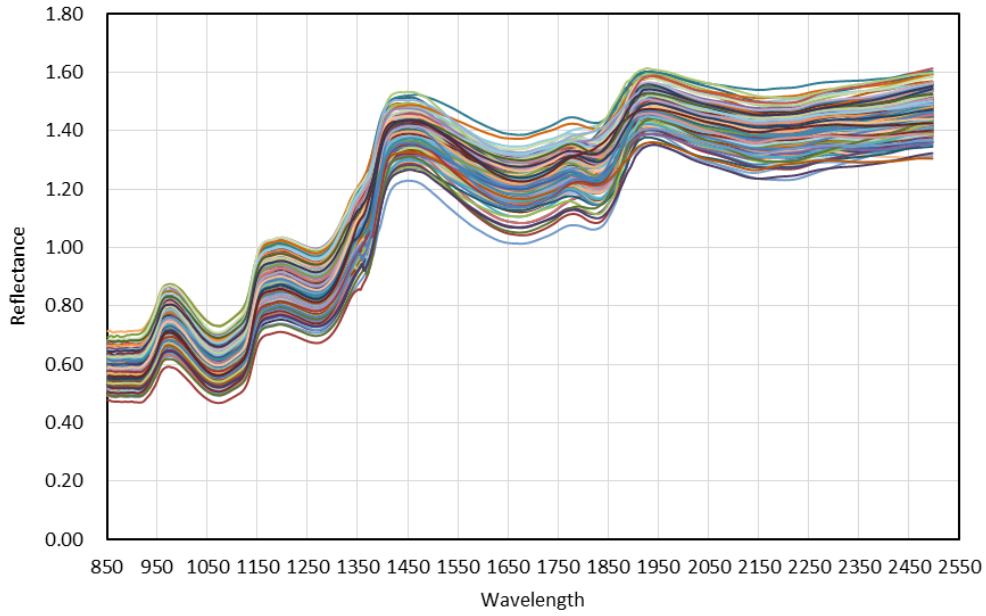

Figure 18. Spectra obtained from reflectance mode ranging from 850-2500 nm of 198 strawberry fruits from 98 genotypes.

4.3.3 Omicsfusion Analysis

Data obtained from invasive and non-invasive measurements were analysed by OmicsFusion. Firmness and juiciness data were submitted as responses and VIS-NIR transmittance, NIR interactance and NIR reflectance data were submitted as predictors.

Poor correlations ($R^{2\ 1}$) were observed between invasive and non-invasive data based on each fruit. Regression coefficients were improved by pooling as described in 4.2.3 for tomatoes. In general, the quality traits could be partially explained by predictors after pooling ($R^{2\ 3} > 0.5$). Transmitted spectra at 935.51 nm

was the best predictor for juiciness. The other two predictors obtained from interactance mode were from an adjacent region, which might also be relevant to strawberry juiciness. Transmittance at 478.63 and 407.47 nm showed best two prediction of disk firmness, followed by interactance at 369.78 nm. Except for the first predictor for juiciness fell in the NIR region, all other predictors were within the visible region.

Table 4. The best three predictors (non-invasive) for quality traits (invasive) determined by OmicsFusion platform for strawberries.

Quality trait	1st Predictor				2nd Predictor				3rd Predictor			
	Method	$R^{2\,1}$	$R^{2\,2}$	$R^{2\,3}$	Method	R^2	R^2	R^2	Method	R^2	R^2	R^2
Juiciness	Trans[4] λ = 935.51	0.07	0.17	0.46	Inter[5] λ = 322.56	0.09	0.13	0.59	Inter λ = 378.37	0.08	0.10	0.55
Disk firmness	Trans λ = 478.63	0.18	0.20	0.76	Trans λ = 407.47	0.15	0.20	0.82	Inter λ = 369.78	0.10	0.15	0.60

1. R^2 per fruit.

2. R^2 per genotype.

3. R^2 per 11 pooled genotypes according to ranked values of invasive measurement.

4. Transmittance.

5. Interactance.

5. Discussion

Texture and taste are very important postharvest quality trait of tomatoes and strawberries to determine their commercial values and consumer preferences. Even though traditional methods are accurate, they are destructive, inefficient, time-consuming and laboratory intensive. Therefore, several non-destructive optical phenotyping methods were used in this study to estimate SSC, acidity, firmness and juiciness of tomatoes and strawberries from a large number of genotypes. Data obtained from both invasive and non-invasive methods were subjected to multivariate regression analysis in order to uncover the best non-invasive predictors. In this section, the identification of key predictors is discussed and an accurate destructive firmness and juiciness method is explained.

5.1 Invasive Firmness and Juiciness Methods

Tomato firmness was evaluated in two invasive methods, namely whole fruit firmness and pericarp disk firmness measurements. The disk method showed more sensitivity and variations than the whole fruit method (Figure 4) and poor correlation was observed between these two methods (Figure 5; R^2 = 0.03). According to Verkerke (communication) these two methods should give a similar trend; however, this was not the case in our result. There were several possible explanations. Errors in disk measurement should be small due to uniformity of cut disks, on the contrary, large errors could occur in whole fruit measurement due to genetic variations in shape, size and number of locular cavities, which made the whole fruit measurement less accurate. By correcting the size variation in whole fruits, regression coefficient between these two methods improved (Figure 6). Additionally, the method to measure disk firmness imitate the bite behaviour, which needs to break cell walls. Disk firmness is only dependent on cell wall structure, whereas whole fruit firmness is dependent on both cell wall structure and turgor pressure (Hertog, Ben-Arie, Róth, & Nicolaï, 2004). Moreover, tomato juiciness showed a better correlation with disk firmness than whole fruit firmness (Figure 8). Therefore, measuring fruit disk is a more reasonable and precise way to quantify fruit firmness. The parameter settings we set in our experiments were good.

In general, tomatoes were less juicy than strawberries (Figure 7 & 15). Strawberries displayed a more constant juiciness levels among genotypes compared to tomatoes. The spectral information provided better predictions for tomato juiciness rather than that of strawberries (Table 3 & 4), which indicated the measurement for tomato might be more accurate. Sometimes after strawberry compression, extra juice was observed on the texturemeter, which suggested a saturation of filter papers. Thus, the insufficient thickness of filter papers was a hamper for strawberry juiciness determination in our study.

5.2 Non-destructive Phenotyping Predictors for Tomatoes

Poor correlations were observed between invasive and non-invasive methods based on each tomato fruit and they were greatly improved by pooling every 11 genotypes with similar invasive values together (Table 3). The VIS-NIR reflectance has been reported to have a poor prediction for SSC and acidity of tomatoes from various genotypes but a good prediction for a single tomato genotype (Clément et al., 2008; Ecarnot et al., 2013; Shao et al., 2007). In our study, 87 round tomato genotypes were used. There were variations in fruit size, shape, colour, surface, mealiness, seed and inner structure (data now shown), which could add errors to spectral information when predicting SSC, acidity, firmness and juiciness. In addition, invasive data showed that the range of values was narrow (Figure 4, 7, 9 & 10), making predictions more challenging. Narrow values could be partly due to the similar mature stage of all fruits since SSC, acidity, firmness and juiciness were all associated with ripening processes. Pooling increased the variability of tested quality attributes and reduced other genotypic variations, therefore, correlation coefficients between invasive and non-invasive methods were significantly improved.

Spectral information obtained from VIS-NIR transmittance, NIR interactance and NIR reflectance were employed to predict SSC, acidity, firmness and juiciness of tomatoes. The best predictors for SSC, acidity, juiciness and disk firmness were generated from NIR interactance and the best predictors for whole fruit firmness were generated from VIS-NIR transmittance (Table 3). In most cases, interactance mode produced the best models and the transmittance mode was better than the reflectance mode. Fruit pericarp was used destructively to quantify SSC, acidity, firmness and juiciness; however, the reflectance mode only acquired information about fruit surface rather than pericarp because the light was not deep enough to penetrate tomato tissues (Clément et al., 2008). What's worse, due to its susceptibility to surface properties reflectance mode is easily affected by genotypic variations in this category. Thus, NIR reflectance did not sufficiently predicted postharvest quality traits in our study. The transmitted spectra carried more information about whole fruit, thus, it provided the best prediction for whole fruit firmness. Even though transmittance mode was less affected by surface properties than reflectance and interactance modes, the amount of light penetrating the fruit was limited and resulted in a more unfavorable signal-noise ratio for transmitted spectra, especially for tomatoes with a heterogeneous internal composition (Schaare & Fraser, 2000), therefore, it was not the best predictors for SSC, acidity, juiciness and firmness, but it can predict them at a sufficient level. In interactance mode, the interacted spectra contain both external and part of the internal information, its active area was mainly the tomato pericarps where the destructive measurements were conducted. Therefore, the interactance spectra carried a lot of information about pericarp and performed the best prediction for tested quality traits. Furthermore, the interactance spectra were less sensitive to specular reflections, which could probably lead to larger errors (Wang, Peng, Xie, Bao, & He, 2015).

5.3 Non-destructive Phenotyping Predictors for Strawberries

Poor correlations were also observed between invasive and non-invasive methods based on each strawberry fruit and the correlations were also substantially improved by pooling every 11 genotypes with similar invasive values together (Table 4). The same reason for pooling has been extensively explained in 5.2 for tomatoes.

Spectral information obtained from VIS-NIR transmittance, NIR interactance and NIR reflectance were employed to predict firmness and juiciness of strawberries. The best predictors for firmness and juiciness were generated from VIS-NIR transmittance (Table 4). The second the third predictors were generated from NIR interactance. When measuring strawberries under transmittance mode, light could be detected at the opposite position of light source; however, when measuring tomatoes, light was also detected around vertical positions. The internal composition of strawberries was homogeneous and the size of strawberries was small, therefore, the transmitted light could penetrate strawberries much easier compared to tomatoes. As the transmitted spectra carried the whole fruit information, transmittance mode was ranked as the best model to predict texture attributes. Similar to tomatoes, the interactance mode was also sufficient to predict strawberry texture attributes.

5.4 Spectral information

In general, the absorbance patterns obtained from our study can be loosely related to the functional groups associated with water and sugars (Alves de Oliveira et al., 2014). All the predictors for tomatoes and strawberries could not be directly reflected from VIS/NIR spectra (Figure 11, 12, 13, 16, 17 and 18), except that the best predictor (935 nm) for strawberry juiciness was viewed in VIS-NIR transmitted spectra with small peaks (Figure 16).

The wavelength of two juiciness predictors for tomatoes and strawberries were close to each other (λ = 919.244 / 935.51 nm), so the region around these wavelengths might be a key predictor for juiciness characters.

Three wavelengths 685, 865 and 985 nm (670-685 nm, 755-870 nm, and 955-1000) obtained from NIR hyperspectral imaging has been reported to predict strawberry firmness (Tallada et al., 2006). The top four to seven predictors of strawberry disk firmness obtained from VIS-NIR transmittance were within 670-685 nm region and more than 50 top predictors of tomatoes disk firmness also fell into these three ranges (data not shown). Our findings confirmed the previous study and addressed the importance of these spectral regions for firmness prediction. The peaks at 680 nm (Figure 11 & 16) were the absorption band of chlorophyll (McGlone et al., 2003). Firmness is related to the cell wall structure of tissues and the most important postharvest process responsible for degrading cell wall structure is ripening (Hertog et al., 2004).

Thus, fruit firmness is indirectly related with ripening (García-Ramos, Valero, Homer, Ortiz-Cañavate, & Ruiz-Altisent, 2005). In addition, chlorophyll content has been reported as an indicator for ripeness, and hence the firmness of tomatoes and strawberries could be associated with chlorophyll content (Tallada et al., 2006). Therefore, it was reasonable that the wavelength predictors for tomato and strawberry firmness were within chlorophyll absorption region. Furthermore, the three top predictors of tomatoes and strawberries firmness showed higher regression coefficient than the ones mentioned above (Table 3 & 4), therefore, the newly discovered wavelengths are highly interesting for further investigation.

Overall, good predictions were found for quality traits of tomatoes and strawberries after pooling. The best predictors for SSC, acidity, juiciness and disk firmness of tomatoes were generated from NIR interactance and the best predictors for whole fruit firmness were generated from VIS-NIR transmittance. The best predictors for firmness and juiciness of strawberries were generated from VIS-NIR transmittance. It is worth noticing that only two juiciness predictors were in the NIR region and all other predictors listed in this report were from visible region, which was different from previous study that the NIR region provided more internal quality information (Butz et al., 2005).

Unfortunately, all the available results are spectroscopy methods with spot measurements, which cannot provide spatial information. Hyperspectral imaging technique can assess the whole fruits and provide spatial information (Wang et al., 2015), which may give a better prediction in the future.

6. Conclusion

The potential of VIS-NIR transmittance, NIR interactance and NIR reflectance to non-invasively predict postharvest quality trait, namely soluble solid content, acidity, juiciness and firmness, of tomatoes and strawberries was investigated. A large number of tomato and strawberry genotypes was used for both invasive and non-invasive methods. Invasive methods to quantify firmness and juiciness were designed in our study. Fruit firmness evaluated by compressing fruit disks was more sensitive and precise than compressing whole fruits. The juiciness method was good enough for tomatoes; but when measuring strawberries, filter papers were not thick enough to absorb all the juice, which enlarged the experimental error. Multivariate regression analysis was performed on invasive and non-invasive data to select characteristic wavelengths as predictors. Low correlations were obtained between invasive and non-invasive data of each fruit for both tomatoes and strawberries, which was due to small variations in tested quality attributes and large genotypic variations in fruit size, shape, internal composition and etc. Correlations were dramatically improved by pooling genotypes with similar tested quality values, separately, because of averaged variations in other physical parameters. Wavelengths obtained by NIR interactance were the best predictors for SSC, acidity, juiciness and disk firmness of tomatoes and that obtained from VIS-NIR transmittance was the best predictor for whole tomato firmness. Wavelength obtained by VIS-NIR transmittance was the best predictor for juiciness and disk firmness of strawberries, but NIR interactance could provide almost equal predictions considering the regression coefficient. NIR reflectance could not give useful predictions as it only had access to fruit surface rather than fruit tissues.

Hyperspectral imaging was also employed in our study, but the data have not been available yet. As spectroscopy only provided point measurements, hyperspectral imaging was expected to bring good predictions since it analyses the entire fruit and takes spatial information into account.

The findings in this thesis provide insights into spectral information that can be used as predictors for non-invasive phenotyping, which can give some references for further research.

7. Further perspectives

In future study, the following aspects are suggested to be taken into consideration:

- The juiciness method for strawberries should be improved by using thicker filter papers.
- Experiments should be carried out with less genotypes but more samples from each genotype. The predictions should be done on all genotypes and all samples from a single genotype respectively in order to compare the applicability of VIS/NIR spectroscopies.
- According to our results, NIR interactance and VIS-NIR transmittance modes seem to be very promising predictors, therefore more efforts should be put on these two modes.
- Increase the light intensity in transmittance mode in order to penetrate the fruit and increase signal to noise levels. But the light intensity should not be too high to overheat the fruits.
- The measurement of spectroscopies is limited in a small area of fruit surface. In addition, NIR spectroscopy lacks the consistent measurement due to spatial variations. Hyperspectral imaging is highly recommended because it offers an improved solution over NIR spectroscopy by analyzing images of the entire fruit, where many images are taken continuously at a range of wavelengths with narrow intervals.
- The further interpretation of the spectra may take place via direct spectrum identification using data bases or increasingly by applying procedures of high-speed chemometrics (Butz et al., 2005).
- The spectral data should be pre-treated and smoothed before multivariate regression analysis.

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Appendix I. Protocol for NIR Hyperspectral Imaging

There are two cameras attached to the hyperspectral imaging system, VIS-NIR camera (red, 400-1000nm) and NIR camera (blue, 1000-1700nm), respectively (Figure A1).

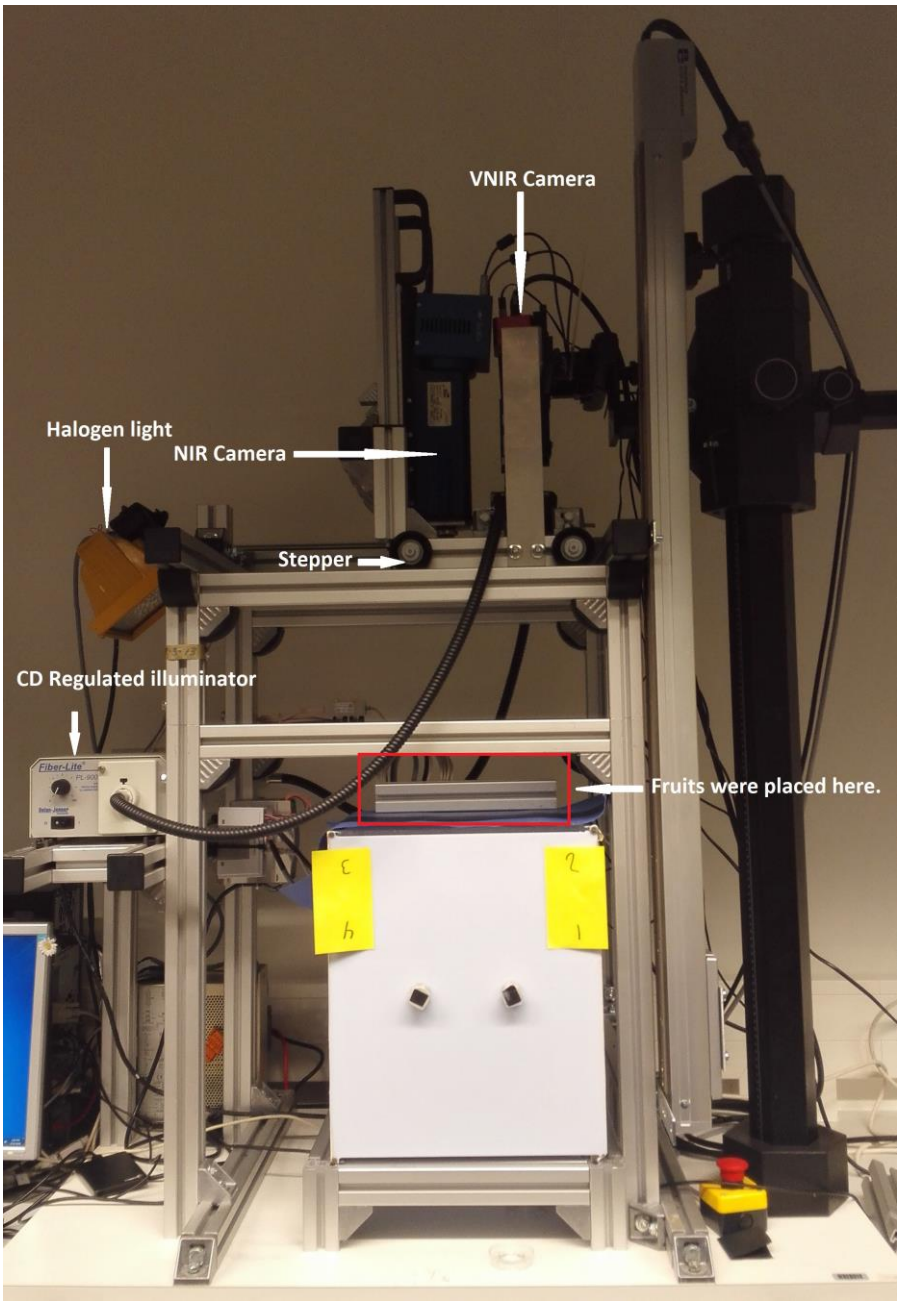

Figure A1. The construction of hyperspectral imaging system.

- Turn on the light 20 minutes before using.
- Turn on the stepper.
- Remove the green calyxes of tomato fruits. Put strawberries on a custom rack with green calyxes down.
- Start with the NIR camera (blue).

- Open the software: Isaac 2.
- Parameter setting:
    - Camera menu → Scan spectral image
    - Camera → NIR
    - Camera: Specin-N17E
    - Spectrum: 900-1700
    - Exposure (ms): 10 (tomatoes) and 20 (strawberries)
    - Stepsize (mm): 0.5
    - Nt of step: 350 (tomatoes) and 450 (strawberries)
    - Stepper table selection: Tripos (horizontal)
- Calibration:
    - Dark reference: use a black card
    - White reference: use a white Teflon plate with 99% reflectance
    - Pay attention: the light in the room should be off and there should not be black lines in the scanned view of white reference.
- Scan the papers indicating fruit position.
- Scan fruits.
- For each scan, the file should be saved:
    - File → save and close specim_1 → D drive →tomato_arnaud → HS-NIR20160517 (Folder name for example)
    - Create a new folder updating the date (Important for Gerrit. Order is HS-NIRyear-month-day with no spaces, see example)
- Close the software, pluck the plug of NIR camera. Turn off the light for NIR camera but keep the power on for ventilation to cool the machine.

Appendix II. Protocol for VIS-NIR Hyperspectral Imaging

- Turn on the light 20 minutes before using.
- Turn on the stepper.
- Remove the green calyxes of tomato fruits. Put strawberries on a custom rack with green calyxes down.
- Start the VIS-NIR camera (red).
- Open the software: Isaac 2.
- Parameter setting:
    - Camera menu → Scan spectral image
    - Camera → VNIR (vis-nir)
    - Spectrum: 400-1000
    - Exposure (ms): 50
    - Stepsize (mm): 0.5
    - Nt of step: 350 (tomatoes) and 500 (strawberries)
    - Stepper table selection: Tripos (horizontal)
- Calibration:
    - Dark reference: with the camera lid closed
    - White reference: with a white Teflon plate with 99% reflectance (Figure A2)

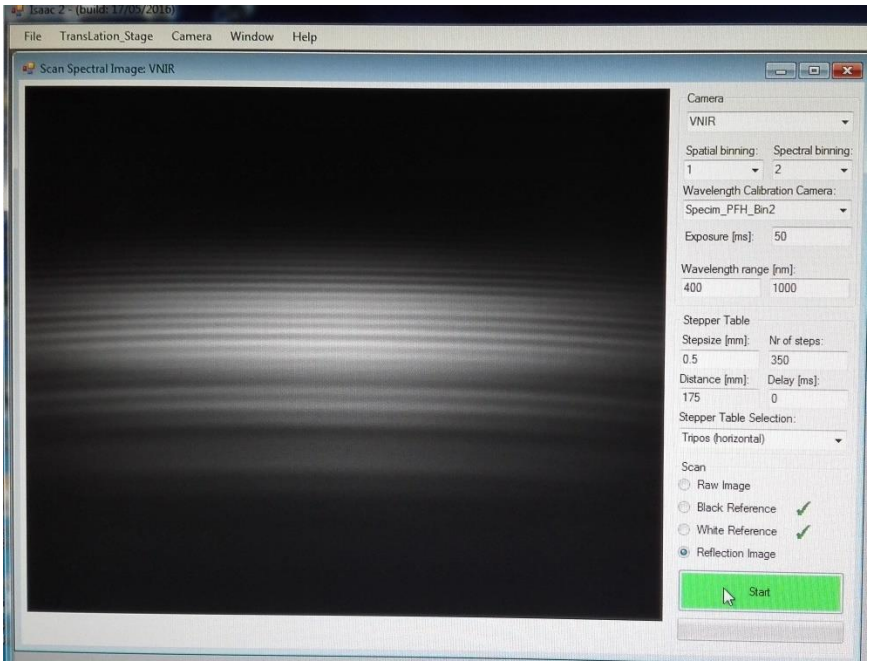

Figure A2. The corrected white reference imaging.

- Scan the papers indicating fruit position in a new folder HS-VNIR20160517.
- Scan fruits (Figure A3).

Figure A3. Example of fruit scanning.

- Save every scan. Create a new folder updating the date (as in NIR)
- After measurements, turn off the light for VNIR camera but keep the power on for cooling.

Appendix III. Protocol for VIS-NIR Transmittance

The equipment is composed by two parts, a DC regulated illuminator (Fiber-Lite® DC-950) and a fiber optic spectrometer with a sensor attached on it (Ocean Optics, SD2000) (Figure A4).

- Turn on the light 20 minutes before measurement. The output of light should be at its maximum value.

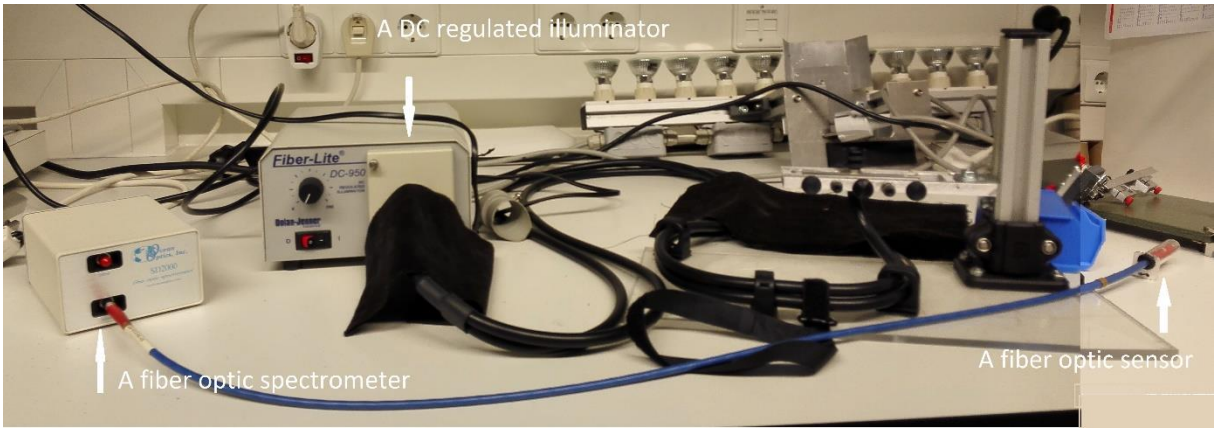

Figure A4. The composition of VIS-NIR transmittance system.

- Open the software, Scan Tool (if it is not on desktop, please find it in C drive/users/public).
- The parameter settings are showed in Figure A5.

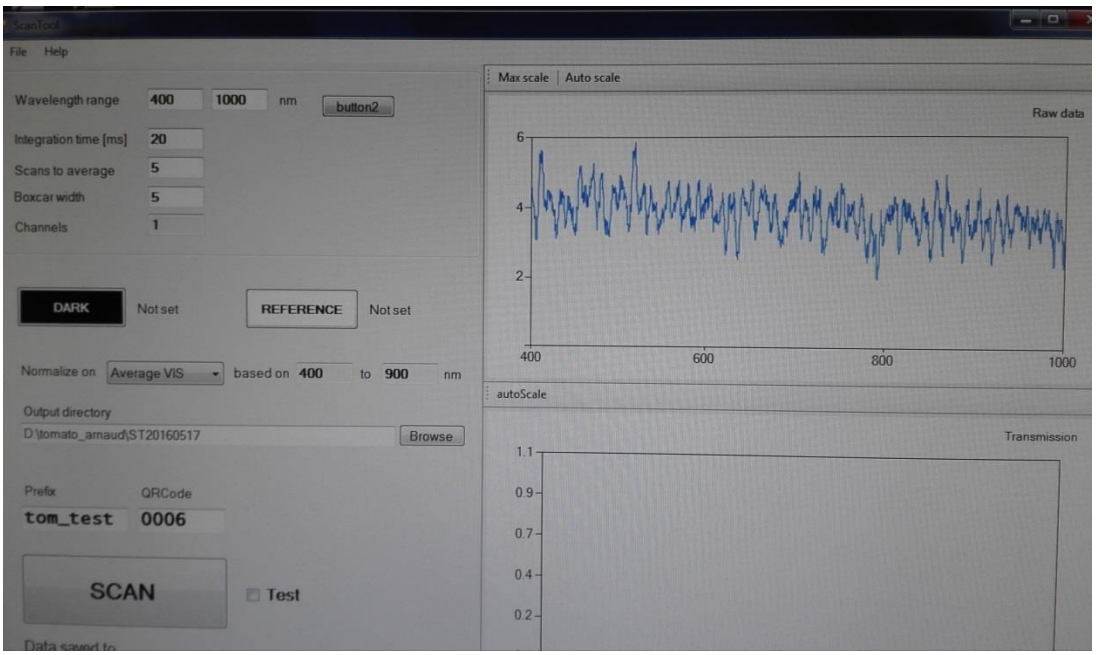

Figure A5. Parameter settings.

- Measure the dark and white reference spectrums.
    - The dark reference spectrum is obtained by covering the fiber optic sensor with a dark card. The correct image of the dark reference should be like the blue waves in raw data window in Figure A5.

- The white reference spectrum is obtained by measuring the transmitted light of a Teflon plate (Figure A6). Do not get saturation. When it is saturated, the background of the output window will become red. The threshold for saturation is 4960.

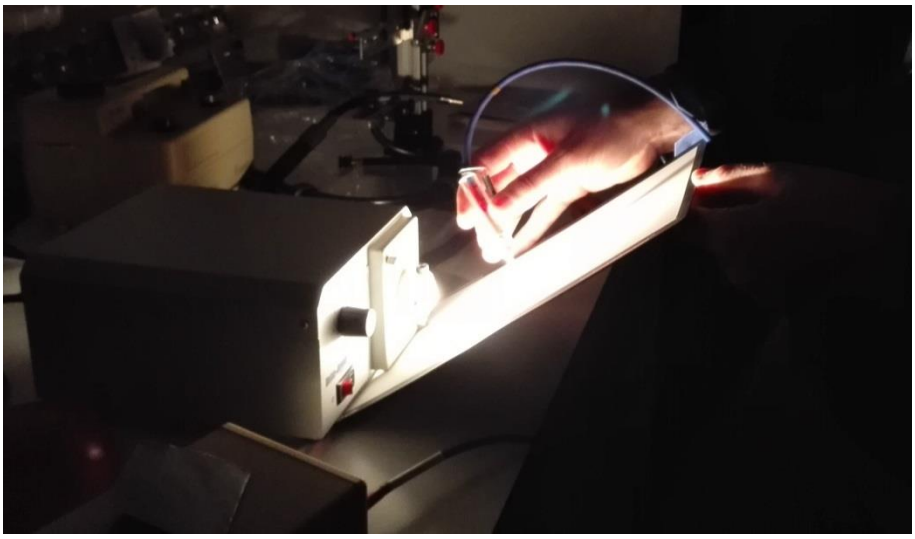

Figure A6. Measuring the white reference spectrum.

- Name the files properly. The data are save at D:\tomato_arnaud\ST20160525. New files will be made every day. ST means spectral transmission.
  - Prefix: the genotype code and the fruit, e.g.01_1_. *01(this is first genotype)_1(this is the first fruit of first genotype)_*
  - QRCode: 0001; the number of scan, it will continue automatically.
- Figure A7 shows how the fruits are attached to the light source and how the sensor is attached to the fruits. Four scans per fruit. Keep the measured max scale above 1000.

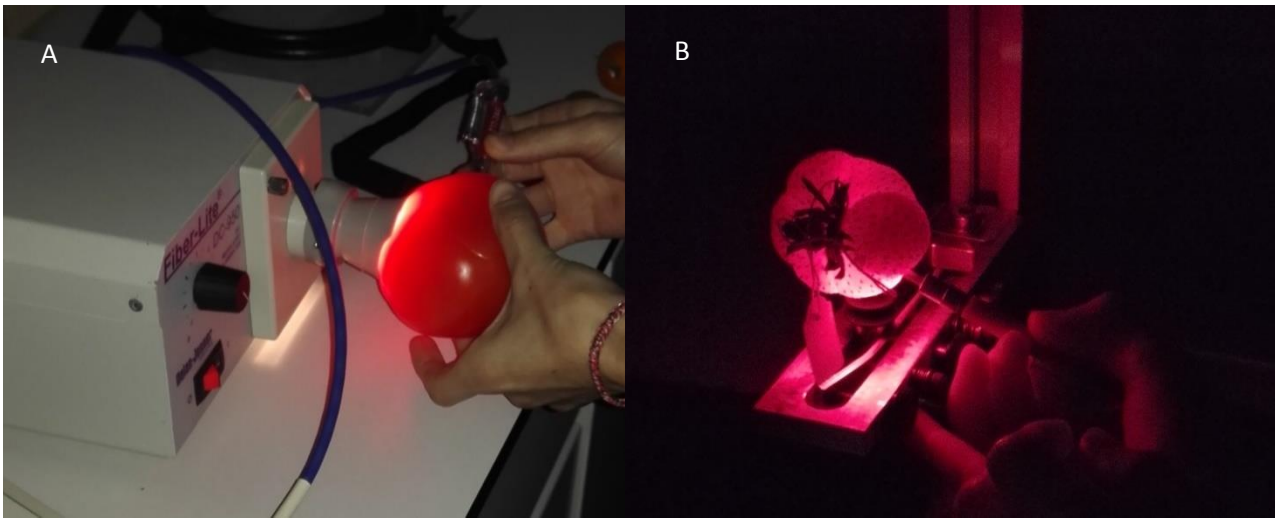

Figure A7. The position of tomatoes (A) and strawberries (B) to the light source and fiber optic sensor. Both the fruits cover the light source. The fiber optic sensor touches the tomato fruit while does not touch the strawberry fruit in case of damaging.

- The upper output window in Figure A5 shows the raw data, which is a live signal. The lower output window shows the final transmittance signal.
- Turn off the light after measurement but keep the power on for a while to cool the equipment (20 minutes).

Appendix IV. Protocol for NIR Interactance

- Close Luxaflex and turn on the lamp to keep the background light the same.
- Turn on a dual fibre light source 15 minutes in advance.
- Open the software, EPPN.
- The strawberry or tomato settings are showed in Figure A8.
- Press the start button ⇨ to start the program.

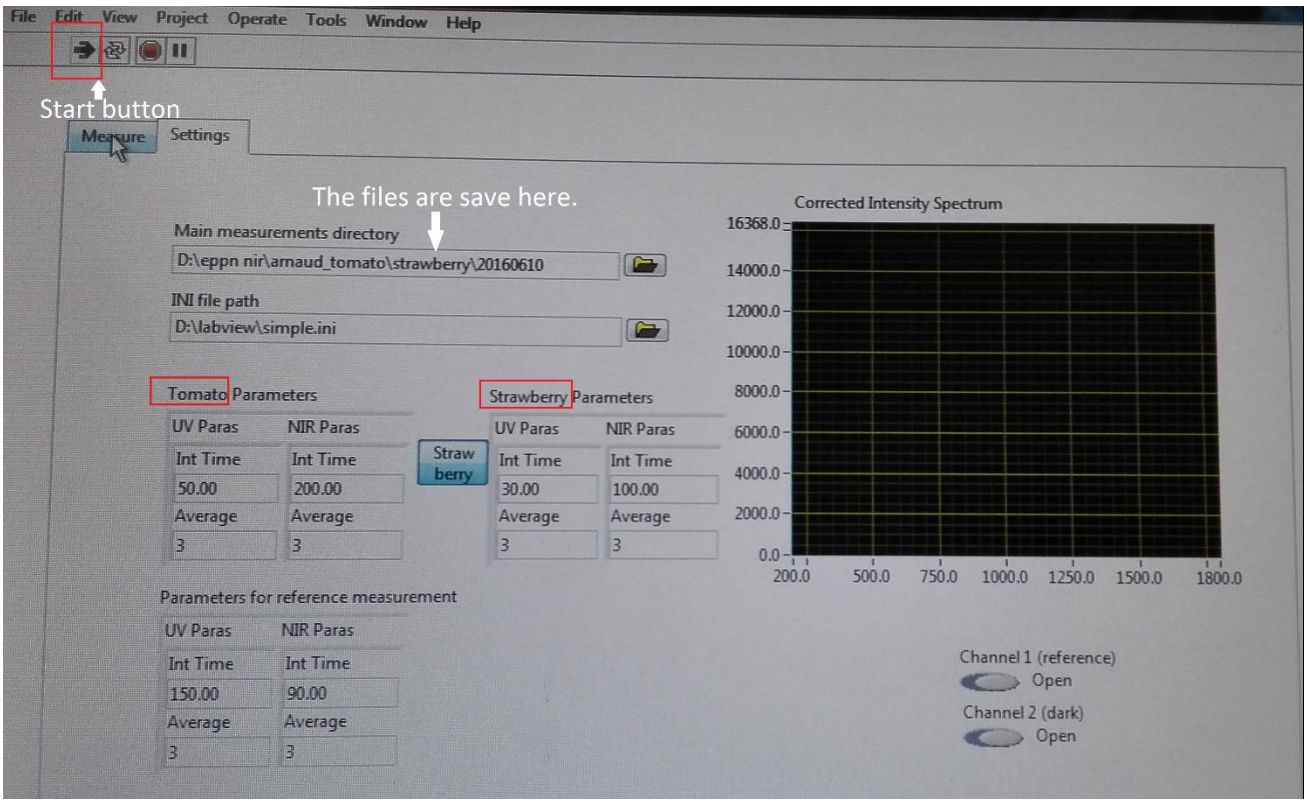

Figure A8. The tomato and strawberry settings.

- Dark and light calibrate:
  - Dark: close light source, fully cover light and sensor with grey disc (10% spectral disc), press TADE DARK → green light on screen.
  - Light: light source distance with disc (50%) and then grey disc 10% spectral disc (Figure A9), press TADE REF → green light on screen .
  - Signals: signal levelling (the peaks) should be 75% of the maximum value.

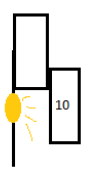

Figure A9. The position of grey disc when doing white calibration.

- Batch name: one fruit per batch and 3 measurements per fruit.
- New measurement press TAKE MEASUREMENT button.
- Make certain to cover the light source and the sensor with fruit surface (Figure A10).
- Randomly choose three spots at the equator to measure.

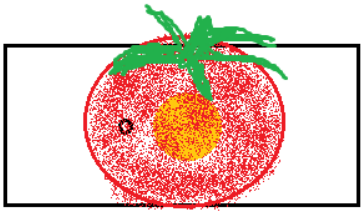

Figure A10. The position of fruits to cover the light sensor.

- At the end switch off the light but leave the computer and the spectrophotometers on.

Appendix V. Protocol for NIR Reflectance

- Calibrate the machine if necessary. The machine will ask for calibration itself.
- Calibration the instrument → scan → options → scan type → background

- Clean the machine with wet tissue to avoid juice or sugar sticking on it.
- Login: no password, just press OK.
- Software: Spectrum, the icon looks like a little red.
- Data were collected at D:\Tomatosphenotyping may 2016
- Setting:
  - Instrument → scan → open → tomato set → apply → change the name of every measured tomato → apply → scan
  - Resolution: 64
  - Duration: scan number 2
  - 3 spots per tomato and 2 spots per strawberry
  - 2 scans per spot
- Name every scan manually.
- Cover the light with each fruit and cover the fruit with a white cap.
- All the tomatoes and strawberries should be at room temperature, because the temperature can influence the spectrum.

Appendix VI. Protocol for Firmness and Juiciness measurement

The firmness and juiciness were determined by a Texturemeter (TA. HD. Plus, Texture Analysis, Stable micro systems).

- Open the texturemeter. When the “busy” button is green, the computer can be turned on.
- Open the software: Textuur
- Calibration → Calibrate height →Return to distance: 20 mm
  - Tomato whole fruit: pure bottom
  - Tomato plugs: 2 filter papers and 2 micaclothes on the bottom
  - Strawberry plugs: 2 filter papers and 2 micaclothes on the bottom
- Load project:
  - Tomato whole fruit: TomatoBite4mm
  - Tomato plugs: TomatoRaquelTest_Noholdingtime
  - Strawberry plugs: TomatoRaquelTest_Noholdingtime
- T.A. setting
  - Tomato whole fruit:
    - Test Mode: compression
    - Pre-Test: 2.00 mm/sec
    - Test speed: 1.00 mm/sec
    - Post-Test speed: 5.00 mm/sec
    - Target mode: Distance
    - Distance: 3 mm
    - Trigger Type: Auto Force
    - Force: 0.049 N
    - 10 % strain
  - Tomato and strawberry plugs:
    - Test Mode: compression
    - Test speed: 1.00 mm/sec
    - Post-Test speed: 5.00 mm/sec
    - Target mode: Distance
    - Distance: 9.7 mm (tomatoes) and 11.7 mm (strawberry)
    - Trigger Type: Button
    - Advanced Options: Off
    - Probe setting: 10.0 mm (tomatoes) and 12.0 mm (strawberry)

- Test Configuration: Name the files and create folders for saving data (Figure A11).

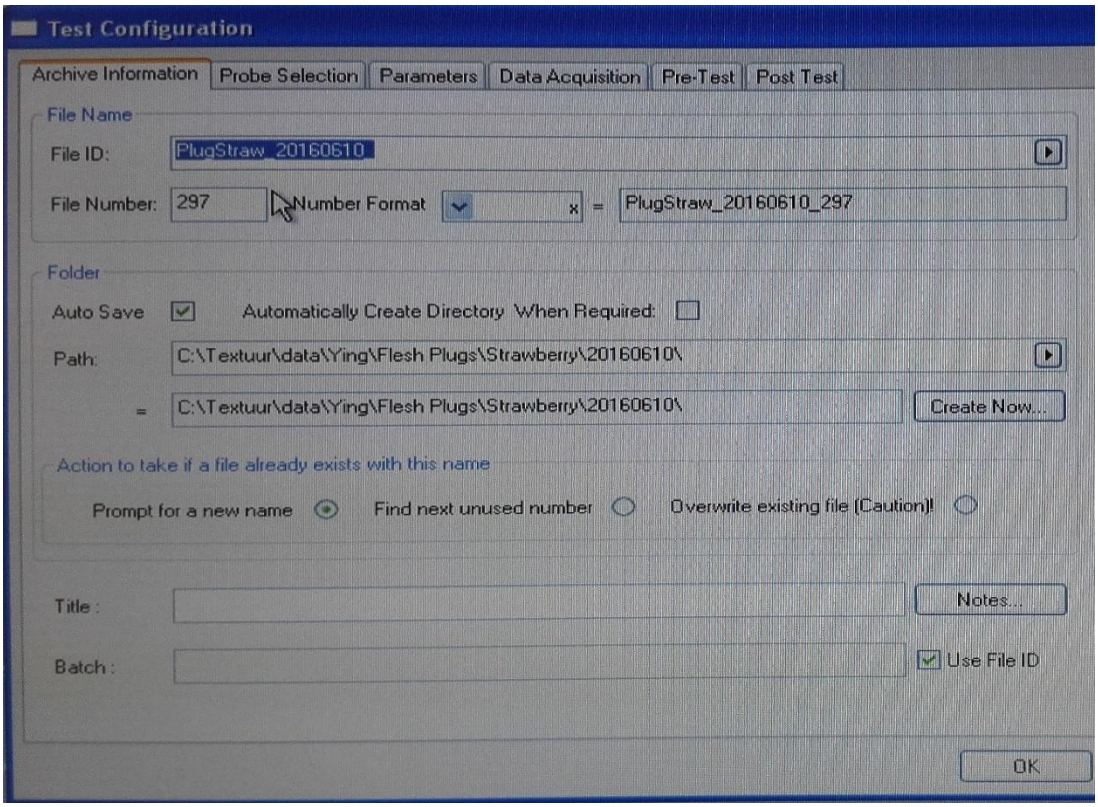

Figure A11. Example of Test Configuration.

- The whole fruit firmness of tomatoes was measured on two points of equator and the plug firmness was measured by compressing two punctured plugs from each fruit (Figure A12).
  - 10% of the tomato fruits were compressed.
  - The plugs were compressed to 1 mm to the bottom.
  - The plugs were placed in the middle of two filter papers and a miracloth.
  - The plugs were punctured from fruits and the strawberry plugs were cut to 1 cm (Figure A13).

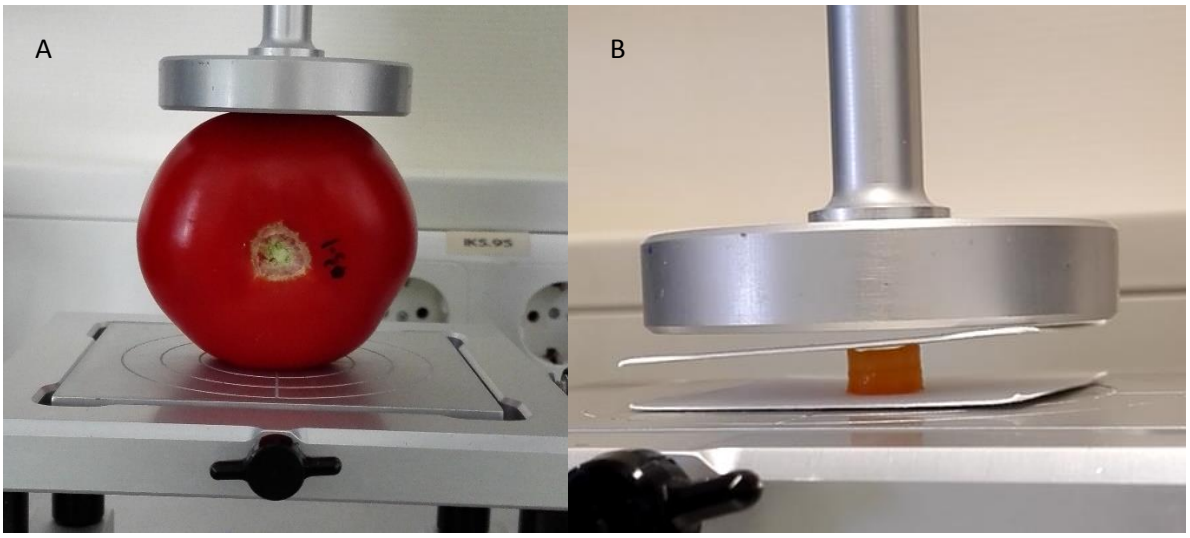

Figure A12. Firmness measurement of whole tomato fruits (A) and tomato/strawberry plugs (B).

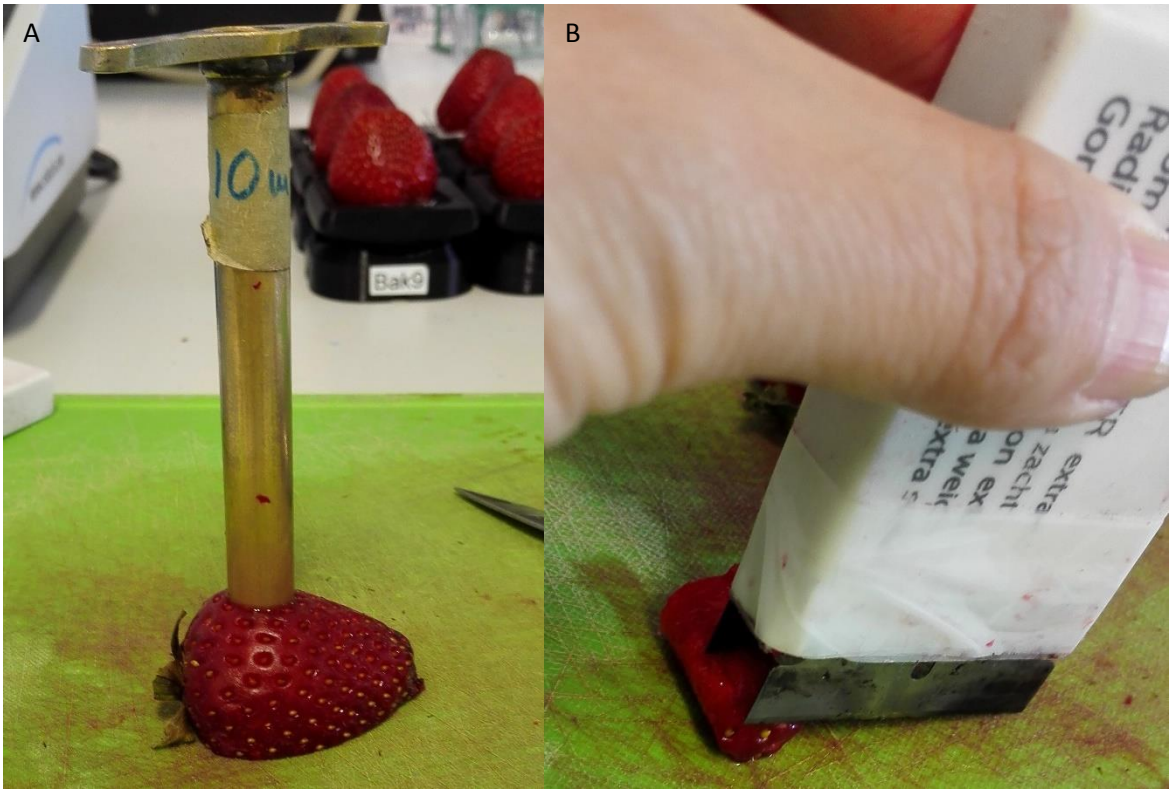

Figure A13. Example of puncturing plugs and cut plugs.

- Juiciness of tomatoes and strawberries was determined by the amount of juice compressed from plugs per fresh weight (Figure A14).
  - The juiciness per fruit was measured in duplicates.
  - Juice weight = the weight differences of two filter papers before and after compression.
  - Juiciness = Juice weight/ Fresh weight

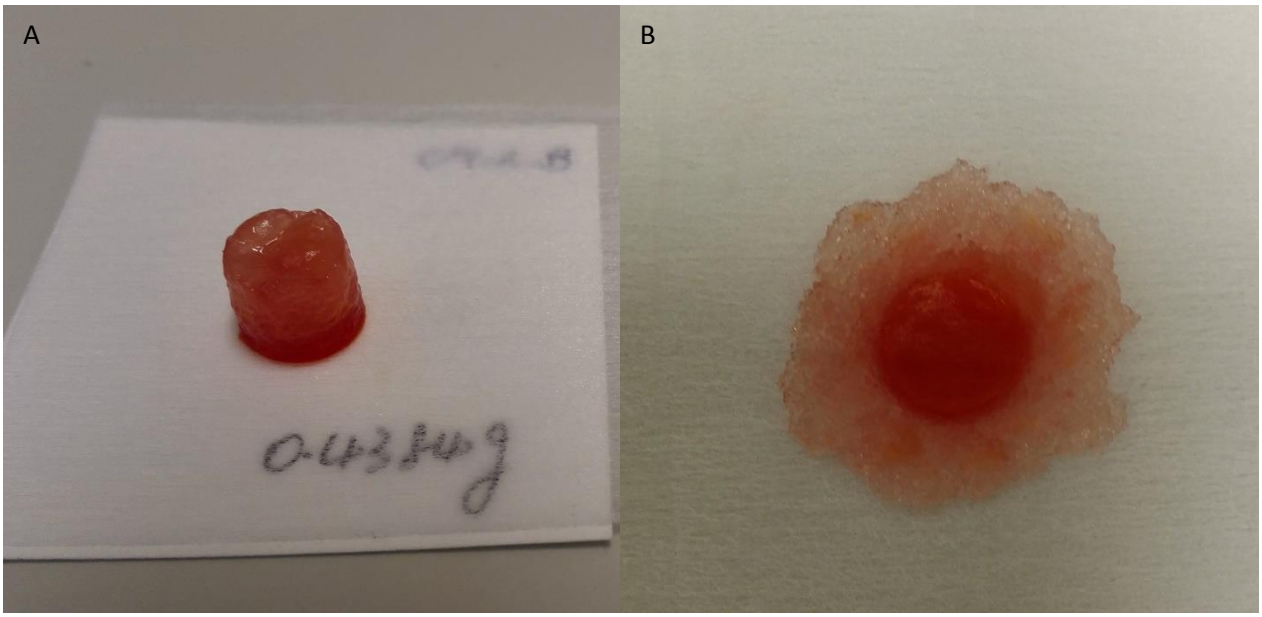

Figure A14.Plugs before and after compression.