ASEAN Journal on Science and Technology for Development

Manuscript 1541

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SPECIAL ISSUE ARTICLE

Non-invasive Digital Refractometer to Measure Maturation of Climacteric Fruits

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Abstract

Sugar content is a superior quality indicator for the post-harvesting and ripening status of climacteric fruits. Total soluble solids (TSS) represented by °Brix index is a measure of the sugar content. The conventional way to measure TSS is by invasive (IN) technique using manual or digital refractometers. While non-invasive (NIN) infrared refractometer devices have been developed in recent years for various agricultural and food science applications, its accuracy to measure fruit TSS directly is not straightforward, as the device is only dedicated to uses for a specific fruit. This study aims to investigate the suitability of the use of a non-invasive refractometer to measure TSS directly for some post harvested fruits. For comparison, a conventional invasive digital refractometer was also used. Red apples and green apples were used as a reference, while tests were done on bananas, mangos, red pears, golden pears and sapodillas. Among the fruit samples, red pear is the only fruit whose TSS can be measured using the non-invasive refractometer accurately, whereas the device is not suitable for the other fruits due to the significant percentage error and inapplicable corrective calibration value which may lead to a limited prediction of TSS content values.

Keywords: Total soluble solids, Infrared refractometer, Invasive, Non-invasive, °Brix index

1. Introduction

Major losses on climacteric fruits occur post-harvest due to the short span of shelf life of the fruits as they ripen. The ripening stage during post-harvesting is monitored to ensure the fruit can be consumed at its optimal sweetness and condition. During ripening process, fruits are developmentally altered biochemically and physiologically to influence the appearance, texture, flavor, and aroma of the fruits which increases the nutritional value and attractiveness of the fruit to the predators for seed dispersal (Alós et al., 2018). In commercial fruit production, fruits are usually harvested before the ripening process occurs at the later stage of maturation, especially in climacteric fruits.

Sugar content is one of the good measures to use for climacteric fruits, due to conversion of starch or carbohydrate into simple sugars during the ripening process. A common way to determine sugar content

Received 21 July 2023; revised 7 October 2023; accepted 15 October 2023.
Available online 14 March 2024

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https://doi.org/10.29037/2224-9028.1541

is by measuring the total soluble solids (TSS), also known as soluble solids content (SSC). Soluble solids refer to the small amount of compounds in the fruit such as dissolved vitamins, carbohydrates, proteins, phytochemicals, phenolics and minerals (Kader, 2008; Tadeo et al., 1987). TSS can be measured using a Brix scale hydrometer or refractometer, where the result will be displayed as °Brix, representing the dry substance content of solution containing mainly sucrose as majority of TSS in fruits consist of sugars (e.g., sucrose, glucose and fructose) and sugar alcohols (e.g., sorbitol and mannitol) (Magwaza & Opara, 2015).

A non-invasive (NIN) technique to measure TSS, where the probing does not require injuring the fruits, has also been explored, using an infrared spectrometer or near infrared transmittance spectroscopy. The non-invasive technique has been used for fruits such as mulberry (Huang et al., 2017), orange (Bizzani et al., 2017) and strawberry cultivars (Li et al., 2011). Its use on other types of fruits is still limited or requires more data. In this work, we investigate the suitability of the use of non-invasive technique for climacteric fruits with thick skin, which are common in Brunei markets.

2. Materials and methods

Measurement of TSS on the fruit samples was done using invasive and non-invasive methods. For the non-invasive method, a calibrated infrared Brix Meter PAL-HIKARi 5 refractometer (°Brix range of 10–18%; Atago, Japan) was used.

2.1. Brix meter calibration

The NIN IR refractometer PAL-3 is designed to measure the TSS of apples. Therefore, before using the device to measure other fruits, calibration is done using red apple (*M.pumila*), green apple (*M.domestica*). The calibrated TSS value $\overline{TSS}_{NIN,c}$ is then used to calculate the % E_r of the measurement with other fruits, following (F2.1) and (F2.2). Table 1 and Table 2 provide the data obtained from the calibration procedure.

2.2. Determination of TSS of various fruits

Fruit samples of banana (*M.acuminata)*, mango (*M.indica*), red pear (*P.communis*), golden pear (*P.pyrifolia*) and sapodilla (*M.zapota)*, were procured from Brunei local markets. These fruits were procured at the same unripe stage, characterised by green colour or hardness. The fruit samples, ten for each fruit, were cleaned thoroughly by washing under running water and petted dry using paper towels as seen in Fig. 1. For each fruit species, out of 10 samples, 5 were used for invasive reading and the remaining 5 were used for non-invasive reading.

Readings were taken from five different areas of the fruit and done in triplicates. The average of the

Fig. 1. Some of the fruit samples used in the experiments.

Table 1. Calibration data with red apple and green apple.

Sample:		Red Apple (M. pumila)				Green Apple (*M. domestica*)			
Parameters:		# Samples	Min Value	Max Value	Mean	# Samples	Min Value	Max Value	Mean
TSS/°Brix	IN	3	10.4	12.7	12.0 ± 0.7	0.5	10.3	10.6	10.5 ± 0.1
	NIN	3	9.3	12.3	11.0 ± 1.1	0.5	11.0	12.1	11.5 ± 0.3

Data is expressed in mean ± SD.

Table 2. Relative percentage error of red apple and green apple (reference fruits).

Sample	$\overline{TSS}_{IN}$/°Brix	$\overline{TSS}_{NIN}$/°Brix	$\overline{TSS}_{NIN,c}$/°Brix	Corrective value	%E_r
Red apple (*M.pumila*)	12.0 ± 0.7	11.0 ± 1.1	13.2	1.1	8.89
Green apple (*M.domestica*)	10.5 ± 0.1	11.5 ± 0.3	9.6	0.9	9.58

Data is expressed in mean ± SD.

readings will be calculated and recorded for future use. The readings were taken in a dim lit or a dark room, to ensure minimal or no light interference. For each fruit, except the bananas, small parts of the fruit from different areas of the fruit were obtained by cutting and the juice was extracted by crushing and the juice was obtained using a dropper to be used for analysis. For bananas, the sample must be first diluted: small parts of the fruit from different areas were obtained by cutting the fruit and the flesh was grounded/mashed, then 0.4 g of fruit flesh was placed in a cuvette and 1.2 mL of distilled water was added using a 5 mL pipette, the mixture was thoroughly mixed, and the mixture was extracted using a dropper to be used for analysis. The results obtained will be multiplied by the dilution factor of 4. The exposed area was sealed with candle wax.

For the conventional, invasive (IN) method, the TSS was measured using a method adopted from Khandaker et al. (2018), with some alterations and done in triplicates, using Digital Hand-held PAL-3 (ºBrix range of 0–93%, Atago, Japan). Readings were taken from five different areas of the fruit. The average of the readings will be calculated and recorded for future use. For banana, dilution was done, and the dilution factor was applied to the TSS readings. The process was repeated for 10–14 consecutive days to measure the changes of TSS as the samples ripened. The corrective calibration value (CCV) was calculated using the formula:

$$CCV = \frac{\overline{TSS}_{IN}}{\overline{TSS}_{NIN}} \qquad \text{(F2.1)}$$

where $\overline{TSS}_{IN}$ is the average TSS of invasive reading and $\overline{TSS}_{NIN}$ is the average TSS measured using non-invasive device. The relative percentage error $\%E_r$ was calculated as:

$$\%E_r = \frac{\overline{TSS}_{NIN,c} - \overline{TSS}_{IN}}{\overline{TSS}_{IN}} \times 100 \qquad \text{(F2.2)}$$

where $\overline{TSS}_{NIN,c}$ is the calibrated non-invasive TSS value.

3. Results and discussion

3.1. Banana (*Musa acuminata*)

For the bananas that we use, it only takes four days for them to ripen (Table 3). Between these four days, increased trends can be seen in both the NIN and IN measurement reading. This increase is due to the conversion of starch into soluble sugars/monosaccharide during the ripening process

Table 3. The average total TSS values with banana.

Day	TSS/°Brix			
	With dilution factor		No dilution factor	
	$\overline{TSS}_{IN}$	$\overline{TSS}_{NIN}$	$\overline{TSS}_{IN}$	Zulkifli
1	10.8 ± 1.62	9.8 ± 0.12	2.70	1.44
2	12.9 ± 0.53	10.3 ± 0.49	3.22	1.93
3	15.1 ± 1.50	11.4 ± 0.57	3.77	2.38
4	14.6 ± 0.56	11.3 ± 0.71	3.66	–

Data expressed in mean ± SD.

(Khandaker et al., 2018). In this experiment, the average total TSS values are presented in Table 3.

The results obtained from this experiment to bananas are consistent with earlier studies, such as those by Subedi & Walsh (2011), Zulkifli et al. (2016) and Khandaker et al., 2018, as summarized in Table 3 with and without considering the dilution factor. After day 4 the fruit skin starts to darken and has large black spots. The TSS can no longer be measured using the NIN refractometer. Experiment by Subedi & Walsh (2011) has shown positive correlation of the TSS of mesocarp and exterior skin colour of the bananas during the ripening process.

A study conducted by Mohd Ali et al. (2018) for TSS_{NIN} using near infrared spectroscopy found $R^2 = 0.81$ for the prediction model for the Brix values, indicating a great potential in predicting sugar content in bananas. In comparison, in this experiment $R^2 = 0.8615$ is found, which demonstrates a possibility of the device in predicting the sugar contents.

3.2. Mango (*Mangifera indica*)

The ripening process of the mango samples that we use continued until day 6. The results obtained from this experiment are consistent with earlier studies, such as those by Gianguzzi et al. (2021) and Wongkhot et al. (2012), as summarized in Table 4. While the results in Wongkhot et al. (2012) show a large increase of the TSS_{IN} value for Chok-Anan cultivar mango, in this study, the TSS_{IN} values do

Table 4. The average total TSS values with mango.

Day	TSS/°Brix			
	$\overline{TSS}_{IN}$	$\overline{TSS}_{NIN}$	Guanguzzi	Wongkhot
1	6.5 ± 0.45	10.6 ± 0.30	6.8	7.09
2	6.7 ± 0.17	10.7 ± 0.16	–	–
3	7.7 ± 0.44	10.9 ± 0.33	–	–
4	8.0 ± 0.45	11.1 ± 0.42	–	–
6	8.1 ± 0.41	11.2 ± 0.18	–	–
8	7.6 ± 0.30	10.7 ± 0.50	9.73	21.20
9	7.5 ± 0.18	9.9 ± 0.52	–	–

Data expressed in mean ± SD.

not seem to change much during post-harvest storage. It is likely due to the different variant of the mangos. After day 6, the TSS value starts declining, which indicates the ripening process has stopped. This decline of TSS value is associated with the breaking down of complex carbohydrate into simple sugars during the ripening process (Islam et al., 2013).

While there is no published studies that could explain why the TSS_{NIN} is quite constant, a study by Subedi & Walsh (2011) to assess the sugar and starch in intact banana and mango by short wave length near infrared spectra (SWNIR) spectroscopy found that the prediction model is only suitable for ripened fruit, with poor prediction across ripening stages, with $R^2 = 0.01$ and $R^2 = 0.8$ at the unripe and ripe stage, respectively. A similar trend can also be seen in comparison for the TSS_{NIN} readings, where $R^2 = 0.0357$ as the mango sample is also measured during its unripe to ripe stage. Therefore, using the NIN IR refractometer PAL-3, accurate *TSS* measurement for mango might only be possible when the fruit is fully ripe, as a minimum *TSS* value of 10°Brix is required for proper detection.

3.3. Sapodilla (M. zapota)

Similar to mangos, the sapodillas (chikoo fruits) that we used also took approximately 6–7 days to ripen fully (see Table 5), although the sapodillas were not at exactly the same ripening stage at the start. This may cause variance in results due to differing *TSS* of each fruit during the IN measurement which might result in the low R^2 value and high variance in the readings from the regression line. After day 6, the fruits became soft and showed indications of spoilage (e.g., unpleasant odor).

For the NIN method, we found $R^2 = 0.5341$ during the ripening process, which is better than the R^2 of the IN readings. However, the NIN readings trendline does not match the IN reading trendline, indicating the NIN results could be inaccurate. The NIN IR refractometer cannot perform measurement at either the spoiled or the hardened areas of the fruit and may produce errors during scanning of the fruit, which occurs from day 2. There are limited studies done on NIN IR refractometer for this fruit, although a reasonable explanation could be as what explained in Li et al. (2016). It stated that fruits with thick skin or heterogeneous internal structure such as passion fruit and tomato are not appropriate for TSS content evaluation using near-IR spectroscopy technology.

Another reasoning factor might be due to the fruit samples being measured during ripening period, when there is incomplete starch to sugar conversion. In a study by Kusumiyati et al. (2018), near-infrared spectroscopy (NIR) is used to determine the fruit quality. The fruits are measured using NIR at 90 days after flowering, and the value $R^2 = 0.78$ is found, which is higher in comparison to the $R^2 = 0.5341$ found in this experiment. Therefore, accurate readings and higher R^2 values might be attainable when the chikoo fruit is ripened and not during ripening stage, as the NIN-IR refractometer only detects TTS values within the range of 10 to 18° Brix.

Table 5. Average TSS with sapodilla.

Day	TSS/°Brix	
	$\overline{TSS}_{IN}$	$\overline{TSS}_{NIN}$
1	9.27 ± 0.21	9.90 ± 0.37
2	9.23 ± 0.12	9.83 ± 0.50
3	9.13 ± 0.21	10.47 ± 0.46
4	9.33 ± 0.17	9.87 ± 0.39
5	9.27 ± 0.24	9.90 ± 0.08
6	9.20 ± 0.08	9.67 ± 0.12
7	9.17 ± 0.17	9.63 ± 0.26
8	9.13 ± 0.25	9.87 ± 0.26
9	Rotten	Continue

Data is expressed in mean ± SD.

3.4. Golden pears (Pyrus pyrifolia)

The ripening duration for the golden pears that were used in this experiment is approximately 8 days for the IN measurement (see Table 6). Beyond that duration, the fruits start to get darken, develop brown spots due to the cutting and release an unpleasant odor which indicates spoilage. Interestingly, for the NIN measurement the fruits continued to ripen and did not show any indication of spoilage. Overall, a slight decrease in trend of both $\overline{TSS}_{IN}$ and $\overline{TSS}_{NIN}$ values could be observed in Table 6, this might be due to the completion of starch hydrolysis (Li et al., 2011), impact bruising (Raquel Scherrer Montero et al., n. d.) and storage temperature (Kishore et al., 2011). The trendline and average

Table 6. Average TSS with golden pear.

Day	TSS/°Brix	
	$\overline{TSS}_{IN}$	$\overline{TSS}_{NIN}$
1	7.77 ± 0.21	11.33 ± 0.34
2	7.10 ± 0.45	11.13 ± 0.33
3	7.37 ± 0.37	12.43 ± 0.12
4	6.77 ± 0.17	11.87 ± 0.46
5	7.30 ± 0.29	12.37 ± 0.81
6	7.27 ± 0.29	12.53 ± 0.69
7	7.43 ± 0.21	12.17 ± 1.03
8	Rotten	

readings of both the IN and NIN methods are very similar, which is a good indicator of compatibility between the NIN-IR refractometer with IN TSS analysis of the golden pear. However, the low R^2 values, i.e., $R^2 = 0.167$ for NIN and $R^2 = 0.21$ for IN, respectively, might indicate that there is high variance in the result. This might be due to the increase in respiration rate of the fruit at room temperature which causes hydrolysis of carbohydrates, reducing the TSS content of the fruit (Devanesan et al., 2011). In addition, the fruits might be at differing maturation stages at the start, which causes variance in the TTS readings.

3.5. Red pear (P.communis)

For red pear it took 8 days to fully ripen, while after that the fruit will become spoiled and can no longer be measured with the NIN IR refractometer. In Table 7, the NIN and IN readings follow the same decreasing trend of TSS, which could be due to the limitation of the NIN refractometer as it can only measure from 10 to 18° Brix, thus giving inaccurate readings for values below 10° Brix. Another reason might be due to the irregular shape of the fruit causing incomplete coverage of the fruit's surface to the sample stage of the refractometer leading to light interference from the surrounding.

Since the fruits are stored at a room temperature, it might cause an increase in respiration rate that causing decrease in monosaccharides in the fruit due to utilization of glucose and fructose (Devanesan et al., 2011). It is also reported in Fukuoka et al. (2009) a correlation could be seen with reduction of sugar content in fruits and cell enlargement by higher temperature during late developmental period in fruits. Overall red pear may be a suitable candidate for non-destructive measurement during its ripening process using the NIN IR refractometer due to similarity of shape to apples, the coordination of trendline of NIN and IN total soluble solid content values and similar total soluble solid range during its ripening period (i.e., 10° Brix-12° Brix).

Table 7. Average TSS with red pear.

Day	TSS/°Brix	
	$\overline{TSS}_{IN}$	$\overline{TSS}_{NIN}$
1	11.2 ± 0.59	11.5 ± 0.54
2	11.1 ± 0.33	11.5 ± 0.66
3	10.5 ± 0.31	12.1 ± 0.09
4	10.4 ± 0.12	11.3 ± 0.05
5	10.8 ± 0.22	9.7 ± 0.08
6	9.5 ± 1.25	10.9 ± 0.22
7	11.1 ± 0.68	10.3 ± 0.96
8	10.1 ± 0.33	10.0 ± 0.22
9	Rotten	

Data is expressed in mean ± SD.

3.6. Applying corrective calibration value to random fruit samples

Due to the difference between the IN and NIN results or values in almost all the fruits measurement, the corrective calibration formula (F2.1) is applied to help synchronize the NIN readings with the IN readings, so that the NIN can be used as a valid estimate to the IN readings whenever required, allowing rapid measurement without injuring the fruit and minimize food wastage.

In Fig. 2, it could be seen that the corrective value of red pear and golden pear (i.e., 0.97 and 0.93), have almost similar to the $\overline{TSS}_{IN}$ value of red apples (i.e., 1.10). This indicates that red pear and golden pear are suitable for the non-destructive measurement using the NIN IR refractometer, as their $\overline{TSS}_{NIN}$ value is very similar to their $\overline{TSS}_{IN}$ readings, and they require only minimal adjustment in corrective calibration value, *CCV* (F2.1) and low $\%E_r$ (F2.2). A possible reasoning for this is because of the similarities in skin thickness and shape configuration between red apples and pears. It was reported in Homutová & Blažek (2006) that the average thickness of apple fruit skin ranges from 33.3 μm to 73.1 μm, depending on the cultivar and ripening period during harvest, while for pear the skin thickness value varied from 1.9 mm to 2.2 mm for Moroccan varieties (Benhoussa, 2019).

For sapodilla, mango and banana, the calibrated values are far from the IN readings which indicates they might not be suitable for the NIN IR refractometer. This is because of the fruit shape and skin thickness. For banana it has an irregular shape and is not spherical, hence the coverage and distance of fruit to sample stage might not be equal, resulting in variance and inaccurate readings. Similarly, for mango and sapodilla, their tougher and thicker skin compared to apple skin may affect the sensitivity and accuracy of the measurement. The thick skin or heterogeneous internal structure of the fruit samples may not be appropriate for TSS content evaluation with near-infrared spectroscopy technology (Li et al., 2016). Therefore, fruits with thick skin and tough flesh may not be suitable for the NIN-IR refractometer.

Table 8 presents the relative percentage error $\%E_r$ of the calibrated NIN results for all fruit samples. Those $\%E_r$ is calculated using (F2.2) to measure the amount of error between the calibrated NIN *TSS* readings and IN *TSS* values to determine whether the corrective calibration values are applicable. As

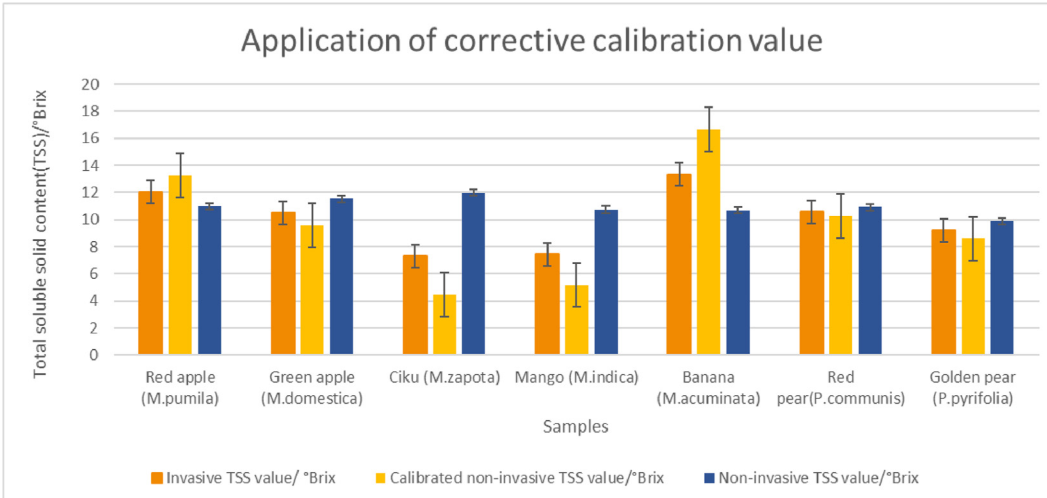

Fig. 2. Application of corrective calibration value with error bars.

Table 8. Relative percentage error of calibrated NIN results for all fruit samples.

Sample	$\overline{TSS}_{IN}$/°Brix	$\overline{TSS}_{NIN}$/°Brix	$\overline{TSS}_{NIN,c}$/°Brix	Corrective value	$\%E_r$
Sapodilla (*M.zapota*)	7.3	12.0	4.4	0.6	64.33
Mango (*M.indica*)	7.4	10.7	5.2	0.7	44.22
Banana (*M.acuminata)*	13.3	10.7	16.7	1.3	19.94
Red pear (*P.communis*)	**10.6**	**10.9**	**10.3**	**1.0**	**3.02**
Golden pear (*P.pyrifolia)*	9.2	9.9	8.6	0.9	7.27

shown in Table 8, Red pear (*P.communis*) and Golden pear (*P.pyrifolia*) have the lowest percentage error of 3.02% and 7.27%, respectively. This may be due to the shape of the pear which is almost spherical or rounded allowing coverage of the sample stage with the NIN IR refractometer evenly, with minimal sunlight exposure, which gives better readings and minimal measurement errors.

Mango and sapodilla have the highest relative percentage error values of 44.22% and 64.33% respectively. For sapodilla, there could be measurement errors due to hardening of the skin and softening of the flesh of the fruit, which may cause difficulty in scanning of the fruit and affect the readings of the NIN refractometer. Therefore, the *CCV* is best applied to fruit samples of red pear and golden pear due to its low relative percentage error value and similar shape configuration to apples, which also results in similar measurement values with the IN method and the NIN refractometer.

4. Conclusions

In conclusion, red pear (*P. communis*) is the most suitable fruit to measure using the NIN method, due to the calibrated NIN value that is the closest to the real IN value and the low relative percentage error value of 3.02%. This device could be used as a non-destructive quality evaluation method to monitor ripening of red pear (*P. communis*) at its maturation or ripening stage to see when it is suitable for harvest. From this study, it is concluded that with few exceptions on red pear and golden pear, the non-invasive, infrared refractometer cannot be used to measure the TSS directly onto thick-skinned climacteric fruits. Thus, this opens a new avenue for further research.

Authors' contributions

JAH: Planning the experiments, designing the study, collected all the data, analysis, writing the first draft and revision; **DK and DSL:** Formal analysis, supervision, contributed to the idea design, review and editing; **AN, NAL and MAAN:** Conducting the experiments and collecting the data; **BFN:** Conceptualization, supervision, design the methodology, idea generation, analysis, and editing. All authors read, approved the final manuscript, and contributed to the writing.

Conflicts of interest

The authors have declared no conflict of interest or perceived conflict of interest for this research article.

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