



Review

# Analytical methods for determination of sugars and sweetness of horticultural products—A review



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ABSTRACT

The determination and quantification of sugars is important for quality control and assurance of horticultural produce. This review discusses analytical methods for determination of sugars and sweetness of fresh and processed fruit and vegetables, including the use of destructive and non-destructive instrumental techniques to evaluate sugar composition and characterize taste profile or sweetness. From the standard hand-held refractometer to the hydrometer, electronic tongue and high pressure liquid chromatography (HPLC) equipped with different detectors, a wide range of devices have been used to determine sugar composition and sweetness of many fruit and vegetable products. Although chromatographic techniques are very accurate and useful, they require extensive sample preparation based on solvent extraction and hence are generally time-consuming and expensive. Visible to near infrared spectroscopy (vis/NIRS) has been proposed as an interesting alternative to traditional methods due to its rapidity, simplicity, cost effectiveness and potential for routine analysis if proper calibration and validation steps were developed. Current trends favour analytical methods that are simple to use, quick and non-destructive. The prospects for using emerging technologies such as hyperspectral imaging and nuclear magnetic resonance for non-destructive assessment of sugar content and sweetness of fresh and processed horticultural food products are also discussed.

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Contents

1. Introduction .....	180
2. Indices used to characterize sugar content and sweetness of horticultural produce—An overview .....	180
2.1. Total soluble solids (TSS) and soluble solids content (SSC) .....	180
2.2. Ratio of soluble solids to titratable acidity (SSC/TA) .....	181
2.3. BrimA .....	182
2.4. Sweetness index .....	182
2.5. Total sweetness index .....	182
3. Analytical methods of measuring sugars and sweetness .....	182
3.1. Destructive methods .....	183
3.1.1. Sensory evaluation .....	183
3.1.2. Hydrometer .....	183
3.1.3. Refractometer .....	183
3.1.4. High performance chromatography .....	183
3.1.5. Electronic tongues .....	185

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3.2. Non-destructive measurement.....	186
3.2.1. Visible to near infrared spectroscopy.....	186
3.2.2. Hyperspectral and multispectral imaging.....	188
4. Future prospects.....	188
5. Conclusion.....	189
Acknowledgements.....	190
References.....	190

## 1. Introduction

Consumer assessment of fruit and vegetable quality which influence initial decision to purchase is often based on external attributes such as appearance, color, shape and size (Opara and Pathare, 2014). However, the decision for subsequent purchases is dependent upon consumer satisfaction based on flavor and internal quality, which are related to soluble solids content (SSC) (mainly sugars), titratable acidity (TA), soluble solids to acid (SSC/TA) ratio and texture (Shewfelt, 2009; Chen and Opara, 2013a,b). With improvement in living standards and income, sensory (taste) quality and sugar content which are often related to internal attributes, have become significant quality parameters in consumer perception of quality and value of fresh and processed horticultural food products (Crowther et al., 2005; Cayuela, 2008; Cayuela and Weiland, 2010).

Sweetness in many fruit and vegetables is a desirable attribute that is often governed, in part, by sugar concentration (Terry et al., 2005; Hong et al., 2014). Therefore, the determination and quantification of sugars and sweetness is of great importance in many fields of plant food sciences research. The sugar content and sweetness of fruit and vegetables is commonly quantified by instrumental assessment as well as sensory evaluation including taste panels (Genizi and Cohen, 1988; Shewfelt, 2009). Soluble sugars, mostly comprised of glucose, fructose and sucrose, may be determined using refractometry or colorimetry. High performance liquid chromatography (HPLC) is the mostly used technique for analysis of individual compounds (Ma et al., 2014). Non-structural carbohydrates may also be separated and determined using HPLC coupled with suitable columns and detectors for quantifying soluble sugars including refractive index detector (RID), evaporative light scattering detector (ELSD) and pulsed amperometric detector (PAD) (Downes and Terry, 2010; Cools et al., 2011; Ma et al., 2014).

The increasing demand for internal quality assurance in the fresh produce industry has spurred the development of a wide range of advanced rapid, real-time, reliable and non-invasive technologies for quality assessment. Conventional laboratory analytical techniques for sugars are destructive, manual, time consuming, require use of hazardous chemicals and are labor intensive (Jie et al., 2014). Visible to near-infrared (vis/NIR) spectroscopy (vis/NIRS) has proven to be the most successful non-destructive, rapid, simple, in real-time, with no use of toxic reagents and with relatively reduced operational costs, hence, these technologies are appropriate for on-line application (Magwaza et al., 2012a, 2014a; Jie et al., 2014).

As an alternative to refractometry and HPLC, a few spectroscopic methods involving nuclear magnetic resonance (NMR), vis/NIRS, hyperspectral imaging, and Fourier transform NIR spectroscopy with attenuated total reflection (ATR-FTIR) have been used to determine the sweetness as an internal quality attribute of fresh produce (Zion et al., 1995; Bureau et al., 2009; Sugiyama and Tsuta, 2010; Pereira et al., 2013; Zhang and McCarthy, 2013).

In this review, we discuss the analytical methods for determination of sugars and sweetness of fresh and processed horticultural products, including several indices used to characterize sugar content and sweetness. The final part of the article illustrates the use of

non-invasive technologies to quantify sugars and discusses future prospects for the application of emerging technologies such as hyperspectral imaging and nuclear magnetic resonance for non-destructive assessment of sugar content and sweetness of fresh and processed fruit and vegetables.

## 2. Indices used to characterize sugar content and sweetness of horticultural produce—An overview

### 2.1. Total soluble solids (TSS) and soluble solids content (SSC)

The sugars and acids, together with small amounts of dissolved vitamins, fructans, proteins, pigments, phenolics, and minerals, are commonly referred to as soluble solids (Tadeo et al., 1987; Ito et al., 1997; Chope et al., 2006; Kader, 1999, 2008a,b). SSC and total soluble solids (TSS) are the most important quality parameters used to indicate sweetness of fresh and processed horticultural food products, in laboratories for research and by industry to determine marketing standards. Given that different researchers in the literature report either TSS or SSC (Table 1), in this review we will be using both terms interchangeably.

TSS can be measured using either a Brix scale hydrometer or a refractometer and reported as “degrees Brix” (°Brix) which is equivalent to percentage (%). In principle, the unit °Brix, which has been in common use in industry for many years, represents the dry substance content of solutions containing mainly sucrose (Wardowski et al., 1979; Echeverria and Ismail, 1987; Dongare et al., 2014). For example, a juice sample that has 25 degree of Brix is assumed to contain 25 g of sugar/100 g of solution (Ball, 2006). However, the above assumption does not hold true in samples of fruit and vegetables because sugars are not the only components contributing to TSS or SSC. Although the term ‘Brix’ is frequently used interchangeably with TSS and SSC, ‘Brix’ technically refers only to the sugar content of fruit juices. Considering that sugars (sucrose, glucose and fructose) and sugar alcohols (e.g. sorbitol and manitol) constitute the majority (approximately 85%) of total soluble solids in many fruits, it is therefore not surprising that both terms have become synonymous. However, this does not hold true for fruit such as limes, in which sugars constitute only 25% of the total soluble solid content (Wardowski et al., 1979). In other types of citrus fruit, nearly 75 to 85% of the total soluble solids of juice is made up of sugars (Wardowski et al., 1979) and higher values of TSS (°Brix or %) correspond with higher sweetness. Therefore, to obtain the sugar concentration in a sample of fruit or vegetable, the TSS values obtained from a refractometer need to be adjusted using a factor, which is based on the percentage contributed by sugars. For instance, the factor will be from 0.75 to 0.85 for citrus fruit, where sugars contribute between 75 and 85% of TSS.

Given that other juice components, beside sugars, contribute to the TSS of fruit and vegetables (Crowther et al., 2005; Chope et al., 2006), it is therefore not surprising that TSS is not always aligned with sensory sweetness. In some fruit, such as strawberries (Kader et al., 2003) and blueberries (Saftner et al., 2008), a low correlation between soluble solids and sweetness has been reported, which has been attributed to the fact that refractometer reading measures the sum total of soluble solids (Kader, 2008a,b). The study by Kader

**Table 1**  
Summary of applications, makes, models and manufacturers of refractometers used in postharvest research of different fruit and vegetables.

Produce	Sugar content expressed as	Type of refractometer	Model, manufacturer and country	Reference
Apple	TSS (%)	Automatic digital refractometer	Atago Tokyo, Japan	<a href="#">Nyasordzi et al. (2013)</a>
	Sugar Content (°Brix)	Hand-held sugar-refractometer	WYT-4, Quanzhou Optical Instruments Company	<a href="#">Liu et al. (2007)</a>
Blueberry Jaboticaba	SSC (%)	Digital refractometer	PR-101α, Atago Co., Tokyo, Japan	<a href="#">Leiva-Valenzuela et al. (2013)</a>
	SSC (%)	Digital hand-held refractometer	PR-101α, Atago, Co. Ltd, Tokyo, Japan	<a href="#">Torres Mariani et al. (2014)</a>
Olive	Sugar Content (°Brix)	Hand-held sugar-refractometer	PR-32α, Atago, Bellevue, WA, USA	<a href="#">Migliorini et al. (2011)</a>
Onion	TSS (%)	Digital hand-held refractometer	Palette 100, Atago Co. Ltd., Tokyo, Japan	<a href="#">Chope et al. (2006)</a>
Orange	SSC (%)	Temperature-compensated refractometer	AO Scientific, Model 10423, Buffalo, USA	<a href="#">McDonald et al. (2013)</a>
	SSC	Temperature compensated refractometer	AO Scientific, Model 10423, Buffalo, USA	<a href="#">Obenland et al. (2009)</a>
Orange and mandarin	SSC (°Brix)	Digital refractometer	DR-A1, Atago Co. Ltd., Japan	<a href="#">Jamshidi et al. (2012)</a>
	SSC/TSS (°Brix)	Digital refractometer	PR-101α, Atago Co., Tokyo, Japan	<a href="#">Wang et al. (2014)</a>
	TSS (°Brix)	Digital hand-held refractometer	PR-32α, Atago, Co. Ltd, Tokyo, Japan	<a href="#">Magwaza et al. (2012b, 2013a)</a>
Pear	Sugar Content (°Brix)	Digital refractometer	WYT-J 0–32% ChongQing, China	<a href="#">Wei and Wang (2013)</a>
Plum	SSC (%)	Digital refractometer	RT-30ATC Instrutherm, São Paulo, Brazil	<a href="#">Pereira et al. (2013)</a>
Pomegranate	SSC (°Brix)	Benchtop temperature compensating refractometer	RFM730, Bellingham and Stanley, UK	<a href="#">Zhang and McCarthy (2013)</a>
Prune	SSC (%)	Temperature compensated refractometer	RFM 100, Bellingham/Stanley Ltd., Atlanta	<a href="#">Slaughter et al. (2003)</a>
Strawberry	TSS (%)	Hand-refractometer	Krüss, Germany	<a href="#">Keutgen and Pawelzik (2007)</a>
Tomato	SSC (°Brix)	Table-top refractometer	ABBE3L, Bausch and Lomb Optical Co., Rochester, NY	<a href="#">Malundo et al. (1995)</a>
	TSS	Digital hand-held refractometer	PR-32α, Atago, Co. Ltd, Tokyo, Japan	<a href="#">Javanmardi and Kubota (2006)</a>
Watermelon	SSC	Digital hand-held refractometer	PR-201α Brix-Meter, Atago, Co. Ltd, Tokyo, Japan	<a href="#">Jie et al. (2014)</a>

TSS, total soluble solids; SSC, soluble solid content.

[et al. \(2003\)](#) showed that anthocyanins and phenolic compounds strongly refract light and contribute up to 32% of the SSC values in produce containing these pigments. Therefore, SSC is probably not a good indicator of sugar concentrations in blueberries and strawberries as it is in other less-pigmented fruit. This proposition is supported by evidence from the work of [Kader et al. \(2003\)](#) who showed that the removal of anthocyanins and phenolic compounds before measuring SSC of blueberry and strawberry fruit with a refractometer increased the reliability of soluble solids as an indicator of sweetness.

In onions, fructose and glucose concentration were positively correlated with likeability ( $r=0.75$ ;  $0.78$ ) and sweetness ( $r=0.77$ ;  $0.78$ ) scores, while sucrose did not appear to affect sweetness ([Terry et al., 2005](#)). However, [Crowther et al. \(2005\)](#) found no relationship between perceived sweetness and individual sugar concentrations in onion tissue while [Chope et al. \(2007a\)](#) showed that TSS was 2-fold higher in onion cultivars with higher total sugars and vice versa. It should also be noted that although TSS was measured on fresh juice and non-structural carbohydrates (NSC) was measured on lyophilized material, a similar trend was observed even when concentrations of total sugars were expressed per gram fresh weight ([Chope et al., 2007a](#)). Results from these studies suggested that TSS is not a good measure of the overall sugar concentration and should not be used to assess perceived sweetness in some onion cultivars.

## 2.2. Ratio of soluble solids to titratable acidity (SSC/TA)

The perception of taste in fresh produce may be affected by other factors such as titratable acidity. In a study of the instrumental and sensory quality characterization of blueberry cultivars, [Saftner et al. \(2008\)](#) showed that blueberry cultivars with high SSC were not

perceived as particularly sweet. This is because other fruit juice components, in addition to sugars, contribute to SSC of the sample. Therefore, a difference in SSC alone does not have practical importance regarding consumer perception of fruit and vegetable sweetness.

The concentration of soluble solids, TA and their ratios are not static, but vary considerably during fruit maturation and ripening. Therefore, these attributes are commonly used as laboratory and commercial indicators of maturity for many horticultural crops ([Genizi and Cohen, 1988](#); [Kader, 1999, 2008a,b](#); [Fawole and Opara, 2013a](#)). In mangoes, for example, the flesh acid concentration is higher in younger fruit and declines during development and the opposite trend is observed with sugars, which accumulate rapidly as the fruit matures ([Ito et al., 1997](#)). As a result, SSC tends to increase and fruit becomes sweeter as the season progresses, while TA decreases and hence the ratio of the two increases. Similar decreasing trend in TA and increase in TSS and TSS:TA was reported in pomegranate fruit by [Fawole and Opara \(2013b\)](#).

Although SSC:TA ratio is currently used as a maturity index for some types of fruit, it has been recognized that this measurement does not always correlate well with the perception of sweetness or tartness in others ([Baldwin et al., 1998](#); [Jordan et al., 2001](#); [Obenland et al., 2009](#)). One difficulty is that the same ratio may be derived from different concentrations of SSC and TA, leading to different flavor perceptions for the same ratio. The second problem is related to the fact that TSS is inversely proportional to the size of fruit or vegetable ([Beckles, 2012](#); [Georgelis et al., 2004](#)). This is evident in tomatoes where TSS ranged from 3 to 5% in large beef-steak tomatoes, 5 to 7% in medium-sized midplum tomatoes and 9 to 15% in cherry tomato fruit ([Georgelis et al., 2004](#); [Beckles, 2012](#)). Furthermore, since TSS is the sum of sugars, acids and other minor components, having acids as part of the numerator (TSS) and the

denominator in the TSS:TA ratio might also be part of the problem with the low reliability of this index.

### 2.3. BrimA

In 2001, Jordan and co-workers investigated the effects of sugar and acids on flavor and developed a new sweetness (or maturity) index called BrimA (pronounced bree-mah) as alternative to brix/acid ratio. BrimA (an abbreviation for Brix minus Acid) measures the balance between Brix (sweetness) and acidity (sourness) (Eq. (1), Jordan et al., 2001; McDonald et al., 2013).

$$\text{BrimA} = \text{SSC} - k(\text{TA}) \quad (1)$$

where  $k$  is a constant that reflects the tongue's higher sensitivity to TA compared to SSC. The index allows smaller amounts of TA than SSC to make the same numerical change to BrimA (Jordan et al., 2001). The constant  $k$  may vary from 2 to 10, depending on fruit species and or cultivar due to their differences in ratios of acids and sugars, with values ranging from 2 for pomegranate (Fawole and Opara, 2013a,b) to 5 for citrus and grapes (Jordan et al., 2001). In oranges, Obenland et al. (2009) modified the formula for BrimA (Eq. (1)) by replacing the value of the constant ( $k$ ) of 5 with 3 and 4 in order to eliminate the generation of negative BrimA values, especially in early season fruit. These authors reported that  $k$  factors of 3, 4 or 5 provided nearly identical values of  $R^2$  as calculated from the linear regression of hedonic score versus BrimA. However, BrimA calculated using  $k$  value of 4 provided a slightly superior predictor of flavor ( $R^2 = 0.565$ ) compared to those calculated with  $k$  value of 3 ( $R^2 = 0.556$ ) and 5 ( $R^2 = 0.560$ ), respectively.

The study by Jordan et al. (2001) demonstrated that fruit flavor was more closely related to BrimA than SSC/TA, and differed with fruit type. Therefore, this index was considered as a superior indicator of eating quality of citrus and grapes than the traditional Brix-to-Acid ratio. Similar observations were reported in a study by Obenland et al. (2009), where BrimA was most closely related to the hedonic flavor score and sweetness. Based on this correlation, these authors developed linear regression model for predicting fruit hedonic score (Eq. (2)) and sweetness (Eq. (3)) and reported  $R^2$ -values of 0.68 and 0.63, respectively. Fawole and Opara (2013a,b) found that BrimA was a good measure of fruit maturity status of 'Ruby' pomegranate for long term storage. After extensive research success, BrimA was adopted in 2012 by the California Department of Food and Agriculture as the standard quality parameter to determine sweetness of navel oranges (Ross, 2012). Since research has shown that BrimA is a consistently better indicator of consumer liking than other commercial maturity standards (e.g. TSS, TA, and brix-to-acid ratio), its future adoption by horticultural industries in different countries is promising. Given the close relationship between BrimA and taste properties of fruit, assessing the correlation of this index with vis/NIR spectrum will be more useful than solely using SSC or TA to characterize fruit taste.

$$Y = 0.142(\text{Color}) - 12.290(\text{BrimA}) + 0.001(\% \text{Juice}) + 4.283 \quad (2)$$

$$Y = 3.144(\text{Color}) - 239.649(\text{BrimA}) + 0.011(\% \text{Juice}) + 37.404(\text{SSC/TA}) + 22.082 \quad (3)$$

### 2.4. Sweetness index

In addition to quantifying TSS, calculating Brix:acid ratio or estimating the BrimA as measures of the sweetness of fresh horticultural produce, the sweetness index (SI) based on the proportion of the individual non-saturated sugar components is one of the common measures of acceptability of horticultural produce (Beckles, 2012). For instance, in strawberries and cherry tomatoes, the sweetness index is calculated based on content and sweetness

properties of individual carbohydrates by multiplying the sweetness coefficient of each sugar (glucose = 1.00, fructose = 2.30, and sucrose = 1.35) with concentration of that sugar (Eq. (4)) (Crespo et al., 2010). In this sweetness estimation approach, the contribution of each carbohydrate is calculated, based on the fact that fructose and sucrose are 2.30 and 1.35 times, respectively, sweeter than glucose; hence, the level of sweetness is expressed using the molar concentration of each sugar (Rosales et al., 2011). Sweetness index is calculated using Eq. (4) after time consuming HPLC analysis of individual sugars, hence, commonly used in laboratories for research purposes (Rosales et al., 2011; Sánchez-Rodríguez et al., 2012).

$$\text{SI} = (1.00[\text{glucose}]) + (2.30[\text{fructose}]) + (1.35[\text{sucrose}]) \quad (4)$$

### 2.5. Total sweetness index

Another index used in the literature to indicate sweetness of horticultural produce is total sweetness index or TSI, where the contribution of each major component of sugar is estimated relative to sucrose, which is assigned an arbitrary value of 1 (Baldwin et al., 1998; Beckles, 2012) (Eq. (5)):

$$\text{TSI} = (1.00 \times [\text{sucrose}]) + (0.76 \times [\text{glucose}]) + (1.50 \times [\text{fructose}]) \quad (5)$$

In tomatoes, Baldwin et al. (1998) reported that TSI and TSI:TA were more closely linked to acceptability based on sweetness as rated by sensory panel compared to TSS or TSS:TA. Therefore, the two indices (TSI and TSI:TA) are considered to be useful predictors of overall acceptability of tomatoes. However, there can be inconsistencies when comparing TSI between different fruit in a batch because fruit with identical total sugar content but relatively more fructose or sucrose taste sweeter than those with higher glucose content (Schaffer et al., 1999; Beckles, 2012). Furthermore, this index requires time consuming HPLC analysis of individual sugars, hence, not commonly in commercial setup.

Acknowledging that the sweetness of horticultural produce is determined by the concentrations of the predominant sugars, Kader (2008a,b) proposed a similar sweetness scale based on different values for the contribution of individual sugars relative to sucrose, resulting in the following order of sweetness: fructose (1.2) > sucrose (1.0) > glucose (0.64). The difference in relative contributions of individual sugars between SI, TSI and the proposal by Kader (2008a,b) lies in the relative importance assigned to the components. However, we did not find any comparative research which evaluates the usefulness of these indices in providing better measures of sweetness of fresh or processed horticultural food products. The use of multiple indices to characterize the same quality attribute (in this case sweetness) presents difficulty in comparing results in published data and thus highlights the need to standardize measurement and analytical approaches to promote traceability and comparability of results both in industry and among researchers.

## 3. Analytical methods of measuring sugars and sweetness

The composition of sugars in food products can be successfully analyzed only if a sensitive, reliable, and rapid analytical method is available (Shanmugavelan et al., 2013). Several instrumental analytical techniques (both destructive and non-destructive) are used to give information on the chemical composition of the sample and, hence, are useful to describe the taste profile of fruit and vegetable products. High pressure liquid chromatography, gas chromatography (GC), colorimetric methods, and other instrumental techniques such as hydrometer, refractometer, and electronic tongue are used to determine the chemical composition of a sample and could be

used to describe the taste of many horticultural products (Kader et al., 2003; Saftner et al., 2008; Shanmugavelan et al., 2013). Given the importance of sugar and sweetness in these food products, it is not surprising that there are several methods that are commonly used to express sugar content. Based on the necessity for sample preparation before analysis, analytical methods for sugar determination may be categorized as either destructive or non-destructive.

### 3.1. Destructive methods

#### 3.1.1. Sensory evaluation

Sensory evaluation is traditionally used to determine the taste of fruit and vegetables (O'Mahony, 1991). In sensory analysis, trained and consumer panels are used to evaluate taste because they give by far the most realistic technique to obtain information on human taste and aroma perception (Beullens et al., 2006, 2008; Rudnitskaya et al., 2006). However, sensory evaluation has some problems, including the correctness of training, standardization of measurements, stability and reproducibility. Other drawbacks of sensory evaluation technique are the high cost and taste saturation of the panelist. According to Shewfelt (2009), a minimum of 24 untrained panellists is essential to have confidence in test results and usually 50–100 panelists are needed to provide adequate information. Furthermore, panelists should represent the demographic profile of potential consumers of the product, in terms of gender, ethnicity, age, etc.

Different scoring scales are used in sensory evaluation to rank samples in order of preference. These scales include hedonic scale where panelist rate product(s) from 1 (extremely dislike) to 9 (extremely like) (Baldwin et al., 1998; Shewfelt, 2009; Obenland et al., 2009, 2010). Another scale that is frequently used in sensory evaluation is known as the 'willingness-to-purchase' scale and ranges from 1 to 5, where 1 is "definitely would not purchase" and 5 is "definitely would purchase" (Malundo et al., 1997). In the acceptability scale, defined by Genizi and Cohen (1988) and Dubost et al. (2003), the panellist score ranges from 1 to 3, where 1 is tastes great, 2 is acceptable and 3 is unacceptable. Although sensory evaluations are useful and provide most realistic information of human taste and aroma perception, they are subjective, not quantitative and do not provide insight into the wider applicability of the results. Due to the subjective nature of the scales used in sensory evaluation, the development of objective analytical methods providing this kind of information is of capital importance for food quality control purposes.

#### 3.1.2. Hydrometer

TSS or Brix of fruit and vegetables are commonly measured by gravimetric methods using the hydrometer (Nor et al., 2014). A hydrometer is a simple but reliable instrument for measuring the density of liquids and is most often calibrated using a standard liquid. A Brix hydrometer measures specific gravity and is calibrated to read directly in units of sugar concentration (degrees Brix, °Brix) at room temperature which is often considered to be 20 °C (Jones, 1995). Although hydrometer is a common technique for quantifying Brix in juice samples of fruit and vegetable products, this measurement technique is inadequate and inaccurate because of manual operating and reading errors. Hydrometer measurements are prone to errors associated with incorrect reading of the meniscus. In addition, large variations in surface tension due to surface contamination could lead to errors in results. As a result, optical techniques have been developed for more accurate analysis of Brix and refractometer is the most commercially acceptable technique.

#### 3.1.3. Refractometer

The refractometer, which optically measures the refractive index of juice, is the standard method used to measure SSC or TSS of

fruit and vegetables. TSS or Brix represents the percentage by mass of total soluble solids of a pure aqueous sucrose solution (Pereira et al., 2013). Several types of refractometers are available in the market, some of which are based on either refraction or critical reflection of light (Meeten and North, 1995; Dongare et al., 2014). Of these, critical angle based refractive index refractometer is more suitable and accurate because it is not affected by suspended solids and color of sample. Because of this advantage, refractive index refractometer is used as a convenient method for measuring Brix of turbid colloidal fluids, such as fruit and vegetable juices (Dongare et al., 2014). The Brix refractometer has advantages over other methods of estimating SSC concentration in that it is inexpensive, readily available, less fragile, and less sensitive to variation in sample temperature and ambient temperature, season of the year and other factors. Refractometers are available in both digital and analog modes. A quick reference table summarizing the makes/models and manufacturers of refractometers used in postharvest research is provided in Table 1. Most digital refractometers have automatic temperature compensation for a specified temperature range (e.g. 10–30 °C) so unlike hydrometers and analog refractometers, there is no need to correct the Brix reading for temperature. However, it is worth noting that refractometer usually have some error if reading is taken too quickly, before temperature equilibration is reached and from particles such a starch settling on the prism, causing variations in refractive index. As one of the advantages of refractometry, the identity of the sugar(s) is irrelevant to its scale, although there are undoubtedly minor variations owing to the specific type of sugar (Ball, 2006).

Results of sugar content are usually expressed as TSS or SSC; however, SSC appears to be the most widely reported terminology. Most researchers refer to soluble solids content (Lu, 2004; Pereira et al., 2013) while some refer to soluble solids concentration (Malundo et al., 1995; Baiano et al., 2012; McDonald et al., 2013), and it is common practice among researchers to use these terms interchangeably. Where appropriate, the terminology used by the researcher have been retained in this review; however, it should be noted that in reality terms, the words 'concentration' and 'content' refer to different measures and thus are not equivalent because while the former refers to the amount (of sugar) per unit of material, the latter refers to the total amount (of sugar) in the material. It is therefore recommended that researchers pay attention to what is measured and report the results using the applicable terminology and appropriate unit of quantification to facilitate correct and accurate comparison of published data in the literature. Furthermore, most researchers use °Brix as the unit of refractometric measurement for TSS or SSC, while others use percentage (%) (Table 1). While both units are equivalent and interchangeable, it is noted that the choice of one unit above the other appears to be a matter of choice among researchers.

#### 3.1.4. High performance chromatography

Amongst the modern techniques of separating naturally occurring compounds, HPLC has been widely accepted to be the most effective and innovative method for carbohydrate analysis (Ma et al., 2014). As a result, numerous procedures have been described using various chromatographic columns and detectors. Extensive research into various HPLC methods for carbohydrate separations has prompted the publication of numerous review articles (Stefansson and Westerlund, 1996; Raessler, 2011). Table 2 summarizes the applications of HPLC to quantify carbohydrates concentration of fruit and vegetables, respectively. These sources show that HPLC has been extensively used for the determination of carbohydrates compounds in foodstuffs.

The typical method of assigning quantity and purity of compounds in a sample using HPLC coupled with photodiode array detectors is not possible for sugars because sugars do not absorb

**Table 2**  
An overview of applications of high performance liquid chromatography to measure sugar concentration of different horticultural products.

Produce	NCS analyte	Column	Mobile phase (eluent)	Detector	Quantification method	Reference
Artichokes	Fructose, glucose, sucrose, kestose, nystose, fructofuranosyl nystose Sucrose, raffinose, starch, inulin fragments, kestose	CarboPac PA100	Gradient: A: water, B: 225 mMol NaOH, C: 500 mMol NaAc	PAD	HPAEC-PAD	Ronkart et al. (2007)
		CarboPac PA100	Gradient: A: 160 mMol NaOH; B: 160 mMol NaOH + 1 M NaAc; C: 1 M NaOH	PAD	HPAEC-PAD	Schütz et al. (2006)
Fruit, vegetables, cereals	Fructose, glucose, sucrose, galactose, maltose, lactose, raffinose	Prevail carbohydrate column	Acetonitrile–water (70:30 v/v)	ELSD	HPLC	Shanmugavelan et al. (2013)
Jerusalem artichoke,	Fructose, glucose, kestose, sucrose, inulin	Hi plex Ca, Hi plex Na	Acetonitrile:Water 3:1		LC–MS	Matias et al. (2011)
Olives	Sugar alcohols, fructose, galactose,	CarboPac PA1	12 mMol NaOH + 1 mMol Ba-acetate		HPAEC-PAD	Cataldi et al. (2000)
Onions	Fructose, glucose, sucrose, fructan	Si NH <sub>2</sub> Spherisorb	Gradient of acetonitrile–H <sub>2</sub> O	ELSD	HPLC	Kahane et al. (2001)
	Fructose, glucose, sucrose	Novapak NH <sub>2</sub> reverse	Acetonitrile:water (80:20)	ELSD	HPLC	Terry et al. (2005)
	Fructose, glucose, sucrose	Waters Carbohydrate	Acetonitrile:Water 80:20	RID or ELSD	HPLC	Davis et al. (2007)
	Fructose, glucose, sucrose	Rezex RCM monosaccharide Ca <sup>+</sup>	Water	ELSD	HPLC	Chope et al. (2007a,b)
Tomato	Fructose, glucose, kestose, nystose, sucrose	Prevail ES	Water:Ethanol 65–85%	RID or ELSD	HPLC	Downes and Terry (2010)
	Glucose and fructose	Sugar Pak	Ethylenediaminetetraacetic acid disodium-calcium salt	RID	HPLC	Georgelis et al. (2004)
	Glucose, fructose, sucrose	Aminex column	Water	RID	HPLC	Beullens et al. (2006)
	Fructose, glucose	Rezex cation exchange	0.007 mol/L nitric acid aqueous solution	CP-AES	HPLC	Paredes et al. (2008)

ELSD, evaporative light scattering detector; RID, refractive index detector; PAD, pulsed amperometric detector; HPLC, High performance liquid chromatography; HPAEC, high-performance anion-exchange chromatography; LC–MS, liquid chromatography–mass spectroscopy.

ultraviolet (UV) or visible wavelengths (Peters et al., 2001; Cools and Terry, 2012). The foregoing literature evidence demonstrates that ELSD is probably the most widely detector for HPLC analysis of sugars in horticultural produce (Chope et al., 2007b; Downes et al., 2009, 2010; Downes and Terry, 2010; Ma et al., 2014). The wide acceptance of ELSD is based on the fact that it does not suffer from limitations such as sample composition, flow rate of mobile phase and temperature of the column compartment. Therefore baseline drift caused by mobile phase and temperature effects can be avoided (Ma et al., 2014). Unlike RID, ELSD does not rely on optical properties of the analytes, but nebulizes the sample and evaporates the mobile phase.

Due to their size, carbohydrate molecules have good light scattering properties, which form the basis of ELSD. The amount of scattered light as a result of particles in the nebulized sample is then detected and presented as a peak (Cools and Terry, 2012). As an added advantage, the detection of ELSD is based on the ability of particles to cause photon scattering, and hence, it can detect most compounds less volatile than the mobile phase (Nogueira et al., 2005; Ma et al., 2014). Based on these merits, ELSD has been proposed as a practical method for screening carbohydrates in real plant samples and therefore been used to detect sucrose, fructose, glucose, mannitol, sorbitol, ketose, xylose, and fructans among others (Downes and Terry, 2010). However, it is worth noting that whilst ELSD is commonly used to quantify sugars, several factors can affect its response, performance and also cause errors in quantification. One of these factors is that ELSD calibration response curve is non-linear (sigmoidal or exponential) (Mathews et al., 2004). However, a linear relationship can be achieved by plotting

the log<sub>10</sub> values of both axes (Mathews et al., 2004; Downes and Terry, 2010; Cools and Terry, 2012). Either a non-linear curve or a converted linear curve can be used to quantify carbohydrates concentration (Downes and Terry, 2010).

Another detector that has been used extensively for the detection of sugars in fruit and vegetable samples is refractive index detector. However, RID is not as sensitive as ELSD (Cools and Terry, 2012). RID work as a differential refractometer that measures changes in the deflection of a light beam due to the difference in the index of refraction of the eluent induced by the solute, exploiting the improbability that solvents and solutes would share the identical refractive index (Raessler, 2011). The drawback of RID is that its signal is highly dependent on wavelength and density of the solute. Furthermore, RID is very sensitive to eluent composition and precludes gradient elution because of baseline drift when using gradient.

Pulsed amperometric detector (PAD) is another commonly used method for detecting sugars and fructans in fruit and vegetable extracts. PAD is considered as the most highly sensitive and reliable detection method for all carbohydrates (LaCourse, 2002). Because of affinity between ionized groups of sugars at alkaline pH and a pellicular quaternary amine stationary phase, PAD has the advantage over ELSD as a high resolution, highly selective and very sensitive technique for determining carbohydrates. Although both ELSD and PAD do not require pre-column or post-column derivatization (Sanz and Martínez-Castro, 2007), ELSD has a disadvantage of low reproducibility, slightly low sensitivity to low molecular weight components and non-linearity of the detector (Davidek et al., 2003; Raessler et al., 2010). Unlike RID and ELSD, PAD is not sensitive

to changes in salt concentration. Therefore, oxide-free detection of aldehyde and alcohol-containing sugar compounds can be executed at gold electrodes in alkaline media (Raessler et al., 2010). Because PAD requires alkaline conditions, it is often coupled with anion exchange chromatography (AEC) which also requires high alkalinity (Zook and LaCourse, 1995).

In addition to chromatographic techniques described above, capillary zone electrophoresis (CZE) has also been used for the determination of main carbohydrates involved in tomato flavor (Roselló et al., 2002). In this comparative study, Roselló et al. (2002) showed that the method based on CZE provided lower limits of detection and higher resolution than HPLC equipped with either refractive index or UV–visible detectors, although no differences in analysis time were observed. A recent approach, reported by Peters et al. (2001) and Paredes et al. (2008) investigated the use of HPLC system inductively coupled with plasma atomic emission spectrometry (ICP–AES) for determining carbohydrates in apple, cranberry, tomato, and orange fruit juices. In this chromatography, the solution leaving the column is directed towards the sample introduction system of the spectrometer. Carbohydrates detection is based on measuring the carbon emission intensity at a specific wavelength (193.03 nm) (Peters et al., 2001). Due to the fact that in ICP–AES carbohydrates are detected by measuring carbon emission intensity, the use of organic compounds in the mobile phase is avoided (Paredes et al., 2008).

Anion-exchange chromatographic (AEC) columns are by far the most common method currently used for the analytical separation of sugar alcohols and carbohydrates. Although AEC columns are widely used, this does not imply that all major problems associated with carbohydrate analysis in plant extracts have been resolved. One of the problems still experienced with AEC chromatographic method is co-elution of sugars, particularly for closely-eluting pairs of sugars, such as rhamnose and arabinose as well as xylose and mannose (Raessler, 2011). In addition, AEC separation has a limited chromatographic resolution for sucrose and the sensitivity of AEC columns to trace amounts of carbonate can cause stability concerns when aqueous hydroxide is utilized as the mobile phase (Sevcik et al., 2011).

Ligand-exchange chromatography (LEC) based on cation exchangers in metal ion forms has also been extensively used in the separation and quantification of carbohydrates. One of the main advantages of LEC is that it offers reasonable resolution of most carbohydrates. However, this advantage is overshadowed by the fact that analyses utilizing LEC are time-consuming. Other disadvantages of the system are its lack of sensitivity depending on temperature and flow rate and its incompatibility with gradient elution when monitored by RID (Ma et al., 2014). In addition to these disadvantages, the combination of LEC and RID escalates these problems because RID is also susceptible to interference from co-eluting sample components.

Extraction of compounds from plant materials is one of the most important steps prior to their determination by HPLC. Conventional extractions are usually time consuming and require relatively large quantities of solvents. In recent years, some novel extraction methods of phenolic compounds have been developed e.g. ultrasonic extraction (Ma et al., 2008), microwave-assisted extraction (Ahmad and Langrish, 2012) and enzyme-assisted extraction methods (Li et al., 2006). NSC contributing to fruit sweetness, such as sucrose, glucose and fructose are easily soluble in polar solvents and therefore can be analyzed directly by HPLC (Raessler, 2011).

Carbohydrates have been extracted using traditional methods such as liquid and solid phase extractions (Sanz and Martínez-Castro, 2007). Solvents and solvent mixtures used for extraction of NSCs are generally based on water, alcohols, (ethanol, methanol), chloroform and mixtures of them. Water or mixtures of alcohol and water are best suited and mostly used for extraction of NSC of fruit

and vegetables (Davis et al., 2007; Downes and Terry, 2010; Cools and Terry, 2012; Magwaza et al., 2013c). Methanol is the more efficacious solvent for extracting NSC in onions, although ethanol is the widely used solvent (Downes and Terry, 2010). Davis et al. (2007) compared the efficacy of three different extraction methods for the sugars and fructans and reported the method utilizing 62.5% (v/v) methanol (O'Donoghue et al., 2004) to be more efficacious than two methods based on ethanol extraction. Higher efficacy for methanol was due to the higher polarity of the methanol solvent, making fructose, glucose and sucrose to be more soluble in methanol-based solutions than ethanol extraction solvents (Davis et al., 2007). A more detailed discussion of the different extraction techniques for carbohydrate analysis have been reviewed elsewhere (Sanz and Martínez-Castro, 2007; Raessler, 2011; Cools and Terry, 2012).

### 3.1.5. Electronic tongues

The information provided in Sections 3.1.1–3.1.4, above show that different instruments are used to determine carbohydrate composition of produce and could be used to describe the taste of a horticultural produce. In some of these techniques, results of sugar concentration do not always correspond to sweetness observed by sensory panels. Therefore, a need was realized in food research for objective high-throughput taste profiling to complement sensory panels. The development of instruments for artificial, bionic senses was derived and inspired by the necessity for simulating human neurophysiology of the senses of taste. Electronic tongues (e-tongue or ET) have proven to be a good alternative for chromatographic techniques in the analysis of fruit and vegetable sweetness (Rudnitskaya et al., 2006; Beullens et al., 2006, 2008). Therefore, the idea of creating an electronic sense of taste gained the interest of many scientists over the past years. As a result, different types of electronic tongues have been developed by several research groups all over the world.

A simple definition of electronic tongue is that of an instrument which closely mimics the organization of human sense of taste (Wei and Wang, 2013). E-tongues are defined as sensor array systems able to detect single substances as well as complex mixtures by means of particular sensor membranes and electrochemical techniques (Woertz et al., 2011). Distinct signals obtained using array of sensor electrodes are saved in the pattern recognition system and later used as fingerprint information to detect substances with similar or different taste properties (Wei and Wang, 2013). Several multi-sensor systems and devices for liquid analysis, such as taste sensor, taste chip, taste sensing system, electronic sensor array system, multicomponent analytical system, or biomimetic sensor array systems are collected under the term “electronic tongues” (Ciosek and Wróblewski, 2007). The main advantages of e-tongues are the low cost, easy-to-handle measurement set-up and speed of the measurements.

Due to their ability to measure and characterize complex liquid matrices, e-tongues have been first used successfully in the area of food industry for example for quality control, comparison of different product qualities as well as comparison to competitive products (Escuder-Gilabert and Peris, 2010; Woertz et al., 2011). The analysis of food products and beverages represent the biggest part of the use of these instruments. As a result, the use of e-tongues for internal quality assessment of food product has been the subject of numerous reviews and research articles in the literature (Vlasov et al., 2002; Ciosek and Wróblewski, 2007).

Different electronic tongues have proven to be successful in discrimination and classification, quality evaluation and control, process monitoring and quantitative analysis of horticultural food products. They have been used for the determination of fruit juice, onions, soft drinks, tea and herbal products, beverages, apples, milk, tomatoes, alcohol, coffee, sake, olive oil, beer, rice, cork, meat, and

**Table 3**  
An overview of different application of electronic tongues to measure sweetness of horticultural produce.

Produce	Measured parameter	Type of electronic tongue	Sensors/detectors	Reference electrode	Reference
Apples	Glucose, fructose and sucrose	University of Saint-Petersburg prototype	15 Potentiometric chemical sensors	Ag/AgCl reference electrode	Rudnitskaya et al. (2006)
Apple juices	Sweetness	ASTREE Liquid Taste Analyzer	7 Liquid potentiometric sensors	Not specified	Bleibaum et al. (2002)
Apricots	SSC and sweetness	Not specified	7 Liquid potentiometric sensors	Ag/AgCl 3 M KCl reference electrode	Kantor et al. (2008)
Pears	SSC	Voltammetric electronic tongue	6 Working electrodes	Ag/AgCl 3 M KCl reference electrode	Wei and Wang (2013)
Tomato	Glucose, fructose and sucrose	Voltammetric electronic tongue	27 Potentiometric sensors	Ag/AgCl reference electrode	Beullens et al. (2006)
Tomato	Glucose and fructose	University of Saint-Petersburg prototype ASTREE Liquid Taste Analyzer	18 Potentiometric sensors  7 Liquid potentiometric sensors	Not specified	Beullens et al. (2008)

SSC, soluble solid content.

soya paste. A quick reference table of applications of electronic tongue systems are summarized in Table 3.

E-tongues are now widely used for analysis of horticultural food products such as fruit, juices and vegetable oil. Beullens et al. (2006) applied an e-tongue consisting of a series of 15 potentiometric sensors and showed that the results for tomato samples were in agreement with those found with HPLC, although e-tongue exhibited low sensitivities for the sugars determination. In a later study, Beullens et al. (2008) evaluated the potential of two types of e-tongues as rapid techniques to analyze taste properties of tomatoes. The first e-tongue (a prototype developed by University of Saint-Petersburg) comprised of 18 potentiometric sensors while the second one (commercially available, ASTREE e-tongue developed by Alpha M.O.S., Toulouse, France) consisted of a set of seven sensors. Both instruments were very well suited to classify tomato cultivars based on their taste profile and according to the correlation loading plot, glucose and fructose were the main compounds that cause this separation. The results showed that the seven-sensor commercial e-tongue predicted the concentration of glucose and fructose better than the 18-sensor instrument but the sensor readings were poorly correlated to the sweetness in tomato as tasted by the sensory panel. The partial least squares (PLS) models for the seven-sensor had slopes close to one ( $>0.90$ ), an offset very close to zero and the correlation coefficient ( $r$ ) values between measured and predicted values were high and close to one. Based on the available literature and commercial use by the industry, it may be concluded that e-tongue is a promising alternative to chromatographic and refractometric methods for sugar profiling. New ideas for the use of e-tongue in future research include a development of a personal taste sensor for finding abnormal ingredients in horticultural food products.

### 3.2. Non-destructive measurement

Nowadays, consumers are capable of distinguishing sensory attributes with a high degree of sensitivity and hence, demanding better quality, consistent supply of quality produce with appropriate taste (Jamshidi et al., 2012). Enhancement and assurance of fresh produce quality could lead to an increased demand and repeat purchases by the consumer. Therefore, there is much incentive to sort and grade fruit based on their internal quality for the fresh market, which lead to increase in profit margins for the industry, through price differentiation for different grades (Mendoza et al., 2014). This would be achieved by implementing appropriate quality control and inspection procedures to sort and grade each individual fruit for internal quality.

In the horticultural industry, the concentration of sugars relative to sweetness of fresh and processed fruit and vegetable products is measured on “representative” samples of a batch of product using conventional and destructive techniques such as refractometry and HPLC (Magwaza et al., 2013a). Although these destructive techniques are widely used, they are sometimes expensive, and require time-consuming and specialized sample preparation. One of the drawbacks is that the results of these tests only reflect the properties of the specific produce being evaluated. The high variability in quality attributes of fruit and vegetables batches, coupled with industry demand for innovative tools for quality measurements, have spurred considerable interest among researchers to search for alternative tools for detecting, sorting, prediction and monitoring of quality during postharvest handling.

The demand for high-quality produce by both consumers and the industry, calls for reliable and rapid sensing technologies for the non-destructive measurement and sorting of horticultural food products. Thus, development of cost effective analytical techniques for non-destructive sweetness analysis is of big practical interest in postharvest technology research. Non-destructive measurement of sweetness would allow the fresh produce industry to deliver superior, consistent fruit to the marketplace and ensure consumer acceptance and satisfaction (Peng and Lu, 2005, 2008). Among many non-destructive sensing techniques that have been developed, optical techniques, especially visible and near-infrared (vis/NIR) spectroscopy (vis/NIRS) show great potential for sorting and grading fresh produce for internal quality (Magwaza et al., 2012a, 2013a).

#### 3.2.1. Visible to near infrared spectroscopy

In the past 20 years, many studies have been reported on predicting SSC and TSS in fruits and vegetables using vis/NIRS technique. The techniques utilize visible and near-infrared (NIR) region of the spectrum, covering approximately 780–2500 nm. NIR spectra are comprised of broad band which arise from overlapping absorptions corresponding mainly to overtones and combinations of vibrational modes involving C–H, O–H, N–H, and S–H chemical bonds (Golic et al., 2003; Magwaza et al., 2012a). The molecular vibrations, particularly by O–H for water and C–H for carbohydrates bonds, are responsible for strong absorption bands in the NIR spectral region by fruit and vegetables (Antonucci et al., 2011). Vis/NIRS measures an aggregate amount of light reflected back from or transmitted through the sample, which is then used to predict certain chemical constituents.

Vis/NIRS-based non-destructive measurements of SSC have been extensively investigated on many horticultural products



**Table 4**  
Summary of applications of visible to near infrared spectroscopy (vis/NIRS) to measure sugar content of fruit and vegetables.

Produce	Measured parameter	Spectrophotometer	Detector	Wavelength range	Data acquisition mode	Accuracy	Reference
Apples	Glucose, Fructose, sucrose	A Bio-Rad FTS 6000 spectrometer	Deuterated triglycine sulphate	3250–800 cm <sup>-1</sup>	Reflectance	R = 0.97	Rudnitskaya et al. (2006)
	SSC	Nexus FT-NIR spectrometer	InGaAs detector	12,500–4000 cm <sup>-1</sup>	Diffuse reflectance	R = 0.91	Zou et al. (2007)
	Glucose, fructose, sucrose	Multi-purpose analyzer (MPA) spectrometer (Bruker Optics)	TE-InGaAs detector	800–2500 nm	Reflectance	R <sup>2</sup> = 0.86 R <sup>2</sup> = 0.76 R <sup>2</sup> = 0.69	de Oliveira et al. (2014a)
		Tensor 27 FTIR spectrometer (Bruker Optics)	Deuterated triglycine sulfate detector	4000–600 cm <sup>-1</sup>	Reflectance	R <sup>2</sup> = 0.94 R <sup>2</sup> = 0.86 R <sup>2</sup> = 0.82	
Apricot	SSC, sucrose, Glucose, fructose	Tensor 27 FTIR spectrometer (Bruker Optics)	Deuterated triglycine sulphate detector	4000–650 cm <sup>-1</sup>	Reflectance	R = 0.96 R <sup>2</sup> = 0.82 R <sup>2</sup> = 0.88 R <sup>2</sup> = 0.72	Bureau et al. (2009)
Jaboticaba	SSC	Spectrum 100 N, (FT)-IR spectrophotometer	Not defined	1000–2500 nm	Diffuse reflectance	R <sup>2</sup> = 0.62	Torres Mariani et al. (2014)
Kiwifruit	SSC	Zeiss MMS1-NIR	Diode array	300–1140 nm	Interactance		McGlone et al. (2002)
Mandarin	TSS	NIR-enhanced Zeiss MMS1 spectrometer	Not defined	720–950 nm	Interactance	R <sup>2</sup> = 0.94	Guthrie et al. (2005)
Mango	SSC	NIRS6500 spectrophotometer	Not defined	700–1100 nm	Interactance	R <sup>2</sup> = 0.84	Saranwong et al. (2004)
Nectarine	TSS	NIRS spectrometer	Silicon photodiode detector	306–1150 nm	Interactance	R <sup>2</sup> = 0.82	Golic and Walsh (2006)
Orange	SSC	QE65000 VIS-SWNIR CCD spectrometer (Ocean Optics)	Back-thinned silicon detector	200–1100 nm	Transmittance, interactance, diffuse reflectance	R = 0.78	Wang et al. (2014)
Peaches	TSS	NIRS spectrometer	Silicon photodiode detector	306–1150 nm	Interactance	R <sup>2</sup> = 0.90	Golic and Walsh (2006)
Plums	TSS	NIRS spectrometer	Silicon photodiode detector	306–1150 nm	Interactance	R <sup>2</sup> = 0.88	Golic and Walsh (2006)
Tomato	Glucose, fructose, sucrose	Bio-Rad FTS 6000 spectrometer	Deuterated triglycine sulphate	3250–800 cm <sup>-1</sup>	Reflectance	R = 0.86 R = 0.77 R = 0.73	Beullens et al. (2006)

SSC, soluble solid content; TSS, total soluble solids; NIR, near infrared; FT-NIR, Fourier Transform; MIR, mid infrared; IR, infrared; InGaAs, Indium gallium arsenide; PbS, Lead selenide; ATR, attenuated total reflection.

(Antonucci et al., 2011; Magwaza et al., 2012a). As such, vis/NIRS has become one of the most used candidates for non-destructive evaluation of sweetness parameters of fresh produce (Walsh, 2005; Golic and Walsh, 2006; Lu et al., 2006; Magwaza et al., 2013a). Several studies of vis/NIR spectroscopy in the spectral region between 400 nm and 2500 nm to measure the sugar content of many fruits including apples, apricot, jaboticaba, kiwifruit, mandarins, melons, mangoes, nectarines, peaches, plums and tomato have been reported in the literature (Table 4).

Golic et al. (2003) used reflectance vis/NIRS to predict individual sugar (glucose, fructose and sucrose) on citrus fruit. This study showed that sucrose was predicted with less accuracy compared to glucose, and fructose. The authors argued that the low predictability of sucrose could possibly results due to the difference in molecular weight of sucrose (MW = 342.30 g/mol) compared to glucose and fructose (MW = 180.16 g/mol). This difference in molecular weight is such that there are 1.89 times fewer number of sucrose molecules than glucose and fructose in the same weight of sample. Therefore, the intensity of the bands associated with hydrogen bonding is smaller in sucrose than in glucose and fructose (Magwaza et al., 2012b, 2013b, 2014b,c).

Jamshidi et al. (2012) investigated the feasibility of reflectance vis/NIRS for taste characterization of 'Valencia' oranges based on taste attributes including soluble solids content (SSC) and titratable acidity (TA), as well as taste indices including SSC to TA ratio (SSC/TA) and BrimA. The authors observed more accurate predictions for SSC ( $r=0.958$ ) and BrimA ( $r=0.918$ ) compared to

TA ( $r=0.858$ ) and SSC/TA ( $r=0.871$ ). The capability of vis/NIRS to predict orange fruit taste based on BrimA index directly and non-destructively was interesting and could be used for taste characterization before or after harvesting.

One of the difficulties of using vis/NIRS to measure fruit quality is the optical thickness of fruit rinds such as those of watermelon and citrus. Due to differences in sweetness and high water content between the center and external parts of fruit flesh, it is necessary in future to design specialized vis/NIRS system equipped with increased light intensity, increased integration time or increased spectrometer aperture/detector size for internal information acquisition. Another disadvantage of vis/NIRS is that a new calibration model is required for each fruit species and cultivar. Considering the variation between growing locations and seasons, it is advisable to continuously upgrade calibration models based on successive seasons and new orchards (Magwaza et al., 2014c). Magwaza et al. (2014c) demonstrated that spiking existing calibration models with a few samples from the target prediction orchard improved model performance and reduced calibration time and costs.

This part of the review has demonstrated that extensive research has been carried out over the past decades on the development of non-destructive or minimally destructive sensors for measurement of SSC. Vis/NIRS technique is well developed and is now being used in packing houses for sorting different types of horticultural products for SSC and other internal quality parameters (Jie et al., 2014). Commercial application of vis/NIRS to fruit sorting was first initiated to sort peaches based on SSC (Kawano, 1998; Golic and

Walsh, 2006), and has been applied to pack-house sorting lines for TSS citrus since the mid-1990s in Japan, and more recently in other countries and fruit types (Kawano et al., 1993; Golic and Walsh, 2006). Although vis/NIRS is a well-established technique for non-destructive assessment of fresh produce, currently, the majority of packinghouses in the world still do not sort and grade individual fruit and vegetables based SSC. Vis/NIRS would present a high potential for in-line commercial measurements if the robustness of multivariate calibration models was improved (Roger et al., 2003; Cozzolino, 2014). Given the recent developments in vis/NIRS technology, adoption of this technology for commercial online sorting systems has a huge potential in the industry. It is expected that in the near future, the industry will be able to interactively sort and grade fruit on the basis of both external appearance and sweetness.

### 3.2.2. Hyperspectral and multispectral imaging

Several imaging techniques have been developed and successfully applied as inspection tools for quality assessment of a variety of fruits (Magwaza et al., 2012a). Multi- and hyper-spectral imaging have emerged as powerful inspection techniques for food and agricultural products (Wu and Sun, 2013a,b). By definition, multispectral imaging involves making images using more than one spectral component of the electromagnetic energy from the same region of an object and at the same scale (Magwaza et al., 2012a; Sugiyama and Tsuta, 2010). On the other hand, hyperspectral imaging, integrates conventional imaging and spectroscopy to attain both spatial and spectral information from an object. Hyperspectral images are made up of hundreds of contiguous wavebands, in both the visible range and NIR regions of the spectrum, for each spatial position of a sample studied and each pixel in an image contains the spectrum for that specific position (Mendoza et al., 2011; Gómez-Sanchis et al., 2008; Magwaza et al., 2012a). Further information on the principles of these technologies can be found in a review by Gowen et al. (2007).

Over the past decade, many studies were reported for quality evaluation of fruits and vegetables quality parameters, such as SSC of apples, dry matter, SSC and acidity of strawberries (ElMasry et al., 2007; Peng and Lu, 2008). Table 5 present an overview of applications of multispectral and hyperspectral imaging systems to assess TSS and SSC of different fruit. For example, Lu (2004) applied hyperspectral imaging technique in the wavelength range of 500–1000 nm, in conjunction with artificial neural network, to predict SSC of apple fruit. The authors reported high prediction with correlation coefficient of 0.88. Sugiyama and Tsuta (2010) used hyperspectral imaging to determine physiological ripeness of melons by mapping sugar distribution at different stages of maturity. Fig. 1 shows the results of visualization of the sugar content corresponding to unripe, mature, and fully mature melons (Sugiyama and Tsuta, 2010). In a recent study, Leiva-Valenzuela et al. (2013) applied hyperspectral imaging technique for predicting the SSC of blueberries in the visible and short-wave NIR region (500–1000 nm). The results from their study demonstrated the feasibility of implementing hyperspectral imaging technique for sorting blueberries for firmness and possibly SSC to enhance the product quality and marketability. Collectively these studies have shown the potential of hyperspectral imaging for measuring physico-chemical properties of agricultural products using spectral information.

The important objective governing non-destructive technology development research in fruit postharvest science is to explore the possibilities of a technology such as vis/NIRS for in-line assessment of quality. While scientific literature is replete with studies exploring feasibility of hyper- and multi-spectral imaging for non-destructive assessment of fruit quality and the technologies is still in development. Their application for in-line and real-time detection of internal quality still needs to be investigated.

## 4. Future prospects

Instrumental methods for quantifying sugars are constantly evolving and so are the ranges of techniques available for data analysis. This constant evolution presents new opportunities for increasingly more inclusive, reliable and sensory-related estimation of sweetness and taste of fruit and vegetable. Some of the prominent modern techniques which have a room for future improvement include mass spectroscopy, HPLC or capillary electrophoresis (CE) coupled with mass spectrometry (MS), hyperspectral and multispectral imaging, and nuclear magnetic resonance (NMR) spectroscopy.

As an alternative to refractometry, which is invasive and time consuming, the use of NMR has been proposed to estimate sweetness in intact fruit relative to the fruit ripeness. NMR has shown potential for future estimation of sugar concentration and sweetness of agricultural products. For example, NMR has been used successfully to estimate SSC of intact prunes (Zion et al., 1995) and sweetness of plums (Pereira et al., 2013), SCC and SSC/TA in pomegranate fruit (Zhang and McCarthy, 2013). Although this method is very promising, it has not been used commercially because the equipment is too delicate and expensive for use in packinghouses. In a study by Zion et al. (1995), the NMR measurement time was approximately 1 s and, according to the authors, this time could be reduced to less than 100 ms, increasing the possibility of this technique for commercial packing line assessment of individual fruit.

Moving forward into the future, there should be a concerted move towards incorporating humans as the ultimate test of sweetness, taste and quality. There is an expense associated with this approach but it is badly needed if the industry goal is to deliver better tasting fruit and vegetables. Sensory panels will not automatically lead to the selection of the “best” fruit but greater frequency of use will help to develop a more accurate, quantitative picture of taste by preference mapping. Another area that still needs further research is developing standardized sweetness index for fruit and vegetables.

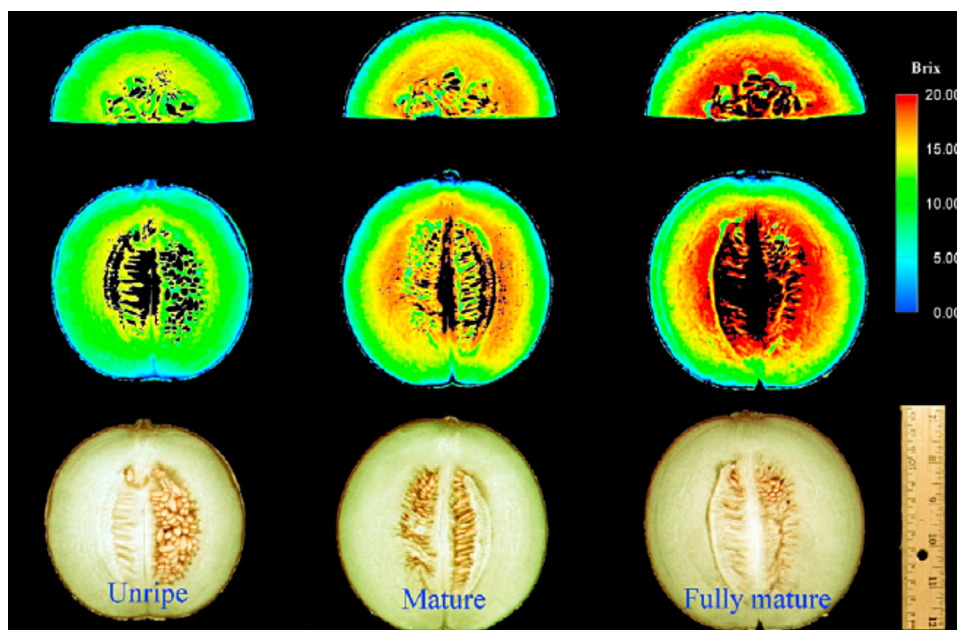
Currently, there is an over-reliance on TSS and TA for assessing quality. Data on fructose, glucose, sucrose levels should be included to complement TSS. This would provide a more accurate description of biochemical changes that are occurring and would be a better indicator of sweetness and quality and may better align with a prediction of human perception of good taste (Baldwin et al., 1998). Further research is still needed to investigate to use vis/NIR spectroscopy for determination of taste attributes (SSC, TA) and to assess the feasibility of using the technique for prediction of taste indices (SSC/TA, BrimA) of fruit directly and non-destructively. Further research is needed to determine how well SSC relates to sugar concentrations in pigmented fruit and the degree to which sugars affect sensory scores for sweetness, flavor and overall eating quality of these fruit. It would also be necessary in future research, for correlating soluble solids to acidity ratio to sensory flavor, to include alternatives, such as subtracting acidity and pigments from soluble solids.

As stated in Section 1, most of the procedures currently used to measure sugars and sweetness of horticultural products are considered to be time-consuming, not cost effective and labor-intensive due to sample pre-treatment and the need for expensive chemicals. Nowadays, the Raman spectroscopy is increasingly used as an analytical technique for the evaluation of food quality (Paradkar and Irudayaraj, 2001; Özbilci et al., 2013). The principle of Raman spectroscopy is based on inelastic scattering of the incident light from a sample, scattering of light due to sample characteristic molecular vibrations and frequency shift of scattered light in a sample (Kneipp et al., 1999). Some of the advantages of Raman spectroscopy over other spectroscopic techniques are non-interference

**Table 5**  
Applications of hyperspectral imaging to quantify the TSS and SSC of selected fruit.

Commodity	Quality parameter	Wavelength range (nm)	Accuracy	Reference
Apple	SSC	680–1060	$r = 0.77$	Lu (2004)
Apple	SSC	450–1000	$r = 0.88$	Peng and Lu (2005, 2008)
Apple	SSC	500–1000	$R = 0.68$ to $0.88$	Mendoza et al. (2011, 2012)
Blueberry	SSC	500–1000	$r = 0.68$ to $0.79$	Leiva-Valenzuela et al. (2013)
Grape	SSC	400–1000	$r^2 = 0.93$ – $0.94$	Baiano et al. (2012)
Strawberries	TSS	400–1000	$r = 0.80$	ElMasry et al. (2007)

R or  $r$ , correlation coefficient between vis/NIRS predicted and measured parameter;  $r^2$ , coefficient of determination.



**Fig. 1.** Representative slice image showing visualization of sugar distribution in immature, mature and fully ripe melons using hyperspectral imaging (Sugiyama and Tsuta, 2010).

from water molecules present in the sample, ease of sampling and measurement, and minimal fluorescence interference of sample matrix varying from sample to sample. Combined with chemometric methods and vibrational spectroscopy, Raman spectroscopy has the potential for both quantitative and qualitative measurements of sugars components and sweetness of horticultural products. For further reading on the use of Raman spectroscopy for identification and quantification of carbohydrates and other plant substances, the reader is referred to a recent review by Schulz and Baranska (2007).

Although work on non-destructive methods to measure quality using vis/NIR-based systems has led to commercial use in a packing line to select fruit with acceptable flavor quality, there is a need for continued development of non-destructive sensing of flavor quality. This should include sensing degree of freshness (time since harvest); use of vis/NIRS to estimate concentrations of flavor-related, non-volatile constituents (e.g. sugars and titratable acids); use of aroma-sensing technology (electronic nose) to detect desirable and undesirable aroma volatiles and taste sensing technology (electronic tongue) (Hong et al., 2014).

Future prospects for vis/NIRS-based imaging systems such as multispectral and hyperspectral imaging as potential non-destructive techniques for fruit and vegetable quality assessment are developing robust models that could be used in commercial packing lines with confidence. While the ultimate goal of any technique is to be universal, it is important to stress that special attention should be paid on model robustness in predicting new populations from different orchards, regions, and production or marketing seasons. Considering the known variation between

cultivars, growing regions and seasons, constant upgrading of calibration models, using fruit from successive seasons and orchard locations is necessary. Spiking existing calibration models with a few samples from the target prediction orchard have been shown to potentially improve model performance (Magwaza et al., 2014c). Development of spiking method described by Magwaza et al. (2014c) is a major advancement towards reducing calibration time and costs.

## 5. Conclusion

This review of the literature has shown that several methods of sugar analysis and quantification of sweetness exist and a new generation of instruments have emerged in recent years. Soluble solid content, usually determined from juice extracted from flesh produce using the refractometric method, is currently the most commonly applied measure of sweetness in fruit and vegetable products. Although new methods are constantly emerging, the use of HPLC has strengthened its position as an analytical technique used during the development and improvement of specially designed columns and more suitable carbohydrates detection techniques such as refractive index detection, evaporative light scattering detection and charged aerosol detection. Documented literature has shown that ligand-exchange and cation-exchange chromatography with refractive index detection, amine-bonded silica gel column, and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) have been successfully applied to quantify carbohydrates. Prior to

the advent of HPAEC–PAD, RID and ELSD used to be widespread tools for carbohydrate analysis by HPLC. RID may suffer from poor sensitivity, specificity, and selectivity depending on temperature, composition, flow rate, and incompatibility with gradient elution of detection. The continued worldwide usage of RID and ELSD is for economic reasons because both detectors are considerably less expensive than PA. As a result, the cost–benefit ratio currently tilts in favour of RID and ELSD. Therefore, in most analytical laboratories, higher sensitivity of PAD, which is not always needed for major carbohydrates (i.e. glucose, fructose and sucrose) is traded for low analysis cost provided by RID and ELSD. Different electronic tongues have also proven to be successful in discrimination and classification, quality evaluation and control, process monitoring and quantitative analysis of horticultural food products. However, these instrumental techniques show some drawbacks because they require laborious and time-consuming sample preparation and skilled people to operate the equipment. There is therefore an increasing trend towards development of non-destructive technologies for sweetness evaluation. As an alternative to refractometry and liquid chromatography, which are invasive and time consuming, a few spectroscopic methods involving NMR, vis/NIRS, and Fourier transform infrared spectroscopy with attenuated total reflection have been used to determine fruit internal quality with respect to sweetness. Although a wide range of destructive and non-destructive methods have been used to measure and quantify the sweet and concentration of sugars in fresh and processed fruit and vegetables, the handheld refractometer is the most commonly used tool, presumably due to its simplicity and low cost compared with more sophisticated and precise bench-top equipment such as HPLC. However, for the identification and quantification of individual (composition) sugars, the most commonly used measurement technique is the HPLC. The HPLC is particularly suitable for cultivar evaluation studies where knowledge of the components of sugars contributing to sweetness is essential.

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