NONDESTRUCTIVE MEASUREMENT OF FRUIT AND VEGETABLE QUALITY

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• Abstract We review nondestructive techniques for measuring internal and external quality attributes of fruit and vegetables, such as color, size and shape, flavor, texture, and absence of defects. The different techniques are organized according to their physical measurement principle. We first describe each technique and then list some examples. As many of these techniques rely on mathematical models and particular data processing methods, we discuss these where needed. We pay particular attention to techniques that can be implemented online in grading lines

1. INTRODUCTION

The late Joseph Juran defined quality as "fitness for use," where fitness is defined by the customer (Juran 1951). This concept was the basis of the ISO 9000 standard that defines quality as the "degree to which a set of inherent characteristics fulfills requirements of the customer" (http://www.iso.org) . Although every actor in a typical horticultural chain can be considered a customer, it makes sense to focus on the consumer, as he or she is the ultimate customer that drives the flow in the chain. For practical use, quality may be described as

some mathematical function of a series of quality attributes; the consumer assesses these quality attributes and consciously or unconsciously assigns a score and then mentally calculates an overall quality score for future purchase decisions (Sloof et al. 1996). The assessment of quality attributes is thus an essential component of the quality appreciation that we focus on in this article.

External quality attributes relate to the appearance of the product and include properties such as color, shape, size, and the absence of surface defects. They determine the purchase behavior of consumers by and large, as these properties may be inspected readily by the eye. As such, most commercial quality systems are based on external quality attributes only. Internal quality attributes include texture properties such as firmness and crispiness, taste, aroma, and absence of internal defects. These attributes determine the organoleptic satisfaction of consumers. But more elusive quality attributes such as freshness, safety, nutritional value and health-promoting properties, chemical residues, production system, authenticity, convenience, and ethical aspects may affect the quality perception of consumers on a more abstract and cerebral level. These attributes may be particularly important but are often difficult to measure and even to define.

Expert and consumer panels are used widely to assess the quality of fruit and vegetables (Meilgaard et al. 2006). The former consist typically of 10—15 experts that are trained to score a list of quality descriptors that they have either derived themselves or obtained from the literature. They can be considered human sensors to quantitatively measure the quality attributes of the product. A series of ISO standards is available to select descriptors, train panelists, evaluate their performance, and statistically analyze the data. Consumer panels consist typically of hundreds of consumers that represent the group of consumers that is considered relevant for the test. They only provide preference scores that can be used to subdivide the consumers into preference segments and associate them, for example, with certain cultivars of a fruit or vegetable species. The preference scores, however, only become meaningful when related to quality attributes measured by trained sensory panels. The advantage of both expert and consumer panels is that they address the quality attributes of fruit and vegetables similarly to the ultimate consumer. However, even when obtained by a well-trained quantitative descriptive expert panel, the scores are prone to large variability

and may drift over time, the capacity of the panel is limited to typically 6--8 objects per session, the procedure is slow, and the cost is high.

Instrumental techniques may be used as alternate methods for measuring quality attributes of fruit and vegetables. These techniques do not suffer from many of the aforementioned disadvantages of human panels. However, their success often depends critically on how their measurement principle mimics humans' perceptions of a particular property. Laboratory colorimeters have been successful in this regard, as they are based on the three-component theory of human color perception that was elaborated by the International Commission of Illumination in 1931 (Smith & Guild 1931). However, there are many quality attributes for which there is no such measurement principle readily available. Mealiness---a texture attribute describing the sensation of dryness and granularity due to cell debonding rather than fracturing during mastication---is such an example. It is a challenge to measure such properties (Barreiro et al. 1998). Instrumental techniques may replace quantitative descriptive panels to some extent; nonetheless, they do not provide an alternative to consumer panels.

Quality attributes can be measured by both destructive and nondestructive techniques. Nondestructive techniques are often fast, reduce waste, and have the particular advantage that the actual measurement does not affect the characteristics of the fruit. The immediate benefit is that such techniques can be used for grading individual fruit and vegetables with respect to quality prior to sale. Because of the large biological variability of the quality attributes of fruit and vegetables, grading individual products is essential to meeting consumers' expectations. Color and size grading by visual inspection has been used for ages to remove products that would not meet the minimal requirements for quality and to simultaneously enhance uniformity. Over the years, this has been automated, and highspeed grading lines using sensors for external quality attributes such as color, size, and appearance are now used widely by growers, cooperatives, and packing houses worldwide. The advent of nondestructive methods to measure internal quality attributes such as texture properties or flavor, as well, opened up exciting new marketing possibilities for horticultural products, provided, of course, that the properties they measure correspond to their human analogs. Nondestructive techniques are also very useful for developing models of changes in quality attributes during postharvest storage, to optimize postharvest processes. As the

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same fruit can be monitored in time, the interfruit variability can be separated easily from the time effect. This improves the estimation of the kinetic parameters greatly (<u>Hertog et al.</u> 2007).

In this article, we review nondestructive techniques for internal and external quality attributes. The text is organized according to physical principle rather than quality attribute, as some measurement techniques can be used to measure widely different quality attributes.

2. OPTICAL TECHNIQUES

2.1. Visible/Near-Infrared Spectroscopy

As with most biological materials, fruit and vegetables are opaque to radiation in the visible (Vis) and near-infrared (NIR) regions of the electromagnetic spectrum. In these media, a complex interplay between absorption and scattering of the electromagnetic waves (light) guides the light-matter interaction. Absorption and scattering depend on the spectral and spatial changes in the complex refractive index at the microstructure level (Bohren & Huffman 1983). The tissue structures made up of the cells and intra/extracellular environments are responsible for the scattering. As photons are most strongly scattered by structures whose size matches the photon wavelength, the nuclei, mitochondria, vesicles, membranes, and cell walls play crucial roles in the scattering of Vis/NIR light by fruit and vegetable tissue. The absorption is caused mainly by the C-H, O-H, and N-H bonds of the main compounds (water, sugars, chlorophylls, carotenoids, etc.). As a photon can be absorbed only if it has the right energy to excite one of the vibrational states of the molecule, each molecule has its own specific absorption spectrum. However, the fundamental vibrations of these bonds occur in the infrared region; thus, the absorption in the Vis/NIR region is caused by overtones and combinations of these fundamental vibrations. This yields absorption peaks in the Vis/NIR region that are broad and overlapping. Moreover, the peaks can shift a few nanometers due to H-bonding. The combination of these effects means that the Vis/NIR spectra of complex mixtures such as fruit and vegetables are hard to interpret. Therefore, advanced chemometric techniques are needed to extract information on the concentrations of the major components from these spectra. As the absorption by water is relatively low in the Vis/NIR range compared to the UV and mid-infrared ranges, the electromagnetic radiation can penetrate quite deep (up to a few centimeters, depending on the wavelength) into biological tissue. Conversely, scattering is remarkably larger, and this enables electromagnetic radiation to diffuse in the sample volume and to be reemitted at the tissue boundaries. A drawback of NIR spectroscopy is that for each fruit species and cultivar, a new calibration model is required, and the calibration models should be based on large datasets incorporating different orchards, seasons, cultivation systems, etc. (Peirs et al. 2002). The prediction accuracy also depends on temperature (Peirs et al. 2003). Finally, the calibration models depend on the spectrophotometer, such that model transfer even between different spectrophotometers of the same brand and type is not straightforward.

NIR spectroscopy has been used successfully to nondestructively measure the soluble solids contents of various fruit, including apple (Lammertyn et al. 1998), cherry (Lu, 2001), kiwifruit (McGlone & Kawano 1998), mandarin (Kawano et al. 1993), melon, and pineapple (Guthrie & Walsh 1997), and peach (Slaughter 1995). The root mean squared error of prediction is typically 0.5--1.0°Bx. Acidity, texture, and other fruit properties are much more difficult to measure by means of NIR spectroscopy; however, some reports have been published in which a reasonable accuracy was obtained (e.g., Peirs et al. 2002). Nicolaï et al. (2007) give a full account of NIR applications in fruit and vegetables. Fruit grading lines equipped with NIR sensors are made available commercially by Aweta (IQA, http://www.aweta.nl), Greefa (iFA, http://www.greefa.nl), Mitsui-Kinzoku (http://www.taste-technologies.com), and others.

2.2. Time- and Space-Resolved Spectroscopy

Advanced techniques, such as space-resolved and time-resolved spectroscopy (SRS and TRS, respectively), improve the classical approach to Vis/NIR spectroscopy (Patterson et al. 1989, Kienle et al. 1996). The main feature of SRS and TRS is their ability to retrieve information on the photon path length in a diffusive medium, which is generally much larger than the geometrical distance between the source and the detector. The typical values of the optical properties of fruit and vegetables in the Vis/NIR region correspond to average photon path lengths, in the order of a few meters.

SRS collects photons at multiple source-detector distances (e.g., in the range 1--10 mm) using an optical fiber arrangement or a camera as detectors (<u>Nguyen et al. 2011</u>). TRS

measures the distribution of photon time-of-flight (related to photon path length by the speed of light in the medium) at the picosecond or nanosecond timescale and at a fixed source-detector distance (e.g., 15 mm). This is done with pulsed laser sources (duration of tens of picoseconds) and fast detection techniques (e.g., time-correlated single photon counting) (Torricelli et al. 2008).

The use of SRS or TRS, in combination with proper models for photon migration, enables the complete optical characterization and simultaneous nondestructive measurement of the optical properties (absorption and scattering) of a diffusive medium. This can be particularly important for most fruit and vegetables because information derived by TRS and SRS refers to the internal properties of the medium and is not so much affected by surface features as is traditional spectroscopy (<u>Cubeddu et al. 2001</u>, <u>Saeys et al. 2008</u>). It is hypothesized that the absorption properties are related to the chemical composition, whereas the scattering properties are related to the microstructural features such as the topology of the intercellular space and the size and shape of the cells. This could enable a means for nondestructively assessing texture, as these features affect the overall mechanical properties. However, other maturity- and ripeness-related features such as biochemical changes in the cell wall--middle lamella complex also affect the mechanical properties, and attempts to predict texture properties based on scattering properties have not been very successful thus far.

TRS has been explored as a potential method for the nondestructive quality evaluation of fruits such as apples, pears, nectarines, etc. (<u>Cubeddu et al. 2001</u>; <u>Nicolaï et al. 2008b</u>; <u>Rizzolo et al. 2009</u>; <u>Eccher Zerbini et al. 2002</u>, 2006). In the case of SRS, measurements based on a multispectral or hyperspectral camera have been used to acquire spatially resolved diffuse reflectance spectra for the prediction of fruit quality attributes (<u>Qin & Lu</u> 2008, <u>Qin et al. 2009</u>).

2.3. Machine Vision

The process of sorting and grading fruit and vegetables at farms, in distribution, and even by consumers still relies mostly on visual inspection. Several researchers have investigated the potential of machine vision to automate this visual inspection and make it more objective (Brosnan & Sun 2004). A machine vision system consists essentially of a digital camera that is connected to a computer and software for image analysis. Monochrome digital

cameras produce a 2D array of intensity values corresponding to the different positions in the image; a digital color image consists of three 2D arrays of intensity values: one for the red band, one for the green band, and one for the blue band. To detect objects (fruit, bruise, stalk, leaf, etc.) in the image, the pixels should be clustered or discriminated based on some features. The simplest way to classify pixels is by placing a threshold on the intensity values of one of the waveband images (red, green, or blue). A major disadvantage of this approach is that a change in the illumination intensity will change the classification result if the threshold is not adapted accordingly. To avoid this problem, it is better to separate the information on the perceived color from the information on the luminosity. This can be achieved with a nonlinear transformation from the red, green, blue (RGB) color space to the L*a*b* color space. In this color space, L* is the axis ranging from black to white, a* is the axis ranging from green to red, and b* is the axis ranging from blue to yellow. This system has become popular in the food industry as it enables the accurate discrimination of red from green independent from the illumination intensity. One such application of this system is for the prediction of the lycopene content in tomatoes (Arias et al. 2000). Wu & Sun (2013) provided a detailed account of color measurement by machine vision.

Alternative segmentation approaches have been proposed that try to exploit the properties of the object's contours, such as concaveness (Bai et al. 2009) or an intensity gradient (Granitto et al. 2005). When the pixels have not all been correctly classified, morphological operations such as erosion and dilatation can be used to remove small groups of pixels that have been misclassified as an object or to fill small holes in detected objects (Gonzalez & Woods 2007). For these detected objects, morphological parameters such as area, largest length, or shortest width can be calculated easily. Alternatively, geometrical models such as spheres, rectangles, or ellipsoids can be fitted to the detected object, thus identifying the shape parameters. Applications of machine vision to fruit and vegetables are vast (see, e.g., Brosnan & Sun 2004). Sorting lines with machine vision systems for detecting color, size, shape, and surface defects are made available by all major sorting line manufacturers.

2.4. Multispectral and Hyperspectral Imaging

Although digital color cameras (RGB) have been designed to acquire the same red, green, and blue bands captured by the human eye, the extraction of quality aspects corresponding to small contrasts in the images (bruises, ripeness stages, etc.) remains very challenging. These very small differences might be detected with more advanced image processing algorithms; however, the computation costs are very often too high for use in the food industry. Instead of trying to detect these very small differences, it might be more efficient to enhance the contrast. Multi- and hyperspectral imaging aims to combine the spectral information content and discriminating power of point spectroscopy with the spatial information content of machine vision. In multispectral imaging, images are acquired at a small number of wavebands (5--10), whereas many more wavebands are included in hyperspectral imaging (Sun 2010).

The data acquired by hyperspectral imaging are called a hypercube. This is a 3D block of data, comprising two spatial dimensions and one wavelength dimension. There are two ways to obtain the data needed for a hypercube. The most straightforward approach is to sequentially acquire the images at different wavebands by placing different filters or a tunable filter in front of the camera. In the second approach, a line spectrograph is placed in front of the camera. This spectrograph consists of a narrow slit and a dispersive element (prism and/or grating). The light entering the slit is split into its wavelength components and projected onto the camera chip. Thus, each acquired image has a spatial axis and a spectral axis. The hypercube is then built by scanning the second spatial dimension line by line. This approach is ideally suited for mounting over a transportation system such as a conveyor belt or for sorting and grading lines. Hyperspectral reflectance imaging is the most common type and is carried out in the Vis/NIR (400--1,000-nm) or SWIR (1,000--2,500-nm) range to detect defects, contaminants, and quality attributes of fruit and vegetables (Nicolaï et al. 2007, Wu & Sun 2013).

Because many, often highly correlated, spectral variables are acquired for every pixel, the same multivariate calibration and classification techniques commonly used in point spectroscopy are also used to convert the acquired hypercubes into virtual images with maximal contrast. Applications in fruit and vegetables include detection of bitter pit and bruises in apples (Nicolaï et al. 2006, Xing et al. 2007) and defects in cherries (Guyer & Yang 2000). Elmasry et al. (2012) review more applications.

3. MECHANICAL TECHNIQUES

Mechanical techniques have been used for many years to gain information about the internal quality of fruit and vegetables in a nondestructive way. These techniques usually focus on estimating a product's firmness and maturity (which are related). In general, mechanical techniques can be divided into two broad classes: a first class using local force-deformation characteristics of a fruit after application of an external force and a second class that focuses on the response of the fruit as a whole after it has been excited. For both classes, several means of inserting energy into the fruit as well as for measuring the resulting response have been described in the literature and are reviewed here. Other mechanical techniques that are reported in the literature are also discussed briefly.

3.1. Impact Analysis

Fruit deform locally after being impacted by an external load and, if the load is below a certain threshold, will eventually converge back to the initial state once this load has been removed. On the basis of Hertz's contact theory (<u>Hertz 1882</u>), the maximal deformation, maximal force, and contact time between impactor and sample provide information about the mechanical properties of the fruit.

The external load is typically provided using a low-mass impact device with a spherical tip made from a material with known and constant physical properties. An integrated force sensor measures the maximal force and the contact time of the impact and is processed further to derive local firmness values. Because mainly force readings are used, fruit must be impacted with a constant energy, and a precise control of the impact device that can handle different sizes and shapes is essential. Different methods based on the above principles have been investigated in great detail under laboratory conditions (Chen et al. 1985) and are translated into commercial sensors that allow both offline and online quality control of fruit and vegetables (e.g., the Sinclair IQ Firmness Tester; see Shmulevich et al. 2003). As an alternative to low-mass impact, the fruit can be dropped from a small height onto a flat surface connected to a force cell, and impact duration can be determined accordingly (De Baerdemaeker et al. 1982, Delwiche et al. 1987). Another method involves the use of compressed air to generate the load on the fruit surface. The main advantage of this method is that it is completely contactless; however, it requires an alternative to a force sensor to derive local fruit response. Precise laser distance sensors can be used for this purpose; their working principle is based on laser triangulation, which can reveal

deformations in the order of magnitude of micrometers (<u>Prussia et al. 1994</u>, <u>Hung et al.</u> <u>1999</u>). On the basis of Hooke's law, the force-deformation relation is translated into local stiffness.

Applications of impact analysis on a broad range of fruit and vegetables from peaches (Delwiche et al. 1987), tomatoes (De Ketelaere et al. 2006), apples (Shmulevich et al. 2003), and kiwi (Ragni et al. 2010) to fruit with more complex shapes such as mangos (De Ketelaere et al. 2006) have been reported. That the method is not sensitive to overall fruit shape is its main advantage over vibration analysis, which is discussed in the next section. In contrast, for products with a high firmness, the deformation after a low-mass impact will be very small and the contact time very short, so that the signal-to-noise ratio is low, pinpointing the main weakness of the methodology.

3.2. Vibration Analysis

Consumers sometimes estimate the ripeness of melons by analyzing the sound that results when they gently tap the fruit: Higher tones are associated with less mature and, hence, harder fruit. This simple phenomenon is useful for studying a broader class of fruit and was first described by <u>Clark & Mikelson (1942</u>). Later, several researchers performed more indepth research and proved that after impact, the fruit will vibrate according to a well-defined pattern of mode shapes, each associated with a certain resonance frequency (Abbott et al. 1968; Finney 1970, 1971; Cooke 1972; Cooke & Rand 1973). Most research has focused on spherical fruit; the (acoustic) stiffness of the fruit *S*, defined as $f_R^2m^{2/3}$, where f_R is the resonant frequency of the vibration in hertz and *m* is the fruit mass in grams, is used as a firmness indicator. If the fruit has an irregular or significantly nonspherical shape, interpreting the vibration response of the fruit is not straightforward (Chen & De Baerdemaeker 1993, Jancsók et al. 2001); in this case, the above formula is not useful as a firmness indicator.

Usually, a small impactor with a high stiffness is used to excite the fruit so that the impact duration is short and a broad range of frequencies is excited (at least as high as the resonant frequency used in the formula for stiffness above). The simplest way to record the vibration of the fruit is with a microphone placed optimally with respect to the impactor, such that the vibration mode shape of interest, usually the spherical mode, is addressed. This setup is used for offline measurements and online grading of up to 10 fruit per second.

Alternative means of registration are accelerometers (Peleg 1993) and piezoelectric transducers (Shmulevich et al. 1996); although, both are contact methods. In an alternate setting, a range of frequencies is scanned using a sinusoidal input, and the detection of the response signal on the other side of the fruit is performed with a pick-up rod or an accelerometer (Finney & Norris 1968). Those methods are more time consuming and require the attachment of sensors to the fruit, which is less desirable for high-speed grading. Laser Doppler vibrometry is one contactless method to measure vibrations of excited fruit (Landahl & Terry 2012); however, because of its high cost, this method will likely be used exclusively by laboratories.

The measurement principle explained above is based on mechanical resonance---the fruit vibrating at its natural frequency after being excited. Before the fruit reaches this stage of equilibrium, transient behavior is observed, during which a surface wave travels along the surface. <u>Sugiyama et al. (2005)</u> used this transient behavior, more specifically the speed of this surface wave, to derive fruit firmness.

There are numerous applications of vibration analysis on fruit and vegetables, but apple (Abbott & Liljedahl 1994), melon (Sun et al. 2010), and tomato (De Ketelaere & De Baerdemaeker 2001) are the most researched specimens, mainly because of their almost spherical shapes, which best facilitate the interpretation of the mechanical resonance pattern observed. Foerster et al. (2013) used different setups for vibration analysis to determine the presence of hollow spears in asparagus. For softer fruit, a large portion of the impact energy will be absorbed during deformation, and only a minor fraction will be transformed into vibration energy; thus, the method is less suitable. The clear advantage of this method is the fact that it is based on the integrated response of the whole fruit, whereas impact analysis is a local method (De Ketelaere et al. 2006). This suggests combining the two techniques in order to cover a wide firmness and shape range. De Ketelaere et al. (2006) already showed the advantage of such a combination by quantifying the repeatability of both methods. More recently, Mendoza et al. (2012) performed a large-scale experiment on apples, concluding that the fusion of signals from different sensors adds prediction power; however, their comparison was between optical and mechanical techniques.

The stiffness is actually a scaled version of the elastic modulus of the fruit; it is a measure of the force required to cause a certain deformation of the fruit while squeezing it. It is,

however, not necessarily related to the firmness as measured with a Magness-Taylor penetrometer---the gold standard for firmness measurements of many fruit, including apple. The latter measures a combination of compression strength and shear properties. This is shown in **Figure 1**, where the Magness-Taylor firmness is plotted against the acoustic stiffness for the apple cultivars Jonagored and Delbard Estival. For the latter, a more or less linear relationship is evident, whereas for Jonagored, there clearly is no correlation between the measurements obtained with both methods (**R**óth 2008). The same holds for impactbased firmness measurements. This is the main reason why, approximately a decade after their commercial introduction, grading lines with vibration-based stiffness sensors have not been a great commercial success.

<COMP: PLEASE INSERT FIGURE 1 HERE>

Figure 1 Magness-Taylor firmness versus stiffness as measured through vibration analysis for apple cultivars (*a*) Jonagored and (*b*) Delbard Estival. For Delbard Estival, an approximately linear relationship is evident, whereas for Jonagored, there is no correlation between the measurements obtained using both methods (\underline{R} 6th 2008).

3.3. Ultrasound

Ultrasound, or ultrasonics, involves sound waves of 20 kHz or more that are indiscernible to the human ear. As with any sound, ultrasound moves through a medium as a series of alternating compressions and decompressions with its velocity being determined by the density and elasticity of the medium (McClements 1997). Absorption and scattering by the medium result in alterations of this velocity and can lead to attenuation of the sound. The technique is relatively simple and cheap in its instrumentation and has been applied to many different food processing operations since the 1970s (Povey & McClements 1988). Given the nature of the sound-medium interaction, both structural and compositional changes can be detected. Ultrasound measurements have been applied to a range of physiochemical measurements, such as firmness (Mizrach 2004), mealiness (Bechar et al. 2005), and chilling injury (Verlinden et al. 2004). Mizrach (2008) reviews the application of ultrasonics in quality evaluation of fresh fruit and vegetables.

Despite the scientific progress made since the 1990s, ultrasonics largely remains a research tool not yet ripe for commercial application in pre- and postharvest quality

management. The main reason is the presence of voids and pores in plant-based food products that, by scattering, attenuate the sound when traveling through the plant tissue, thus complicating the interpretation of the data (<u>McClements 1997</u>). Also, because of the large mismatch in acoustic impedance between the air and ultrasound probe, either direct contact between the probe and the product or the use of a gel between both is required.

4. X-RAY RADIOGRAPHY AND TOMOGRAPHY

4.1. Principle

X-rays were discovered by W.C. Roentgen in 1895 and cover wavelengths between 10 and 0.01 nanometers, corresponding to a frequency of 3×10^{16} Hz to 3×10^{19} Hz and energies in the range 120 eV to 120 keV. Although X-rays interact with a material similarly to other types of electromagnetic radiation, most imaging applications are based on the absorption of the X-ray photons by the material, which depends on the local density, the atomic number, and the energy of the X-rays.

To produce X-rays, electrons are typically accelerated in a vacuum tube through a potential difference and directed onto a specific metal target. The electrons that hit the target release X-rays as they slow down (braking radiation or bremsstrahlung). The X-ray photons produced in this manner have a continuous energy spectrum from near zero up to the energy of the electrons. Additional photons at specific energy levels are emitted through X-ray fluorescence when orbital electrons are knocked out of the inner electron shell of the metal atom and electrons from higher energy levels fill up the vacant positions. After passing through the object, the X-rays enter crystal scintillators that convert them to flashes of light that are detected and processed electronically to produce an image (<u>Barrie Smith & Webb</u> 2010).

4.2. X-ray Radiography

In X-ray radiography, a single image of transmitted X-rays through an object is acquired. The resulting image is thus superimposed information or a projection of the 3D object volume in a 2D plane (Salvo et al. 2003). According to the Beer--Lambert law, the ratio of the number of transmitted to incident photons is proportional to the integral of the absorption coefficient of the object along the path that the photons follow through the sample. When the object contains a feature that is sufficiently large and has sufficiently different absorption properties than those of the surrounding material in the object, the element will become visible on radiographic images. Radiography equipment is available commercially for industrial use and is used mainly for the detection of foreign objects with high contrast in foods. Radiography has the advantage that it is fast and can be implemented inline on sorting lines (Jiang et al. 2008, Hansen et al. 2005, Kim & Schatzki 2001). The drawback is that the success of the method depends on sufficient contrast between the features to be detected in the scanned product and their environment. The detection resolution of conventional radiography equipment is also limited to approximately 1 mm.

X-ray radiography has been used to investigate internal disorders in fruit and vegetables (Haff & Toyofuku 2008). Using radiography methods, Hansen et al. (2005) successfully detected larval feeding damage caused by codling moths. Jiang et al. (2008) developed an inline X-ray scanner for automatically detecting infestation damage due to pests inside fruits. Kim & Schatzki (2000) and Shahin et al. (2001) developed a radiography-based algorithm for sorting water core--affected apples that was estimated to be fast enough for sorting lines, providing constraints on apple orientation with respect to the X-ray beam; the classification success rate was dependent on the severity of the disorder. A similar conclusion was found for detecting center rot in onions (Tollner et al. 2005). Radiographybased systems also were developed to scan quality features of nuts (Kim & Schatzki 2001). Recent advances in X-ray radiography methods are dedicated to improving image contrast (Nielsen et al. 2013, Haff 2008), more accurate and fast image segmentation methods (Mathanker et al. 2010), image texture analysis (Toyofuku & Schatzki 2007), and machine learning classification (Mathanker et al. 2011). Although most applications rely on the absorption contrast between defects and the product, in transmission radiography, newer developments such as phase contrast and dark-field imaging using grating interferometry have been proposed (Nielsen et al. 2013).

4.3. X-ray Computed Tomography

X-ray computed tomography (CT) uses a mathematical algorithm to compute a 3D image (tomogram) from multiple radiographs of the object taken from different angles (<u>Salvo et al.</u> <u>2003</u>). X-ray CT was developed in the late 1970s and enables the nondestructive visualization of the internal structure of objects. These first, mainly medical, CT scanners

had a pixel resolution in the order of 1 mm. In the 1980s, after some technological advances toward microfocus X-ray sources and high-resolution detection systems, it was possible to develop micro-CT systems that could achieve a pixel resolution in the micrometer range, which is 1,000 times better than that of the medical CT scanners. The advantage of medical CT scanners is that the source-detector assembly rotates at high speeds around the object, whereas the latter is translated through the gantry system. In this way, 3D images can be recorded within seconds. This comes, however, at a high construction cost to accurately control moving mechanical and electronic parts. Micro-CT systems, however, usually have fixed source-detector assemblies with a rotation stage on which the object can be mounted. This makes construction simpler and reduces the costs. Medical and micro-CT systems use microfocus X-ray sources that produce a polychromatic, divergent beam that puts constraints on the achievable image resolution, field of view, and image quality. Synchrotron radiation sources, however, can deliver a very high flux at small source size, resulting in high contrast and resolution. The parallel beam produced by synchrotron radiation with good spatial coherence makes a quantitative reconstruction, free of artifacts, possible. These conditions, however, can only be achieved at one of the few large-scale synchrotron facilities available.

Early CT research aspired to determine maturity or ripeness-related parameters of fruit (Brecht et al. 1991, Tollner et al. 1992), but a good correlation was found only between X-ray absorption and water content, or density as a derivation thereof. More successful applications of X-ray CT addressed the detection of internal defects. Lammertyn et al. (2003a,b) used X-ray CT to study the development of core breakdown disorder in pears and were able to visualize both tissue browning and cavity formation at better resolution but lower contrast with X-rays than with magnetic resonance imaging (MRI). X-ray CT also showed evidence of codling moth feeding tunnels in apples, as well as in cherries (Hansen et al. 2005). Donis-Gonzalez et al. (2012b) explored a medical CT as a first step to an inline sorting system for discriminating chestnuts with decayed tissue and voids. Further progress will be required in hardware, image reconstruction, and image processing algorithms to achieve sufficiently fast and affordable inline CT systems for product quality evaluation (Donis-Gonzalez et al. 2012a).

Micro-CT to visualize the 3D microstructure of fruits and vegetables was attempted in the early 2000s but suffered from low contrast and limited resolution (Kuroki et al. 2004; <u>Mendoza et al. 2007, 2010</u>). A breakthrough in 3D tissue visualization was achieved using synchrotron X-ray CT in phase contrast mode (Verboven et al. 2008). Together with the latest developments in microfocus X-ray systems to improve resolution and absorption contrast, this has triggered a more widespread use of micro-CT for microstructure analysis in relation to understanding the internal quality of fruit and vegetables (Verboven et al. 2013, Herremans et al. 2013, Musse et al. 2010) and modeling for optimization of postharvest storage and processing (Abera et al. 2013, Datta et al. 2012, Ho et al. 2011). Micro-CT is typically done on excised samples, and, although live tissue can be imaged, it does not qualify as a nondestructive technique. However, new large format detectors with up to $14,450 \times 14,450$ pixels enable image acquisition with small intact fruit within the micrometer resolution range.

5. MAGNETIC RESONANCE IMAGING

MRI is a nondestructive, nonintrusive spectroscopic technique based on the interaction of electromagnetic radiation in the radiofrequency range with matter. Often protons (¹H nuclei) are targeted, such as those present in the water of fruit or vegetables, where the spins of the protons within the material are aligned by applying briefly a strong magnetic field. By monitoring the proton dynamics afterward, information on the spatial distribution of proton density, relaxation parameters (T_1 and T_2 values), and self-diffusion parameters inside the sample can be obtained. The relaxation and self-diffusional properties often provide complementary information and enhanced contrast and are linked to proton mobility (<u>Barrie Smith & Webb 2010</u>).

MRI is particularly suitable for biological materials (given that protons are abundant therein), mainly in water but also in fat, oil, or salt, and it allows one to distinguish these components. Furthermore, MRI is sensitive to several quality parameters affecting the produce, particularly those that affect the water concentration or mobility (e.g., internal browning). Its nondestructive character makes it particularly attractive for scanning intact fruit and vegetables but also for monitoring their quality over time, for example, during storage. For these purposes, the spatial resolution is sufficient (typical slice thickness ≈ 100 -

-1,000 μ m, resolution in 2D slice \approx 10--50 μ m; see, e.g., Clark et al. 1997, Defraeye et al. 2013). The image acquisition speed is, however, relatively low, compared to other techniques such as X-ray imaging, and is strongly determined by the required image quality and whether 1D or 2D imaging is performed. The sensitivity to water is high in MRI (dynamic range), but it is used predominantly to investigate relative differences or changes in water content instead of quantification, given that the latter is not straightforward (Mariette et al. 2012, Van As & van Duynhoven 2013), due to temperature changes and other components containing protons. MRI has been used successfully in the past to measure several quality parameters of fresh fruit and vegetables, including the presence of internal defects, such as voids/cavities (Saito et al. 1996), worm damage, pits (Chen et al. 1989), bruising (McCarthy et al. 1995, Zion et al. 1995), water core disorder in apples (Wang et al. 1988, Cho et al. 2008), internal browning in apples (Clark & Burmeister 1999, Gonzalez et al. 2001, Chayaprasert & Stroshine 2005, Cho et al. 2008, Defraeye et al. 2013), mealiness of apples or peaches (Barreiro et al. 2000, Marigheto et al. 2008), core breakdown in pears (Wang & Wang 1989; Lammertyn et al. 2003a,b), and chilling injury in citrus fruit or zucchini squash (Wang & Wang 1992, Hernández-Sánchez et al. 2004).

MRI has also been used to measure physical properties, such as size, shape and volume, and has been correlated with firmness, soluble solids, or acid content (Abbott et al. 1997, Clark et al. 1997, Létal et al. 2003). A distinction between ripe and immature fruit and vegetables could be made, for example, by measuring the free water content in tomato or pineapple (Hall et al. 1998, Chen et al. 1989) or the oil content in avocado (Chen et al. 1989). Pathogen infection has also been successfully detected (Hall et al. 1998). In Figure 2, CT and MRI images of a healthy apple and one with severe water core symptoms---a disorder characterized by water-soaked regions in the fruit---are compared. The affected regions are clearly visible in both images, although the imaging principle is very different: In CT, the contrast between healthy and affected tissue is due to the increased density in the latter due to the water soaking; in MRI, the affected area lights up because the water mobility is very different from that of healthy tissue.

<COMP: PLEASE INSERT FIGURE 2 HERE>

Figure 2. X-ray CT (left column) and MRI (right column) images of Ascara apples without (a,b) and with (c,d) water core. Both techniques are capable of detecting the water core region inside the fruit; however, the contrast in the MRI images is better due to the particular pulse sequence

used (<u>Herremans et al. *submitted*</u>). Abbreviations: CT, computed tomography; MRI, magnetic resonance imaging.

MRI shows large potential for online grading, sorting, or quality evaluation of fresh produce (Ruiz-Altisent et al. 2010). Due to the high volume and low value of fruit and vegetables, and the high cost of MRI equipment and its relatively low acquisition speed, industrial implementations of MRI were nonexistent until very recently (Ruiz-Altisent et al. 2010, McCarthy & McCarthy 2013). Research efforts are being directed toward developing cost-effective but fast equipment that can achieve realistic throughputs. In particular, the focus is on hardware, namely the use of cheaper, faster, low-field, wide-bore MRI scanners (Chayaprasert & Stroshine 2005, Hernández-Sánchez et al. 2007, Milczarek & McCarthy 2009, Van As & van Duynhoven 2013) and smaller, mobile (1D or 2D) systems (Danieli et al. 2009, 2010), but also on faster pulse sequences (e.g., gradient echo method; see Abbott et al. 1997). These developments improve the potential for MRI to become a cost-effective tool for quality evaluation of fresh produce.

6. MASS SPECTROMETRY

6.1. Gas Chromatography-Mass Spectrometry

A common analytical technique in flavor research is conventional gas chromatographymass spectrometry (GC-MS). This technique is often combined with headspace solid-phase microextraction (HS SPME), a rapid, simple, and inexpensive extraction and concentration technique for volatile compounds (<u>Kataoka et al. 2000</u>). GC-MS devices are commonly equipped with low-cost single quadrupole mass analyzers that can be operated in full scan mode (FSM) or selected ion monitoring (SIM) mode (<u>Biniecka & Caroli 2011</u>). In fruit flavor research, FSM is most often used, given that it provides information on the full aroma profile. SIM mode is typically applied for trace analysis or when the researcher is interested in the quantification of only a few volatile compounds (e.g., off-flavor, contaminants) (<u>Koch et al. 2010</u>). A mass analyzer gaining particular interest in flavor research is the timeof-flight (ToF) mass analyzer (<u>Biniecka & Caroli 2011</u>). The main advantages of ToF instruments over quadrupole instruments are the higher sensitivity in FSM and mass spectral continuity. Hence, more complex volatile mixtures can be analyzed because deconvolution of coeluting peaks is more straightforward (<u>Glinski & Weckwerth 2006</u>, <u>Biniecka & Caroli 2011</u>). Furthermore, because of the high scanning rate of ToF instruments (500 scans s⁻¹ versus 50 scans s⁻¹), these mass analyzers are often combined with comprehensive 2D gas chromatography (GCxGC). In GCxGC, two GC columns with different stationary phases are connected in series through special interfaces. Coeluting peaks in the first dimension undergo additional separation on the second column (<u>Dallüge et al. 2003</u>). Because the second column is typically a short narrow-bore capillary column, a fast scanning mass analyzer is needed to obtain sufficient data about the eluting peaks. GC-ToF-MS and GCxGC-ToF-MS have, for instance, been applied to analyze the aroma of apples and grapes (<u>Rocha et al. 2007</u>, <u>Aprea et al. 2011</u>).

Although used extensively with fruit and food in general, the total runtime of conventional GC-MS methods might be long. Therefore, the application is not feasible for high-throughput purposes. Although the theoretical background for fast GC-MS was already established in the 1960s, the lack of adequate instrumentation to meet fast GC-MS requirements hindered routine application until recently (Mondello et al. 2004, Korytar et al. 2002). The primary aim of fast GC-MS is to maintain sufficient resolving power during a shorter analysis time than that for conventional GC-MS. This can be achieved by manipulating numerous analysis parameters, such as column length, column internal diameter, stationary phase, film thickness, carrier gas, linear velocity, oven temperature, and temperature ramp rate (Korytar et al. 2002, Vandendriessche et al. 2013). The advantages of fast GC-MS over conventional GC-MS in fruit flavor research have been shown, for instance, for strawberry (Chen et al. 2007, Vandendriessche et al. 2013).

A typical fruit or vegetable headspace may contain as much as 100 or more individual volatile components. To relate the headspace composition to the aroma as perceived by a sensory panel, advanced chemometric techniques such as principal component regression (PCR) or partial least squares (PLS) are necessary (see below). This is somewhat similar to how the human brain processes and integrates signals from individual olfactory neurons. The analysis is complicated by the fact that the human olfactory system is preferentially selective with respect to certain high impact components. For example, β -ionone imparts a characteristic aroma to tomatoes even at very low concentrations. Additionally, sulfides, typically present in the headspace of *Brassica* species, have very low aroma thresholds.

6.2. Advanced Mass Spectrometry Techniques

Although high-throughput flavor analysis can be achieved with HS-SPME-fast-GC-MS, the necessary preconcentration of the aroma compounds and chromatographic separation make this technique still too time-consuming To reduce the analysis time, several direct inlet MS techniques have been developed.

In headspace fingerprint mass spectrometry (HFMS), the headspace of a sample is injected into the ionization chamber of a mass spectrometer without prior chromatographic separation (Saevels et al. 2004). This is typically implemented by means of a short capillary column that is operated at an elevated temperature so that a broad, featureless peak is obtained. The spectrum resulting from simultaneous ionization and fragmentation of the mixture of molecules introduced constitutes a "fingerprint" of the actual aroma. These fingerprints can then be used in combination with advanced chemometric techniques to discriminate samples. Saevels et al. (2004) used this technique to measure ripeness of apple fruit. HFMS was also used to fingerprint the aroma profile of tomato cultivars (Berna et al. 2004) and the evolution of aroma production in strawberries during superatmospheric oxygen storage (Berna et al. 2007).

Taylor et al. (2000) coupled a modified atmospheric pressure chemical ionization (APCI) source to a mass analyzer for measuring in vivo aroma release from food. The device consists of a sample inlet and an ionization source typically containing a corona discharge. During ionization, first initial reactant ions (H₃O⁺) are formed. In a second phase, the volatile compounds are ionized by the transfer of protons from the reactant ions (Jublot et al. 2005). One of the advantages of APCI-MS as a technique for aroma analysis is that it can cope with water and air, allowing fruit headspaces to be directly introduced in the ionization source. In addition, because it is a soft ionization technique, the main and usually only ion formed is the protonated intact molecule. As such, volatile mixtures can be resolved entirely by mass, and a temporal separation by a GC column is unnecessary (Jublot et al. 2005, Taylor et al. 2001, Boukobza & Taylor 2002) as well as to analyze strawberry, red bell pepper, cucumber, kiwi, and lettuce volatiles (Friel et al. 2007, Surawang et al. 2005, van Ruth et al. 2003, Garratt et al. 2005, Watson et al. 2002). Proton transfer reaction-mass spectrometry (PTR-MS) is a related method and has been used to evaluate the volatile

compounds of a wide variety of fruit and vegetables (<u>Biasioli et al. 2003</u>, <u>Lokke et al. 2012</u>, <u>van Ruth et al. 2003</u>, <u>Farneti et al. 2012</u>, <u>Cappellin et al. 2012</u>).

Selected ion flow tube-mass spectrometry (SIFT-MS) is based on the chemical ionization of the aroma compounds by the initial reactant ions H_3O^+ , NO^+ , and O_2^+ coupled with fast flow-tube technology and mass spectrometry. The power behind SIFT-MS over other chemical ionization techniques such as APCI-MS and PTR-MS is the use of three reactant ions. In this way, more information about the analyte molecules is provided, so that identification is more straightforward, and isobaric compounds can be easily distinguished from each other (Spanel & Smith 1999). Absolute concentrations of the aroma compounds can be calculated in real time without the need for calibration and standards. Concentrations are determined using ion-molecule reaction rate coefficients, flow-tube geometry, ionic reaction time, measured flow rates, and pressure (Spanel & Smith 1999, Smith & Spanel 2011). SIFT-MS is typically used for targeted analysis, because previous identification of the aroma compounds in the sample and knowledge about their reaction with the reactant ions is necessary (Azcarate & Barringer 2010). SIFT-MS has been successfully used to monitor volatiles in peppers (Azcarate & Barringer 2010, Wampler & Barringer 2012), tomato (Xu & Barringer 2009), tomatillo (Xu & Barringer 2010), and strawberry (Ozcan & Barringer 2011).

In addition to the above-mentioned, most popular direct inlet MS techniques, desorption electrospray ionization (Joyce et al. 2013), extractive electrospray ionization (Gu et al. 2012, Chen et al. 2007), and direct analysis in real time (Li 2012) have been used for the analysis of volatile compounds in fruit. In addition, multicapillary column chromatography, coupled with ion mobility chromatography, has as well been proven to be a very good technique for aroma fingerprinting (Vandendriessche et al. 2012).

7. GAS SENSORS AND ELECTRONIC NOSES

An electronic nose is a biomimetic instrument that aims to perceive an aroma in a similar way as the human olfactory system. It comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern-recognition system (Gardner & Bartlett 1994). The volatile components interact with the gas sensors and produce a physical response that is transduced into an electrical signal for further processing. Similar to human

olfactory receptors, the sensors are semiselective. This feature is essential, as it involves combining several sensors to discriminate more aromas than the number of sensors used. The ideal sensors for integration in an electronic nose meet the following criteria: They must provide high sensitivity toward chemical compounds (similar to that of the human nose (down to 10^{-12} g/ml), low sensitivity toward humidity and temperature, medium selectivity, high stability, high reproducibility and repeatability, short reaction and recovery times, easy calibration, and small dimensions; must respond to different compounds present in the headspace of the sample; must be robust and durable; and must make it easy to process data output(Nicolaï et al. 2008a. Different sensor principles have been used in electronic noses, including metal-oxide semiconductors, metal-oxide semiconductor field-effect transistors, conducting organic polymers, quartz microbalances, surface acoustic wave sensors, and colored dyes (Nicolaï et al. 2008a, Berna, 2010). Immobilized olfactory neurons of rodents have been explored recently as bases for a bioelectronic nose (Micholt et al. 2013). Signal drift remains an important problem in all sensor types.

Electronic noses have been successful in monitoring the aroma of melons (Benady et al. 1995), pears (Oshita et al. 2000), nectarines (Di Natale et al. 2001), apples (Saevels et al. 2003, 2004), tomatoes (Berna et al. 2004), mangos (Li et al. 2009), and citrus (Pallottino et al. 2012). Most applications aim to discriminate cultivars or ripeness stages, or to detect fungal infection. The New Zealand company ripeSense (http://www.ripesense.com) developed a disposable ripeness sensor. The sensor is initially red and graduates to orange and finally yellow as a response to volatiles generated during ripening. It can be integrated into a package and gives the consumer an idea of the ripeness of the fruit. Similar sensors are likely to emerge within the next couple of years.

8. CHEMOMETRICS

The signals generated by many of the aforementioned techniques are multivariate and consist of an array of many highly correlated variables. Appropriate statistical techniques are required to process such signals. This is often called chemometrics.

The original variables are typically projected onto a lower number of uncorrelated latent variables. The most popular method for performing this dimensionality reduction is principal component analysis (PCA). When the aim is to predict the concentration of a component of interest, the regression is performed with the latent variables instead of the original variables, to avoid problems in the estimation of the regression coefficient. In principal component regression (PCR), a principal component analysis is performed, and only the most important principal components are used in the regression to predict the component of interest. Although this is a quite straightforward approach, it has been shown that the definition of the principal components aiming to explain maximal variance in the spectral data, while neglecting the covariance with the component of interest, is suboptimal. Therefore, partial least squares (PLS) regression, which defines the latent variables based on the covariance between the spectral data and the component of interest, has become by far the most popular regression method, especially in NIR spectroscopy (Næs et al. 2004). When the aim is not to predict concentrations, but rather to separate the samples into different classes, multivariate discrimination methods such as the PCA-based soft independent modeling of class analogies and the partial least squares discriminant analysis are most commonly used.

9. CONCLUSIONS

Nondestructive techniques are now available for many quality attributes. Techniques for external attributes such as color, size, and absence of external defects are now widely used on commercial sorting lines. Although several techniques are available for internal quality attributes, their success depends critically on how closely they mimic the perceptions of humans. This is an issue particularly for quality attributes such as texture and flavor that are typically perceived by humans in a destructive way. For example, the firmness of a fruit is assessed while chewing by biomechanical sensors in the jaw; sweetness is perceived through sugar receptors in the taste buds on the tongue; the retronasal aroma perception is due to the interaction of olfactory neurons in the nose cavity with volatiles that are liberated through chewing.

Nondestructive measurement of quality attributes makes sense only when the resulting information is used for improving quality. This implies not only improving postharvest handling and storage processes but also major changes in our current quality systems and commercialization models, which are almost exclusively based on external quality

attributes. This will require radical changes in the ways in which fruit and vegetables are commercialized.

SUMMARY POINTS

- 1. The success of techniques for measuring the quality attributes of fruit and vegetables often depends critically on how their measurement principle mimics the way humans assess a particular property.
- 2. Nondestructive techniques for fruit quality attributes allow grading individual fruit and vegetables prior to commercialization.
- Nondestructive techniques are best for modeling changes of quality attributes during postharvest storage of shelf life for optimizing postharvest processes as they allow removing inter fruit variability.

4. Many nondestructive techniques measure a complex signal that needs to be related to the quality attribute of interest via chemometrics techniques.

5. Techniques for external attributes such as color, size, and absence of external defects are used widely on commercial sorting lines for fruit and vegetables. Nondestructive sensors that allow grading based on sugar content, dry matter, and firmness are now also available commercially.

6. Grading of fruit and vegetables based on quality attributes requires major changes in the current quality systems and commercialization models that are now almost exclusively based on external quality attributes.

FUTURE ISSUES

- 1. Will time- and space-resolved spectroscopy enable more accurate measurements of quality attributes of fruit and vegetables than NIR spectroscopy?
- 2. What is the relation between mechanical properties measured through impact or vibration analysis and attributes measured by destructive instrumental methods or sensory panels?
- Is it possible to improve hardware and software so that real-time X-ray tomography and MRI at commercial grading line speeds become available for reasonable costs?
- 4. Is it possible to reduce drift and improve reproducibility of electronic noses based on improved sensor designs?

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KEY TERMS AND/OR ACRONYMS

APCI: atmospheric pressure chemical ionization CT: computed tomography FSM: full scan mode GC-MS: gas chromatography-mass spectrometry HFMS: headspace fingerprint mass spectrometry MRI: magnetic resonance imaging NIR: near infrared PCA: principal component analysis PCR: principal component regression PLS: partial least squares RGB: red, green, blue SRS: space-resolved spectroscopy ToF: time of flight TRS: time-resolved spectroscopy Vis: visible