Methods for Quality Assessment of Citrus Fruit

Subjects: Food Science & Technology

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Citrus spp. are spread mainly in the Mediterranean basin and represent the largest fruit source for human consumption. Postharvest losses, mainly due to diseases and metabolic disorders of fruits, can cause severe wastage, reaching 30 to 50% of the total production. Preserving quality and extending shelf life are essential objectives for postharvest technological innovation, determined by the proper handling, treatment, storage and transport of harvested produce. Moreover, the application of novel sustainable strategies is critical for the reduction of synthetic fungicide residues on fruit surfaces and the impact on the environment caused by waste disposal of fungicides. In modern citrus packinghouses, advanced systems for sorting and measuring the external and internal quality of large amounts of citrus fruits rely on automated and reliable inspection systems based on computer vision techniques, equipped with electronic sorting devices capable of examining fruit images at a very high speed and measuring external properties, such as colour, size and the presence of damage or defects.

Keywords: sustainable strategies ; innovative technologies ; fruit quality ; shelf life ; postharvest ; modified atmosphere packaging (MAP) ; cold storage room ; precooling

1. Visible and Near-Infrared Reflectance Spectroscopy (Vis/NIR)

Aside from the investment costs for the equipment, Vis/NIR analyses do not require trained staff, chemicals, or additional materials, allowing the definition of these techniques as sustainable or “non-polluting”. The ease of management and the high accuracy of the tools composing the Vis/NIR equipment (e.g., optical fibers) make this technique suitable for constructing at-line, online and inline sensors in production plants to control industrial processes and food quality. However, the accurate prediction of an unknown measure depends on the calibration model used. If an algorithm does not describe with very high accuracy the correlation occurring between the wavelengths and the independent variable, it cannot be exploited on a further batch. For example, Matera et al. [1] tested the accuracy of Vis/NIR techniques in detecting the Imazalil content in water solution. Imazalil is a fungicide used to control postharvest losses due to *Penicillium* spp. in citrus by dipping the fruits in an aqueous solution containing Imazalil. Its concentration, related to the amount of fruit treated throughout the day, can be dramatically reduced. Therefore, the efficacy of a spectroscopy-based control system was evaluated to monitor fungicide concentration online during treatment. The results showed that not only was a different accuracy level obtained when using Vis rather than NIR (highest accuracy with NIR) but also that data pretreatment (such as normalization, first or second derivative degree) hugely affected the prediction capabilities of the algorithms (PCR, PLS, SVM, ENSEMBLE).

Vis/NIR techniques applied to fruits have some drawbacks [2]. When a light beam hits a fruit or any other biological sample, the incident radiation may be specularly reflected, absorbed or transmitted. The relative contribution of each phenomenon depends on the item’s light-scattering properties related to the sample’s microstructure and chemical composition [3].

In thin-skinned fruits, most of the light beam interactions occur on the flesh, and the skin has mainly a modulation effect upon the spectra. In contrast, in citrus fruits, the interactions occur between flavedo and albedo, and few photons probe the flesh. Considering this, amongst the Vis/NIR spectra-acquiring methods (transmittance and reflectance), in citrus, the reflectance mode is easier to handle, with higher signal levels than the transmittance mode [4]. However, the assessment of quality indexes in citrus fruits through Vis/NIR spectroscopy depends on the interplay between pulp and skin biochemistry and their optical properties, and, to date, the efforts to correlate optical properties and quality indexes have been evident only for titratable acidity, total solid soluble content, pH and firmness [5][6][7].

2. Hyperspectral Imaging Analysis

Hyperspectral imaging analysis (HSI) is a non-destructive optical analysis technique. However, unlike other optical technologies that can only scan for a single colour, HSI can distinguish the entire colour spectrum in each pixel, simultaneously considering the spectral and spatial information of samples. In HSI, each image pixel contains spectral information, which is added as a third dimension of values to the two-dimensional spatial image, generating a three-dimensional data cube, sometimes referred to as a data hypercube or an image cube [8]. Amongst spectral imaging, hyperspectral imaging has been widely used to estimate food quality in terms of surface damage (bruises, chilling and insect bites), surface quality (firmness, moisture content, hardness, rottenness and fungal presence) and assessment of some biochemical components in vegetable products [9].

HIS has also been exploited for citrus fruits analysis, with successful results in the detection of external defects in oranges, such as insect damage, wind scarring, thrips scarring, scale infestation, canker spot, copper burn, heterochromatic stripe, phytotoxicity and stem-end rot [10]; citrus black spot in 'Valencias' oranges [11]; pectin content in 'Lanelate' oranges [12]; and solid soluble content [13] and pesticide residue [14] in 'Navel' oranges. As for Vis/NIR analysis, data pretreatment prior to calibration is also required for HIS to leverage the data set.

The direct model calibration building suffers from some drawbacks, as some peaks or wavelengths in the spectra are convoluted due to both the chemical complexities of foodstuffs and errors in the measurements (light scattering, temperature drift, electrical noise).

Some preprocessing techniques are used to remove irrelevant information that can degrade the performance of the numerical algorithm used to develop the calibration model.

These include MSC (Multiplicative Scattering Correction), SNV (Standard Normal Variate), first and second derivatives filters (Savitzky–Golay), de-trending, scaling, and normalization [15]. Several attempts using HIS have been successfully carried out to discriminate between 11 types of pathological decay in citrus, corresponding to a classification success rate of around 89% for detecting rottenness [16]. The findings carried out by the investigation mentioned above suggest that imaging technology coupled with multi-sensors (temperature, humidity, CO_2/O_2) [17] can be an effective tool for the effective control of safety and quality across the logistic of citrus fruits.

3. Raman Spectroscopy

Raman spectroscopy is a non-invasive optical technique that is easy to conduct and only requires a very compact set-up, which makes it portable. Raman spectroscopy measures inelastic light scattering based on a monochromatic source, providing information on the chemical composition by recording the molecular vibrations of the constituent components (spectral footprint). It can detect subtle molecular and biochemical changes in tissues [18] and can be conducted on living organisms, allowing the characterization of the chemical structure of tissues and distinguishment between normal and diseased tissues [19][20][21]. Liu et al. [22], using this technique coupled with partial least squares discrimination analysis (PLS-DA), reached a correct recognition rate of 100% in detecting citrus greening bacterial disease (HLB), the most severe citrus disease caused by phloem-limiting bacteria.

4. Nuclear Magnetic Resonance

One of the most critical features of NMR is measuring water content and distribution, which is very useful for assessing ripeness, defects or decay in fruits and vegetables. High-resolution images can contribute to the evaluation of maturity and quality parameters. However, they can also help the understanding of physiological processes, such as water transport and the presence of water-soluble metabolites [23].

NMR can be applied to a wide range of liquid and solid matrices without altering a sample or producing hazardous wastes.

Chen et al. [24] showed the reliability of NMR imaging in detecting textural changes under various conditions, such as dry regions in 'Washington' oranges and 'Valencia' oranges; bruising in apples, peaches, Asian pears and onions; worm damage and stage of maturity in Asian pears, red/green tomatoes and pineapples; the presence of void spaces in potatoes, cucumbers and tomatoes; and the presence of seeds and pits in olives and prunes. Butz et al. [25] also reviewed the use of NMR to measure water states and water-related properties of fruits, including apples, pears, peaches, kiwi fruits and oranges. Therefore, there is ample evidence of the applications of NMR for assessing or inspecting quality parameters of a variety of fruits, such as ripeness, defects and decay, as well as differentiating between unaffected tissue, brown tissue and cavities during various conditions, such as postharvest, storage and transportation.

In addition to its ability to assess the physical properties of certain foods, NMR can also be used to investigate chemical attributes related to volatile organic compounds (VOCs) [26].

NMR spectroscopy combined with statistical analysis techniques is one of the promising tools for food quality control. One such combination is Spin Generated Fingerprint Profiling (SGFP) with NMR, which fully automatically performs sample transfer, measurement, data analysis and reporting for quality control of fruit juices [27]. Having established a spectral database, NMR enabled the simultaneous identification and qualification of 28 different compounds in a mixture. It also allowed the detection of fraudulent addition of sugar, citric acid, lemon juice and galacturonic acid (an indicator of an exhaustive enzymatic treatment). SGFP represents a heterogeneous collection of statistical models which can be applied consecutively to one single spectrum, such as specific models for multi-fruit type separation, fruit type differentiation between citrus varieties (e.g., citrus sinensis and citrus reticulata), differentiation of product categories (e.g., orange juice and orange juice made from concentrate) or the characterization of compositional differences between two groups of similar products [28].

5. Nanosensors for Early Detection

By deploying the above-mentioned spectroscopy- and image-based analytical techniques (NIR/VIS, HIS, Raman and NMR) coupled with proper image processing techniques, it has been possible to correlate optical properties with several parameters to detect or predict the behaviour of many quality parameters, mainly chemical and physicochemical, and, in some works, the extent, at an early stage, of pathological decay in oranges [29][30][31][32][33] or freezing damage in sweet lemons [34] and oranges [35][36].

Early detection of a pathological condition has many advantages, such as early warning for planning actions to mitigate colonization and thus postharvest waste and loss of crops.

In the last few decades, many advances have been made in relation to quickly and accurately detecting hidden fungal colonization in *Citrus*, in the field, during handling or storage early on. These advances encompass the development of nanosensors for the identification of fungal attack before the appearance of visible signs of infection on citrus surfaces using an array composed of electrochemical or biological receptors.

Various nanosensors are being developed to meet the different requirements in food quality inspection and food processing control. The most common nanosensors for food and agriculture applications include optical sensors, electrochemical nanosensors, e-noses, e-tongues and biosensors [37].

For instance, a range of VOCs accompany upcoming pathological decay in citrus fruit, such as limonene, b-myrcene, a-pinene, sabinene, acetaldehyde, ethanol, ethylene, and CO_2, which can be detected to follow the extent of the decay [38].

VOCs were easily distinguished using an electrical sensor array for early-stage on-farm detection of HLB [39].

Limonin is a VOC responsible for the bitter taste of citrus fruits, such as oranges, grapefruits, etc. An abnormally high level of limonin indicates HLB, which results in stunted tree growth and affects fruit quality in terms of nutritional value, taste, texture and aroma. Ceria nanoparticles are microsensors based on the quantification of limonin to detect HLB using an organic electrochemical transistor platform [40]. As it is challenging to identify HLB-infected trees because they may remain asymptomatic for months to years after infection, the finding of this research is particularly interesting as the sensor can be exploited to correlate VOCs with the decay index at harvest, which is relevant to deciding postharvest destinations and treatments of fruits.

The electronic nose (e-nose) relies on a portable device equipped mainly with broad-spectrum chemical sensors. With the support of a pump, the sample’s headspace is passed over the e-nose’s detector, which generates signals acquired by a computer and processed by pattern-recognition algorithms, providing a fingerprint of the volatiles present in the analyzed sample [41].

Research on citrus fruits using e-nose technology has been concerned with the identification and characterization of different citrus cultivars and varieties, postharvest quality monitoring, disease identification through the detection of specific volatile biomarkers in the early stages of deterioration and metabolic changes as a result of fruit respiration, transpiration and/or fermentation during storage [42][43][44].

The aromatic variation of oranges was successfully studied for one month using e-nose, principal component analysis (PCA) and partial least squares discriminant data analysis (PLS-DA). The research produced evidence of the excellent sensitivity and resolution of e-nose sensors in measuring aromas of decay in oranges and predicting storage days correctly [45].

However, although postharvest fungal disease detection in citrus fruits under cold storage conditions is crucial, few works have been carried out at present. For instance, the e-nose may be successfully applied as a reliable, non-destructive technology to identify *P. digitatum* in ‘Valencia’ oranges during cold storage [42][46] and to discriminate between uncontaminated lemons and lemons contaminated with *P. digitatum* spores [47].

The electronic tongue (e-tongue) is a multisensory device for the rapid qualitative assessment of different liquid food products based on chemical sensor arrays, a signal trapping device and software which converts the signals into appropriate results. Among its various uses, e-tongue has excellent potential for studying the shelf life of different products. A study carried out on Satsuma mandarins [48] showed that the e-nose and e-tongue systems, combined with some algorithms, could provide a fast and objective detection system to trace fruit quality and, in particular, to discriminate different ripening stages and trace internal quality changes (i.e., ascorbic acid, soluble solids content, total acid and sugar/acid ratio). Raithore et al. [49] successfully used an e-tongue to differentiate between orange juice made from healthy fruit and fruit affected by HLB disease. Orange juice made from the fruit over the harvest season and from fruit harvested from healthy or HLB-affected trees were separated by harvest maturity, disease state and disease severity using an e-tongue system.

Biosensors rely on a biologically active element, such as an antibody, enzyme, oligonucleotide or receptor blocked on a specific array, which is highly selective due to the possibility of tailoring the specific interaction with the analyte. The interaction analyte bioelement produces a biosignal (optical, electrochemical or colorimetric) that is converted into a measurable signal by a transducer. With respect to biosensors, extremely interesting is the capability to detect early on fungal mycotoxin presence, which is potentially harmful to public health [50]. However, little evidence of biosensors’ exploitation for the early detection of pathological or physiological alterations in citrus fruits has been reported in the literature, owing to different effectiveness and sensitivity results.

Electrical bioimpedance spectroscopy measurements of the flavedo, albedo and pulp in ‘Star Ruby’ grapefruit were carried out for early freeze-damage detection in grapefruit, showing a Correct Correlation Rate of 100% [51]. Chalupowicz et al. [52] used a bacterial cells-based biosensor to follow up *P. digitatum* colonization in Valencia. That approach was based on bacterial luminescent responses to changes in volatile organic compounds (VOCs) following infection, enabling fungal infection detection on the third day of infection, before the appearance of visible signs.

Wang et al. [39] reported the application of electrical biosensor arrays based on single-walled carbon nanotubes (SWNTs) decorated with single-stranded DNA (ssDNA) for the detection of four VOCs—ethylhexanol, linalool, tetradecene and phenylacetaldehyde—that serve as secondary biomarkers for the detection of infected citrus trees during the asymptomatic stage of HLB.

Biosensors are particularly useful in the citrus industry and are expected to address the severe and urgent needs encountered there by providing fast, simple and cost-effective methods for detecting pathological diseases, especially those caused by unculturable microorganisms.

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