EMERGING TECHNOLOGIES FOR NON-DESTRUCTIVE QUALITY EVALUATION OF FRUIT

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A B S T R A C T

The development of science and technology, and especially of information technology, has made available many non-destructive methods for analysis of materials, which can also be applied to fruits. The fruit industry needs non-destructive techniques for on-line sorting and certifying high quality fruit. Electronic noses, acoustic methods and optical methods like near infrared spectroscopy are mentioned, and their applicability for fruit analysis is discussed. The principles and the application of time-resolved reflectance spectroscopy (TRS) are more deeply described. TRS can measure separately and independently the optical properties (absorption coefficient μ_a and transport scattering coefficient μ_s') at selected wavelengths in highly diffusive media. Absorption coefficient is related to chemical compounds, like pigments, while scattering coefficient is related to tissue structure and to air and water distribution. TRS has recently gained increasing use in biomedicine for the non-invasive investigation of biological tissues. It has also been used for optical characterization of fruit. TRS measurement is not influenced by color or other surface characteristics, because it probes a volume at a depth of 1-2 cm. Recent experiences of application with fruit are reported. In pears it was possible to detect brown heart (by μ_a at 720 nm) and mechanical injury (by μ_s' at 720 nm), which were not visible externally. In apples, the scattering coefficient was related to pectin composition and to firmness after storage. In nectarines, by using μ_a at 670 nm, high and low flavour fruit were selected and the softening during shelf life was predicted. The absorption at about 670 nm, near the chlorophyll peak, is also useful to evaluate maturity in apples and pears.

Key words: time-resolved reflectance spectroscopy, electronic nose, Near Infrared spectroscopy, maturity, firmness, soluble solids, quality

INTRODUCTION

The development of science and technology, and especially the development of information technology, has made available many non-destructive methods for analysis of materials, which can also be applied to fruits. The fruit industry needs non-destructive techniques for on-line sorting and certifying high quality fruit. But there is a gap between what industry wants to measure, and what is actually measurable by these non-destructive methods. Industry wants to measure and certify fruit quality. In general, quality means meeting the expectations of consumers regarding safety, storability and sensory properties of products. Sensory properties are appearance (color, shape, and defects), texture (crispness, juiciness, firmness, toughness, mealiness, etc.) and flavour (taste and aroma). Sensory quality can be measured, as such, only with sensory tests. Otherwise other properties which give rise to sensory stimuli can be measured, such as sugar content for sweetness.

Methods to measure fruit quality can be destructive or non-destructive. Sensory analysis for texture and flavour is intrinsically destructive. Among instrumental measurements, all the most used and well-known conventional measurement methods, such as firmness measurement by penetrometer test, the analysis of soluble solids by refractometer, and chemical methods are destructive. Only the measurement of epidermal color by a colorimeter, and the evaluation of appearance by means of image analysis are non-destructive.

With destructive methods, a sample of fruit must be measured in order to estimate the quality of a batch: besides the economical loss, due to fruit destruction, there is also the problem of how the sample is representative of the whole batch.

Non-destructive methods overcome these problems, as they can be applied for fruit by fruit selection and grading, so overcoming possible discrepancies between different batches and samples of fruit, without destroying a certain amount of sample fruit.

Non-destructive methods can be classified according to the principle used to detect fruit properties: mechanical, acoustic, optical, other. A review of some recent developments in non-destructive methods can be found in Nicolaï et al. (2005).

The ‘Black Box’ approach vs measurement of fundamental properties

Many non-destructive methods follow a “black box” approach to estimate the fruit characteristics. First a set of samples is measured both non-destructively and with known, conventional methods. Then, data are processed by some of the many methods offered by statistics and information technology, in order to find a relationship between the conventional measure and the multiple response of the non destructive method. Afterwards, the relationship found is applied to predict the value of the characteristic in new sets of samples. The electronic noses and tongues and the widespread Near InfraRed Spectroscopy (NIRS) methods are of the “black box” type. Other techniques, such as some of the mechanical methods, or optical methods such as time-resolved spectroscopy, allow to measure fundamental, well defined properties of fruit

Mechanical non-destructive methods

Mechanical non-destructive methods aim at measuring texture characteristics, mainly firmness. They have been reviewed by Shmulevich (2004). These methods use low mass impact tests: impact parameters are detected by accelerometers, or resonance frequency is detected by a microphone. The resonance frequency generally changes with ripening, and measures a property of the whole fruit. The results of impact tests are very sensitive to variations in fruit location and impact angle, and to deviations of fruit from spherical shape. Moreover the results are very much affected by water content. While the aim is to replace the conventional Magness-Taylor pressure test by a non-destructive test, it is obvious that the two types of test measure different properties. In fact all non-destructive mechanical methods measure stiffness and elastic properties which are more related to turgor pressure and water loss, than to mechanical strength of cell wall and middle lamella, as in the case of penetrometer (Hertog et al., 2004).

Gas sensors arrays: electronic noses

The electronic noses try to simulate the functioning of the olfactory system. They are made of an array of chemical and electronic sensors with partial specificity and of a system of pattern recognition, able to recognize simple and complex odours (Garner and Bartlett, 1993). There are many types of sensors, which, based on different principles, react with a change in their properties: metal oxide semiconductors of different types and conducting organic polymers change their electrical properties when absorbing volatile compounds; sensors based on quartz crystal microbalance change their mass, so changing their resonance frequency, which is measured. Different types of sensors differ according to repeatability, to reaction and recover time, to selectivity and to sensitivity to humidity. Electronic noses can recognize classes of compounds. Each sensor reacts to a different set of volatile compounds; the pattern of the combined responses of all the sensors gives a “fingerprint” of a compound or a mixture. The electronic nose cannot analyze and determine the different volatile compounds, like a gas chromatograph, because its response is not unique. It is useful to detect deviations from a standard, whose fingerprint is well known, or to follow changes in time (Riva et al., 2005).

Optical techniques

Near infra-red reflectance spectroscopy

Among optical techniques, continuous-wave near infra-red reflectance spectroscopy (NIRS) is gaining increasing popularity for the quality assessment of fruit, as in the last two decades these techniques have provided encouraging results for estimating several quality parameters in fruit (Birth and Olsen, 1964; Fukuda and Kubota, 1979; Throop et al., 1989; Upchurch et al., 1997; Lammertyn et al., 2000; Schaare and Fraser, 2000; Clark et al., 2003ab; Slaughter et al., 2003; Saranwong et al., 2004; Peirs et al., 2005). NIRS has been used for estimating soluble solids content, but also other properties: dry matter, firmness, acidity. Generally the estimation for soluble solids is satisfactory and consistent, while for firmness and acidity the results are more variable. NIRS has also been used to predict optimum harvest date of apples from the changes in soluble solids, after correction for seasonal effects (Peirs et al., 2005). For NIRS, the use of appropriate methods for processing data is crucial. Spectral data can be pre-processed by smoothing, normalizing and calculating the first or second derivative. In order to calibrate the system for prediction purposes, a model with selected variables by multiple linear regression can be used, or the whole spectrum can be used by performing data reduction by linear combination of variables with principal component regression (PCR) or partial least squares (PLS). Calibration must be followed by a validation procedure. Calibration models must be based on a large number of different samples, and must be updated for every different condition, including season, variety, and temperature. With the increasing number of calibration samples and of different conditions, the robustness of the calibration model increases. Selection lines with NIRS require manual positioning of fruit, in order to obtain reliable results, so they have high costs. Penetration depth is relatively small in reflectance (1-5 mm) (Lammertyn et al., 2000), while for transmission measurements a high power illumination is required.

Near infra-red multispectral scattering

With a different approach, Lu (2004a) proposed to utilize light scattering by multi-spectral imaging. A sharp, focused broadband light beam illuminated a portion of the fruit, generating backscattering images at the surface of the fruit. Spectral scattering images were acquired for four different wavelengths in the range 680-940 nm. Scattering images were averaged radially, so producing one-dimensional spectral scattering profiles, which were then input into a back-propagation neural network for predicting apple fruit firmness and soluble solids content. Using the ratios of the scattering profiles, firmness predictions in different experiments had correlations between 0.76 and 0.87 (Lu, 2004b).

Continuous wave (CW) optical methods, like the ones described above, measure the diffusely remitted intensity which is not only strongly dependent on the color of the skin (thus masking information from the pulp), but it is also determined by both the absorption and the scattering properties of the sample in such a way that it is not feasible to separate the effects of these properties. Light absorption is determined by the constituents of the pulp that produce characteristic spectral features in the visible and near infrared region of the spectrum. Conversely, light scattering is due to the microscopic changes in refractive index caused by membranes, air vacuoles, or organelles which deviate the photon paths and are ultimately responsible for light diffusion.

To separate these two effects, time- or frequency-resolved methods are required. In contrast to the "black box" approach, these methods measure well-defined, fundamental optical properties of fruit pulp.

Time-resolved reflectance spectroscopy

Principle

Time-resolved reflectance spectroscopy (TRS) is a non-destructive method for optical characterisation of highly diffusive media. It has recently gained increasing use in biomedicine for the non-invasive investigation of biological tissues (Yodh and Chance, 1995). Similarly, it has been used for optical characterisation of fruit (Cubeddu et al., 2001ab). In TRS, a short laser light pulse is injected into the medium to be analyzed. Due to photon absorption and scattering events, the diffusely reflected pulse is attenuated, broadened and delayed. The absorption coefficient μ_a and the transport scattering coefficient μ'_s are simultaneously and independently estimated by fitting the time distribution of the diffusely reflected light pulse, detected by time-correlated single photon counting techniques, with a theoretical model of light propagation. In TRS, light penetration into a diffusive medium depends on the optical properties of the medium and on the source-detector distance. In most biological tissues such as fruit and vegetables the depth of the probed volume is of the same order as the source-detector distance, which is 1-2 cm (Cubeddu et al., 1999). Consequently, the measurements probe the bulk properties, not the superficial ones, and may provide useful information on internal quality. The novelty with TRS is the use of a pulsed laser source, and the detection of the temporal distribution of re-emitted photons. This allows one to measure separately both μ_a and μ_s' in the pulp of the fruit averaged over the probed medium, while CW techniques are intrinsically dependent on the coupled effect of both of them. These optical parameters carry quite distinct information about the tissue, since absorption is determined by pigments (chlorophyll, anthocyanins) or key constituents (water, sugars), while scattering is caused by the dielectric constant mismatch in the tissue, and is more related to the cellular structure. Thus, direct measurement of both

μ_a and μ_s', as provided by TRS, can provide more valuable information on the probed medium. The time required for one TRS measurement is now one second with a manual portable prototype, but the technique could be adapted for on-line measurement, reducing acquisition time to ten milliseconds without loss of accuracy.

Applications

Some applications of TRS have been studied in apples, pears and nectarines in collaboration with researchers of the Department of Physics of Politecnico of Milan, who developed the method.

In apples, scattering at 750 nm (μ'_s750) was correlated to internal space volume and to firmness and pectin composition: at harvest, μ'_s750 was directly related to water soluble pectin (W) and inversely to oxalate fraction (O) and residual insoluble pectin (R); after storage it was inversely related to firmness, R and protopectin index (R/W+O) (Vanoli et al., 2006). In 'Conference' pears, the development of over ripening and of bruises (translucent tissue) was detected by the scattering coefficient μ_s' at 720 nm. The absorption coefficient μ_a at 720 nm discriminated between sound and brown heart affected tissue in the intact fruit (Eccher Zerbini et al., 2002).

Chlorophyll absorbs light with a peak at 672 nm. The use of absorption at a wavelength near the chlorophyll peak (between 630 and 690 nm) as a maturity index has been checked in different fruit species, by measuring μ_a at harvest and then checking fruit quality after storage and shelf life. 'Jonagored' apples were harvested on two dates and classified at harvest by μ_a at 630 nm (μ_a630). The absorption coefficient μ_a630 was significantly higher in first harvest apples. Apples with higher μ_a630 had lower fruit mass and lower per cent blush. Fruits classified as more mature by TRS had less titratable acidity at harvest and more soluble solids after storage; according to sensory analyses, these fruits were significantly sweeter, more aromatic and pleasant. However flesh firmness was not affected by maturity classification (Vanoli et al., 2005). As regards Abbé Fétel pears, at harvest, fruit with high μ_a690 (i.e. less mature) had lower mass, soluble solids and titratable acidity, whereas skin color (hue), starch and firmness were not different between the maturity levels. Significant correlations were found between μ_a690 and soluble solids (r=0.72), acidity (r=0.49) and mass (r=0.54) (n=30 fruits) (Eccher Zerbini et al., 2005; Eccher Zerbini et al., 2004).

In nectarines of two sizes (A and B) classified at harvest by μ_a670, on the average fruit mass was significantly lower in fruit with higher μ_a670. Smaller size (B) fruit had a significantly higher μ_a670 than larger size (A) fruit. In fruit with lower μ_a670 at harvest (more mature), the soluble solids content and per cent blush was higher, firmness and acidity lower than in fruit with high μ_a670. As regards sensory attributes, more mature fruit (lower μ_a670 at harvest) were perceived significantly less firm and more juicy, sweet, pulpy and aromatic and were more appreciated by the assessors (Eccher Zerbini et al., 2003). Sugar and acids composition was also affected by μ_a (Jacob et al., 2006).

Perhaps the most interesting result obtained so far from the use of μ_a 670 for the evaluation of maturity, is its predictive power as regards softening of nectarines. The measurement of the absorption coefficient at 670 nm (μ_a670) at harvest, by means of TRS, allowed to assess the maturity of individual fruits (Eccher Zerbini et al., 2005bc). Even though firmness is a destructive measurement, by selecting at harvest fruit of the same maturity level, it was possible to follow softening in different fruit of the same maturity. Softening occurred earlier in more mature fruit (lower μ_a at harvest) and later in less mature fruit (higher μ_a at harvest), with the same sigmoid pattern in time. Softening of nectarine fruit during the shelf life at 20°C was fitted by a logistic model with μ_a and time as independent variables, which explained 85% of the variation in fruit firmness in 'Spring Bright' and 75% of the variation in fruit firmness in 'Ambra'. The parameters of the model were practically the same for the same cultivar in two seasons despite different climatic conditions. The differences between cultivars were limited. With this model, it is possible to predict the onset of softening and the softening rate at 20°C in nectarines, if their μ_a is measured at harvest.

Other techniques

Magnetic resonance imaging

Other techniques have been proposed for the non-destructive assessment of quality in fruit or vegetables. Magnetic resonance imaging can provide high resolution images of internal structures of intact fruit, but with a high equipment cost and low speed of measurement (Wang and Wang, 1989; Clark et al., 1997; Clark and Burmeister, 1999).

DISCUSSION

Non-destructive methods measure different properties from conventional methods. In some cases, such as NIRS, the properties measured are not well defined and conventionally measured properties are empirically predicted by calibration models.

Compared with other optical techniques, TRS has the merit of measuring well-defined, physically based properties. Moreover, TRS explores a volume within the fruit, without being affected by surface properties. TRS technique, by means of the scattering coefficient μ_s', allows a description of the virtual appearance of the internal tissue in the intact fruit to a depth of about 2 cm, of the presence of defects and of their position inside the fruit, as it can be visually assessed only after cutting the fruit. By using the coefficient of absorption μ_a at a wavelength near 670 nm, the maturity of individual fruit can be measured, which is one of the main determinants of the postharvest quality. The maturity level can be discriminated by TRS even if fruit are extensively covered by intense blush.

Possible applications of this non-destructive technique for commercial purposes that until now can be envisaged are: a) to detect internal defects in fruit, even following up their formation; b) to grade fruit according to maturity, predicting their potential shelf life in the case of nectarines. So a great deal of potential applications can be envisaged for this technique when a simpler instrumentation will be available, also for on-line grading of fruits.

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TECHNOLOGIE DO NIEDESTRUKCYJNEj OCENY JAKOŚCI OWOCÓW

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STRESZCZENIE

Rozwój nauki i techniki, a szczególnie techniki informacyjnej przyczynił się do udostępnienia wielu niedestrukcyjnych metod do analizy materiałów, które mogą być również zastosowane do owoców, jako że sektor owocowy potrzebuje technik niedestrukcyjnych do sortowania i poświadczania wysokiej jakości owoców. W pracy wspomniano o elektronicznym nosie, metodach akustycznych i optycznych, takich jak spektroskopia bliskiej podczerwieni oraz przedyskutowano ich zastosowanie do analizy owoców. Dokładniej opisano podstawy i zastosowanie spektroskopii fali odbitej analizowanej w funkcji czasu (TRS). TRS może mierzyć oddzielnie i niezależnie właściwości optyczne (współczynnik absorpcji μ_a i współczynnik rozpraszania μ_s') materiałów o wysokiej zdolności rozpraszania przy wybranych długościach fali. Współczynnik absorpcji wiąże się ze składem chemicznym, jak barwniki, podczas gdy współczynnik rozpraszania powiązany jest ze strukturą tkanki i rozmieszczeniem powietrza i wody. TRS zyskuje w ostatnim czasie coraz większe zastosowanie w biomedycynie do nieinwazyjnego badania tkanek biologicznych. Metoda ta używana była również do optycznej charakterystyki owoców. Na pomiary metodą TRS nie maja wpływu kolor i inne cechy powierzchni, ponieważ badana jest objętość na głębokości 1-2 cm. Przedstawiono ostatnie doświadczenia z zastosowaniem tej metody na owocach. U gruszek możliwe jest wykrywanie zbrązowień wewnętrznych (wykorzystując μ_a przy 720 nm) i uszkodzeń mechanicznych (μ_s' przy 720 nm), które nie są widoczne na zewnątrz. W jabłkach, współczynnik rozpraszania związany był ze składem pektyn i jędrnością owoców po przechowywaniu. U nektaryn, biorąc pod uwagę μ_a przy 670 nm, wybrano owoce bardzo aromatyczne i o słabym aromacie oraz przewidziano mięknięcie miąższu w temperaturze pokojowej. Analiza absorpcji przy długości fali około 670 nm, w pobliżu piku charakterystycznego dla chlorofilu, jest również przydatna do określania dojrzałości jabłek i gruszek.

Słowa kluczowe: spektroskopia fali odbitej w funkcji czasu, elektroniczny nos, spektroskopia bliskiej podczerwieni, dojrzałość, jędrność, związki rozpuszczalne, jakość