Cutting Edge Technologies in Postharvest Research: Journey to the Centre of the Fruit

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Abstract

Food microstructure is at the base of many food quality properties. The EU project InsideFood focuses on the application of high technological techniques to inspect internal quality of fruit. Tomographic techniques such as magnetic resonance imaging, X-ray computed tomography, optical coherence tomography and confocal microscopy can be used to obtain information about the 3-D microstructure of the fruit which is believed to affect quality attributes such as texture. Optical techniques such as spatially or time resolved reflectance spectroscopy may also be used to obtain information about fruit microstructure. This microstructural information can be incorporated in multiscale simulation models to predict the cellular gas concentrations in fruit. Such models aid towards a better understanding of, for instance, controlled atmosphere storage of apple and postharvest behaviour of fruits and vegetables in general.

INTRODUCTION

Food microstructure is defined as the organisation of food constituents at the microscale and their interaction. Most solid foods, including fruits and vegetables are microstructured. Many food properties relevant to the postharvest behaviour and the processing behaviour of fruits and vegetables are related to their microstructure. Examples include gas and water transport properties of fruit, colour as related to light scattering properties just beneath the surface of the food and fruit firmness as related to the overall tissue structure. Until recently, the measurement of food microstructure was essentially based on light or electron microscopy. Because of the often considerable sample preparation time, cost and complexity of the equipment, such techniques were mainly used for academic purposes but seldom in a food industrial environment. However, recently advanced tomographic and spectroscopic techniques have emerged that allow food scientists and engineers to inspect the food microstructure non-destructively. The European FP7 project InsideFood focuses on integrated sensing and imaging devices for designing, monitoring and controlling microstructure of foods using

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these techniques as they offer a vast potential for use as innovative sensors for both off-line and on-line measurement of food microstructure.

TOMOGRAPHY

X-Ray Computed Tomography (X-Ray CT)

X-ray CT is a relatively new technique developed in the late 1970s, which enables the non-destructive visualisation of the internal structure of objects. The technique of X-ray (micro)-CT is based on the interaction of X-rays with matter. When X-rays pass through an object, they will be attenuated in a way depending on the density and atomic number of the material in relation to the used X-ray energies. By using projection images obtained from different angles, a reconstruction can be made of a virtual slice through the object. When different consecutive slices are reconstructed, a 3D visualisation can be obtained. μCT as non-invasive technique has been applied to the study of the internal 3D structures of several food products and fruits. Kuroki et al. (2004) obtained 3D spatial information about gas-filled intercellular spaces in cucumber fruit. Babin et al. (2005) studied the microstructure of cellular cereal products captured by synchrotron radiation μCT. Leonard et al. (2008) used μCT on processed banana. Synchrotron radiation μCT has been applied with success to more difficult products such as apple and pear, with resolutions below 1 micron (Mendoza et al., 2007; Verboven et al., 2008; Ho et al., 2011). A new challenge in technology is the nano-CT system opening up a new era in X-ray imaging with a spatial resolution in the range of hundreds of nanometers.

The fact that with micro- and nano-CT the object can be scanned under normal environmental conditions without any coating, vacuum treatment or other preparation techniques makes it an interesting tool suitable as a reference to interpret the signals from other methods. Computed tomography on dense foods with high water content and little differences in attenuation coefficients between the phases is a difficult task. This poses limitations to the achievable contrast resolution with nano- or micro-CT imaging. On water-containing foods, the challenge for X-ray CT imaging is to find the right scanning protocol avoiding drying out of the sample and to subsequently segment the image.

Optical Coherence Tomography (OCT)

OCT is a relatively young and promising contactless high-resolution imaging technique, which has been introduced for biomedical diagnostics applications, like e.g., the detection of retinal diseases. For OCT measurements, the sample is commonly illuminated with light in the near infrared. The backscattered and -reflected photons from the sample are collected and brought to interfere with a reference beam. From the interference pattern, the location of the scattering sites within the sample can be determined. Because of the coherent detection of the backscattered photons, the method is very sensitive and even suitable for turbid materials - the penetration depth is several times higher than that obtained with, e.g., confocal microscopy. For OCT the depth resolution is decoupled from the lateral resolution. The depth resolution is dependent on the spectral width of the used broadband light. The lateral resolution depends on the focusing/imaging optics. Furthermore, the wavelength of the imaging light can be chosen, for example to increase the penetration depth, or alternative physical phenomena can be used to gain additional material contrast (e.g., polarisation-sensitive OCT to detect the orientation of fibrous tissue). Since OCT detects inhomogeneities in the refractive index of materials, complementary information can be obtained, e.g., to X-ray CT, where the contrast is related to the density distribution.

A variety of novel developments and instrumental extensions of OCT have been presented recently (Stifter, 2007), and the high potential for other applications such as in the field of non-destructive testing and evaluation has started to be recognised. However, alternative OCT applications remain to date in the minority when compared to their biomedical counterparts, where imaging down to the cellular level has even been demonstrated.

Nuclear Magnetic Resonance Spectroscopy and Imaging

Nuclear magnetic resonance (NMR) studies magnetic nuclei such as protons by aligning them with an applied constant magnetic field and perturbing this alignment using an alternating magnetic field (Hills et al., 2004, 2005).

In NMR relaxation, the different time constants for the nuclei to return to their thermodynamic rest state after an electromagnetic pulse is applied is measured. The time constants are related to, amongst others, concentrations of the magnetic nuclei but also their mobility. 2-dimensional NMR relaxometry has been used to study complex microstructured systems by providing detailed "relaxation spectra" giving separate peaks for water in different pores and compartments in microstructured systems. Each peak in the 2D spectrum is characterized by a particular proton longitudinal and transverse relaxation time (T_1 and T_2 respectively), which differ according to the local water content and size/nature of the pore or compartment. Peaks from solutes and biopolymers also arise and their relaxation times give additional information on their dynamic state. Other types of 2D relaxation and diffusion experiments are possible and these give information on localized water transport through the microstructure (Marigheto et al., 2005, 2006).

High Resolution, slow Magic Angle Spinning NMR spectroscopy has only recently been developed as a probe for complex foods. Conventional high resolution NMR spectrometers have very limited use in complex food analysis because the magnetic field susceptibility discontinuities across phase boundaries in microstructured foods create strong localized magnetic field gradients when the sample is placed in the strong magnetic field of the NMR spectrometer. These gradients broaden the spectral lines and prevent clean spectral resolution. Slow Magic Angle Spinning (MAS) removes these field gradients by rotational averaging and restores the narrow spectral lines.

Magnetic Resonance Imaging (MRI) greatly broadens the range of applications devoted to the inspection of food products and, particularly, of fruit and vegetables. MRI provides a picture that contains combined spectroscopy and relaxometry information both spatially resolved. The studies performed up to date are a demonstration of the potential of these techniques for the internal quality monitoring even under online conditions: maturity in avocados, pit detection in cherries and olives, internal browning in apples, internal breakdown in pears, freeze injury and seed in citrus. Nondestructive detection of the browning disorder (browning) has been made possible by magnetic resonance imaging (MRI) and X-ray CT (Lammertyn et al., 2003a,b).

On-line MRI, as a macroscopic technique, has shown to correlate well with the internal organization of tissue for high magnetic fields strength (4,7 Tesla). Available and renewed grading lines have been designed for easy and quick mounting and decoupling, and therefore can be transported and tested in commercial equipments with lower field strengths (<0.5 Tesla). Shifting from high to low magnetic field strengths requires the reformulation of sequence parameters for each application as to optimize signal to noise ratio and contrast.

SPECTROSCOPY

Time (TRS) and Space Resolved (SRS) Diffuse Reflectance Spectroscopy

A common feature of most food and feed is their opacity to visible light. The majority of biological materials can in fact be modelled as a diffusive medium. In these media, due to the microscopic spatial changes in the refractive index, light undergoes multiple scattering events and its overall distribution (i.e., attenuation) is determined by the interplay between light scattering and light absorption. Scattering is very much dependent on the microstructure of the product. Classical VIS/NIR spectroscopy can only provide information on light attenuation, but does not allow the non-destructive assessment of both the absorption and scattering coefficients in diffusive media. On the contrary, time-resolved diffuse reflectance spectroscopy (TRS) and space-resolved diffuse reflectance spectroscopy (SRS) provide a complete optical characterisation with the simultaneous non-invasive measurement of the optical properties (absorption and scattering) of diffusive media (Cubeddu et al., 2001; Bevilacqua et al., 1999; Lu et al., 2006). This can be of special interest for most fruits and vegetables as well as for other foods (e.g., meat, fish, and cheese), 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 the case for continuous wave spectroscopy (Cubeddu et al., 2001; Saeys et al., 2008).

From the optical parameters information can be obtained on the physical properties (Nicolaï et al., 2007), such as the density (or number) and size of particles or droplets (in case of emulsions). Those parameters are important for the processing of food and subsequently for the quality of the derived food products. These techniques are totally non-invasive since the optical radiation in the 600-2000 nm spectral range (red and near infrared, NIR) is non-ionising. Moreover, very limited energy (average power of few mW, pulse duration of hundreds of ps) is typically employed in TRS systems, therefore no chemical, mechanical or thermal damage can occur to the sample. TRS and SRS have the capacity to probe internal microstructural properties with minimal influence from the optical properties of the surface, allowing for non-destructive applications (Thueler et al., 2003; Xing et al., 2006; Nicolaï et al., 2007).

The main advantages of these spectroscopic techniques are their absolute non-invasiveness, their potential to access both the structure properties and the composition and the capacity to probe the layers below the skin (e.g., membranes below the eggshell or fruit flesh below the skin); TRS and SRS have the potentiality for non-contact measurements, therefore on-line applications are feasible. Similarly, using advanced optoelectronic devices can develop robust and sensitive sensors and systems. The key issue will be to obtain insight in the complex correlation of physiological-structural parameters, which contribute to the information entangled in optical parameters, with respect to the definition of food quality and safety.

INTEGRATIVE DATA ANALYSIS

The large amount of data and the large variation in data types generated by different techniques require advanced procedures for data analysis.

Image Processing

The tomographical imaging methods generate 3D data stacks with sizes up to several Gigabytes for one image. Image processing for structure analysis first requires segmentation to convert the grey-scale image into a black and white image by determining the population assignment (e.g., void space or solid material) for each voxel in the image. For this, a common practice is to select a simple global threshold, which often is set to match a predetermined bulk measurement of porosity. However, this procedure is very subjective and may lead to biases when one is trying to segment a stack of X-ray images. The distinction between the void and solid phases in tomographic and radiographic images is frequently not sharp (i.e., do not show a bimodal distribution). Moreover, the resulting binary images of plant tissue using global thresholds are noisy and the average porosity is highly dependent on the selected threshold value (Mendoza et al., 2007). Other thresholding methods, e.g., that developed by Oh and Lindquist (1999) can be used to perform segmentation of the noisy images into distinct phases.

Quantitative Analysis

The segmented image is the basis for further quantitative analysis. Since the reconstructed 3D binary image consists of a cubic array of small voxels identified as either pore or cellular material, they can be used to calculate the bulk porosity and phase fractions of the sample. The images can be used to analyze the path of networks of microstructural phases such as the pore space, which is important for mass transport (Ho et al., 2006). The medial axis construction can be used as the base from which to calculate the spatial position of phases in the sample and other geometrical features such as: specific surface area, distributions of disconnected volumes, connectivity of phases and tortuosity (Mendoza et al., 2007).

Multivariate Statistics

Microstructure features that are measured using the various techniques or computed using image processing software can be related to quality attributes of the food such as measured with reference methods like parallel plate compression or penetrometry. As the microstructure features are essentially multidimensional, multivariate techniques are required. Multivariate data analysis generally involves data reduction. It reduces high dimensionality in a multivariate problem where variables are partly correlated, allowing the information to be displayed in a smaller dimension (Naes et al., 2004). There are many multivariate techniques to choose from: principal components analysis (PCA), principal components regression (PCR), partial least squares (PLS), canonical discriminant analysis (CDA), feature weighting (FW) and cluster analysis (CLA). The above multivariate techniques are all linear as a model is calculated using linear combinations of input data. Nonlinear techniques include artificial neural networks (ANN) and kernel based techniques.

Physical Modelling

Physical modelling provides a tool to use the structural measurement data in a dynamic way by in silico simulation of the relevant processes. Two important aspects are 3D microscale water status and transport, which determines to a large extent food texture, and light propagation in the complex 3D microstructure, which allows us to better understand the optical properties of the food materials in relation to texture quality of the microstructured foods.

Macroscopic continuum models in which the underlying structural complexity of the tissue has not been taken into account explicitly, have been developed successfully to describe transport of metabolic gasses in pear (Lammertyn et al., 2003a,b; Ho et al., 2006) and water in apple and pear (Veraverbeke et al., 2003a,b; Nguyen et al., 2006). The inherent assumption is that the material parameters are independent of the spatial scale. Food material parameters, such as the moisture diffusion coefficient of the tissue, should therefore be considered as apparent material parameters: they incorporate both actual physical constants such as the diffusion coefficient of water molecules in air but also the microscale geometry of the tissue and the intracellular space. A modelling approach in which the water transport is solved on the true microscopic structure results in more reliable and physically meaningful models, which would provide better insight and facilitate the development of improved and novel methodologies for safeguarding product quality during storage and processing (Fig. 1).

CONCLUSIONS

The European FP7 project InsideFood focuses on integrated sensing and imaging devices for designing, monitoring and controlling microstructure of foods. The non-invasive sensors for characterising microstructure studied in the project are either based on tomography (X-ray nano- and microtomography, nuclear magnetic resonance spectroscopy, magnetic resonance imaging, optical coherence tomography) or spectroscopy (time- and space-resolved reflectance spectroscopy). The techniques are correlated to understand the effect of microstructure on water and solute status, texture and optical properties and internal defects of food. To this end, data analysis algorithms are developed, including image processing, modelling and multivariate statistics. The research is aimed to bring closer to the market on-line sensors for microstructure analysis and to provide tools for process design and optimization.

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Figures

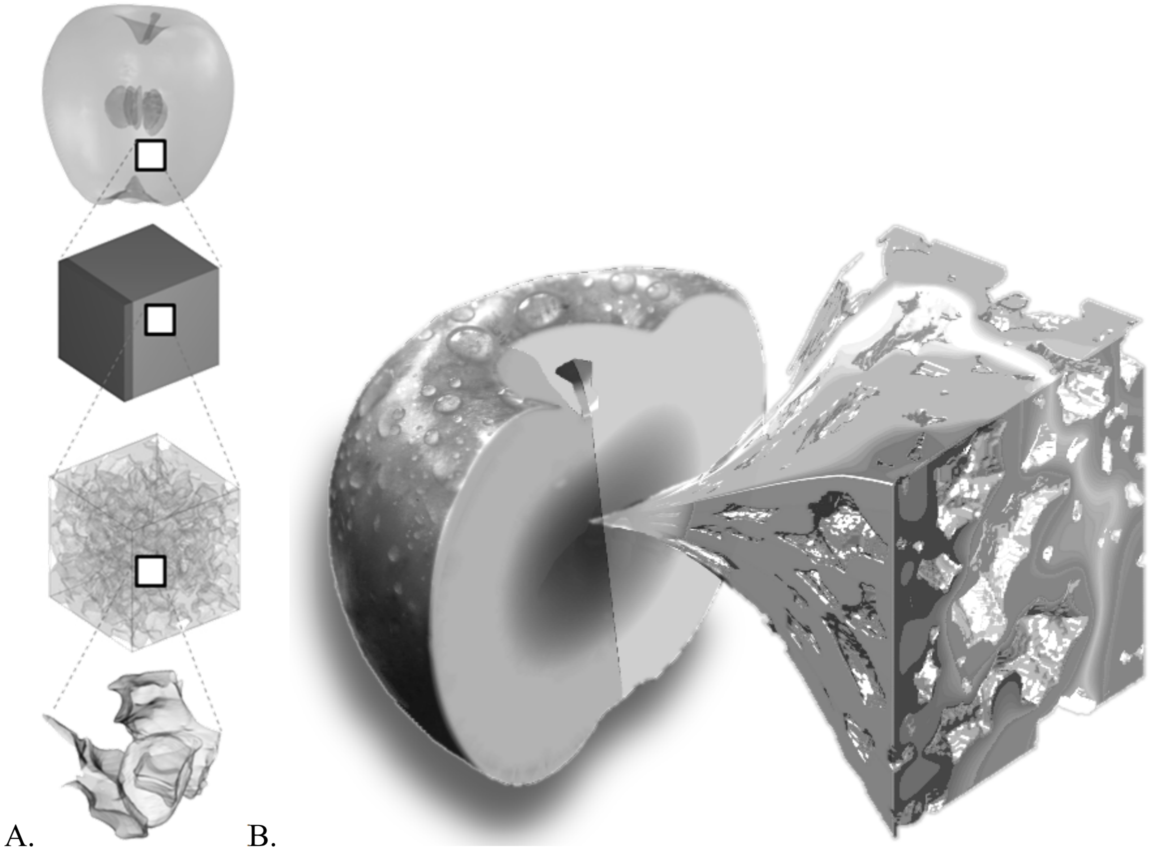

Fig. 1. The availability of 'inexpensive' optical techniques for measuring fruit microstructure enables the development of real life 3D geometrical models of increasing levels of complexity (A). These can be subsequently used to model and improve understanding of biophysics such as gas transport, water loss, photosynthesis or mechanical deformation. Fig (B) provides an artist impression of simulated O_2 and CO_2 gas distributions in apple based on either a macro or microscopic geometrical model of apple(tissue) (After Ho et al., 2011).