

Chapter 2

Quantitative Measurement of the Pore Structure

This is a special area of research in which there are numerous techniques, equipment and software that allow determination of the pore structure with respect to the number of pores, pore size and shape distributions (Kocer et al. 2007; Hicsasmaz et al. 2003), number of cell faces, cell wall thickness, pore interconnectivity and polydispersity index (Trater et al. 2005). Pore structure analysis is a high technology field in which microscopic techniques such as light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) besides numerous image analysis techniques primarily used to visualize the human body for purposes of medical diagnosis are well-adapted. X-ray microtomography (Frisullo et al. 2012; Trater et al. 2005) is one such technique in which 2-D sections can be imaged non-destructively, and then a qualitative 3-D reconstruction can be performed. 2-D micro-tomography images and related software allow measurement of cell wall thicknesses with accuracy. Magnetic resonance imaging (MRI) (Wagner et al. 2008; Ishida et al. 2001), SEM and environmental scanning microscopy (ESEM) (Stokes and Donald 2000) also provide data on the pore structure, SEM being one of the most widely used methods. Ultrasound (Lagrain et al. 2006) is used in microstructure measurements. Mercury porosimetry (Hicsasmaz and Clayton 1992) and liquid extrusion porosimetry (Datta et al. 2007) are quantitative methods which do not use imaging techniques, but that use the capillary penetration technique.

2.1 Capillary Penetration Techniques

2.1.1 Mercury Intrusion Porosimetry

Mercury porosimetry is a capillary penetration technique used to predict the pore volume intruded/extruded corresponding to a certain capillary pressure. In mercury porosimetry, relatively higher capillary pressures are used due to high surface tension and contact angle of the non-wetting mercury. Detection of capillaries with

diameters greater than 200 μm is not possible using mercury porosimetry. In mercury porosimetry, the sample cell is first evacuated, and then the pressure is increased incrementally, meanwhile monitoring the mercury filled specimen pore volume continuously upon each increment in pressure (Hicsasmaz and Clayton 1992). If capillaries with diameters larger than 200 μm occupy considerable volume, then mercury penetration is accomplished in a very short incremental pressure range and a sound structural analysis cannot be performed.

2.1.2 Liquid Extrusion Porosimetry

In liquid extrusion porosimetry, the specimen is first imbibed in a special liquid with very low surface tension, and the capillaries are emptied progressively from largest to the smallest by incremental increase in pressure, meanwhile monitoring the emptied pore volume continuously. The membrane arrangement used is such that, extruded liquid can pass through the membrane selectively at gas pressures applied within the range of the test (Datta et al. 2007).

2.2 Imaging Techniques

2.2.1 Destructive Techniques

SEM is well-suited for quantitative analysis of the pore structure, since it allows a wide range of magnification, a high depth of field, and produces digital data fit for image analysis. SEM combines the best aspects of light microscopy and TEM (James 2009). SEM is widely used for micro-structural analysis. However, analysis of many sections is required to get statistically relevant results, and the information on total pore volume and pore size distribution is not reliable due to the discontinuous nature of 2-D sections (Gropper et al. 2002; Autio and Salmenkallio-Martilla 2001). This is because 2-D sections are prepared by cutting to expose the cross-section. Cutting is a destructive technique which may in turn alter the structural features of the specimen.

Individual pores are sliced off-center and the diameters depend on the depth of the cut (Scanlon and Zghal 2001; Campbell and Mougeot 1999). Therefore, micro-structural data obtained from SEM sections are semi-quantitative. Reliable assessments on micro-structure can be made by combining the results of SEM analysis with those from capillary penetration techniques. Destructive nature of SEM images does not allow quantization of pore interconnectivity and cell wall thickness. These are in fact the most important features of the microstructure that influence mechanical strength, bulk infusibility by liquids and effective diffusivity of gases.

It is difficult to obtain adequate contrast between the air and solid phases using light microscopy and SEM. Although lighting and the angle of illumination are important, even careful arrangement proves to be inadequate for specimens with thin cell walls. Cell walls on the 2-D section can be painted to enhance contrast when light microscopy is used (Dogan et al. 2013; Kocer et al. 2007; Hicsasmaz et al. 2003). However, even careful painting can introduce extra artifacts. Therefore, these techniques provide semi-quantitative results on pore size and shape distributions and their relationship with processing parameters and ingredient mixtures.

Imaging by SEM requires special preparation of samples like food materials that contain water. SEM is well-suited for dried food samples (Bai et al. 2002) which further results in shrinkage, and thus alteration of the microstructure. The dried sample is sputtered by a metal coating to enhance conductivity, and thus to obtain high quality images. Cryo-SEM is an alternative to avoid the disadvantages of drying. In this case, cold-stage imaging is performed on frozen samples, but formation of ice crystals cause artifacts, and makes it difficult to perform quantitative analysis. ESEM (Electroskan SEM) allows imaging of hydrated samples. This is possible due to the design of ESEM which allows the electron chamber under high vacuum, while keeping the specimen chamber under moderate vacuum.

2.2.2 Non-invasive Techniques

X-ray microtomography is a non-invasive technique allowing visualization of the internal structure of a sample based on the local variation of the X-ray attenuation coefficient. X-ray microtomography allows scanning of the whole sample. This technique is quite new in the field of food engineering (Leonard et al. 2008; Babin et al. 2006; Haedelt et al. 2005; Trater et al. 2005; Lim and Barigou 2004; van Dalen et al. 2003). The general methodology involves targeting the specimen with a polychromatic X-ray beam. X-rays transmitted by the specimen fall on specially-designed X-ray scintillators that produce visible light, which is recorded by a charge-coupled device camera. Transmitted intensity is related to the integral of the X-ray attenuation coefficient (μ) along the path of the beam according to the Lambert–Beer’s law. The X-ray attenuation coefficient depends on the density (ρ) and atomic number (z) of the material and on the energy of the incident beam (E) given by:

$$\mu = \rho \left(a + \frac{bz^{3.8}}{E^{3.2}} \right) \quad (2.1)$$

where a and b are constants (Leonard et al. 2008).

A tomographic scan is accomplished by rotating the specimen between 0 and 180° about an axis perpendicular to the X-ray beam while collecting radiographs of

the specimen at small angular increments. The radiographs are then reconstructed into a series of 2-D slices using the back-projection software (Brun et al. 2010) that maps estimates of attenuation coefficients, which depend on density variations within the specimen. The series of slices, covering the entire sample, can be reconstructed into a 3-D image that can either be presented as a whole or as virtual slices of the sample at different depths and in different directions. Reconstruction of cross-sections at depth increments as low as 1 μm , and along any desired orientation is possible using special software (Brun et al. 2010). The resulting 3-D and/or 2-D reconstructed images are formed of two grey levels corresponding to the two phases of the porous material. The low grey levels (dark voxels) correspond to the pore space, and the high grey levels (bright voxels) correspond to the solid cell walls.

MRI is ideally suited for non-invasive imaging of water, and has been applied to food materials (Horigane et al. 2006; Troutman et al. 2001; Cornillon and Salim 2000). MRI imaging is not a quantitative method of microstructure measurement, but it is very powerful in following moisture migration problems. The effect of micro-structure on moisture migration can further be studied by relating MRI images with X-ray microtomography images (Weglaz et al. 2008). MRI is based on nuclear magnetic resonance (NMR) signals sampled in the presence of magnetic fields to obtain spatial resolution depending on the NMR properties of the specimen (proton density, T1 and T2). Proton density is the NMR property of the specimen that originates from the protons of carbohydrates, proteins and fats in the solid matrix. Differences in T2 decay signals describe the water of hydration, free water and the lipids. Short T2 decays correspond to the water of hydration, while long T2 decays correspond to free water. Time domain signals sampled under the influence of magnetic field gradients are converted into 1-D, 2-D or 3-D images called the “k-space”. The MR image is obtained after a series of complex Fourier transforms. The key issue in the MRI technique is to collect enough data points in the “k-space”, i.e. resolution in time and space that can describe the specimen (Weglarz et al. 2008).

2.2.3 Quantitative Image Analysis

Measuring the pore structure parameters such as the total pore volume, pore size distributions, polydispersity index, pore shape factors, interconnectivity of the pores and cell wall thicknesses requires the use of special image analysis software regardless of whether the images are obtained by destructive or non-invasive techniques. Image analysis software is used to perform an action called thresholding on the images to convert grey level images to binary images. The software automatically determines the threshold grey tone by maximizing the entropy of the histogram, s , given by (Leonard et al. 2008; Kocer et al. 2007; Hicsasmaz et al. 2003):

$$s = - \sum p_i \log(p_i) \quad (2.2)$$

where p_i is the probability of any pixel grey value in the image. Then, the value of 1 is assigned to all pixels whose intensity is below the threshold grey tone, and the value of 0 is assigned to all the others. An average threshold grey value among the analyzed 2-D sections needs to be defined to carry out quantitative image analysis on 3-D reconstructions. Thresholding results in a binary image regardless of whether quantitative image analysis is carried out on 2-D sections or 3-D reconstructions. The binary image is further filtered to remove the black spots (Leonard et al. 2008; Kocer et al. 2007; Hicsasmaz et al. 2003). Colored parts of the binary image correspond to the cell walls, black parts correspond to the pores. Then, the pore structure parameters are evaluated depending on the capabilities of the image analysis software.

Image analysis software should have special capabilities to increase contrast between the gas and solid phases before quantitative analysis of images obtained by 2-D destructive techniques is performed. Image analysis software use techniques such as image subtraction and edge detection that involve complex mathematical methods such as Fourier transforms. However, these enhancement methods can also introduce extra artifacts. Objective quantitative measurement of the pore structure parameters is not possible using 2-D images obtained from 2-D destructive techniques as a result of the contrast problem, and high section to section variability.

X-ray microtomography is a powerful tool for quantitative analysis of the pore structure. Trater et al. (2005) defined a representative volume element and suggested a method to calculate various pore structure parameters. Defined representative volume element was divided into a number of equidistant 2-D slices. The pore volume (V_i) of the selected representative volume element was found as;

$$V_i = t \left(\sum_{n=1}^{N_i-1} \frac{A_{v,n,i} + A_{v,n+1,i}}{2} \right) + \frac{t}{3} (A_{v,1,i} + A_{v,N_i,i}) \quad (2.3)$$

where $A_{v,n,i}$ is the pore area on slice n , t is the constant spacing between any two slices, and N_i is the number of slices that pore i appears. Diameters of the pores (D_i) in the representative volume element were calculated assuming spherical geometry:

$$D_i = 2 \left(\frac{3V_i}{4\pi} \right)^{1/3} \quad (2.4)$$

The average pore diameter (D_{mean}) was calculated as the mean diameter of all the pores within the representative volume element:

$$D_{\text{mean}} = \frac{\sum_{i=1}^M D_i}{M} \quad (2.5)$$

where M is the number of pores in the representative volume element. The weighted average pore diameter (D_{wt}) was calculated as:

$$D_{wt} = \frac{\sum_{i=1}^M D_i V_i}{\sum_{i=1}^M V_i} \quad (2.6)$$

where the individual pore volumes (V_i) are the weighting factors. Porosity of the representative volume element (ε) was calculated as:

$$\varepsilon = \frac{\sum_{n=1}^T \sum_{i=1}^{M_n} A_{v,n,i}}{\sum_{n=1}^T \sum_{i=1}^{M_n} A_{v,n,i} + \sum_{n=1}^T A_{s,n}} \quad (2.7)$$

where $A_{v,n,i}$ is the pore area on slice n , M_n is the total number of pores on a slice, $A_{s,n}$ is the total solid area on a slice and T is the number of slices in a representative volume element. The average cell wall thickness (t_{wall}) was evaluated as:

$$t_{wall} = \frac{\sum_{n=1}^R A_{s,n} / \sum_{i=1}^{M_n} P_{n,i}}{R} \quad (2.8)$$

where $P_{n,i}$ is the total perimeter of pore i in slice n . Fractional cell interconnectivity (φ) was calculated as:

$$\varphi = \frac{\sum_{i=1}^M \left(\frac{\sum_{n=1}^{N_i} l_{n,i}}{\sum_{n=1}^{N_i} P_{n,i}} \right)}{M} \quad (2.9)$$

where $l_{n,i}$ is the total length of line segments needed to close the discontinuous portions on each pore i in slice n , $P_{n,i}$ is the total perimeter of pore i in slice n , N_i is the number of slices that pore i appears and M is the number of pores in the representative volume element. Weighted fractional cell interconnectivity was evaluated as:

$$\varphi_{wt} = \frac{\sum_{i=1}^M \left(\frac{\sum_{n=1}^{N_i} l_{n,i}}{\sum_{n=1}^{N_i} P_{n,i}} \right) V_i}{\sum_{i=1}^M V_i} \quad (2.10)$$

where the pore volumes (V_i) are the weight factors. Polydispersity index (I) was calculated as

$$I = \frac{\sum_{r=1}^R D_r N_r / \sum_{r=1}^R N_r}{\sum_{r=1}^R D_r^2 N_r / \sum_{r=1}^R D_r N_r} \quad (2.11)$$

2.3 Applications of Pore Structure Analysis to Food Materials

Quantitative pore structure analysis has been widely used to evaluate the micro-structure of porous foods and lately 3-D imaging techniques are applied to study the pore structure of foods, and to evaluate the effect of processing on structure-related characteristics of food materials.

Drying studies followed by tomographic image analysis showed that increase in the drying temperature resulted in an increase in porosity due to the development of higher pressure gradients within the sample resulting in higher rates of moisture loss (Leonard et al. 2008). This semi-quantitative approach can be used to compare the pore structures developed during various drying techniques. Continuous monitoring of the pore structure coupled to a pore network model can be used to control drying so as to manufacture dried food products with the optimum pore structure characteristics.

Baked and extruded foods are characterized by pore sizes within a few orders of magnitude (Dogan et al. 2013; Kocer et al. 2007; Hicsasmaz et al. 2003). Thus, it is very difficult to obtain objective quantitative results. Large range of pore sizes result in large specimen-to-specimen variations requiring a vast number of 2-D sections to be analyzed. Non-invasive image acquisition offers vast opportunities for objective quantitative evaluation of baked and extruded food materials.

Images of baked and extruded foods obtained by light microscopy (Kocer et al. 2007; Hicsasmaz et al. 2003) and X-ray microtomography (Trater et al. 2005) showed a bimodal distribution of pore sizes in which larger pores were located towards the interior, while relatively smaller pores were abundant towards the crust. This is due to the faster cooling of the crust since the temperature gradient at the interface with the ambient is very high leading to high convective heat transfer rates, thus faster cooling and setting in the crust region.

Similar behavior was observed in Supercritical Fluid Extrusion (SCFX) extrudates with practically a non-porous outside layer, a large number of small cells towards the surface, and a small number of large cells towards the center. SCFX is a process in which supercritical CO₂ (SC-CO₂) is used as the blowing agent instead of steam (Cho and Rizvi 2009). CO₂ loss mainly happens from the surface and a high concentration gradient at the interface with the ambient causes the rate of CO₂ loss to increase. After the initial stage of CO₂ loss the SCFX surface acts like a seal, since diffusion distance through the surface increases due to a large number of small cells. This further allows the CO₂ bubbles towards the center to increase in size forming fewer pores with larger size. SC-CO₂ forms gas bubbles at the die exit upon expansion to the ambient. Bubbles towards the center expand freely, whereas the nuclei at the surface are restricted by the die surface. Besides, nuclei towards the center have more time to expand (Potente et al. 2006). Finally, SCFX extrudates require post-processing treatment, since SCFX is a low temperature processes that does not allow structure setting. Setting occurs during

drying upon simultaneous heat and mass transfer. The surface sets first, limiting bubble growth at the surface.

Images of 2-D slices of SCFX-extrudates obtained by X-ray microtomography showed that the pores are elongated in the longitudinal direction, whereas they are spherical in the radial direction (Cho and Rizvi 2009). This finding is also true for steam extrudates (Karkle et al. 2012; Robin et al. 2010; Trater et al. 2005), and probably occurs due to the orientation of the shear field as the screw pushes the material through the barrel towards the die and out of the die to the ambient resulting in elastic recovery (Moraru and Kokini 2003; Pai et al. 2009). Image analysis on 3-D reconstructed images of SCFX extrudates showed that increase in the volumetric flow rate of the melt due to an increase in SC-CO₂ level caused this type of anisotropy to increase. Anisotropy diminished with addition of fibrous materials into the feed mix (Karkle et al. 2012; Parada et al. 2011). This is because elastic recovery at the die exit reduces, and alignment of the fibers in the direction flow hinders radial extension.

Porosity of baked and extruded products increases and their bulk density decreases with increasing expansion (Dogan et al. 2013; Kocer et al. 2007; Trater et al. 2005; Hicsasmaz et al. 2003). More expanded products are characterized by large pore sizes and lower polydispersity indeces (Kocer et al. 2007; Trater et al. 2005) due to the coalescence of the gas bubbles promoting interconnectivity. 3-D image analysis by Trater et al. (2005) showed that coalesce of gas bubbles upon expansion causes a decrease in the number of pores. Addition of fiber into the feed mixture caused a larger number of cells, but with similar expansion characteristics (Karkle et al. 2012; Parada et al. 2011). Hindered radial expansion by fiber alignment favored more nucleation sites (Chanvrier et al. 2007) increasing the number of cells, but decreasing cell diameters as observed from 2-D sections of X-ray microtomography (Karkle et al. 2012). On the other hand, both the number of pores and the pore size increased by addition of proteins in the extrusion feed mix (Mesa et al. 2009; Cheng et al. 2007). Protein-protein interactions strengthen cell walls supporting expansion, while foaming properties of proteins lead to more nucleation sites for water vapor increasing the number of pores.

Quantitative analysis of X-ray microtomography images (Trater et al. 2005) showed that the cell wall thickness increases with expansion which is contrary to the conventional logic that the cell walls should stretch and get thinner with increasing expansion. This finding is supported by the fact that cell wall thickness increases as the number of pores decrease due to the coalescence of gas bubbles during expansion. This indicates that contractile forces due to surface tension and elasticity cause the ruptured walls to be absorbed into the intact cell walls causing the cell wall thickness to increase. Quantitative image analysis of 3-D reconstructed images (Trater et al. 2005) indicated low degree of pore interconnectivity. Contrary to Trater et al. (2005), Cho and Rizvi (2009) found that cell walls become thinner as expansion increases. This is probably due to the use of different image analysis software and methodology. Measurement of microstructure on 3-D reconstructed images from X-ray microtomography depends on the capabilities of

the image analysis software besides standardization and validation of the methodology.

Image analysis on 3-D reconstructed images of SCFX extrudates (Cho and Rizvi 2009) showed that increase in the volumetric flow rate of the melt due to an increase in SC-CO₂ level caused more pore interconnectivity. Similar results were obtained for steam extrudates indicating that increase in expansion leads to the increase in pore size (Dogan et al. 2013; Trater et al. 2005).

Pore Structure in Food

Simulation, Measurement and Applications

Gueven, A.; Hicsasmaz, Z.

2013, VIII, 50 p. 8 illus., Softcover

ISBN: 978-1-4614-7353-4