Use of non-invasive X-ray microtomography for characterizing microstructure of extruded biopolymer foams

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Abstract

Understanding foam microstructure formation is important for a priori design and engineering of new biopolymer-based products for both food and industrial applications. However, this has been hindered by unavailability of an imaging technology to characterize the cellular structure of foams accurately. This study investigated a non-invasive imaging technology, X-ray microtomography (XMT), for visualization and measurement of microstructural features of biopolymer foams. Brittle corn starch foams with two levels (5% and 15%) of whey protein concentrate (34% protein) factorialized with two moisture contents (26% or 34%) were produced using extrusion. XMT allowed non-invasive imaging of sample cross-sections at various depths, and facilitated accurate and hitherto impossible measurements of features like true cell size distribution (bi-modal), average diameter (0.58 to 2.27 mm), open wall area fraction (0.068 to 0.099), cell wall thickness (0.09 to 0.15 mm), and true void fraction (0.63 to 0.84). Results indicated XMT is superior to conventional imaging techniques for characterizing foam microstructure.

Keywords: X-Ray; Microtomography; Microstructure; Biopolymer; Foams; Imaging; Extrusion

1. Introduction

1.1. Foaming of biopolymeric materials

Foaming, or incorporation of bubbles in materials, has a wide range of applications. Foams of industrial materials like plastics or metals are used for producing lighter structures, saving on material costs, engineering better acoustic and thermal insulation properties, improving structural strength, preventing fracture propagation and providing a cushioning effect. Foaming is undertaken in food (or biological) materials as well, for a variety of reasons. In food materials, foaming is integral to introducing zest and zip to beverages (beer, champagne, soft drinks, etc.), improving aroma (cappuccino coffee), and creating the right texture (ice cream, whipped cream, bread, cereal, etc.) (Niranjan, 1999). Several food foams, such as puff-dried fruits and vegetables, popcorn, breads, cakes, puffed breakfast cereals, and expanded snack products, have matrices consisting of biopolymers like starch, protein and cellulose. This is a very important category of value-added foods, and a general term for such products is biopolymer foams. Despite several innovations in foaming technology for biopolymers, a basic understanding of the dynamics of structure formation is lacking (Campbell & Mougeot, 1999), and a priori design of products continues to be illusive and at best intuitive. This is true not only for traditional food foams, but also for biopolymer-based industrial foams where precise control of microstructure is crucial.
Steam-based extrusion has gained widespread use in the last several decades for continuous processing of a variety of biopolymer foams, ranging from snacks and breakfast cereals for human consumption to pet-food and biodegradable packaging materials (Della Valle, Vergnes, Colonna, & Patria, 1997; Fang & Hanna, 2000; Kokini, Chang, & Lai, 1992). This process involves application of a combination of heat, moisture and shear to a mix of biopolymers, like starch and protein, to form a viscoelastic melt in the extruder barrel. This melt is pressurized in the barrel, heated to temperatures above 100 °C and forced to exit the extruder die, where a sudden pressure drop rapidly converts the pressurized liquid water into steam. This causes the melt to explosively expand and attain a cellular structure. Extrudate structure is stabilized as the matrix transforms to a glassy state on cooling and drying. More recently, another technology called super-critical fluid extrusion (SCFX) has been developed for continuous processing of novel biopolymer foams. SCFX utilizes supercritical CO₂ as the blowing agent rather than steam.

The dynamics of biopolymer foaming during extrusion, and the resultant foam microstructural features (such as average cell size and cell size distribution, cell wall thickness, and open wall area ratio) control product attributes like void fraction, water absorption, flavor retention, and most significantly, mechanical strength or texture (Djelveh, Cornet, & Gros, 1999; Gibson & Ashby, 1997; Niranjan, 1999; Wilkinson, Dijksterhuis, & Minekus, 2000). However, just like other foaming methods for biopolymers, extrusion processing is still considered to be more an ‘art’ than ‘science’, and one of the primary reasons of this gap in scientific understanding is the lack of a suitable imaging technology that can help in characterizing the cellular structure of extruded foams in an objective and accurate manner. This study focused on addressing this challenge by evaluating X-ray microtomography (XMT) as a novel imaging technique for effectively characterizing microstructure of starch-based foams.

1.2. Traditional imaging methods versus XMT

Important microstructural features of any foam include cell size distribution, average cell wall thickness, average number density of cells and presence or absence of an interconnected network of cells (often measured as open cell number or volume fraction). However, few studies have attempted to study the microstructure of food foams in an objective manner. More often than not, in the case of biopolymer foams, researchers report a few cross-sectional images and discuss the microstructure qualitatively without actually making any quantitative measurement of key features (Autio & Salmenkallio-Martilla, 2001; Gropper, Moraru, & Kokini, 2002; Lai, Davis, & Hoseney, 1985; Lee, Ryu, & Lim, 1999; Owusu-Ansah, van de Voort, & Stanley, 1984). Microstructure has remained a gray area mainly because of the inadequacy of current imaging techniques like digital video imaging, light microscopy and scanning electron microscopy (SEM). These techniques are 2-D and destructive in nature because sample preparation involves cutting to expose the cross-section to be

<table>
<thead>
<tr>
<th>Nomenclature</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>List of symbols</td>
<td></td>
</tr>
<tr>
<td>$\theta$</td>
<td>open wall area ratio</td>
</tr>
<tr>
<td>$\theta_{wt}$</td>
<td>weighted open wall area ratio</td>
</tr>
<tr>
<td>$\rho_c$</td>
<td>piece density of expanded extrudate</td>
</tr>
<tr>
<td>$\rho_s$</td>
<td>density of solid material (cell walls) in extrudate</td>
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<tr>
<td>$v$</td>
<td>imaging void fraction measured via XMT</td>
</tr>
<tr>
<td>$v'$</td>
<td>physical void fraction measured via extrudate densities</td>
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<tr>
<td>$A_{s,m}$</td>
<td>cross-sectional solid area of slice $m$</td>
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<td>$A_{v,m,i}$</td>
<td>cross-sectional void area of cell $i$ in slice $m$</td>
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<td>$D$</td>
<td>average cell diameter</td>
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<tr>
<td>$D_i$</td>
<td>diameter of cell $i$</td>
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<tr>
<td>$D_r$</td>
<td>average diameter of cells in the $r$th range of cells</td>
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<tr>
<td>$D_{wt}$</td>
<td>weighted average cell diameter</td>
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<tr>
<td>$f_o$</td>
<td>open cell volume fraction</td>
</tr>
<tr>
<td>$I$</td>
<td>total number of cells in the VOI</td>
</tr>
<tr>
<td>$I_{m}$</td>
<td>total number of cells in the slice $m$</td>
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<td>$I_{m,i}$</td>
<td>total length of line segment(s) used to close discontinuous portions cell $i$ in slice $m$</td>
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<tr>
<td>$M_i$</td>
<td>number of slices on which cell $i$ appears</td>
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<tr>
<td>$N_{cell}$</td>
<td>cell density (cells/cm³ of unexpanded material).</td>
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<tr>
<td>$N_r$</td>
<td>number of cells in the $r$th range of cells</td>
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<td>$P_{index}$</td>
<td>polydispersity index</td>
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<td>$P_{m,i}$</td>
<td>total perimeter of cell $i$ in slice $m$</td>
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<td>$V_i$</td>
<td>total interior volume of cell $i$</td>
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<td>wet basis</td>
</tr>
<tr>
<td>$X_w$</td>
<td>moisture content</td>
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Important microstructural features of any foam include cell size distribution, average cell wall thickness, average number density of cells and presence or absence of an interconnected network of cells (often measured as open cell number or volume fraction). However, few studies have attempted to study the microstructure of food foams in an objective manner. More often than not, in the case of biopolymer foams, researchers report a few cross-sectional images and discuss the microstructure qualitatively without actually making any quantitative measurement of key features (Autio & Salmenkallio-Martilla, 2001; Gropper, Moraru, & Kokini, 2002; Lai, Davis, & Hoseney, 1985; Lee, Ryu, & Lim, 1999; Owusu-Ansah, van de Voort, & Stanley, 1984). Microstructure has remained a gray area mainly because of the inadequacy of current imaging techniques like digital video imaging, light microscopy and scanning electron microscopy (SEM). These techniques are 2-D and destructive in nature because sample preparation involves cutting to expose the cross-section to be
viewed, which can alter structural features. Also, the 2-D image of a sample cross-section does not give accurate information on cell size distribution, as cells are generally sliced off-center and the diameters measured from the image depend on the depth of cut (Campbell & Mougeot, 1999; Scanlon & Zghal, 2001).

Apart from cell size distribution, which is probably the single most important microstructural feature of foams, the degree of interconnectedness between cells and average cell wall thickness also significantly impact important properties like mechanical strength, bulk infusibility of liquids, and effective diffusivity of gases or liquids. However these features are impossible to ascertain using destructive, 2-D imaging techniques. Indirect techniques like gas (air, nitrogen or helium) pycnometry have been used to measure the open cell volume fraction ($f_o$, ratio of volume of interconnected pores to total pore volume) of various biopolymer foams (Bhatnagar & Hanna, 1996; Hicsasmaz & Clayton, 1992; Jones, Chinnaswamy, Tan, & Hanna, 2000). These studies reported predominantly interconnected and open cell structures for white bread ($f_o > 97\%$), butter cookies ($f_o > 84\%$) and cereal foams expanded by extrusion or gun puffing ($f_o > 84\%$). However, this technique can lead to many inaccuracies in measurement. For example, adjoining cells that are closed and not interconnected might be counted as interconnected if there is a small crack in the cell wall, allowing gas to penetrate. In addition, the pressure applied for penetration of gas into the porous solid might cause further cracks in the walls, especially when cell walls are very thin and fragile, as in highly expanded extrusion puffed foams.

Another problem in SEM or optical imaging is obtaining adequate contrast between air and solid phases, for which the ‘lighting’ and angle of illumination play an important role. Often, segmentation techniques like image subtraction and edge detection are employed to enhance distinction between the two phases and extract relevant features from any image. These techniques use mathematical treatments like Fourier transforms and other algorithms which are not only very complex and require a large amount of computational time, but might lead to incorrect or varying results depending on the treatment applied (Barrett & Peleg, 1992; Barrett, Normand, Peleg, & Ross, 1992; Gao, Tan, Shatatdah, & Heymann, 1999; Hall & Bracchini, 1997; Smolarz, Van Hecke, & Bouvier, 1989; Tan, Zhang, & Gao, 1997).

To overcome the drawbacks of previously-used methods, non-invasive imaging technologies need to be explored for characterizing biopolymeric foam microstructure. Low resolution X-ray tomography or computerized axial tomography (CAT scanning) is widely used in medicine to image body tissues in a non-invasive manner. This technology has its origins in the 1970s and conventionally has a resolution of $\sim 100 \mu m$, which is not sufficient to explore the microstructure of many types of foam.

In the past few years, use of very high energy X-rays from high intensity synchrotrons, or other sources, has allowed resolutions of 1 $\mu m$ and enabled the use of XMT in other fields like geology and metallurgy for visualization of internal microstructure of very opaque objects like rocks or metallic foams (Coker, Torquato, & Dunsmuir, 1996; Nieh, Kinney, & Wadsworth, 1998). In order to distinguish it from CAT scanning, this technology will be referred to as high-resolution XMT. Some researchers have used CAT scanning or XMT for imaging the microstructure of food foams, but with very limited results (Falcone et al., 2004; van Dalen, Blonk, van Aalst, & Hendriks, 2003; Whitworth & Alava, 1999).

Two types of XMT are common, one based on absorbance of X-rays and the other based on the phase shift (or phase contrast) of incident X-rays produced by an object (Falcone et al., 2004; Snigirev, Snigireva, Kohn, Kuznetsov, & Schelokov, 1995). This study focused on evaluation of the absorbance XMT technique to determine microstructural features of expanded extrudates. The general methodology involves targeting the specimen with a polychromatic X-ray beam with high spatial coherence. The X-rays not absorbed by the specimen fall on specially-designed X-ray scintillators that produce visible light, which is then recorded by a charge-coupled device (CCD) camera. A tomographic scan is accomplished by rotating the specimen 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 back-projection software that maps estimates of attenuation coefficients, which depend on density variations within an object. 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. Manipulation of XMT data using special software also allows reconstruction of cross-sections at depth increments as low as 1 $\mu m$, and along any desired orientation of the ‘plane of cut’. A series of non-invasive XMT slices of the same sample in any direction can provide much more information than just one SEM or optical imaging picture. The true 3-D shape of the cells can also be visualized from its 2-D slices.

It is clear from the above discussion that XMT is an important tool that holds great potential for imaging biopolymeric or food foam structures. The objective of this work was to evaluate the use of XMT and develop methodology for characterizing microstructure features (such as cell diameter distribution, degree of inter-
connectivity of cells, cell wall thickness, and void fraction) in extruded biopolymer foams.

2. Materials and methods

2.1. Materials

Native corn starch (≈25% amylose, Cargill Gel 03547, Cargill, Inc., Minneapolis, MN) was used as the base ingredient for all extrusion runs. Whey protein concentrate with 34% protein (WPC-34) was obtained from Foremost Farms USA (Baraboo, WI).

2.2. Extrusion

A Wenger TX-52 twin-screw extruder (Wenger Manufacturing, Sabetha, KS) equipped with a 3.3 mm circular die was used to extrude all materials. Prior to extrusion runs, the extruder was calibrated to ensure that selected water injection and feed rates were accurate. Before collecting samples for each treatment, extrusion conditions were allowed to stabilize for approximately 10 min. Once the conditions stabilized, data and samples were collected for 3 to 5 min per run. Samples were dried at 100 °C for 10 min immediately following extrusion, and were subsequently cooled with ambient air.

2.3. Experimental design and statistics

A simple 2 × 2 factorial design with 3 replicates was used for producing brittle extruded foams with varying microstructure. The treatments comprised of two levels (26% and 34%, wb) of in-barrel moisture content (Xw) and two levels (5% and 15%) of WPC-34 in the feed mix. The XMT scans and analyses (in duplicate) were performed on only one of the three replicates. Calculation of standard deviation (SD) and coefficient of variation (CV) for imaging data were performed using Microsoft Excel. The SAS System for Windows (Version 8.2; SAS Institute, Cary, NC) was used to determine correlations between microstructural parameters.

2.4. Image acquisition and processing

A desk-top XMT system (Model 1072, 20–100 kV/0–250 μA, SkyScan, Aartselaar, Belgium) set at 40 kV/100 μA (to obtain optimum contrast between solid and gaseous phases) was used to scan all samples. Maximum sample field of view for this instrument was 20 mm × 20 mm, and maximum resolution less than 5 μm. The sample size (height ranging from 10 to 18 mm) for this study resulted in feature resolution between 10 and 20 μm for all samples. A 12-bit, 1024 × 1024 pixel, cooled CCD camera was used to collect the X-ray data. The initial X-ray radiographs or raw images (Fig. 1(a)) were obtained by placing the sample on a stage and rotating it through 180° (0.9° per image capture). Image reconstruction was accomplished using Volumetric Reconstruction for MicroCT Instruments (Version 2.1) software provided by Skyscan. This reconstruction software used a filtered back-projection algorithm utilizing cone-beam (Feldkamp) reconstruction, with a reconstruction time of 4.7 s per cross-section. A set of 2-D images, or “slices” of infinitesimal thickness, taken perpendicular to the axis of extrusion and covering the entire cylindrical sample was obtained after reconstruction. A representative slice is shown in Fig. 1(b). Due to their time-consuming and cumbersome nature, all measurements were limited to an appropriate volume of interest (VOI) consisting of 15 consecutive

![Raw image](image1)

![Representative slice after reconstruction](image2)

![Volume of interest](image3)

Fig. 1. Schematic showing the construction of the VOI for XMT measurements.
slices from the central portion of each sample (Fig. 1(c)). Each VOI contained the entire sample cross-section, and ranged from 1.6 to 7.2 mm in height, depending on size of cells (larger cells required more height to cover entire cells). This VOI, with the slices separated by a constant distance \( t \) (dependent upon sample size), was used for all further analyses. The number of cells analyzed ranged between 33 and 63, depending on cell size (for samples with larger cells, fewer numbers of cells were analyzed). Thresholding was carried out on each 2-D image, with a threshold value of 35 set to differentiate between void volume and solid material. Subsequently, a median rank filter was applied in a 3 \times 3\) pixel matrix area using two to three iterations, depending on image quality. Filtering was followed by a smoothing operation and then measurements were carried out on image features.

### 2.5. Measurement of microstructural features

For sample cross-sections exposed by each slice (\( m \)), 2-D cell perimeters (\( P_{m,i} \)), 2-D cell void areas (\( A_{v,m,i} \)), and total solid area (\( A_{s,m} \)) were obtained using image analysis software (Scion Image for Windows\textsuperscript{\textregistered}, Scion Corp., Frederick, MD). Three-dimensional data were obtained by integrating the 2-D data over the 15 consecutive slices in the VOI. Calculations of various microstructural features are described in detail below.

**Cell volume** \((V_i)\). Interior volumes of cells contained in the VOI were calculated by integrating the 2-D cell void areas \( (A_{v,m,i}) \) over the VOI.

\[
V_i = t \times \left( \sum_{n=1}^{M_i-1} A_{v,m,i} + \frac{A_{v,m,i+1} + A_{v,m,i+2}}{2} \right) + \frac{t}{3} (A_{v,1,i} + A_{v,M_i,i}), \quad (1)
\]

where \( A_{v,m,i} \) is the 2-D void area of cell \( i \) in slice \( m \); \( t \), distance between slices (constant for any given sample), and \( M_i \), number of slices on which cell \( i \) appears (\( \leq 15 \)).

As the VOI was a subset of the total product volume, portions of some cells were not accounted for while calculating their volume. As shown in Fig. 2, fractional cells were cells that had \( \leq 1/2 \) of their estimated total volume present in the VOI. Partial cells were defined as those cells that had \( >1/2 \) of their estimated total volume contained in the VOI, but were not wholly contained in the VOI. Volume measurements made on all fractional and partial cells within the VOI were reported as whole cell volumes for sake of simplicity.

**Cell diameter** \((D_i)\). Interior diameters of individual cells based on the void volume of the cell were calculated assuming spherical geometry.

\[
D_i = 2 \times \left( \frac{3V_i}{4\pi} \right)^{1/3}. \quad (2)
\]

**Average cell diameter** \((\bar{D})\). Average cell diameter for any sample was calculated as the arithmetic average of diameters of all cells within the VOI.

\[
\bar{D} = \frac{\sum_{i=1}^{I} D_i}{I}, \quad (3)
\]

where \( I \) is the total number of cells in the VOI.

**Weighted average cell diameter** \((\bar{D}_w)\). Weighted average cell diameter for any sample was calculated by weighting each cell diameter with the corresponding cell volume.

\[
\bar{D}_w = \frac{\sum_{i=1}^{I} D_i V_i}{\sum_{i=1}^{I} V_i}. \quad (4)
\]

**Imaging void fraction** \((v)\). The ratio of void volume of the sample to that of the total volume was calculated from 2-D void area and solid area data integrated over the whole VOI.

\[
v = \frac{\sum_{m=1}^{15} \sum_{i=1}^{M_i} A_{v,m,i} + \sum_{m=1}^{15} A_{s,m}}{\sum_{m=1}^{15} \sum_{i=1}^{M_i} A_{v,m,i} + \sum_{m=1}^{15} A_{s,m}}, \quad (5)
\]

where \( A_{v,m,i} \) is the 2-D void area of cell \( i \) in slice \( m \); \( I_m \), total number of cells in slice \( m \), and \( A_{s,m} \), total 2-D solid area of slice \( m \).

**Average wall thickness** \((t_{wall})\). Average cell wall thickness for any sample was calculated from 2-D solid area and 2-D cell perimeter data integrated over the VOI.

\[
t_{wall} = \frac{\sum_{m=1}^{15} (A_{s,m}/P_{m,i})}{15}, \quad (6)
\]

where \( P_{m,i} \) is the total 2-D perimeter of cell \( i \) in slice \( m \).

**Open wall area fraction** \((\theta)\). The ratio of open or broken cell wall area of any sample to total cell wall area was calculated from 2-D measurements on cell perimeters integrated over the whole VOI.

\[
\theta = \frac{1}{I} \sum_{i=1}^{I} \left( \frac{\sum_{j=1}^{M_i} l_{m,j}}{\sum_{j=1}^{M_i} P_{m,i}} \right), \quad (7)
\]

where \( l_{m,i} \) is the total length of the line segment(s) used to close the discontinuous portion(s) of 2-D perimeter of cell \( i \) in slice \( m \) (Fig. 3).
Weighted open wall area fraction \( (\theta_{\text{wt}}) \). Weighted open wall area fraction was calculated by weighting each cell open area with the cell volume.

\[
\theta_{\text{wt}} = \frac{\sum_{i=1}^{j} \left( \sum_{r=1}^{R} \frac{\ell_{w,i}}{D_{r} N_{r,i}} \right) V_{i}}{\sum_{i=1}^{j} V_{i}}.
\]

Polydispersity index \( (P_{\text{index}}) \). This parameter was used as a measure of the uniformity of cell size distribution \((\text{Goel} \& \text{Beckman, 1994})\). \( P_{\text{index}} \) varies between 0 and 1, with a value of 1 representing a completely homogeneous cell size distribution.

\[
P_{\text{index}} = \left( \frac{\sum_{r=1}^{R} D_{r} N_{r}}{\sum_{r=1}^{R} N_{r}} \right)^{-1} \left( \frac{\sum_{r=1}^{R} D_{r} N_{r}}{\sum_{r=1}^{R} D_{r} N_{r}} \right)^{-1},
\]

where \( R \) is the number of ranges over which the spread of cell diameter values were divided; \( D_{r} \) average diameter of cells in the \( r \)th range of cells, and \( N_{r} \) number of cells in \( r \)th range.

2.6. Physical analysis of samples

**Moisture content.** Moisture content of samples was obtained by drying 1 g of ground product in a forced air oven for 24 h.

**Piece density** \( (\rho_{p}) \). Individual volumes of 10 pieces were first calculated from their respective lengths, and major and minor diameters (assuming a cylindrical geometry with elliptical cross-section). Piece density \( (\rho_{p}) \) was then obtained by dividing mass of each piece by its volume, and averaging over the 10 pieces. Extrudate solid density \( (\rho_{s}) \) was obtained by measuring the volume of a known mass of ground extrudate with a helium pycnometer (Model NVP-1, Quantachrome, Boynton Beach, FL), and dividing the extrudate mass by its measured volume. All density measurements were adjusted to 0% moisture basis to eliminate effects of water on density.

**Physical void fraction** \( (v') \). For comparison with imaging void fraction \( (v) \), physical void fraction was calculated.

\[
v' = \frac{(\rho_{p} - \rho_{s})}{\rho_{s}}.
\]

Cell density \( (N_{\text{cell}}) \). \( N_{\text{cell}} \) is an indirect measure of number of cells per unit volume of unexpanded extrudate \((\text{Shimbo, Baldwin,} \& \text{Suh, 1995})\).

\[
N_{\text{cell}} = \frac{(\rho_{p}/\rho_{s}) - 1}{\pi D_{r}^3/6}.
\]

**Scanning electron microscopy.** As a reference, scanning electron microscopy (SEM) was performed on four of the samples that were previously scanned using XMT. Prior to SEM analysis the samples were cut using a razor blade and sputter coated with gold-palladium using a Desk II Sputter/Etch Unit (Denton Vacuum, LLC, Moorestown, NJ). The SEM used was model S-3500N, with an absorbed electron detector model S-6542 (Hitachi Science Systems, Ltd., Hitachinaka, Ibaraki Pref 312-0033, Japan).

3. Results and discussion

Typical cross-sections obtained by XMT for the four treatments under consideration are shown in Fig. 4. Three-dimensional imaging measurements of microstructural parameters, obtained from integrating 2-D data over the VOI, are shown in Table 1. Coefficients of correlation between various parameters are listed in Table 2. Table 3 shows the standard deviation (SD) and coefficient of variation (CV) for the microstructural parameters as measures of variability between duplicate samples from the same treatment.

3.1. Comparison between XMT and SEM imaging

SEM images of exposed cross-sections of the same samples are shown in Fig. 5. From visual inspection, it was apparent that XMT images were much better suited for image analysis due to their sharp depth of focus and greater contrast between solid and void areas in the cross-section. Moreover, the physical cutting of the samples for obtaining SEM images led to partial damage as indicated by the cracks and open walls in the images.

The greatest advantage of XMT, however, was the ability to generate multiple 2-D cross-sections, which facilitated imaging measurements at incremental depths. Fig. 6 shows the change in cross-sectional area for a few cells with increasing depth of slice within the VOI for a typical treatment. The manner in which cells were
microscopy would be inadequate to accurately characterize the true 3-D cell size distribution of the foam.

When corrections were made to account for the inaccuracies arising from partial or fractional cells within the VOI, estimates of cell size ($D$ and $D_{wt}$) were larger (up to 12%) although trends remained the same. Trends among other parameters measured (except $\theta$ and $\theta_{wt}$) were also maintained when corrections were applied. However, these corrections led to a significant portion ($V_{error}$ of 9.9% to 39%) of the void volume within the VOI being excluded (Table 1). Due to the above reasons and their complicated nature, it was concluded that these corrections need not be applied to XMT measurements of cell structure.

### 3.2. Cell diameter distributions

Cell diameters ($D$) for all treatments had a bi-modal distribution (Fig. 7). This was confirmed by a visual inspection of the 2-D slices (Fig. 4) that indicated a preponderance of larger cells in the interior of the extrudates, as compared to smaller cells in the exterior. This might be a result of a combination of post-extrusion convective heat transfer and differential diffusion of steam to the ambient, which would lead to faster setting of cell structure and lesser expansive force closer to the extrudate surface. Alavi, Gogoi, Khan, Bowman, and Rizvi (1999) observed similar results for extrudates expanded by supercritical CO$_2$, which had relatively large interior cells surrounded by a layer of smaller exterior cells.

Average cell diameter ($\bar{D}$) and weighted average cell diameter ($D_{wt}$) ranged between 0.582 and 2.27 mm, and 0.793 and 4.97 mm, respectively (Table 1). The poly-

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Table 1

<table>
<thead>
<tr>
<th>WPC, %</th>
<th>$X_{wt}$, %</th>
<th>$\bar{D}$, mm</th>
<th>$D_{wt}$, mm</th>
<th>$\theta_{wt}$</th>
<th>$t_{wall}$, mm</th>
<th>$P_{index}$</th>
<th>$v_i$</th>
<th>$v_p$</th>
<th>$V_{error}$</th>
<th>$N_{cell}$, cells/cm$^3$(×10$^4$)</th>
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<td>0.15</td>
<td>0.76</td>
<td>0.71</td>
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<td>4.97</td>
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<td>0.58</td>
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<td>0.099</td>
<td>0.086</td>
<td>0.09</td>
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Table 2

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<th>$N_{cell}$</th>
<th>$D$</th>
<th>$D_{wt}$</th>
<th>$\theta$</th>
<th>$\theta_{wt}$</th>
<th>$t_{wall}$</th>
<th>$P_{index}$</th>
<th>$v_i$</th>
<th>$v_p$</th>
<th>$V_{error}$</th>
<th>$N_{cell}$</th>
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<tr>
<td>$N_{cell}$</td>
<td>$-0.82^a$</td>
<td>$-0.72^a$</td>
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<td>0.75$^a$</td>
<td>$-0.82^a$</td>
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<td>$v_i$</td>
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<td>0.93$^a$</td>
<td>$-0.12$</td>
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<td>$-0.81^a$</td>
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<tr>
<td>$P_{index}$</td>
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<td>$-0.83^a$</td>
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<td>$-0.18$</td>
<td>$-0.76^a$</td>
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<tr>
<td>$t_{wall}$</td>
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<td>$-0.59$</td>
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<tr>
<td>$\theta_{wt}$</td>
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<td>0.13</td>
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</tr>
<tr>
<td>$\theta$</td>
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<td>$-0.25$</td>
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<td>1</td>
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</table>

$^a$ Significant correlation ($p < 0.05$).
Table 3

Standard deviation (SD) and coefficient of variation (CV) for all imaging measures

<table>
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<tr>
<th>Measure</th>
<th>Treatment</th>
<th>5% WPC, 26% $X_w$</th>
<th>5% WPC, 34% $X_w$</th>
<th>15% WPC, 26% $X_w$</th>
<th>5% WPC, 34% $X_w$</th>
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<tr>
<td></td>
<td>SD</td>
<td>CV</td>
<td>SD</td>
<td>CV</td>
<td>SD</td>
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<td>$D$</td>
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<td>$D_{wt}$</td>
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<td>0.804</td>
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<td>$\theta$</td>
<td>0.042</td>
<td>52.2</td>
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<td>53.9</td>
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<td>$\theta_{wt}$</td>
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<tr>
<td>$t_{wall}$</td>
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<tr>
<td>$P_{index}$</td>
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<td>0.145</td>
<td>19.16</td>
<td>0.018</td>
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<td>5.73</td>
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<td>$N_{cell}$</td>
<td>142.8</td>
<td>6.91</td>
<td>732.6</td>
<td>21.76</td>
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</table>

Fig. 5. Scanning electron micrographs of extrudates. (a) 5% WPC, 26% $X_w$, (b) 5% WPC, 34% $X_w$, (c) 15% WPC, 26% $X_w$, (d) 15% WPC, 34% $X_w$.

Fig. 6. Evolution of cell areas over slices 1–9 for cells 2–5 and 19 from 5% WPC, 26% $X_w$ treatment. The XMT images below show cross-sections of cells 2, 3 and 19 as they appear on slices 3, 5 and 7.
dispersity index ($P_{\text{index}}$) for all treatments ranged between 0.679 and 0.899. Significantly negative correlations ($p < 0.05$) were observed between $P_{\text{index}}$ and $D$ (Table 2). This could be indicative of cell coalescence taking place in the extrudate leading to a great degree of non-uniformity in cells diameters, and thus lower $P_{\text{index}}$. Coalescence takes place when cells continue expanding and cell walls become progressively thinner until they rupture, thus combining adjacent cells into one. Schwartzberg, Wu, Nussinovitch, and Mugerwa (1995) had a similar observation with regards to evolution of cell structure during popcorn puffing.

The cell diameter distributions and $P_{\text{index}}$ data for extruded biopolymer foams obtained in this study are in contrast with the very limited literature data that is available, namely, Barrett et al. (1992) for steam-expanded and Alavi et al. (1999) for CO$_2$-expanded extrudates. The aforementioned studies reported uni-modal log-normal (steam) or normal (CO$_2$) cell diameter distributions, and $P_{\text{index}}$ much lower (0.29 for steam) or higher (>0.90 for CO$_2$) than that in current study. Although these studies utilized other raw materials and one of them had a different blowing agent, the contrast in cell size distributions in comparison with the present study was more likely because the earlier studies involved 2-D image analysis of just one slice from each sample.

### 3.3. Cell density and void fraction

Void fraction ($v$) calculated from image analysis ranged between 0.629 and 0.842, and had a strong correlation ($p < 0.05$) with cell diameter ($D$), although it was observed that a close to fourfold increase in $D$ led to only about 33% increase in $v$. Cell density ($N_{\text{cell}}$) ranged between $0.207 \times 10^4$ and $2.38 \times 10^4$ cells/cm$^3$, and was negatively correlated ($p < 0.05$) with $D$ and $v$. These data confirm the occurrence of cell coalescence, and indicated that simultaneous cell expansion and coalescence contributed to increase in cell size and reduction in number of cells, as overall sample expansion increased.

The imaging void fraction $v$ was in good agreement with the physical void fraction $v'$ (<2.0% difference) for low moisture treatments, but was substantially higher (>20% difference) than the latter for higher moisture treatments. Clearly, $v$ was a more accurate measure of void fraction than $v'$, because the former was direct measured from 3-D image analysis, while the latter was calculated indirectly from density data for expanded extrudates and solid matrix.

### 3.4. Cell wall thickness and open wall area fraction

Average cell wall thickness ($t_{\text{wall}}$) of the extrudates, as measured by 3-D XMT, ranged between 0.09 and 0.15 mm (Table 1). Wall thickness is a parameter rarely reported in studies related to biopolymer foams. Like other microstructural parameters, in the past, 2-D SEM images of foams were used to study cell wall thickness and results were limited to only qualitative comparisons between treatments due to the tedious nature of measurements. Nonetheless wall thickness is an important parameter to study and quantify, as it affects...
mechanical properties like compression modulus and crushing stress (Gibson & Ashby, 1997; Gogoi, Alavi, & Rizvi, 2000; Ryu, Neumann, & Walker, 1993). Gibson and Ashby (1997) used mathematical models based on cubical cell geometry to describe the role of wall thickness in determining various mechanical properties of foams. Ryu et al. (1993) observed that increasing amounts of shortening in extruded wheat flour produced extrudates with thicker cell walls and higher breaking strength, although cell size and expansion also appeared to be different.

In the current study, measurement of average wall thickness ($t_{\text{wall}}$) and its relationship with other microstructural parameters (Table 2) provided insights into the evolution of foam structure. $t_{\text{wall}}$ was positively correlated with cell diameter $D$, which was apparently contrary to conventional logic which dictates that as cells expand their walls or membranes would stretch and become progressively thinner until they rupture, coalesce with adjacent cells. In actuality, this might indeed be the case but $t_{\text{wall}}$ could still increase if ruptured walls absorbed into intact walls due to contractile forces caused by surface tension and elastic stresses, as reasoned by Schwartzberg et al. (1995). This reasoning is supported by the strong negative correlation ($p < 0.05$) of $t_{\text{wall}}$ with cell density $N_{\text{cell}}$, indicating that lower $N_{\text{cell}}$ or greater coalescence led to increased $t_{\text{wall}}$.

In general, the degree of inter-connectivity of cells impacts important properties of foams like mechanical strength, bulk infusibility of liquids, thermal and acoustic insulation, and effective diffusivity of gases or liquids. Open wall area fraction ($\theta$), which is a direct measure of the degree of inter-connectivity of the cellular structure, ranged from 0.068 to 0.099 for the starch-based extrudates examined in this study. The volume weighted open wall area fraction ($\theta_{\text{wt}}$) ranged from 0.086 to 0.120. These results indicated that the majority of cells were partially or completely closed, and contradicted the widely accepted notion based on previous studies that steam-based extrusion leads to highly inter-connected, open cell structures (Bhatnagar & Hanna, 1996; Hicsasmaz & Clayton, 1992; Jones et al., 2000). These studies principally relied on measurement of total open cell volume using gas pycnometry, which has inherent limitations and inaccuracies as discussed earlier. In a previous study on starch-based supercritical fluid extrudates, Gogoi et al. (2000) estimated that the majority of cells were closed (number fraction of 64–85%). However, the method used by Gogoi et al. (2000) was based on counting of cells that appeared to have open walls by visual observation of one SEM image from each sample, with no regard to the size of the open area.

It is apparent from Table 2 that $\theta$ and $\theta_{\text{wt}}$ did not correlate well with any of the other cell structural features including cell diameter, wall thickness, void fraction, and polydispersity index. Table 3 describes the variability (standard deviation SD and coefficient of variation CV) for all imaging measurements. CV for all measurements, except for $\theta$ and $\theta_{\text{wt}}$, was reasonably low (2.31–16.19%). The fact that measurements of cell $\theta$ and $\theta_{\text{wt}}$ had the highest variability (CV of 7.460–88.8%) may explain their lack of correlation with other measured parameters. The extent of cell wall rupture differs from one sample to another depending on local tensile and failure stresses, which in turn are a function of the composition of cell wall material and its moisture content (Schwartzberg et al., 1995). The exceptionally high variability in $\theta$ and $\theta_{\text{wt}}$ may reflect the inherent heterogeneity of material properties, which is a common feature of biological ingredients like starch and whey protein. The high variability and poor correlation of $\theta$ or $\theta_{\text{wt}}$ with other parameters might be resolved in future studies by increasing the number of samples per treatment on which XMT measurements are performed.

4. Conclusions

Results from this study indicate that X-ray microtomography presents a viable method of accurately determining the structure of food foams. XMT has several advantages over current imaging methodologies, as it is non-invasive, leads to good contrast between gas and solid phases thus aiding in measurements, and can provide 3-D visualizations of products. XMT was able to accurately capture several features of cellular structure, which was not possible with methods employed in the past. This imaging technology has the potential of providing new insights into internal structure of starch-based foams and would aid in understanding the dynamics of various foaming processes, and modeling of mechanical properties and other quality attributes important in various food and industrial applications.

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References


