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CONFOCAL LASER SCANNING MICROSCOPY IMAGING OF DEEP-FAT FRIED BATTER COATING

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ABSTRACT Porosity and pore size distribution are very important microstructural properties of fried foods needed in process optimization and product development. Confocal laser scanning microscopy provides a means of imaging food samples to qualify and quantify specific components identified by staining, and also produces images of higher resolution compared to the conventional light microscopy. The objectives of this study were to characterize pore properties and quantify fat distribution of deep-fat fried chicken nuggets batter coating using confocal laser scanning microscopy imaging. Chicken nuggets were fried at three temperatures namely 170, 180 and 190°C. Detached batter coatings were stained non-covalently with drops of 0.005% solution of Nile Blue A, cryosectioned to 60 µm and 2-D images were obtained at fluorescence and reflection modes of the microscope. The images were quantitatively analyzed for fat and pore characteristics. Fat distribution was significantly affected by frying temperature and time and it decreased within the depth of the coating's thickness. The correlation coefficients between fat distribution obtained from the image analysis and fat content obtained by the conventional method ranged between 0.60 – 0.79 and the relationship was only significant ($P<0.05$) at two temperatures, 180 and 190°C. Porosity ranged from 4.97 to 32.7% and was significantly influenced by frying temperature. Pore size varied between 1.2 and 523 µm. There was formation of small and big pores with frying time. The results show that fat distribution and pore properties of fried chicken nuggets batter coatings are influenced by the frying conditions, and that fat content can be predicted from fat distribution data at elevated frying temperatures.

Keywords: Porosity, Fat distribution, Stain, Confocal, Microscopy, Imaging.

INTRODUCTION Fried foods continue to attract much demand because of their peculiar organoleptic properties of such as good mouth-feel, distinct flavor, unique taste and palatability, which make them irresistible. Application of batter and breading coating is one of the means devised to reduce fat uptake during the frying operation aside from the fact that they add more value such as improved texture, appearance, taste and volume, to fried foods. Its mechanism of mass transfer control involves film formation on the substrate as a result of structural changes during heating. Batter and breading are made from combination of different ingredients including flours, starch, hydrocolloids, bread

crumbs, water and seasoning. Carbohydrate and protein constituents of the system gel when water and/or heat are applied. The structure that is developed during processing, to a large extent, defines some of the quality attributes of the coating system. Food structure can be characterized in terms of density, porosity, pore size distribution and specific volume (Aguilera, 2005; Boukouvalas et al., 2006). A good understanding of these physical properties of foods, especially at microscopic scale, forms the basis for optimization of the processes that lead to their formation and also sets the stage for development of new and higher quality products. Deep-fat frying itself, leads to structural modification which affects the mechanism of mass transfer. The significance of microstructural changes to oil absorption during frying has been discussed by Baumann & Escher (1995) and Mellema (2003).

There are a number of innovative approaches that are being applied in the study of food surface and its internal morphological details namely magnetic resonance imaging, X-ray computed tomography, atomic force microscopy, scanning electron microscopy and confocal laser scanning microscopy (CLSM) (Dürrenberger et al., 2001; Kawas & Moreira, 2001; Kim et al., 2007; Adedeji and Ngadi, 2009; Wagner et al., 2008). CLSM is used for acquiring images to differentiate macro and microscopic components of materials based on their fluorescence intensity, which could be natural (autoflurorescence) or imparted by application of stains. CLSM is a type of light microscopy (LM) but differs in that its source of illumination is a laser instead of visible light used in LM and the system has capability to acquire images at different depth within the sample, creating a means to build 3-D images. Examples of stains/dyes used in food application include Nile Blue, Nile Red and Congo Red, used for marking fat (van Dalen, 2002); Rhodamine B, Fluorescein-Isothiocynate (FITC) and Safranin O, are used for staining proteins (van de Velde et al., 2003); and carbohydrates are marked by FITC, and Safranin O in the absence of protein (Dürrenberger et al., 2001; Tromp et al., 2001). Structural images of food in 3-Dimension can also be acquired by CLSM in the reflection.

CLSM has been used to study fried foods microstructures by different authors. These studies are limited to subjective analysis and in some cases surface changes. Bouchon and Aguilera (2001) used CLSM to study fried potatoes and were able to show that oil is located in the interior pockets or around intact cells. Pedreschi and Aguilera (2002) also used CLSM to elucidate oil distribution and cell wall structure of fried potato. Bouchon et al. (2003) equally employed CLSM to ascertain that oil is mainly located in the crust region of fried potatoes. So far, there has not been any effort on application of CLSM for quantitative analysis of pore and fat distribution in fried batter coating. The objective of this study was to characterize microstructural properties and quantify fat distribution in deep-fat fried chicken nuggets batter coatings using confocal laser scanning microscopy.

MATERIALS AND METHODS

Commercially produced chicken nuggets were procured from a local manufacturer (Olymel, St-Jean-sur-Richelieu, QC, CA) and stored in the deep-freezer at -50°C prior to use. The composition of the breading/batter coating of the chicken nuggets included wheat flour, wheat crumbs, spices, guar gum and salt. They were placed in a refrigerator at 4°C for 4 h before frying. The dye used for staining fat prior to CLSM observation, was Nile Blue A (N0766, Sigma-Aldrich, Oakville, ON.) and its molecular weight was 732.85.

Frying. Chicken nuggets samples were fried in fresh canola oil at three temperatures namely 170, 180 and 190°C for a time interval ranging between 0 and 240 s in a Henny Penny Computron 7000 pressure fryer (Model 500C, HP Corporation, Eaton, OH, USA). Fried samples were immediately removed from the oil and the surfaces blotted with tissue paper to remove surface oil and were allowed to cool at ambient temperature.

Sample Preparation and Imaging. Nile Blue A (0.005%) solution was prepared by dissolving it in demineralized water according to van Dalen (2002) method. The fried samples were covalently stained by adding one or two drops of the stain to the sample's surface. The stained samples were kept for 12 h at refrigeration temperature (8°C) for the dye to spread within the sample's matrix especially to areas where fat is located (van de Velde et al., 2003). The chicken nuggets samples were cut into cube shapes (0.5 x 0.5 x 0.5 cm). They were quick frozen in liquid nitrogen and were cryosectioned to 60 µm thickness at -20°C in a Cryotome (Shandon Cryotome, Thermo Scientific, Waltham, MA, USA). They were subsequently placed on to microscope slides (SuperFrost Fisherbrand, Fishcer Scientific, ON, CA).

Images were acquired under a confocal microscope (Bio-Rad Radiance 2100, Hemel Hempstead, UK), equipped with an argon (Ar) laser and coupled on to a fluorescence microscope (Nikon Eclipse E800, Hertfordshire, UK). Fluorescence images were acquired at the excitation-emission wavelength of 488-570 nm to show fat distribution, and greyscale images at reflection/trans mode, to show structures of the sample that include pore distribution. Stack of twelve 8 bits 2-D images were obtained in each mode with a resolution of 1024 x 1024 pixels (pixel size of 1.197 µm) in TIF format.

Imaging Analysis. In order to obtain fat distribution, stack of fluorescence images were read into an image processing software, ImageJ (Nation Institute of Health, MA, USA). Automatic thresholding were carried out using the Otsu algorithm (Otsu, 1979). Two steps of morphological operations, erosion and dilation, were carried out to remove noise from the images. In order to compute the fat distribution, the binarized images (12 two-dimensional images from each treatment) were read into the MATLAB (R2006a, Version 7.2.0.232) workspace and the white spotted areas that correspond to fat were computed as a percent of the ratio of the area delineated as fat (white) and the total area of the entire image.

In order to delineate between the pores and the rest of the sample, the trans images were loaded onto ImageJ software, the pore areas were manually selected. All the selected areas were subsequently "filled" with black color before thresholding. Triangle algorithm (Zack et al., 1977) in ImageJ software gave a better segmentation of the pores from the rest of the sample with minimal noise. Porosity was computed from the binarized images using MATLAB. Pore size distribution within each image was computed by using the *bwlabel*, *regionprops* and *hist* functions in MATLAB.

Fat content. Fat content was determined the conventional way by following the protocol recommended by AOAC Method 960.39 (AOAC, 1990). Solvent extraction was carried using petroleum ether in a Soxhlet extraction system (SER 148, Velp Scientifica, Usmate, Italy). Between 3 – 5 g of ground freeze-dried fried batter coating were placed in thimble and Petroleum ether was used to extract fat from them. Fat content was determined as the ratio of the mass of extracted fat to the mass of dry sample. The process took 2 hrs.

Statistical Analysis. All images were acquired in replicate of three per frying time and a minimum of 12 images per replicate. Analysis of variance was performed at 5% probability to test the effect of treatments on the variation observed in the dependent variables and where there is statistical significance, mean separation was performed using Duncan multiple range test. All statistical analyses were performed on SAS system (Version 8.2, SAS Inst., Cary, USA).

RESULTS AND DISCUSSION

Fig. 1 show images of unfried chicken nuggets coating, both at the reflection mode showing pore distribution (Fig.1A) and at fluorescence mode, showing fat distribution (Fig.1C). The binarized image of the unfried chicken nuggets coating in Fig. 1B shows that there are few pores present within the sample matrix prior to the frying process. In Fig. 1C, the red color shade show how fat is distributed within the food matrix, which is shown more clearly when the image was binarized (Fig.1D). Similar images for chicken nuggets coating fried at 180°C for 240 s are shown in Figure 2. Pores are clearly seen in the trans images after frying for a long period. Figures 2B and 2D show the binarized images of the trans and fluorescence images. These binarized images were those further analyzed to obtain the quantitative data presented below. Images presented in Figure 3 are those of the chicken nuggets batter coating fried at 180°C for various time intervals. These images show an increase in the degree of connectivity between adjacent pores and formation of bigger pores with frying time. The change in structural formation of the sample could be attributed to mass transfer processes and physicochemical transformations such as gelatinization of starch and denaturation of proteins, all induced by the elevated frying temperatures (Kassama and Ngadi, 2004; Moreira, 2006). These patterns persisted at all frying temperatures. The change in fat distribution with frying

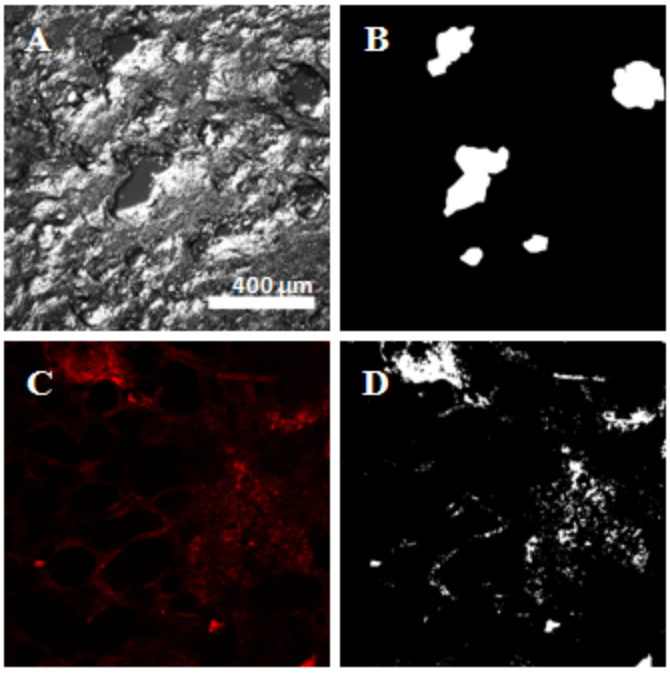

Figure 1. CLSM images of unfried chicken nuggets coating: A - grayscale image obtained in trans mode of the microscope, B - binarized image of A, C - image obtained at fluorescence mode and D is the binarized image of C.

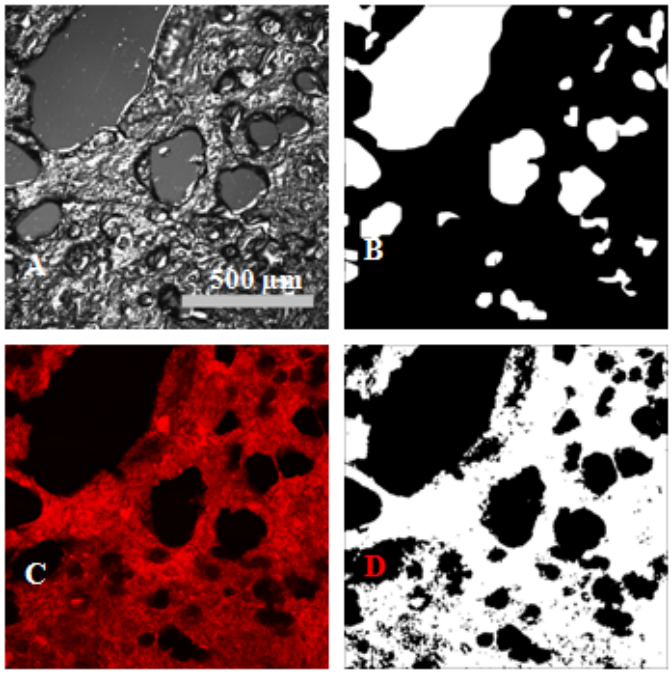

Figure 2 Images of chicken nuggets coatings fried 180°C for 240 s: A is the grayscale image obtained at the reflection/trans mode of the microscope, B is the binarized image of A showing pore distribution, C is the fluorescence image showing fat spread and D is the binarized image of C.

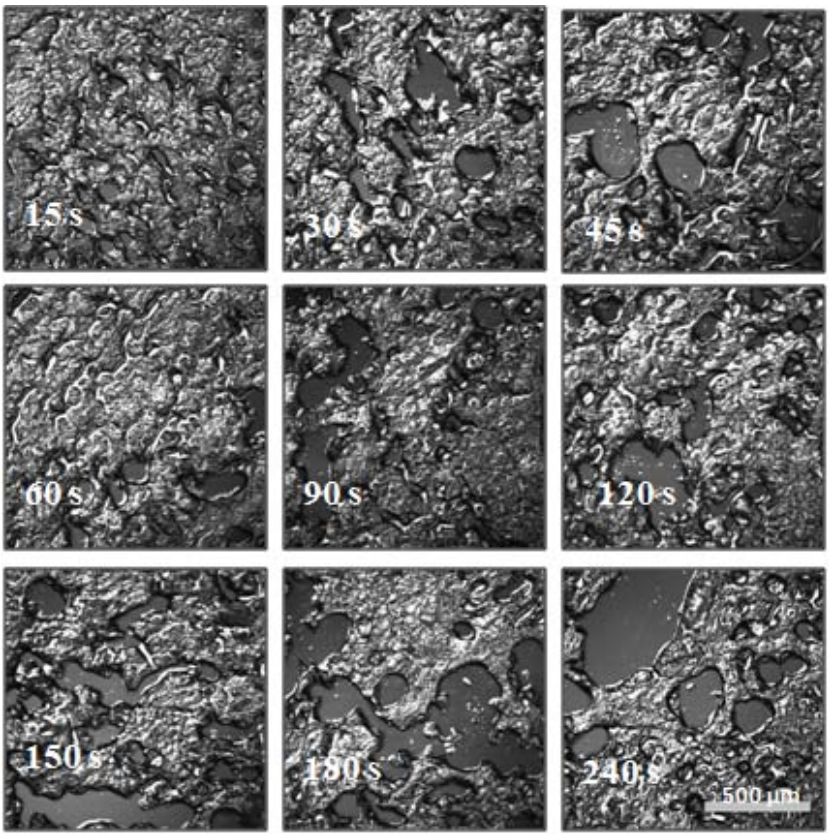

Figure 3 Grayscale images of chicken nuggets batter coating fried at 180°C obtained at the trans mode of the confocal laser scanning microscope.

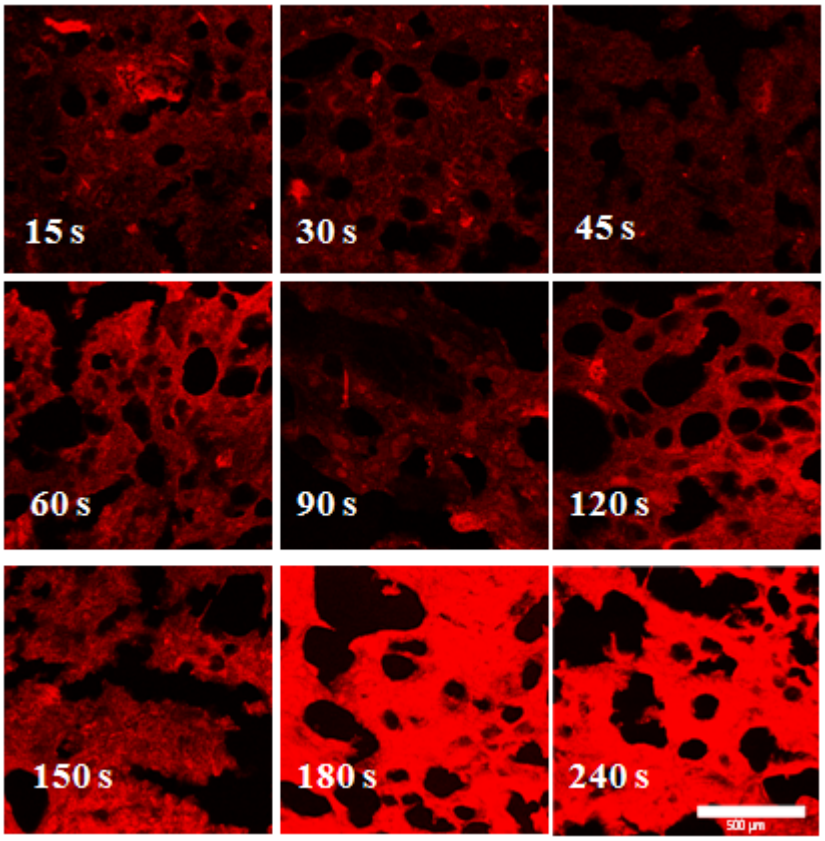

Figure 4 Two-dimensional images obtained in the fluorescence mode for samples fried at 190°C at different frying time.

time is shown, in Figure 4, as increase in dye color intensity with time, indicating increased fat absorption with frying time. The intensity increased with frying temperature meaning an increase in fat content of the sample with higher temperatures (Figures not shown). The quantitative data of fat distribution as a function of frying time is shown in Figure 6. The percent fat distribution within the frying temperature ranged between 11.52 and 59.3%. Analysis of variance (ANOVA) showed that there was significant ($P < 0.05$) effect of frying temperature and time on the variation shown in fat distribution. Mean separation showed that fat distribution at 170°C frying temperature was statistically different from those at 180 and 190°C. Pearson correlation between the fat content obtained the conventional way and fat distribution from CLSM imaging gave correlation coefficient of 0.60, 0.79 and 0.79 at frying temperatures of 170, 180 and 190°C, respectively, with a 5% probability of error (Figure 7). There was poor correlation between fat content and distribution at 170°C. However, fat content data at 180 and 190°C strongly fitted the data of fat distribution obtained from CLSM. The reason for the poor relationship at 170°C is not clear. The goodness of fit obtained at 180 and 190°C indicates that fat content can be predicted with a high degree of accuracy at these temperatures. Fat distribution within the depth of the batter samples fried at every frying temperature show a diminishing intensity of the dye within the depth of the 60 µm thick sample. This corroborates the assertion by some authors that fat intrusion into product during frying is limited to the crust (Pedreschi et al., 2008; Saguy et al., 1997).

Porosity of the samples expressed as the ratio of area of pores to the area of the whole sample is shown in Figure 8. Porosity ranged between 4.97 and 32.7% for all the samples

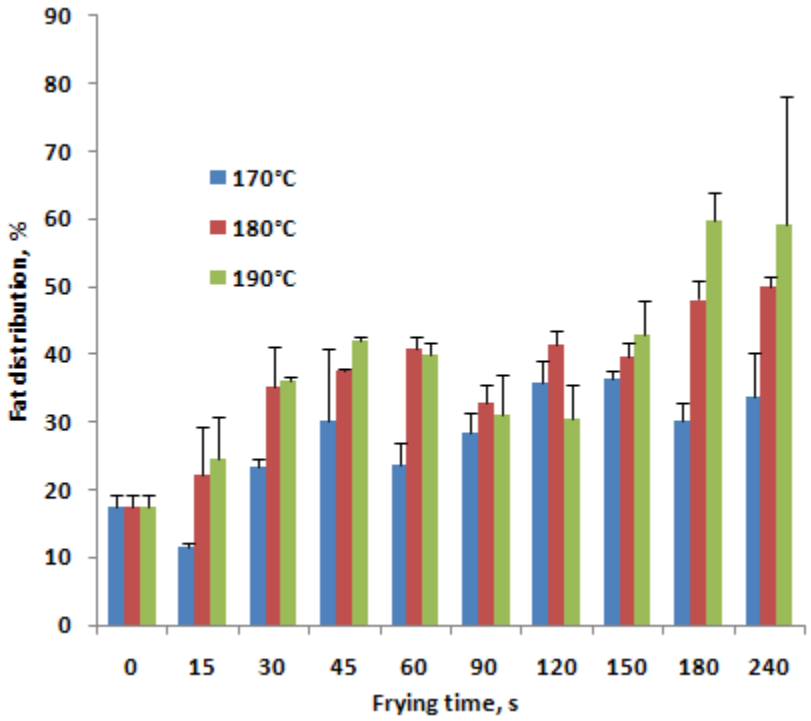

Figure 5. Graph of fat distribution as a function of frying time for deep-fried chicken nuggets breading coating.

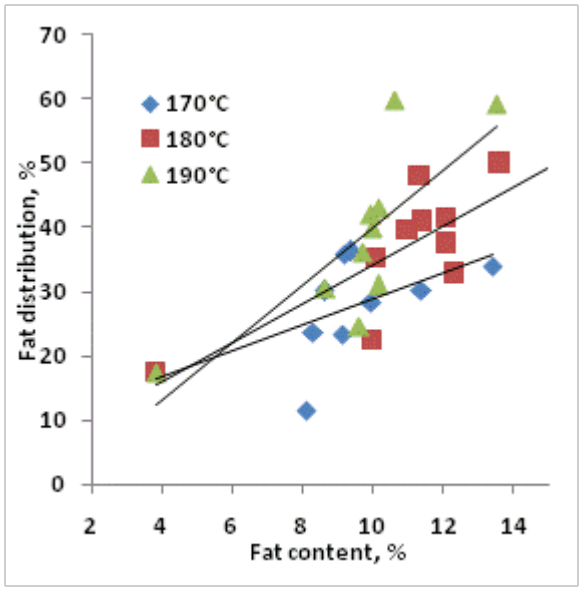

Figure 6 Graph of fat content from conventional method against fat distribution obtained from CLSM image analysis.

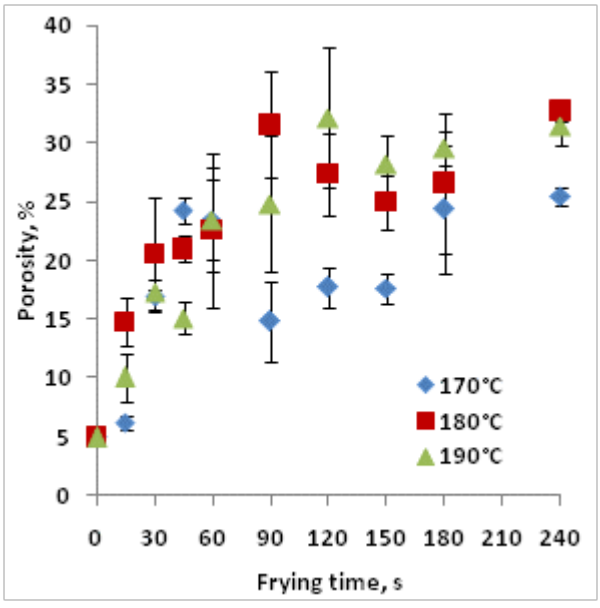

Figure 7 A plot of porosity versus frying time for deep-fat fried chicken nuggets coating at different frying temperatures.

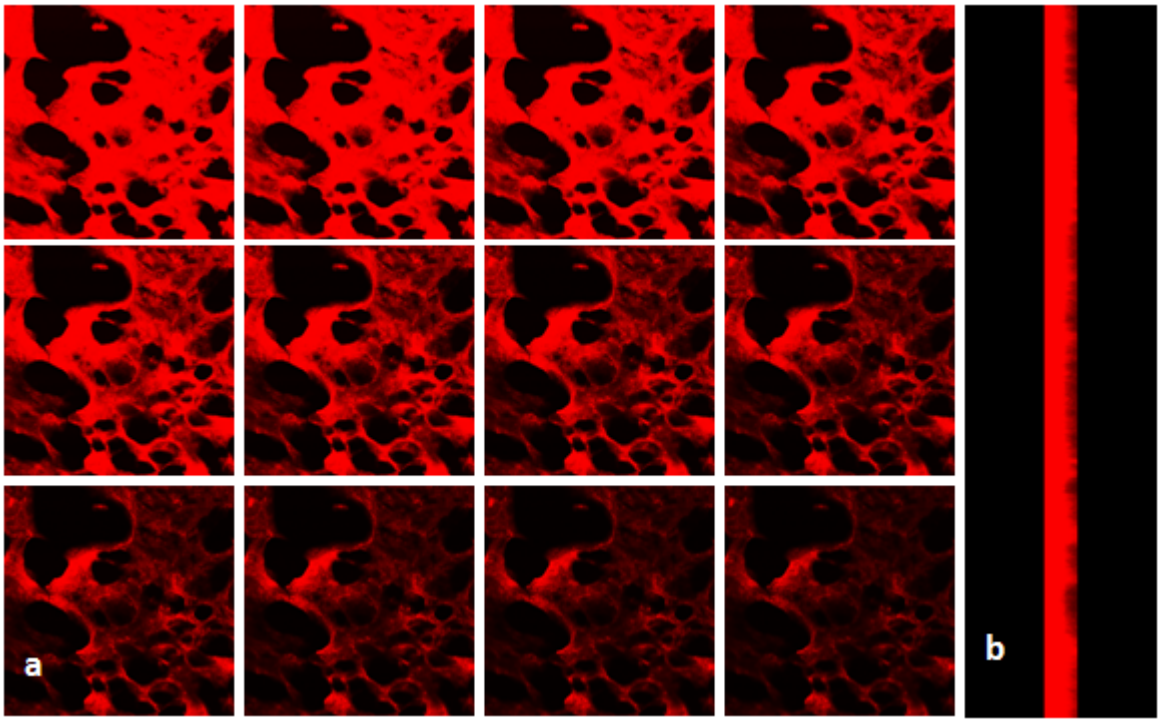

Figure 8. Display of stained sample (fried at 190°C for 180 s) showing diminishing intensity toward the batter core, indicating fat distribution within the depth of the sample. (b) shows a cross-sectional view of the sample from its 3-D image.

fried over a period of 240 s and at three temperatures. Adedeji and Ngadi (2009) used x-ray micro-computed tomography (CT) to obtain porosity in the range of 7 – 14% for fried chicken nuggets batter. Dogan et al. (2005) obtained a porosity ranging from 23 to 40% for fried batter formulated with soy and rice flour using nitrogen stereopycnometer. The variation between these authors' results and this study could be attributed to the difference in sample composition and scale of measurement of the methods used (Fehrmann et al., 1980; Skyscan, 2008).

Pore size distribution for the chicken nuggets batter coating, fried at 180°C for different frying times, is shown in Figure 9, where pore size in pixel was plotted against pore count. The pore size in this graphs are presented as the number of pixel that correspond to a particular area of the pore. Each pixel equals 1.197 x 1.197 µm; therefore the area corresponds to 1.43 μm^2. If pores are assumed to be circular, using the area formula for cycle, pores that contain 1 – 150,000 pixels would have pore diameters in the range of 1.2 - 523 µm, which is the pore range for the samples studied (Figure 8). Almost 70% of the pores in the unfried sample are less than 100 µm. Also, there were formation of smaller pores (< 42 µm) and bigger pores (270 - 523 µm), while medium size (191 – 270 µm) pores diminished as frying progressed as seen after 120 to 240 s of frying.

CONCLUSION Fat distribution and pore characteristics of deep-fat fried chicken nuggets batter coating were obtained using CLSM. Images showing fat distribution as function of frying time, temperature and product depth were presented. There was significant effect of frying temperature and time on fat distribution. Also, there was a good correlation between fat distribution from CLSM images and fat content determined

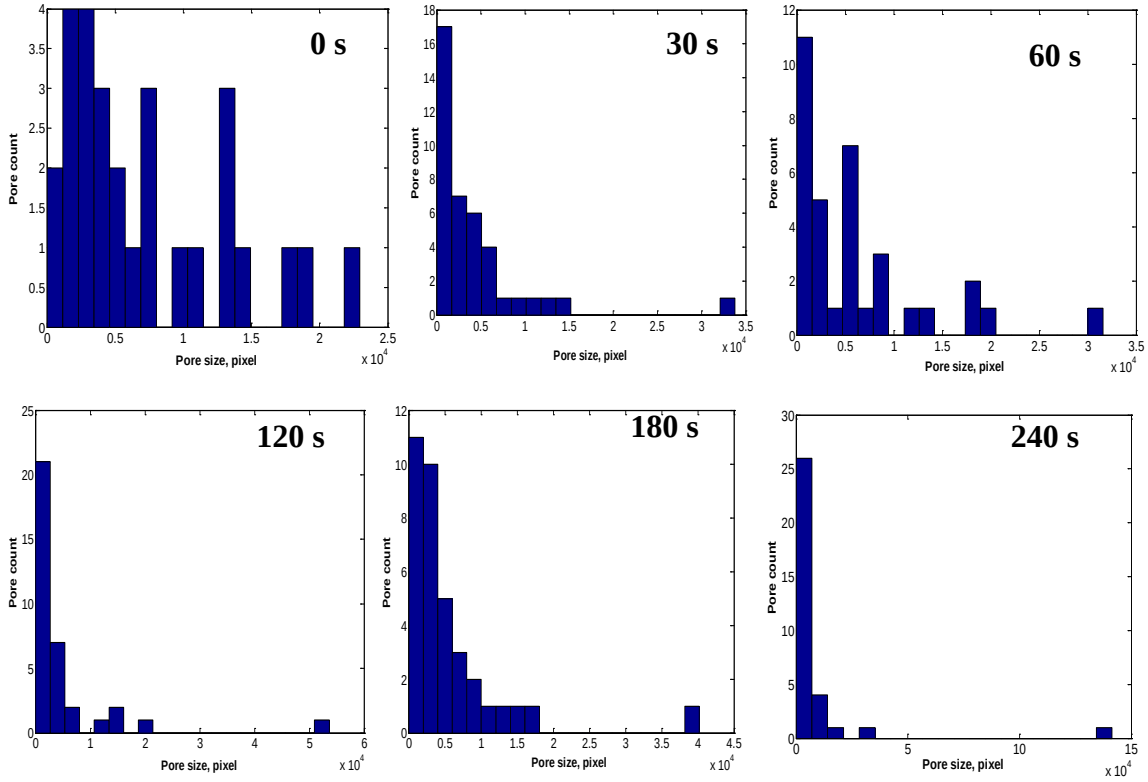

Figure 9 Pore size distribution for chicken nuggets coating fried at 180°C at times ranging from 0 to 240 s. Each pixel is equal 1.197 µm.

by the conventional method at two frying temperatures, 180 and 190°C. Porosity ranged from 4.97 to 32.70%, and was significantly ($P < 0.05$) influenced by frying temperature. Pore size ranged approximately between 1.20 – 523 µm. Frying led to the formation of smaller and bigger pores.

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