EVALUATION OF DIFFERENT TECHNIQUES FOR MICROSTRUCTURAL CHARACTERIZATION OF DEEP-FAT FRIED FOODS

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ABSTRACT

This study investigated the use of different techniques in evaluating the microstructural characteristics of deep-fat fried chicken nuggets, and considered the effect of frying conditions and batter formulation. Mercury intrusion porosimetry was used to obtain porosity, pore size distribution and pore shape for freeze dried deep-fat fried chicken nuggets batter coating. Porosity was found to range between 40 - 69%. Frying time and temperature significantly (P < 0.05) influenced porosity. Porosity showed a high positive and negative correlation with moisture and fat contents, and the correlation coefficients was between 0.88 – 0.96 and 0.78 - 0.8, respectively. Pore volume, pore area and mean pore diameter varied between 0.54 – 1.5 cm³/g, 2.53 – 15.5 m²/g and 0.25 – 8.32 µm, respectively. Hysteresis phenomenon showed that pore shapes other than cylindrical structure were present within the samples.

Porosity of preformed and lab formulated deep-fat fried chicken nuggets batter coatings were investigated using helium pycnometry. There was significant (P <0.05) effect of frying temperature and batter formulation on porosity. Porosity at 170°C was significantly different from those at 180 and 190°C, while porosity of batter with 100% wheat flour was significantly lower than the other formulated batters. However, porosity increased with frying time and temperature, and it ranged from 2.15 – 47.9% and 9.96 – 54.8% for the preformed and the formulated batter, respectively. Porosity demonstrated significant (P < 0.05) positive and negative correlation with fat uptake and moisture loss, respectively for all batter coatings.
Images (3-D) of deep-fat fried chicken nuggets batter coating and core were obtained by X-ray micro computed tomography (CT). Porosity for the batter coating and the chicken core varied from 7 – 14.1% and 4.4 – 7.7%, respectively. Frying time significantly (P < 0.05) influenced porosity, fragmentation index (degree of interconnectivity) and pore count. Porosity and pore count increased with frying time while pore interconnectivity decreased. Pore shapes were between cylindrical and spherical structure for the coating, while the core pores showed cylindrical shape. Pore size distribution showed that micropores (< 100 µm) increased in volume and number with frying time.

X-ray microCT was also used to investigate the effect of batter formulation on pore characteristics. There was significant (P < 0.05) effect of batter formulation on porosity, fragmentation index and pore count. Pore shape of all the formulations remained relatively the same with structure model index from 2 – 3, indicating cylindrical shape. Porosity ranged between 18.2 – 32.1%. The addition of carboxymethyl cellulose (CMC) caused about 25% decrease in porosity, though not significantly. The addition of rice flour into the batter formulation resulted in significant increase in porosity when compared to batter with 100% wheat flour without CMC inclusion. The addition of CMC resulted in decreased interconnectivity between the pores while rice flour addition caused increase pore connectivity. Other properties of the batter such as viscosity, batter pick-up, fried batter color, texture, moisture and fat content were significantly (P < 0.05) influenced by batter formulation. Some correlations were shown between the physico-chemical properties of the batter system and porosity.
Fluorescence and reflective images of the deep fat-fried chicken nuggets batter coating were obtained in 2-D using confocal laser scanning microscopy (CLSM). There was a decrease in fat intensity from the surface towards the core of the sample. Porosity and fat distribution were significantly influenced by frying temperature and time. Porosity ranged between 5–33%, while pore size varied from 1.20 – 523 µm. There was formation of smaller (pores ≤ 42 µm) and bigger (pores ≥ 270 µm) pores, while medium size (191 – 270 µm) pores diminished with frying time. There was a strong correlation (r = 0.79) between fat distribution obtained from CLSM imaging and fat content obtained by the conventional method at 180 and 190°C, indicating a reasonable capacity to predict one from the other.

All the techniques used effectively evaluated the microstructural properties of fried foods studied within their limit of measurement. None of the techniques could provide full microstructural properties of the fried foods studied. It is therefore suggested that various techniques be combined in order to obtain a complete data of the pore characteristics of fried foods.
RÉSUMÉ

Cette étude a testé l'utilisation de différentes techniques dans l'évaluation des caractéristiques microstructurales de friture profonde de pépites de poulet panées tout en considérant l'effet des conditions de friture et la formulation de la pâte.

Un porosimètre d’intrusion de mercure a été utilisé pour mesurer la porosité, la distribution de la taille des pores et la forme des pores dans des pépites de poulet panées lyophilisées. La porosité a varié de 40 à 69 %. Le temps et la température de la friture ont affecté significativement (P < 0.05) la porosité. La porosité a montré une haute corrélation positive et négative avec le contenu en humidité et en graisse. Les coefficients de corrélation étaient entre 0.88 – 0.96 et 0.78 – 0.8, respectivement. Le volume, la surface et la moyenne des diamètres des pores ont été entre 0.54 – 1.5 cm³/g, 2.53 – 15.5 m²/g et 0.25 – 8.32 µm, respectivement. Le phénomène d’hystérèse a montré que les pores n’ont pas tous une structure cylindrique.

La porosité de pépites de poulet panées frites préformées et formulées au laboratoire a été mesurée en utilisant un pycnométre en hélicium. L’effet de la température de friture et la composition de la pate sur la porosité était significatif (P<0.05). La porosité à une température de friture de 170 °C a été significativement différente de celles à des températures de 180 et 190°C, alors que la porosité de la pâte avec 100 % de farine de blé a été significativement inférieure aux autres pâtes formulées. La porosité a augmenté avec le temps et la température de friture. Elle varie de 2.15 à 47.9 % et de 9.96 à 54.8 % pour la pâte préformée et la pâte formulée, respectivement. La porosité a montré
une corrélation positive et négative significative (P < 0.05) avec l’absorption d’huile et la perte d’humidité, respectivement.

Des images en 3-D du revêtement et du noyau des pépites de poulet panées frites ont été obtenues par X-ray micro computed tomography (CT). La porosité du revêtement et du noyau des pépites a varié de 6.99 à 14.1 % et de 4.45 à 7.69 %, respectivement. Le temps de friture a influencé significativement (P<0.05) la porosité, le degré de l’interconnectivité et la dimension des pores. La porosité et la dimension des pores a augmenté avec le temps de friture alors que l’interconnectivité des pores a diminué. La structure des pores du revêtement était cylindrique et sphérique, tandis que celle du noyau était cylindrique. La distribution de la taille des pores a montré que les micropores (< 100 µm) ont augmenté en volume et en nombre avec le temps de friture.

Des Rayons MicroCT X ont été utilisés pour tester l'effet de la formulation de la pâte sur les caractéristiques des pores. La formulation de la pâte a influencé significativement (P < 0.05) la porosité, l’index de fragmentation et le nombre des pores. La forme des pores de toutes les formulations est restée la même avec une structure modélisée index de 2 à 3, indiquant une forme cylindrique. La porosité a varié entre 18.2 et 32.1 %. L’ajout de carboxymethyl cellulose (CMC) a causé une diminution de porosité d’environ 25 %. L’ajout de la farine du riz dans la formulation de la pâte a augmenté significativement la porosité comparée à la pâte formée de 100 % de la farine de blé sans CMC. L’ajout de CMC a entraîné une diminution de l’interconnectivité entre les pores tandis que l’ajout de la farine du riz a causé une augmentation de la connectivité des pores. D’autres propriétés de la pâte comme la viscosité, la couleur de la pâte frites, la texture, la teneur en humidité et en matières grasses étaient significativement (P < 0.05).
influencées par la formulation de la pâte. Des corrélations ont été observées entre les propriétés physico-chimiques de la pâte et la porosité.

Les images fluorescentes et réfléctives de la friture profonde des revêtements de pépites de poulet ont été obtenues en 2 dimensions à l’aide de la microscopie confocale laser à balayage (MCLB). Une diminution de l’intensité de la graisse de la surface vers le noyau de l’échantillon a été observée. La porosité et la distribution de la graisse ont été influencées significativement par le temps et la température de la friture. La porosité était entre 4.97 et 32.7 %, tandis que la taille des pores était entre 1.20 et 523 µm. Il y avait une formation de petits pores (≤ 42 µm); et de grands pores (≥ 270 µm), tandis que les pores de taille moyenne (191-270 µm) ont diminué avec le temps de friture. Une forte corrélation ($r = 0.79$) a été observée entre la distribution de la matière grasse obtenue à partir de l’imagerie MCLB et le contenu en matière grasse obtenu par la méthode conventionnelle à 180 et 190°C, indiquant une capacité raisonnable pour prédire l’une de l’autre.

Toutes les techniques utilisées ont évalué efficacement les propriétés microstructurelles des aliments frits étudiés au sein de leur limite de mesure. Aucune technique n’a pu fournir les propriétés microstructurelles complètes des aliments frits étudiés. Il est donc suggéré que plusieurs techniques soient combinées afin d’obtenir des données complètes des caractéristiques des pores des aliments frits.
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CONTRIBUTION OF AUTHORS

The contributions made by the different authors are as follows: Mr. Akinbode A. Adedeji is the principal author of this work. He is the Ph.D candidate who planned, designed and executed the various stages of experimentation, data analysis, manuscript writing and revision for scientific publications. Prof. Michael O. Ngadi is the thesis supervisor, who guided the candidate in the process of planning, experimental design and execution of various stages of the work which include all mentioned above. Prof. Ngadi also corrected, edited and reviewed all the manuscripts sent for publications. Dr. Laura Li Liu helped in the area of developing part of the Matlab codes for image analysis of microscopy images and also helped to edit and review this aspect of the manuscript.
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\( \Delta P \)  Pressure difference (Pa)

\( \mu \)  Linear attenuation coefficient (cm\(^{-1}\))

\( a \)  model constant

\( A_1 \)  Pore area before dilation (m\(^2\))

\( A_2 \)  Pore area after dilation (m\(^2\))

\( b \)  model constant

CLSM  Confocal laser scanning microscopy

CT  Computed tomography number

D  Pore diameter (m)

\( D(v) \)  Pore size volume distribution function

\( dP \)  Pressure differential (Pa)

FC  Fat content

FI  Fragmentation index

I  Transmitted radiation

\( I_o \)  Incident Radiation

k  Constant in CT equation

K  Consistency index (Pa\(\cdot\)s\(^n\))

l  Distance travelled by radiation in the sample (cm)

MC  Moisture content

MIP  Mercury intrusion porosimetry

P  Pressure applied (Pa)
$P_1$ Initial sample chamber pressure (Pa)

$p_1$ Pore perimeter before dilation (m)

$P_2$ Final sample chamber pressure (Pa)

$p_2$ Pore perimeter after dilation (m)

$r$ Pore radius corresponding to $P$ (m)

$r_{pore}$ Pore radius (m)

$S$ Pore surface area before dilation (m$^2$)

$S'$ Pore surface area after dilation (m$^2$)

SAS Statistical analysis software

SMI Structure model index

$v$ Pore volume at Pressure $P$ (ml)

$V$ Structure volume (m$^3$)

$V_c$ Chamber volume, (m$^3$)

$V_p$ Pore volume (ml)

$V_R$ Expansion Chamber volume (m$^3$)

$V_{SP}$ Volume of sample (m$^3$)

$v_t$ Total pore volume (ml)

$x$ Image coordinate

$\gamma$ Surface tension (N/m)

$\gamma^n$ shear rate (1/s), $n$ is flow behavior index

$\varepsilon$ Porosity (%)

$\rho$ Specific component density (g/ml)

$\rho_{ap}$ Apparent density (g/ml)
\[ \rho_b \quad \text{Bulk density (g/cm}^3) \]

\[ \rho_s \quad \text{Solid density (g/cm}^3) \]

\[ \tau \quad \text{shear stress (N/m}^2) \]

\[ \Theta \quad \text{Contact angle between liquids (degrees)} \]
I. GENERAL INTRODUCTION

1.1 Background

Deep-fat fried (DFF) foods are very popular food because of their unique quality characteristics such as smooth mouth-feel, distinct flavor, palatability and aesthetic appeal. The snack food industry is a multi-billion dollar industry worldwide, which keeps growing, and deep-fat fried foods constitute a high percentage of the market output. In 2007, fried foods comprised more than 65% of total retail sales of snack foods in Canada (Agriculture and Agri-Food Canada, 2009). However, health concern about the consumption of fried foods is growing and consumers’ awareness of this has posed more challenges to further understand the mechanism of mass transfer in fried foods with the ultimate aim of reducing the amount of oil entrained. Fat uptake has been described as a surface phenomenon involving equilibrium between adhesion and drainage of oil during the cooling period (Ufheil and Escher, 1996). Therefore effective control of the activities at the surface level will help improve the quality of fried foods greatly especially from the nutritional point of view. There are a number of approaches being used for mass transfer control in fried products among which is the application of food coatings, which form barriers to mass transfer. Food coatings have been applied successful in reducing fat uptake in fried foods (Singthong and Thongkaew, 2009; Adedeji and Ngadi, 2009; Rimac-Brncic et al., 2004; Fiszman et al., 2003; Mallikarjunan, et al., 1997; Pinthus et al., 1993). The formulation of these coatings however, is extremely flexible (Xue and Ngadi, 2006). For example, breading/batter ingredients often contain varying amounts of flours, water, hydrocolloids and seasoning. During frying, several physical and chemical
changes occur, such as protein denaturation, starch gelatinization, bonding of polar components, and migration of moisture and fat. The later has been reported to cause pore formation, and this plays a significant role in mass transfer. The unique texture of the surface developed during frying does not only control fat uptake but also contributes to the crispiness that make coated fried foods appealing. Other interactions of the batter constituents influence the surface structure formation during frying (Aguilera and Hernandez, 2000). All these factors affect the complexity of deep-fat frying in which conventional instrumental analytical methods of property evaluation may not be sufficient to explain (Aguilera and Stanley, 1999). There is therefore the need to explore the use of modern techniques of analysis such as imaging and advanced microscopy to understand changes at microscopic level during frying so as to improve the quality of DFF foods.

The mechanism of mass transfer during deep-fat frying is yet to be properly defined and understood (Gamble et al., 1987; Bouchon et al., 2003). A lot of assumptions are made in developing mathematical models that describe the mechanism and properties of DFF foods due to the complexity of the process. Porosity, pore size, shape, interconnectivity, heterogeneity, and dimensionality have all been correlated with mass transfer in DFF products (Lorén et al., 1999; Gamble and Rice, 1988). However, research activities have not fully explored the relationship between microstructural formation, mass transfer and functional properties of porous food system like coated DFF products.

A lot of changes taking place during food processing that define the overall quality and acceptance such as structural transformation, happen at the micro level (Aguilera, 2005). The study of food microstructures has the potential to provide a lot
more information for use in product development and quality assurance than thought possible. For example if structural modifications (e.g. pore development) during processing (drying, frying etc) are accounted for in diffusion models especially in effective diffusivity ($D_{eff}$) computation, a better model with good predictive and descriptive ability could be developed (Aguilera, 2005). It is interesting to note that majority of the elements that critically influence transport behavior, physical, rheological, textural and sensorial properties of foods exists below 100 µm (Lorén et al., 1999; Aguilera, 2005). Microstructural characterization could also be a key to developing products with optimal quality in terms of oil content, mechanical and organoleptic properties. Therefore a good insight into structural transformation under different processing conditions could help in understanding the relationship between structure and DFF foods.

Microscopy provides a means of visualizing structural and spatial configuration of food matrices at the micro level between a scale range of $10^{-9}$ to $10^{-3}$ m. Advanced microscopy techniques allow non-invasive, real time, 3-D rendering of images, and provide a contrast between components for both quantitative and qualitative assessment. A number of microscopy techniques are available for studying food properties, for example, light microscopy (LM), electron microscopy (EM), confocal laser scanning microscopy (CLSM) and atomic force microscopy (ATM). Also, advances in computer technology have made possible the development of softwares for better image processing and quantitative image analysis. Other imaging techniques include X-ray computed tomography (CT) and magnetic resonance imaging. These techniques have the capability of rendering 3-D images of the internal morphology of the food materials, thereby,
providing more details than when compared to other techniques. X-ray CT especially has ability to differentiate between sample components based on their attenuation and it has been applied to study a couple of food materials such as bread, fried potato, aerated chocolate, mousse, marshmallow and muffin (van Dalen et al., 2007; Miri et al., 2006; Lim and Barigou, 2004).

Microscopy techniques in combination with other physical methods such as mercury intrusion porosimetry (MIP), Fourier transform infra-red spectroscopy (FTIR), magnetic resonance imaging (MRI) and X-ray CT scanning for studying changes in foods during processing can promote better understanding of the structural role of food components and their effect on the overall microstructure of the complex food system (Aguilera and Stanley, 1999; Hermansson et al., 2000; Bouchon and Aguilera, 2001).

Microstructural study of food materials have been somewhat disregarded up until recently when it became clear that the future of fabricated foods will depend on a better understanding of the food system at micro level. The potential contribution of microstructural characterization to production of novel, healthy, appealing and high quality DFF foods is significant (Bouchon et al., 2003). There is therefore the need for more research effort to establish these, making use of enormous science and technology from biology and material science (Aguilera, 2005).

1.2 Hypothesis

Based on the foregoing discussions, it was hypothesized that batter formulation and processing conditions would have significant effect on microstructural properties of deep-fat fried foods and different measurement techniques such as microscopy, X-ray micro-
computed tomography, pycnometry and porosimetry could provide valuable insights on development of microstructure in the products.

1.3 General objective

The general objective of this study was to evaluate the microstructural changes in breading/batter system of deep-fat fried foods as affected by components interaction and processing conditions. Also, to make a comparison of the results of various methods for evaluating fried foods microstructural characteristics namely mercury intrusion porosimetry (MIP), helium pycnometry, confocal laser scanning microscopy (CLSM) and X-ray computed tomography (CT) techniques. The outcome is anticipated to enhance quality evaluation and provide ready tools for novel fried foods development, better modeling and optimization of deep-fat frying of coated foods.

1.4 Specific objectives

The specific objectives for this project are:

1. To study the effect of processing conditions (frying temperature and time) and batter formulation on microstructural changes in the breading coating of deep-fat fried chicken nuggets using mercury intrusion porosimetry technique and helium pycnometry.

2. To explore the use of X-ray microCT for characterization of pore properties of breading and chicken core of deep-fat fried chicken nuggets.
3. To determine the influence of batter formulation (flour level and type) on pore properties of deep-fat fried food coating.

4. To quantitatively evaluate microstructural property and fat distribution in deep-fat fried chicken nuggets coatings using confocal laser scanning microscopy.

5. To compare the microstructural properties of deep-fat fried chicken nuggets coating obtained by mercury intrusion porosimetry, pycnometry, X-ray microCT imaging and confocal laser scanning techniques.
II. GENERAL LITERATURE REVIEW

2.1 Deep-Fat Frying

Deep fat frying is one of the oldest methods of cooking that probably originated from the Mediterranean (Varela, 1988). Foods are fried primarily to cook and to make them more desirable, palatable and digestible. Frying is a process that involves simultaneous heat and mass transfer in which frying oil is the medium of heat transfer into the food, while moisture migrate out and oil is absorbed into the food (Budžaki and Šeruga, 2005; Krokida et al., 2000). Usually, foods to be fried are immersed in hot oil at a temperature range between 120 to 180°C depending on the raw material and the final product desired (Costa and Oliveira, 1999). Many physicochemical changes take place during frying such as starch gelatinization characterized by swelling of starch granules; protein denaturation; browning; crust formation, which develops as a result of drying out of the surface of the fried product; flavor components formation that characterizes fried foods; shrinkage and swelling. These physical and chemical changes lead to structural transformations at both the macro and micro level.

Mass transfer phenomena in frying involves the outflow of moisture and intrusion of fat caused by transfer of heat energy to the product and its surface characteristics during and after frying, although the process is still not clearly understood. During deep-fat frying, heat is transferred by convection from the oil to the surface of the food and then into the core by conduction. The moisture from the food escapes through weak crevices and forcefully dug pores created by pressured moisture in the food. With this moisture loss, the surface temperature rises reaching closer to that of the frying oil and
this subsequently leads to crust formation. Although, some oil may replace some of the water removed, it has been shown that overpressure development during frying prevents substantial amount of oil absorption (Mellema, 2003). Gamble and Rice (1987) were among the first that attempted to describe the mechanism of mass transfer in fried products. They concluded that most of the oil drawn into potato when removed from the frying oil is due to condensation of vapor that creates a vacuum effect thereafter. They also proposed that oil absorption and moisture loss in fried potato chips is a simultaneous phenomenon. Also, Ufheil and Escher (1996) showed that oil is not absorbed during the frying process but rather, after frying by the adhesion to the surface of the product. They demonstrated this by frying potato slices in dyed oil and measured the amount of dyed oil in the sample during and after frying by spectrophotometric method. Moreira et al. (1997) reported that only 20% of oil is absorbed during frying of tortilla chips while the remaining 80% is on the surface. They also showed that overall, about 64% of the total fat content of the tortilla chips were absorbed during the cooling stage. Consequently, Moreira and Barrufet (1998) concluded that oil absorption mechanism in tortilla chips is a capillary force driven phenomenon. Bouchon et al. (2003) stated that there are three fractions of oil that can be identified as a result of different mechanisms of fat absorption. These include structural oil (STO), which represents oil absorbed during frying; penetrated surface oil (PSO), which represents the oil sucked into the food during cooling after removal from the fryer and surface oil (SO), which is the oil that is left on the surface of the product. Some authors have proposed that moisture loss is a diffusion driven phenomenon where fluid concentration gradient is the driving force for mass movement in fried products (Adedeji et al., 2009; Chen and Moreira, 1997; Ngadi and
Correira, 1995); while others have described the mechanism as pressure driven capillary force process in which pressure developed in the product during frying leads to substantial mass flow especially during the cooling period (Moreira et al., 1997).

Fried foods are high in calorie and their consumption has generated a lot of concern among consumers especially in the last couple of decades where changes in our social behavior have compelled more and more people to eat out than ever before. The trend today is towards the consumption of healthier foods with low calorie content. Fat reduction in fried foods has been the target of many research activities and a couple of approaches have been applied successfully. These include physical and chemical treatments such as pre-drying (Debnath et al., 2003; Moyano et al., 2002; Gupta et al., 2000), microwave pretreatment (Adedeji et al., 2009), blanching (Rimac-Brncic et al., 2004), and soaking in NaCl solution (Bunger et al., 2003). Fat absorption in frying is affected by a host of factors like frying time, frying temperature, initial moisture content of the product, oil quality, product geometry, interfacial tension, post-frying treatment and surface condition of the food (Rice and Gamble, 1989; Gamble and Rice, 1988; Adel-aal and Karara, 1986; Fan and Arce, 1986; Mittelman et al. 1982; Pinthus and Saguy, 1994; Lulai and Orr, 1979; Ng, et al., 1957). Mass transfer, especially fat uptake, during frying is partly a surface phenomenon (Bouchon et al., 2003; Ufheil and Escher, 1996) and the process has been linked directly to the product’s structural configuration especially at the surface (Bouchon et al., 2003). The condition of the surface determines to a great extent the level of fat absorbed and moisture loss. Modification of the surface by the application of edible coating and batter system has been reported to reduce fat
absorption (Adedeji and Ngadi, 2009; Pedreschi et al., 2005; Mellema, 2003; Mallikarjunan et al., 1997).

2.2 Food Coatings

Coatings are applied to foods for various reasons such as preservation from spoilage, characteristic flavor and texture development, mass transfer control during processing etc. There are several examples which include milk products, hydrocolloids and batter systems. Food coatings are applied to DFF foods primarily to reduce fat uptake and to add value (Garcia et al., 2004; Fiszman et al., 2003; Mohamed et al., 1998; Pinthus et al., 1993). Batter and breading systems are the common type of food coatings used in DFF foods. Usually, the batter is made up of flours, starch, water, hydrocolloids, flavoring, seasoning and other ingredients depending on the manufacturer’s choice. It is often in the form of a thick liquid or semi-liquid system into which food is dipped prior to frying. The breading comprises of breadcrumb or cracker grits applied dry to form a suitable texture. The batters are generally classified into two basic categories namely puff/tempura batter and adhesive/interface batter (Loewe, 1990). The adhesive batters are often used with the breading coating to serve as a gumming agent to enable the breading to evenly coat the surface of the food material. Puff/tempura is usually liquid dough, chemically leavened, which can by itself serve as the outside coating to a food material.

The functionality of the batter system is affected by a number of factors such as viscosity, water holding capacity, thermal properties and product components. The formulation is also extremely flexible and changes the product’s malleability during product development. However, recent studies have established strong relationship
between formulations, functional properties of coating system and their overall qualities like pick-up values, adhesion power, texture and appearance (Dogan et al., 2005; Xue and Ngadi, 2006). The formulation of the breading and batter system is becoming less of an art and more of science due to research activities aimed at understanding the nature of the components, and their interaction during preparation and processing.

Flours are the major functional ingredients of breading and batter used in DFF foods. Wheat flour is the traditional flour for batter formulation primarily because of the viscoelastic characteristic of its gluten content; however other flours such as rice, soy and corn flours are increasingly being used as substitutes because they improve the overall functionality of the formulation such as water holding capacity, viscosity, color etc. The addition of other flours has been found to increase the marketability of the products by making battered foods more appealing to the Asian market where flour based products are major staples (Loewe, 1993). Also, whole rice flour based - batter could serve as an alternative food for individuals with gluten allergies (Mukprasirt et al., 2000a). The significance of substitution is seen in the overall quality of the product for example, as seen in the case of reduced fat uptake when certain level of rice flour is added to batter formulation (Dogan et al., 2005; Mukprasirt et al., 2000a,b; Shih and Daigle, 1999). Although, rice flour has less adhesiveness and thickening properties compared to wheat flour, its other functional properties make it the flour of choice as wheat substitute. Corn flour is also used because of its ability to reduce puffing, increase crispiness and to impart a desirable yellow color to the food. The coloration is indicative of carotene content of the flour (Salvador et al., 2002).
A combination of the different flours may be used to improve the overall quality of batter.

Hydrocolloids are added to the breading/batter system to serve two main purposes: to control viscosity and the water holding capacity of the system (Rimac-Brnčić et al., 2004). This helps create a network of structure that minimizes mass transfer during frying resulting in reduced oil uptake and the formation of a more desirable texture (Dogan et al., 2005). Hydrocolloids form gels when heated, but return to their original viscosity when cooled (Dziezak, 1991). The gelation is what promotes the barrier-resistant effect to oil uptake and moisture loss (Meyers, 1990; Mellema, 2003). The type of hydrocolloids often used in DFF foods are of the cellulose derivatives such as methylcellulose (MC), carboxyl methylcellulose (CMC), hydroxypropyl methylcellulose (HPMC), and others like Xanthan gum, Guar gum etc (Rimac-Brnčić et al., 2004). The performance of hydrocolloids is determined by their ability to interact with the other food components. Another property of hydrocolloids is that they are indigestible and their inclusion in batter coating has significant effect on the rheological and textural properties of fried products (Meyers, 1990). Of particularly interest is the ability of the food structures to change during the addition of different colloidal bodies. The knowledge of components interaction and structural (macro and micro) changes during processing is said to be very useful in optimizing food production processes and in the development of products with desired organoleptic properties (Langton et al., 1997). Few studies have explored the use of microstructural characterization in understanding the relationship between hydrocolloids and other components on functional properties of DFF foods.


2.3 Food Microstructures

Food components are transformed by mechanical, biological, chemical or thermal means during processing. Although some of these transformations may be visible to the human eye, a huge amount occurs at such small scale to be recognized by the human eye. For example, during frying, the starch in the food swells or gelatinizes, carbon dioxide (CO$_2$) gas may be produced and moisture evaporates forcing its way out. These changes combined create a network of structure within the fried food that defines its quality in terms of aesthetical, sensorial, nutritional and textural properties (Donald, 2004; Stanley, 1987). In order to effectively describe, predict and control the behavior of food materials, a thorough understanding of the way in which components are organized is needed (Aguilera and Stanley, 1999; Bouchon et al., 2003; Aguilera, 2005). The emphasis in the food industry since the turn of the century has been on the product rather than the process alone. Understanding how product components transformation occurs during processing and its effect on the quality are therefore important in developing products that will meet consumers’ need. The field of material science has long ago established a strong link between structure and properties, and some of these knowledge form the foundation for the development of the field of food product engineering (Aguilera, 2000).

The demands in today’s food market are higher quality, variety, more healthy and innovative food items. The future of fabricated foods would depend to a great extent on the understanding of structural configuration at the micro level, since most elements that determine food properties exist at this scale. This has made the knowledge of chemical composition, qualitative assessment and macroscopic analysis grossly inadequate to develop an efficient perception of how these factors exist and transform during
processing and storage (van Dalen et al., 2003). Most elements that determine and control
textural, sensorial, rheological, mechanical and transport properties of foods exist at a
range below 100 µm. Thus, the need to understand how these properties relate to these
elements such as colloidal particles, structural characteristic like porosity, pore
connectedness and distribution (Sanguansri and Augustin, 2006; Aguilera, 2005).

In porous material such as fried foods where a clear understanding of the transport
phenomena form the bed rock for process optimization, a knowledge of the food
microstructures is very important in developing the related equations. Food
microstructures such as porosity also have significant effect on the food’s physical
properties such as mass diffusivity, thermal conductivity and thermal diffusivity
(Rahman, 2001). Mechanical and textural properties of food are also correlated to the
porosity (Huang and Clayton, 1990). Porosity and crispiness of snack foods by extrusion
or frying are also highly related (Hussain et al., 2002). Kassama and Ngadi (2004)
determined the effect of processing conditions (frying temperature and time) on porosity
of fried chicken meat and related them to oil absorption and moisture loss ratio. They
further used empirical models to fit the data of porosity and fat content and obtained a
good correlation. It is therefore, important to determine the formation of pores in foods
during frying because of its importance in quality characterization and process design.

2.3.1 Structure - Relationship to physical properties and transport phenomenon
during frying
The structural formation of food components determines their physical properties such as
mechanical, optical, electrical, thermal and rheological properties (Barbosa-Cánovas et
al., 2006; Aguilera, 2005). Foods dried or fried are synonymous with crispy texture and crunchy feel during eating. The loss of moisture during the elevated processing temperature leads to significant moisture loss, leaving a porous and dehydrated product. For example, when a gel is formed from a combination of starch and protein heated at elevated temperatures, different mechanical properties are obtained for different ratios of starch and protein. Aguilera (2000) reported the use of video microscopy in providing information for model development in order to predict mechanical properties of different composite mix. There is also a significant change in thermal properties of food product as a result of the structural transformation. For example a highly conductive material would show different thermal conductivity for different degree of porosity (Barbosa-Cánovas et al., 2006).

Transport phenomena are significant in food engineering applications. The impact of structure in transport phenomena has been reported by Aguilera (2000, 2005); Saravacos and Maroulis (2001); Roca et al. (2008). Bouchon et al. (2003) suggested that the microstructure of potato formed during frying (i.e. the mean pore size, interconnectivity, and permeability) of the crust region might be the single most important factor modulating fat absorption. Mathematical models have been used immensely to describe, predict and interpret the mechanisms of mass transport, however most of these models, empirical and theoretical alike, have excluded the microstructural parameters in their configuration. This has brought a lot of inconsistencies in the proportionality constant of the models, such as the effective moisture diffusivity ($D_{eff}$) of Fick’s diffusion model, obtained by different researchers on the same product under similar environment (Saravacos and Maroulis, 2001). The Fickian diffusion model is
used extensively by engineers to predict mass transport in foods, although the issue of structural influence on the constant of proportionality is not defined. However, the use of this model without resolving the involvement of structure in describing mass transport is being questioned (Aguilera, 2005).

A number of techniques have been used to elucidate the microstructural properties of foods and these include microscopy (Ferrando and Spiess, 2000); X-ray computed tomography (Falcone et al. 2004; Trater et al. 2005; Leonard et al. 2008); Magnetic resonance imaging (Ramos-Cabrер et al. 2005); computer vision technique (Hullberg and Ballerini 2003; Du and Sun 2006); pycnometry and porosimetry (Taiwo and Baik, 2007; Kassama and Ngadi, 2005a; Rahman et al. 2002). Each technique has its limitations. For example, porosimetry can only be used for bone-dry samples and while it does not produce any images, it produces pore characteristics at a range as low as 0.005 μm. Confocal laser scanning microscopy and X-ray CT techniques produce visual images in 3-D, and are applicable in both qualitative and quantitative assessment of food products microstructures.

2.3.2 Microscopy and food imaging

Microscopy is a method for producing visible images of structures or details too small to otherwise be seen by the human eye, using a magnification tool (Flint, 1994). Many microscopy approaches have been adapted for food application, the earliest types being the light microscopes. The technology is relatively available and inexpensive and was used effectively for determining adulteration in foods as far back as early 19th century (Aguilera and Stanley, 1999; Kaláb et al., 1995; Flint, 1994). Microscopy is native to
biological and medical sciences but its application for food study became more prominent in the 1930s when a book titled “The structure and the composition of foods” was published by Winton and Winton in a four series publication between 1932 and 1939. However, the emergence of the electron microscopes in the early 20th century revolutionized the way biological microstructure was examined. The electron microscope compared to the light microscope has higher resolution and magnification (Table 2.1) but sample preparation has always posed a major challenge. Recent advances include the use of lasers, video microscopy, confocal illumination and tandem scanning (Ferrando and Spiess, 2000; Aguilera and Stanley, 1999). Some of these new powerful microscopes have reduced or removed the constraints found in the early microscopes like tedious sample preparation operation, limitation to 2-D imaging and production of artifacts in images.

Table 2.1 Comparisons between different microscopes.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>LM</th>
<th>SEM</th>
<th>CLSM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>$10^{-6}$</td>
<td>$10^{-9}$</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td>Magnification</td>
<td>$10^3$</td>
<td>$10^1$ to $10^5$</td>
<td>$10^3$</td>
</tr>
<tr>
<td>Lens</td>
<td>Glass</td>
<td>Magnetic Lens</td>
<td>Beam Splitter</td>
</tr>
<tr>
<td>Specimen - preparation</td>
<td>Minimal</td>
<td>Complex</td>
<td>Minimal</td>
</tr>
<tr>
<td>- thickness (μm)</td>
<td>10-100</td>
<td>1-10</td>
<td>10-200</td>
</tr>
<tr>
<td>- environment</td>
<td>Ambient</td>
<td>Vacuum/Gaseous</td>
<td>Ambient</td>
</tr>
<tr>
<td>Illumination source</td>
<td>Visible light</td>
<td>Electron beam</td>
<td>Laser</td>
</tr>
<tr>
<td>Image display</td>
<td>2-D</td>
<td>3-D</td>
<td>3-D</td>
</tr>
</tbody>
</table>

Adapted from Stanley and Tung (1976).
Other imaging methods which have been introduced to study food structures include X-ray CT technique used primarily for *in vivo* human imaging in radiology has been adapted successfully to study agricultural produce (Dull, 1986; Self et al., 1993, Tollner, 1993; van Dalen et al., 2003, 2007). Nuclear magnetic resonance (NMR) or Magnetic resonance imaging (MRI) is another technology currently being explored for non-invasive structural study of food products (Mariette, 2009; Fukuoka, 2002).

2.3.2.1 Light microscopy (LM)

LM is a technique that uses the transmission or reflection of visible light from a sample through a series of lenses to bring magnified images of the subject to the human eye(s), photographic plate, or to a digital screen (Caprette, 2005). Information so obtained have been used to augment those from macroscopic evaluation. The limitations of this technique include tedious sample preparatory step, artifacts in the images acquired, production of 2-D images and the ineffectiveness of the subjective method of observation due to error of the human interpretation. The introduction of advance method of image acquisition using photomicrography, digital imaging and image analysis has reduced the error associated with subjective assessment of micrographs. The magnification obtainable with light microscope is between 10 and 1500 times, and a resolution that is about $10^3$ smaller than unaided eye (> 0.2 μm) (Blonk and van Alast, 1993). Examples of light microscope are bright field microscope, polarizing microscope, and fluorescence microscope. Light microscopy often requires sample preparation such as fixation, cryo-sectioning and staining. Some advantages associated with the use of LM include the observation of samples under environmental conditions; the ease of selective staining;
and the handling of samples under different conditions such as dried, frozen, wet and multiphase samples (Blonk and van Alast, 1993).

2.3.2.2 Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy is a system classified under light microscopy with an enhanced ability to provide an optical sectioning of the sample and includes a component (pin-hole), which eliminates out-of-focus images. CLSM uses laser beam as source of illumination unlike LM and SEM which uses visible light and electron beam, respectively. CLSM operates by sequentially scanning the focal plane of the objective lens by a laser beam, which in turn scans the sample and collects the long wavelength fluorescence generated by the illuminated object through a pin-hole detector (Fig. 2.1).

Figure 2.1 A schematic diagram of confocal laser scanning microscope (Source: http://www.zeiss.com).
The pin-hole eliminates blue-images (out-of-focus images) from reaching the detector to provide an excellent resolution. An optical section of stained sample is obtained through point-by-point scanning of the sample in the x and y coordinates within the focal plane. The movement of the focal point of the objective lens through the focal plane of sample provides for the acquisition of several 2-D images that could be reconstructed in stack using image analysis software to produce a 3-D image. This microscopy technique can be operated in two modes namely epi-fluorescence (needed for samples that were stained) and epi-reflection (used for auto-fluorescence samples) modes (Dürrenberger et al., 2001). CLSM is a powerful optical tool for visualizing structures of biopolymers mixtures like gels and emulsions, and food structures generally (Tromp et al., 2001; Blonk et al., 1995).

The application of CLSM to food imaging is quite new, as traditional light microscopy and other methods pose some limitations especially in sample preparation, affecting the quality and details of image so produced (Kalab et al., 1995). CLSM produces images from a single focal plane of sample with random thickness. The technique produces contrast based on fluorescence emission from different components of the sample. The fluorescence could be auto-fluorescence or from a staining agent (van de Velde et al., 2003). When samples are stained with fluorescent dye, they spread based on their accessibility and affinity. The use of multiple labeling is now a common approach to permit simultaneous assessment of several individual components in one sample. Samples could either be covalently or non-covalently labeled, that is, mixed with or dropped on the sample, respectively. Among the stains used for biopolymers like carbohydrates (CHO) are Fluorescein 5-Isothiocynate (FITC) (CHO; protein),
Rhodamine B (protein) and Nile Blue/Nile Red (fat) (Blonk and van Aalst, 1993; van de Velde et al., 2003). CLSM requires minimal sample preparation and sectioning at temperature range between –20 and 30°C depending on the sample’s content. Mukprasirt et al. (2000a) used CLSM to study the interaction effect of batter components on its adhesion property. Also, biopolymer mixtures behavior was studied using CLSM by van de Velde et al. (2003) and Tromp et al. (2001). Bouchon and Aguilera (2001) used CLSM to study fried potatoes and were able to show that oil is located in the interior pockets or within the surrounding intact cells. Pedreschi and Aguilera (2002) also used CLSM to elucidate oil distribution and cell wall structure of fried potato. Bouchon et al. (2003) employed same system (CLSM) to ascertain that oil is mainly located in the crust region of fried potatoes. Pedreschi et al. (2008) studied topography, oil distribution and uptake on the surface of fried potato slices using CLSM and made similar conclusion as the other authors that oil is located on the surface in fried potato.

2.3.2.3 X-ray computed tomography (CT) scanning

X-ray CT scanning is a method that was developed in 1970s, which was primarily used in the medical field to scan soft tissues and bones in human body non-invasively (Trater et al., 2005; Ketcham and Carlson, 2001). The most used CT scanner in food application is the microtomography, which is also referred to as X-ray micro computed tomography or X-ray microCT (Lim and Barigou, 2004; van Dalen et al. 2003). The high resolving power of X-ray microCT to the single digit microns makes it a good tool for imaging intrinsic elements of heterogeneous system such as food. The fact that it requires no or minimal sample preparation; it acquires images under natural conditions of temperature
Figure 2.2. A schematic diagram of X-ray imaging system (Ngadi et al., 2009).

and pressure; it is a non-invasive/non-destructive imaging technique and its 3-D rendition of images makes it a versatile tool in food imaging applications.

In order to produce images, X-rays generated by an X-ray tube at very high energy levels, in the range of 10-100 keV are first focused on to the sample. The X-ray beam then traverses the sample, probing at axial and lateral resolution up to the micrometers capturing images at different angles (Fig. 2.2). For more X-ray imaging, the sample is rotated at an angle perpendicular to the beam. The projected X-ray energy is either scattered or absorbed based on the compositions of the material. The attenuation of the incident radiation for a monoenergetic beam is defined by Beer Lambert’s law:

$$I = I_0 \exp \left( - \int \mu(x)dx \right)$$  \hspace{1cm} (2.1)

where $I$ and $I_0$ are the transmitted and incident radiations, respectively, $\mu$ is the linear attenuation coefficient (cm$^{-1}$), $x$ is the coordinate of the image in the beam direction and $l$
(cm) is the distance traveled by the radiation inside the sample. Images are captured on to a sensitive X-ray camera, which produces an enlarged image of each projection as 2-D radiographs. These projections are then reconstructed by an algorithm to form a 3-D structure of the object. An example of the reconstruction algorithms is the filtered back-projection algorithm applied by Sasov and van Dyck (1998) for the development of a desktop X-ray CT system. Images produced translate into a map of spatial distribution of the linear attenuation, \( \mu \) i.e. contrast in the images are produced based on the density of the sample, which also determines the amount of absorbed or scattered X-ray energy. The ability to discriminate between similar components (bulk density.linear attenuation) of an image depends on the accuracy of the \( \mu \) of the pixels (Falcone et al., 2006; Denison et al., 1997). In order to quantify normalized linear attenuation of individual components of a digital image, the following expression referred to as CT number is applied:

\[
\rho = 1 + \frac{CT}{k}
\]  

(2.2)

where \( \rho \) is the specific component mass density (g/ml), \( CT \) is computed tomography (CT) number/value and \( k \) is a constant. When \( k \) is 1000, the CT number is referred to as “Hounsfield unit” (Falcone et al., 2006; Kim et al., 2007). Hounsfield unit is an arbitrary scale in which for example CT number of air and water are -1000 and 0, respectively. Fat ranges from -200 to -5. (Barcelon et al., 1999; Tollner et al., 1989; Kim et al., 2007).

Digital images of the types produced by X-ray CCD (charge coupled device) cameras are also represented in shades of white and black referred to as grayscale. The number of bits determines the range of pixel representation in this format. For 8-bits images, the range used is from 0 (dark/black) to 255 (white); for 12-bits, 0 to 4095 and
Table 2.2 General classification of CT scanners

<table>
<thead>
<tr>
<th>CT Scanner type</th>
<th>Scale of observation</th>
<th>Scale of resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>m</td>
<td>mm</td>
</tr>
<tr>
<td>High-resolution</td>
<td>dm</td>
<td>100 µm</td>
</tr>
<tr>
<td>Ultra-high-resolution</td>
<td>cm</td>
<td>10 µm</td>
</tr>
<tr>
<td>Microtomography</td>
<td>mm</td>
<td>µm</td>
</tr>
<tr>
<td>Nanotomography</td>
<td>µm</td>
<td>nm</td>
</tr>
</tbody>
</table>

Source: Ketcham and Carlson (2001).

for 16-bits images 0 to 65,535. The intensity (grayscale index) of black and white in an image is a function of the product density. X-ray CT has been used non-invasively to distinguish changes during ripening of peach, which has potential in robotic harvesting and sorting of peach based on maturity (Barcelon et al., 1999). Lim and Barigou (2004) used X-ray microCT to study the microstructure of a number of cellular food products, acquiring useful quantitative data about the cell network, cell distribution and extent of anisotropy of microstructure. van Dalen et al. (2003) obtained 3-D images and quantitative data of several foods items using X-ray microCT imaging. They showed how this technique can be used to monitor structural changes during the cooking of a rice kernel. Miri et al. (2006) presented pore characteristics of fried potato strips as affected by frying condition using X-ray microCT techniques.

X-ray equipment are primarily classified based on their resolving power (Ketcham and Carlson, 2001). The most suitable class of X-ray CT system for imaging internal morphology of food is the microtomography and nanotomography which have the capability of scanning to few micron levels, targeting food elements that require detailed study, which mostly exist at this scale (Table 2.2).
2.3.3 Image acquisition and analysis

Images can be acquired using traditional photography, digital cameras and scanner microscopes. The use of photography though inexpensive is laborious and comes with array of limitations. Digital image acquisition has taken over a substantial segment of the scientific imaging (Russ, 2005). The commonest is the CCD (charge coupled device) camera which converts optical brightness from an object into electrical amplitude signals using a plurality of CCDs, and then replicates the image of the object using the electric signals without time constrain. CCD was first introduced in 1969 and a basic CCD consists of a series of closely spaced metal-oxide-semiconductor capacitors (MOS), each one corresponding to a single image pixel. In its most basic form a CCD is a charge storage and transport device: charge is stored on the MOS capacitors and then transported across these capacitors for readout and subsequent transformation to a digital image (Farid, 2009). Modern microscopes and table top X-ray scanning systems are equipped with high resolution CCD cameras for image acquisition. Scanning microscopes is a form of image acquisition technique that produces images by raster scanning and can capture images directly for computer storage. Example of systems that make use of this technique are the confocal laser scanning microscope that employs a mirror to deflect laser beam across the axis of the image and scanning electron microscope that uses magnetic fields to deflect an electron beam. Another example is the atomic force microscope (AFM) that produces image by scanning the surface of the sample. Digitally acquired images such as those from MRI, X-ray CT and direct CCD cameras are prone to noise production. Images have to be processed in order to improve their quality and obtain the required information (Du and Sun, 2004).
Image processing enhances the visual appearance of images, allows the observation of detailed structures, facilitates direct measurement, and isolation of important details (Russ, 2005, 2007). As the human eye is incapable of objective and quantitative determinations of image features observed under the microscope or acquired from X-ray CT scanning, image processing through electronic devices is essential. Computer technology and mathematics have played a great role in recognizing, differentiating and quantifying images. Some of the technologies have the capability to perform segmentation on grayscale and color images. There are also a number of softwares that have been developed for image processing, and these have a wide application from biology to material science and food system.

Some of the softwares used in image processing perform various functions such as color improvement, noise removal, 3-D reconstruction of 2-D images, image segmentation and the quantification of parameters like volume, area and dimensions. Examples of these softwares include ImageJ (National Institute Health, MD, USA); DIPlip, a customized software, that works on the platform of MATLAB, developed by a group of scientists in the Netherlands for 3-D image analysis (van Dalen et al., 2003); Fovea Pro and The Image processing Tool Kit (Russ, 2005); MATLAB image processing toolbox (The Mathwork, Inc, Natick, MA, US) etc. Other customized softwares include Nrecon, CTan and CTvol (Skyscan Kontich, Belgium). Nrecon’s algorithm is based on an improved filtered back-projection algorithm for reconstruction of X-ray images; CTan analyses X-ray microCT acquired grayscale images in 2-D and has capability for selection of region of interest (ROI), image smoothing, binarization, erosion, dilation, skeletonization and development of 3-D model; CTvol software is used for 3-D image
visualization and processing. Several combinations of these customized softwares may be used at various stages of X-ray CT image processing.

2.3.3.1 Segmentation and binarization

Segmentation is an image processing step used for selecting pixels that constitute structure of interest from the rest of the image on the basis of their unique brightness or color range (Leung and Lam, 1996). The aim of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze (Russ, 2005; Da Fontoura and Marcondes, 2001; Shapiro and Stockman, 2001). There are basically three types of segmentation namely classification, edge-based and region-based segmentations. Classification segmentation is based on some similarity measure between pixel values e.g. thresholding; edge-based segmentation – this searches for edges in the image often used as borders between regions, and region-based involves region growing, merging and splitting (Baltes, 2009). Thresholding is a major type of segmentation and involves a process of delineation between object of concern in an image by setting its pixels to white color and the remaining background to black (Lee et al., 1998). However, the showing of the binarized image depends on the inversion scale. The object and background could be white and black, respectively, or vice versa. Manual thresholding is considered more accurate based on visual inspection by a human subject; however in most cases this should be avoided if possible (Russ, 2007). Manual thresholding is time consuming and incompatible with automatic processing, in addition to the fact that different results could be obtained at different times and by different people. A number of algorithms have been developed to facilitate the automatic detection
of threshold point in images and these include background symmetry algorithm, Isodata algorithm, triangle algorithm; histogram derived and Otsu algorithm (Russ, 2005; Otsu, 1979; Ridler and Calvard, 1978).

2.3.3.2 Dilation and erosion
Dilation and erosion are image processing steps performed on binarized images to remove errors at the pixel level following the thresholding step. Dilation simply refers to the addition of pixels to increase the size of an object while erosion involves the removal of pixels for the reduction of size. Dilation and erosion are referred to as morphological operations because they alter the shapes of features to produce smoother object boundaries (Russ, 2005). When erosion is preceded by dilation, the process is referred to as closing and the reverse as opening. The two do not produce the same result. These processing steps could be performed as many times as required to obtain a clean image.

2.3.4 Porosimetry
Porosimetry is a technique used for determining pore characteristics of a material. The principles of porosimetry are based on the non-wetting property of the liquids used, the non-reactive nature of the fluids with other materials, the capillary law governing liquid penetration into small pores and the relationship between the intrusion and extrusion pressures and the pore diameter. The pore diameter is calculated using the Washburn expression (Giesche, 2006; Micromeritics, 1999):

\[
D = -\frac{4\gamma \cos \theta}{P}
\]  
(2.3)
where $D$ is the pore diameter, $P$ is given as the pressure required to force the non-wetting liquid into a pore, $\gamma$ is the surface tension of intruding liquid and $\theta$ is the angle of contact between the liquid and the sample. The negative sign of the equation 2.3 is indicative of contact angle greater than 90°. Examples of liquids often used are mercury and Galwick™. Mercury has been used in many studies because of its strong cohesive force and high surface tension, which gives it the ability to resist wetting. Surface tension of mercury at 25°C is given as 484 dynes/cm (0.484 N/m) and it demonstrates high contact angle with solids, hence its choice in most porosimetry studies (Schoonman et al., 2001). The contact angle for most food material ranges between 130 - 140° (Mikijelj et al., 1991). MIP principles are based on the assumptions that the pores are cylindrical in shapes. This assumption is generally accepted to simplify what would have been a complex problem (Ngadi et al., 2001). Pore size distribution is determined based on the relationship between the pore radius and pore volume (Eq. 2.4) (Ritter and Drake, 1945):

\[
D_v(r) = \frac{P}{r} \frac{d(v_r - v)}{dP}
\]

(2.4)

where $D_v(r)$ is the pore size volume distribution function, $P$ is the applied pressure at the point when mercury is forced into the sample, $r$ pore radius that correspond to $P$ every corresponding, $v_r$ is the total pore volume and $v$ is the pore volume at pressure $P$.

The porosity, pore volume, pore area and pore size distribution of foods have been measured using porosimetry technique (Datta et al., 2007). Farkas and Singh (1991) used mercury intrusion porosimetry (MIP) technique to determine the porosity of freeze-dried chicken meat and reported a value of 64%. Ngadi and Kassama (2001) and
Kassama and Ngadi (2005a, b) also used MIP to study pore characteristics of meat patties and fried chicken meat.

### 2.3.5 Pycnometry

Pycnometry is a fluid displacement method which is used for pore volume determination, based on the ability of the gas (Helium) used to penetrate the tiny crevices or the irregularities of the surface of a material (Micromeretics, 1992). First, a porous material is placed into the pycnometer and a pressure of about (20 psig) 137.9 kP is applied to the chamber containing the sample. Next, a valve that connects a second chamber of known volume to the first is released to allow expansion of the gas into the second chamber. The reduction in pressure is noted and used to calculate the volume of the sample based on gas law, excluding the open pores. The following expression is used for calculating the volume of the porous sample:

\[
V_{SP} = V_C + \frac{V_R}{1 - \left(\frac{P_1}{P_2}\right)}
\]  

(2.5)

where \(V_{SP}\) is the volume of the sample, \(V_C\) is the volume of the chamber that contains the sample, \(V_R\) is volume of the second empty chamber, \(P_1\) and \(P_2\) are the pressures before and after expansion into the second chamber. Apparent density can be calculated from the volume obtained by dividing the mass of the sample by \(V_{SP}\) of material that excludes open pores but includes closed pores. To obtain solid density, the material is ground up to remove all closed pores and the sample is analyzed as a particulate solid to obtain a different \(V_{SP}\).
2.3.6 Pore development

Pores are developed during food processing operations such as drying, extrusion and frying as a result of changes in properties of the material. They are very important attributes of foods because they influence the food qualities, the transport phenomena that govern absorption, the conductivity and permeability (Dullien, 1992). Pores exist in various forms within the food matrix and can be either closed or open pores (Fig. 2.3). Closed pores are completely inaccessible and behave as part of the solid material. Open pores consist of blind pores, free pores and linked/interconnected pores, which are usually accessible to the surface of the solid (Ziaiifar et al., 2009; Giesche, 2006). Pores are generally defined in terms of size (diameter of the entry point), area, volume, distribution, interconnectedness and volume ratio (porosity).

![Figure 2.3 Schematic representations of different types of pore in a porous material (Adapted from Ngadi et al., 2009).](image)
Porosity is determined as a ratio of the volume of the accessible pores present in a sample and the overall volume (which may or may not include open pores) of the sample:

\[ \varepsilon = \frac{V_p}{V_{SP}} = 1 - \frac{\rho_b}{\rho_s} \]  \hspace{1cm} (2.6)

\[ \varepsilon = 1 - \frac{\rho_b}{\rho_{ap}} \]  \hspace{1cm} (2.7)

where \( V_p \) is the pore volume, \( \rho_b \) is the bulk density, \( \rho_s \) is the solid density and \( \rho_{ap} \) is the apparent density. Porosity could either be determined in absolute (Equation 2.6) or apparent porosity terms (Equation 2.7). In determining absolute porosity all the pores both open and closed are included (Karathanos and Saravacos, 1993) while for apparent porosity the closed pores are excluded in the calculation (Kassama and Ngadi, 2005b).

Bulk density is measured by liquid displacement and it is the fraction of the mass of sample and the total volume that includes all the pores. Kassama and Ngadi (2005b) used water displacement method to measure bulk density of fried chicken meat. Krokida et al. (2000) used n-heptane for French fries; Vélez-Ruiz and Sosa-Morales (2003) used turnip seeds for doughnut; toluene displacement method was applied by Moreira et al. (2009) on fried potato chips; Hicsasmaz and Clayton (1992) used fine sand to measure the bulk densities of various foods. Maneerote et al. (2009) used volume displacement method, by weighing the pressed down weight of fried rice cracker in a cylinder of known volume to obtain bulk density. Apparent density is a measure of sample mass compared to its volume, excluding the open pores, while solid density would measure the same mass-volume ratio but excludes all pores.
2.4 Use of imaging techniques and other physical methods for effective study of food microstructures

Due to the complexity in the configuration of food, there is usually the need to combine different techniques to obtain meaningful results and information to fully understand food microstructures (Hermansson et al., 2000). As the preparation steps introduce some degree of distortions into samples, it is necessary to use several imaging techniques during food processing (Kalab et al., 1995). Also, in order to be able to cover the minute scales of $10^{-9}$ to $10^{-3}$, a number of different imaging methods might be required (Datta et al., 2007; Hermansson et al., 2000; Schoonman et al., 2001; Aguilera and Stanley, 1999). X-ray microCT and newly developed X-ray nanotomography have resolving power of up to $10^{-7}$ (Skyscan, 2008). Certain porosimeter have capability to measure structures (pores) as small as 0.005 µm (Micromeritics, 1999). The use of microscopy and other physical techniques such as X-ray CT-scanning and MIP has been shown to enhance accuracy of information on food microstructures. Datta et al. (2007) combined MIP and microscopy to obtain full information on pore characteristics of bread from different baking methods. Hermansson et al. (2000) in their review paper on new approaches to characterizing food microstructures presented a work where a combination of CLSM and microscopy techniques were effective in showing that emulsifier addition had pronounced effect on the kinetics of gel formation and structure over long scales. Thus, the use of different techniques for the characterization of food microstructures will facilitate the comparison of results over wider scale of measurement and allow the complete understanding of structural composition of food.
CONNECTING TEXT

Microstructural characteristics are very important in defining fried foods attributes such as the texture, volume, aesthetic appeal, heat and mass transfer, nutritional composition, thermal characteristics and organoleptic properties. In particular, it was noted that information on these characteristics of breaded fried foods are scarce. In chapter 3, mercury intrusion porosimetry method was used to characterize the microstructural properties of deep-fat fried chicken nuggets coating. The effect of frying temperature and time were evaluated.
III. CHARACTERIZATION OF PORE PROPERTIES OF
DEEP-FAT FRIED CHICKEN NUGGETS COATING USING
POROSIMETRY TECHNIQUE

3.1 Abstract

The objective of this study was to characterize the pore properties of deep-fat fried chicken nuggets coating under different processing conditions namely frying temperatures (170, 180 and 190°C) and time (0 to 240 s) using porosimetry technique. Porosity range obtained was between 40 - 69 %. Porosity decreased with frying time. The main effect of temperature on porosity was significant (P<0.05). Porosity showed a high positive and negative correlation with moisture and fat contents and the correlation coefficients ranged between 0.88 – 0.96 and 0.78 - 0.80, respectively. Bulk density increased with frying time while apparent density was relatively the same. Pore distribution showed bimodality. There was no significant effect of temperature on pore size distribution. Over 70% of the pore volume is made up of pores greater than 1 µm. Pore volume ranged between 0.5 and 1.5 cm³/g and it decreased with frying time. Mean pore diameter was between 0.006 and 389 µm while with frying time, it ranged between 0.2 - 8.3 µm. Total pore area was between 2.5 - 16.5 m²/g. Hysteresis phenomenon showed that some of the pores were not perfectly cylindrical in shape.
3.2 Introduction

Deep-fat fried foods remain a major part of our diet although there are concerns about consumption of high calorie content foods. Fried foods are easy to prepare and they possess unique flavor resulting in a huge multi-million dollar market that keeps growing (Agriculture and Agri-Food Canada, 2009; Garcia et al., 2004; Suderman, 1996). The need to reduce fat uptake has become the target of several research studies. Techniques that have been investigated include pre-drying, precooking (par-frying, microwave and oven cooking), and application of surface coating (Adedeji et al. 2009; Pedreschi and Moyano, 2005; Krokida et al. 2001). Some of the coatings used include flour base products such as wheat, rice and corn flour; starch; seasoning agents; and hydrocolloids (cellulose derivatives: carboxymethyl cellulose (CMC), methyl cellulose (MC), hydroxypropyl methylcellulose (HPMC) and others like guar gum, xanthan gum) (Rimac-Brnčić et al., 2004). Food coatings are usually made of batter and/breading system. Batters are leavened flour paste, while breading is made of bread crumbs or ground biscuit. Batter adds value to fried products by the development of unique surface texture, flavor and coloration. They also provides protective barriers to fat absorption and moisture loss during frying due to their increased water holding capacity and structural transformation such as gelation.

Food structure is developed naturally or by processing. Structure of food has been related to quality change experienced during food processing such as drying and deep-fat frying, especially at the microscopic scale (Aguilera, 2005; Donald, 2004; Mellema, 2003; Krokida and Maroulis, 2001; Mallikarjunan, et al., 1997). There has been renewed interest in studying microstructural properties of food such as porosity and pore size.
distribution primarily because of their importance in defining food quality, in better understanding of transport properties of foods and optimization of various processes that lead to production of porous foods (Witrowa-Rajchert and Lewicki, 2006; Aguilera, 2005; Rahman et al., 2002; Pimuths et al., 1995a).

There are several techniques that have been used to study pore characteristics of foods. These include pycnometry (Taiwo and Baik, 2007; Kassama and Ngadi, 2005a, 2004, 2003; Rahman and Sablani, 2003; McDonald and Sun, 2001); microscopy (Liang et al., 2006; Bouchon and Aguilera, 2001); X-ray computed tomography (van Dalen et al., 2007, 2003; Kim et al., 2007; Miri et al., 2006; Lim and Barigou, 2004; Barcelon et al., 1999); magnetic resonance imaging (MRI) (Wagner et al., 2008; Bows et al., 2001) and porosimetry (Kassama and Ngadi 2005b, Rahman and Sablani, 2003; Rahman et al., 2002; McDonald and Sun, 2001; Ngadi et al., 2001; Karathanos et al., 1996; Karathanos and Saravacos, 1993).

The objectives of this study were to elucidate pore characteristics of deep-fat fried chicken nuggets coating using mercury intrusion porosimetry and to relate these pore properties to pretreatment condition, moisture and fat contents.

3.3 Principle of mercury intrusion porosimetry (MIP)

The principle of porosimetry is based on capillary law, non-reactive and non-wetting characteristics of certain liquid such as mercury. Mercury liquid will not penetrate a pore until certain pressure is applied. The relationship between the applied pressure and the diameter of the pore is described by the Young-Laplace equation also referred to as Washburn expression (Giesche, 2006; Dullien, 1992):
\[ \Delta P = \gamma \left( \frac{1}{r_1} + \frac{1}{r_2} \right) = \frac{-2\gamma \cos \theta}{r_{\text{pore}}} \]  

(3.1)

where \( \Delta P \) is the applied pressure, \( r \) is the radius of the pore (\( r_1 \) and \( r_2 \) are the curvature of the liquid interface), \( \gamma \) is the surface tension of the liquid given as 0.485 N/m, and \( \theta \) is the contact angle between the intruding liquid and the wall of the material. The contact angle is usually between 130° and 140° for most food materials and mercury (Giesche, 2006). The negative sign is indicative that the contact angle is greater than 90°. A major assumption in mercury intrusion porosimetry is that the pore shape is cylindrical. This assumption is generally accepted to simplify what would seemingly have been a complex problem (Ngadi et al., 2001). According to Eq. 3.1, the pressure differential is usually proportional to the surface tension of mercury, the contact angle between the material of interest and the intrusion fluid, mercury, and inversely proportional to the radius of the pore. Pore size distribution is determined based on the relationship between the applied pressure, pore radius and pore volume (Eq. 3.2) (Ritter and Drake, 1945):

\[ D_v(r) = \frac{P}{r} \frac{d(v_t - v)}{dP} \]  

(3.2)

where \( D_v \) is the pore size volume distribution function, \( P \) is the applied pressure at the point when mercury is forced into the sample, \( r \) pore radius that corresponds to \( P \), \( v_t \) is the total pore volume and \( v \) is the pore volume at pressure \( P \).

Porosimetry, unlike some other techniques, provides information on bulk and apparent densities, pore size distribution, pore area, pore shape (hysteresis phenomenon) and a wide range of porosity between 0.005 to 360 µm. Porosimetry has been used extensively to study pore characteristics of dried foods (Rahman et al., 2002; Karathanos et al., 1996). The method has also been used for pore characterization of certain
fresh/moisture and partially dried foods (Rahman et al., 2005; McDonald and Sun, 2001) even though explanation on how evacuation of moisture present in the sample was done was not given, and this is a major requirement for effective functioning of the MIP system (Giesche, 2006; Micromeritics, 1999).

3.4 Materials and methods

3.4.1 Materials

The chicken nuggets used for this study was obtained from a local manufacturer (Olymel, Boucherville, QC, CA). The composition of the breading and batter coating of the chicken nuggets included wheat flour, wheat crumbs, spices, guar gum and salt. The chicken nuggets samples were kept at sub-freezing temperature (-50°C) prior to use and brought to refrigeration condition (4°C) for about 4 hours and then transferred to room temperature for about 30 min before use.

3.4.2 Sample preparation

Chicken nugget samples were fried in fresh canola oil at 170, 180 and 190°C for 9 time intervals between 0 and 240 s in a Henny Penny Computron 7000 Pressure Fryer (Model 500C, HP Corporation, Eaton, OH, USA) that has a capacity for 30 L. Three pieces of chicken nuggets with an average volume of 75 cm³/nugget were fried in each run, giving oil to product ratio of approximately 130:1. Excess oil on the surface was wiped off using paper towel. Fried samples were allowed to cool under ambient conditions for 20 min. The coating of the fried chicken nuggets samples was carefully peeled with hand, and quick frozen in liquid Nitrogen. The F-frozen samples chicken nuggets samples were then
kept in the deep-freezer at -40 °C for 24 hours before they were freeze-dried using a laboratory freeze-dryer (Thermo Savant Modulyod-115, NY, USA) for 36 hours.

3.3.3 Mercury intrusion porosimetry

Pore structure characteristics were determined following the methods described by Ngadi et al. (2001) and (Micromeritics, 1999). Freeze-dried fried chicken nuggets coatings were weighed into the penetrometer of the porosimeter (Auto Pore III series 9400, Micromeritics Inst Co., Norcross, GA, USA). The porosimeter is capable of measuring more than 0.005 µm pore sizes and operates at maximum pressure of 228 MPa (33000 psi). The penetrometer is made of glass material in a cylindrical shape with an enlargement at one end in bulb shape, which comes in various sizes for different sample structure (powder or solid). The particular one used for this study had a 15 cm³ bulb volume, a total stem volume of 1.131 cm³ and a maximum measurable intrusion volume of 1.057 cm³. The size of the penetrometer was chosen to ensure maximum measurement accuracy since it was estimated that the total pore volumes of freeze-dried coating samples should be within 25 to 90% of the maximum measurable intrusion (stem) volume of the penetrometer. The amount of pore volume equivalent to a pore size was obtained by Eq. 3.2 above. A plot of cumulative pore volume over the pore size range of the coating is presented as the pore distribution graph. Porosity (ε) was obtained by subtracting the ratio of apparent and bulk volume from one (Eq. 3.4):

$$
\varepsilon = 1 - \frac{V_{app}}{V_{bk}} = 1 - \frac{\rho_{bk}}{\rho_{app}}
$$

(3.4)
where $V_{app}$, $\rho_{app}$, $V_{bk}$ and $\rho_{bk}$ are apparent volume and density, bulk volume and density, respectively. In order to measure pore properties such as porosity and pore size distribution, freeze-dried coating samples were weighed, placed in the penetrometer and loaded in the low-pressure port of the porosimeter. After running the sample at the low-pressure port, the penetrometer assembly was then transferred to the high-pressure port according to the standard protocol (Micromeritics, 1999). The generated data were output to a computer system interphased with the equipment.

### 3.3.4 Moisture content

Moisture content was determined as the mass of moisture in the sample to the mass of the dry matter of the sample (dry basis, db), following a modified procedure described by Bradley and Vanderwan (2001). Fried samples taken at different time intervals were heated in an oven (Isotemp 700, Fisher Scientific, Pittsburgh, PA) at 105°C for 24 hours and the difference in weight before and after was used for computation.

### 3.3.5 Fat content

Fried chicken nuggets coating samples were freeze-dried and ground in a coffee grinder (Bodum 5678-57, C-Mill, USA). Fat content was determined following the protocol of AOAC 960.39 (AOAC, 1990). Samples The freeze-dried fried breading coating samples (3 to 5 g) were each weighed into a thimble for fat extraction in a solvent extractor (SER, Velp Scientifica, Usmate, Italy) using petroleum ether. Fat content was determined as the ratio of the mass of extracted fat and dry matter of the sample. The whole process of extraction took approximately two hours.
3.3.6 Statistical Analysis

All treatments were applied in triplicate; linear regression and analysis of variance were carried out at 5% probability on SAS Version 8.2 (SAS, Inst., Cary, USA) (SAS, 1999) software. Correlation analysis was performed on Microsoft excel spreadsheet (Microsoft-office, 2007).

3.4 Results and discussion

3.4.1 Porosity

Porosity values for deep-fat fried and freeze-dried chicken nuggets coating samples obtained using MIP are presented in Fig. 3.1. Porosity ranged between 40 - 69 %. The control/initial porosity obtained in this study is comparable to those obtained for products

![Figure 3.1 A plot of frying time against porosity of chicken nuggets coating obtained from MIP methods.](image-url)
processed under similar condition by other authors such as Farkas and Singh (1991), King et al. (1968), and Kassama and Ngadi (2005b) who reported porosity values of 65%, 70-80% and 71%, respectively for dried chicken meat. There was a significant (P < 0.05) effect of frying time on porosity of fried samples. Frying temperature equally affected porosity significantly (P < 0.05). Mean separation showed that there is significant difference (P<0.01) between porosities at 170°C, compared to the values at 180 and 190°C. However, there was no interaction effect of temperature and time on pore development in fried batter coating of chicken nuggets. There was a general decrease in porosity with frying time. The batter coating material comprised of various ingredients including water and air pockets prior to frying. Some of the water was evaporated during frying, creating pores on its way out of the product. Parts of these pores were filled subsequently with fat as frying progressed. There is also complex expansion and collapse of the physical matrix of the batter system. Normally, these phenomena could translate to increase in porosity during frying. However, it has to be noted that the fried samples were freeze-dried prior to porosity measurement in order to create the required vacuum in the mercury porosimetry (MIP) system (Giesche, 2006 and Micromeritics, 1999). Pores spaces in fried samples are occupied by moisture, fat and air. Freeze-drying removed moisture but not fat from the pore spaces. Thus, the MIP measurements accounted for the spaces occupied originally by air and the spaces occupied by the water that was removed by freeze drying. The high porosity recorded initially was attributed largely to water which had been removed during the freeze drying step. The longer the frying time, the lower the amount of water available for removal during the freeze drying
Figure 3.2 Correlation graph between porosity of chicken nuggets coating and moisture content (a) and fat content (b). $r = 0.90$, 0.88 and 0.96 for MC, and 0.78, 0.85 and 0.78 for FC at 170, 180 and 190°C, respectively. MC – moisture content; FC – fat content; db – dry basis.
step resulting in the decreasing trend in porosity. Kassama and Ngadi (2005b) also reported a decreasing trend in porosity of deep-fat fried freeze-dried chicken meat. Apparently, fat intrusion into the sample during the frying should also contribute to the decrease in porosity (Pinthus et al., 1995a). This interrelationship between pore development, moisture and fat transfer are illustrated in Figs. 3.2a and 3.2b where moisture loss and fat gained were plotted against porosity, respectively. There were linear correlations between porosity versus moisture and fat contents. Decreasing moisture content resulted in decreasing porosity (correlation coefficient was between 0.88 – 0.96 for the different frying temperatures). Fat content presents a negative correlation with

![Figure 3.3 Frying time effect on pore size distribution as a function of cumulative volume when frying temperature was 180°C.](image)

- Figure 3.3 Frying time effect on pore size distribution as a function of cumulative volume when frying temperature was 180°C.
Figure 3.4 Effect of frying time on percent pore volume distribution (180°C frying temperature) of chicken nuggets coating.

Porosity and the correlation coefficient, r ranged from 0.78 to 0.85. All were statistically significant at P < 0.05.

3.4.2 Pore size distribution

Pore size distribution curves for chicken nuggets coating fried at 180°C are shown in Figs. 3.3 and 3.4. The mean pore size ranged between 0.006 to 389 µm. Similar range was obtained at the other temperatures. Final pore volume ranged between 0.54 - 1.5 cm³/g (Fig. 3.3). There has not been any report in the literature on pore volume of the coating materials. However, Farkas and Singh (1991) obtained a cumulative
Figure 3.5 Graph of pore size distribution against incremental pore volume, showing peaks of volume concentration among pore sizes for sample fried at 180°C.

Volume range of 0.3 and 1.4 ml/g for air-dried and freeze-dried chicken meat, respectively. Kassama and Ngadi (2005b) had reported a range of 0.34 – 2.08 cm³/g for fried chicken meat. Ngadi et al. (2001) presented pore volume range of 0.19–0.37 cm³/g for oven-cooked soy extended beef patties. McDonald and Sun (2001) reported values of 0.63 and 0.71 cm³/g for vacuum tumbled and non-vacuum tumbled cooked beef samples, respectively. There was a general decrease in pore volume with frying time. This is in concert with the porosity trend obtained as discussed previously. A similar trend was reported by Kassama and Ngadi (2005b) for fried chicken meat. Over 70% of the pore
volume was constituted by pores greater than 1 µm (Fig. 3.4). A plot of incremental pore volume as a function of pore size distribution is shown in Fig. 3.5. Peak points in the curves indicate prevalence of certain pore sizes within the distribution (Rahman and Sablani, 2003). There are two peaks (bimodal distribution) shown in most of the frying curves, the first around 0.3 - 6 µm pore diameter and the second between 6 and 389 µm, depending on the frying time. Adedeji and Ngadi (In press) also reported bimodal pore distribution, with two peaks around 94 to 169 µm and 544 to 582 µm for deep-fat fried chicken nuggets batter coatings formulated with different levels of wheat and rice flours. Datta et al. (2007) studying bread baked under different heating modes, also reported bimodality distribution of pore sizes relative to volume. Orr (1980) in his study on a variety of porous food materials reported a bimodal distribution for grains with two maxima at 0.8 and 25 µm and concluded that pores of majority of foods are larger than 0.1 µm. Karathanos and Saravacos (1993) reported bimodal peaks (1-3.5 and 6-8 µm) for granular, unpressed Amioca starch, containing 11% moisture when low intrusion pressure was applied and a third one (3 nm) was shown in the range of high intrusion pressure. Adedeji and Ngadi (In press) also reported bimodal pore distribution, with two peaks around 94 to 169 µm and 544 to 582 µm for deep-fat fried chicken nuggets batter coatings formulated with different levels of wheat and rice flours. The authors applied the X-ray µCT technique and peak pore size ranges were different from those obtained in this study probably due to difference in scale of measurement and samples’ characteritics. There was a diminishing effect of frying time on micro pores (<10 µm) constituent of the sample. After 60 s of frying the position of the first peak moved from around 0.1 µm
Table 3.1 Pore characteristics of chicken nuggets coating fried at 180°C obtained from MIP.

<table>
<thead>
<tr>
<th>Frying Time, s</th>
<th>Bulk Density, g/cm³</th>
<th>Apparent density, g/cm³</th>
<th>Total Pore Area, m²/g</th>
<th>Average Pore Diameter, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.46(0.05)</td>
<td>1.48(0.01)</td>
<td>2.53(0.02)</td>
<td>6.23(0.66)</td>
</tr>
<tr>
<td>15</td>
<td>0.52(0.06)</td>
<td>1.21(0.11)</td>
<td>4.94(0.04)</td>
<td>0.95(0.06)</td>
</tr>
<tr>
<td>30</td>
<td>0.51(0.07)</td>
<td>1.26(0.12)</td>
<td>4.70(0.07)</td>
<td>0.33(0.17)</td>
</tr>
<tr>
<td>45</td>
<td>0.55(0.02)</td>
<td>1.25(0.05)</td>
<td>9.17(0.09)</td>
<td>0.25(0.04)</td>
</tr>
<tr>
<td>60</td>
<td>0.74(0.04)</td>
<td>1.40(0.31)</td>
<td>3.77(0.03)</td>
<td>7.60(0.01)</td>
</tr>
<tr>
<td>90</td>
<td>0.61(0.10)</td>
<td>1.40(0.51)</td>
<td>2.96(0.14)</td>
<td>4.58(0.72)</td>
</tr>
<tr>
<td>120</td>
<td>0.78(0.04)</td>
<td>1.35(0.01)</td>
<td>4.18(0.02)</td>
<td>8.32(0.39)</td>
</tr>
<tr>
<td>150</td>
<td>0.72(0.03)</td>
<td>1.26(0.08)</td>
<td>16.53(0.13)</td>
<td>5.29(0.12)</td>
</tr>
<tr>
<td>180</td>
<td>0.75(0.03)</td>
<td>1.32(0.09)</td>
<td>16.24(0.06)</td>
<td>6.92(0.15)</td>
</tr>
<tr>
<td>240</td>
<td>0.75(0.05)</td>
<td>1.26(0.04)</td>
<td>9.71(0.87)</td>
<td>4.19(0.07)</td>
</tr>
</tbody>
</table>

Numbers in parenthesis are standard deviation.

towards the 10 µm mark and this pattern of distribution was shown up to 240 s frying time (Fig. 3.5).

Bulk density of the samples ranged between 0.5 - 0.8 g/cm³ while the apparent density is from 1.2 to 1.5 g/cm³ (Table 3.1). There was an increase in bulk density with frying time indicating an increase in mass per unit volume of coating sample as a result of fat intrusion during frying. Kassama and Ngadi (2005b) reported similar trend for freeze-dried fried chicken meat. Apparent density remained relatively unchanged during the frying operation ranging between 1.21 and 1.48 g/cm³. Taiwo and Baik (2007) reported apparent density in the range of 1.03 – 1.28 g/cm³ for fried sweet potato. The range obtained in this study is also within the range reported by Kawas and Moreira (2001) for fried tortilla chips and by Kassama and Ngadi (2005b) for fried chicken meat. The increasing bulk density with frying time explained the decreasing porosity with frying time as reported earlier. The average pore diameter varied with frying time between 0.25
- 8.32 µm and did not show any particular pattern with frying time. Kassama and Ngadi (2005b) had reported a range of 0.25 and 1.17 µm for chicken meat fried for up to 360 s. Total pore area was between 2.53 - 16.53 m²/g. There was a general increase in pore area with frying time. Kassama and Ngadi (2005b) had reported a range of 5.86 - 8.24 m²/g for deep fat fried chicken meat. The difference in sample composition could be the reason for the difference seen. Analysis of variance showed that influence of frying temperature on the cumulative pore volume distribution for deep-fat fried chicken nuggets coating was insignificant (P<0.05) (Fig. 3.6). The pore size distribution for the chicken nuggets

![Figure 3.6 Temperature effect on pore volume distribution in deep-fat fried chicken nuggets coating fried for 240 s.](image)

Figure 3.6 Temperature effect on pore volume distribution in deep-fat fried chicken nuggets coating fried for 240 s.
coating fried at 170 and 190°C followed similar trends described for 180° frying temperature above.

3.4.3 Hysteresis

The hysteresis phenomenon is shown for both control (unfried) and fried (15 and 240 s at 180°C) chicken nuggets coating (Fig. 3.7). The occurrence of hysteresis is as a result of change in advancing and receding contact angles of mercury and the material during

![Figure 3.7 Hysteresis phenomenon in deep-fat fried chicken nuggets coating pore. EMV: Percent entrapped mercury volume.](image-url)
intrusion and extrusion process, respectively, such that the amount of mercury forced out during extrusion is less than amount forced in during intrusion even though equal pressure was applied. The “ink bottle” and “energy barrier (snap-off)” phenomena have been described to influence the development of hysteresis trend in mercury intrusion porosimetry. The “ink bottle” phenomenon describes the occurrence of pores that are wider at the base than at the throat, while the “energy barrier” occurrence describes the difference between the intrusion and extrusion pressure which occurs as a result of varied structural dimensions of pores (Giesche, 2006). These two phenomena confirmed the likelihood of different geometry formation aside cylindrical shape in biological materials. However, the evaluation of percent entrapped mercury indicates that minimal disparity exists between intrusion and extrusion volumes (Fig. 3.7). Hence, justifying the assumption of cylindrical shape made in Eq. 3.1. Kassama and Ngadi (2005b) reported similar result for fried chicken meat. Tsakiroglou et al. (1998), Mason and Morrow (1984, 1991, 1992) all used systems with model pores to confirm and explain cause of hysteresis phenomenon in MIP analysis of non-axisymmetric pores. The occurrence of hysteresis phenomenon was evident in all the other coating samples fried at every temperature.

3.6 Conclusion

Pore characteristics of deep-fat fried chicken nuggets coating were determined by mercury intrusion porosimetry. Although frying temperature affected porosity of fried chicken nugget coating, it did not affect pore volume distribution. The decreasing trend shown in porosity distribution with frying time is attributable to the freeze-drying step
required in MIP measurement and this is a major limitation of the technique in that it cannot be used for measuring pore properties of hydrated materials directly without accounting for the moisture removed through freeze-drying. There was a bimodal pore volume distribution indicating different points of pore prevalence in the distribution in the samples. More than 70% of the pore volume is constituted by pores greater than 1 µm. Bulk density decreased with frying time while apparent density remained somewhat the same. There was hysteresis phenomenon in the distribution which showed that some of the pores are not completely cylindrical.

3.7 References


In chapter 3, porosity and pore size distribution of chicken nuggets breaded/batter coating were obtained. The effect of frying conditions was significant on the variation shown in pore development, while moisture loss and fat uptake influenced porosity significantly. A major constraint in the application of mercury intrusion porosimetry to fried products is that the samples must be bone-dry before they are tested. And this was found to produce an opposing trend in porosity obtained. It is therefore imperative to use another method in order correlate the two results. In chapter 4, a gas displacement method (helium pycnometry) was used to study porosity of breading/batter coating of chicken nuggets under different frying conditions, and also the effect of batter formulation on porosity was also considered.
IV. POROSITY DETERMINATION FOR DEEP-FAT FRIED COATINGS USING PYCNOMETER

4.1 Abstract

The effect of processing conditions such as frying time and temperature, and batter formulation on pore development in deep-fat fried chicken nuggets coatings were studied using helium pycnometer method. Chicken nuggets with preformed and lab prepared batter coatings were fried at temperatures between 170 and 190°C for a time range between 0 and 240 s. There was a significant (P<0.05) effect of frying temperature and batter formulation on porosity. Porosity increased with frying time and temperature, and ranged between 2.15 - 47.9% for the preformed batter and 10 – 55% for the formulated batters. Apparent and bulk densities of the preformed batters increased and decreased with frying time, respectively, but both declined gradually with increasing frying temperature. As the level of rice flour in the formulation increased, apparent and bulk densities also increased. Batter formulation and frying temperature significantly (P < 0.05) influenced the variation in moisture and fat content. Porosity demonstrated positive and negative correlation with fat uptake and moisture loss, respectively, for all the batter coatings.
4.2 Introduction

The food market today is driven by consumers’ demand for convenient and healthy food items that require minimal preparation, appealing to the eye and contain fewer calories. Fried foods are among the most consumed the world over and the market keeps increasing. The challenge for food manufacturers is to reduce drastically the amount of fat absorbed into food during the frying process while other qualities of the food are preserved. In order to reduce fat content of fried foods, various researches have targeted modification of food material by pre-drying the product, pre-heating in the microwave or oven, parfrying and applying coating among others (Adedeji et al., 2009, Krokida et al., 2001, Mallikarjunan et al., 1997, Pedreschi and Moyano, 2005). Food coatings are applied because of their functional properties. They act as a barrier to mass transfer during processing such as baking and frying by forming a continuous film of gel on the surface of the product. For example they are used for reducing moisture loss, to preserve juiciness of the product, and fat uptake in fried food items such as onion rings, squid rings, fried chicken drumsticks and chicken nuggets, potato chips (Adedeji et al., 2009, Ling et al., 1998, Moreira et al., 1999, Sanz et al., 2004). Apart from mass transfer control, they also add value to the product in terms of volume, flavor formation and crispy texture development. Food coatings are usually made of batter and/breading system. Batters are either leavened flour paste or interface/adhesive batter applied solely to the surface of the product, while breading is made of bread crumbs or ground biscuit applied to food after the initial dipping of food in more fluid unleavened batter slurry (Loewe, 1990). Food coatings are made from array of ingredients like flours, starch, hydrocolloids, water, seasoning agents and spices. The interactions of these components
during processing form unique structures that define some of the food quality attributes. Wheat is the traditional flour for batter preparation. Other flours such as corn, soy and rice flours are being added to modify the functional and quality attributes of the batter. For example, rice flour addition into batter is reported to reduce fat uptake (Dogan et al., 2005, Mohamed et al, 1998, Shih and Daigle, 1999). The formulation of batter for food application is very flexible. However, some recent researches have focused on understanding and establishing the relationship between their functional properties and formulation composition (Xue and Ngadi, 2006, Akdeniz et al., 2005, 2006, Lee and Inglett, 2007).

Deep-fat frying involves simultaneous heat and mass transfer. The application of heat leads to structural changes as a result of moisture migration from within the food into the frying medium, swelling and collapsing of the food components such as starch and protein (Hussain et al., 2002, Rahman, 2001). The degree of structural modification influences the mass transfer process, it is therefore important to characterize food structures such as porosity and pore distribution in order to optimize mass migration during frying (Aguilera, 2005).

Porosity is a major microstructural property of foods. It influences quality properties of foods such as texture, mechanical, sensory, and transport phenomena during processing such as drying, rehydration and frying (Huang and Clayton, 1990, Ziaiifar et al., 2009, Kassama et al., 2003, Gogoi et al., 2000, Vincent, 1989). Pores occur naturally while some are formed during processing such as drying and frying. Pore development during frying has been attributed mainly to trapped vapor within the food matrix that gets superheated and forces it way out into the frying oil (Kawas and Moreira, 2001, Pinthus
et al., 1995a, Ziaiifar et al., 2009). Pore formation in fried foods depends on processing conditions such as frying temperature and time, food composition, pre and post heating treatments (Ngadi et al., 2001, Kassama and Ngadi, 2005, Hussain et al., 2002). It has been shown that there is a strong correlation between porosity and the amount of fat absorbed during frying (Moreira et al., 1997, Pinthus et al., 1995a, Kassama and Ngadi, 2005). Therefore it is important to have a clear understanding of porosity change in foods during processing.

There are several methods available for determining food porosity. Examples include microscopy, magnetic resonance imaging (MRI), X-ray micro-computed tomography (CT), porosimetry and gas displacement methods (e.g. helium pycnometry) (Taiwo and Baik, 2007, Karathanos et al., 1996, van Dalen et al., 2007, Wagner et al., 2008, Kassama and Ngadi, 2005). Helium pycnometry method is a simple procedure that takes advantage of the small atomic diameter (0.22 nm) and non-reactive characteristics of helium gas to determine products volume, excluding both the surface and connected interior pores, and this can be applied in the computation of porosity (Micromeritics, 1992, Rahman et al., 2002, Ayral et al., 1992, Krus et al., 1997). The objective of this study was to evaluate the effect of frying time and temperature, and batter formulation on pore development in deep-fat fried chicken nuggets batter coating using pycnometry method.
4.3 Materials and methods

4.3.1 Materials and sample preparation

Two groups of batters were used in this study. The first was industrially preformed chicken nugget coating (Olymel, Boucherville, QC, CA). The composition of the preformed batter included wheat flour, wheat crumbs, spices, guar gum and salt. The samples were kept at sub-freezing temperature (-50°C) prior to use and brought to refrigeration condition (4°C) for about 4 hours before use. The second form of batters were formulated in our laboratory with wheat and rice flours at different ratios namely 100:0, 70:30, 50:50, 30:70 and 0:100. The wheat flour (Five Rose All Purpose Flour, Les Cuisines Five Roses kitchens, Montreal, QC, Canada) was purchased from a local grocery store in Montreal, Canada. There properties were provided by the manufacturers as follows. The wheat flour particle size and protein content were 24 µm and 13.3%, respectively. The long grain rice flour (particle size, 105 µm and protein content, 7.2%), RL-100, used was supplied by Rivland Partnership (Riceland Foods, Arizona, USA). Set amount of salt - 1%, carboxymethyl cellulose CMC (TIC Gums Inc, Maryland, USA) - 1% and NaHCO₃ (baking powder) - 0.5% were added to the wheat-rice flour mixture. Flour mixture and water were added at the ratio 1:1.5, respectively. The mixture was thoroughly mixed together using a kitchen mixer (B-Speed Mixer, Black and Dekker, ON, CA). Cut chicken breasts were dusted with flour before dipping into the batter for better pick-up.
4.3.2 Frying

Preformed chicken nuggets were fried in fresh canola oil in a programmable fryer (Henny Penny Computron 7000 pressure Fryer, Model 500C, HP Corporation, Eaton, OH, USA) at temperature ranging between 170 – 190°C for 10 time intervals between 0 and 240 s while chicken nuggets coated with formulated batters were fried at 180°C for the same time intervals. Excess oil on the surface was mopped off using paper towel. Fried samples were then allowed to cool under ambient conditions. Surface coating of the samples was carefully peeled by hand and cut into rectangular shape that weighed between 3 – 5 g.

4.3.3 Apparent density

Apparent volume of the weighed batters was measured in a helium pycnometer (Model 1305 Multivolume, Micromeritics Instrument Corporation, Norcross, GA) (Appendix 5.1). Each sample was placed in the 35 cm³ sample chamber of the pycnometer and was subjected to cyclic action (purging) by pressurizing and depressurizing with helium gas prior to analysis in order to expel all the air and vapor trapped in the pores and crevices. The analysis was conducted at ambient temperature with pressure of up to 135 kPa (19.5 psi). The systems valves were closed initially to allow equilibration to atmospheric pressure. Then the valve that leads to the sample chamber was opened to allow helium gas to enter up to 19.5 ± 0.2 psi (135 ± 1.37 kPa) and the valve was closed for between 15 to 30 s to allow the gas to penetrate the sample thoroughly before pressure ($P_1$) reading was made. The second valve that leads to the expansion chamber from the sample chamber was then opened to allow trapped helium gas to flow in and pressure
(P₂) reading was also taken after equilibration for 15 – 30 s. To determine the sample volume, the following equation (4.1) was used. Detailed procedure is provided in the standard protocol manual of Micromeritics (Micromeritics, 1992). Each sample was measured thrice and three replicates per treatment were used.

\[
V_{SP} = V_c - \frac{V_R}{(P_1/P_2) - 1}
\]

(4.1)

Where \( V_{SP} \) is the volume of the sample; \( V_c \) is volume of the sample cell with the empty sample cup in place; \( V_R \) is the volume of the expansion chamber; \( P_1 \) is sample chamber initial pressure with the expansion chamber valve closed; and \( P_2 \) is the final chamber pressure with the expansion chamber valve open. Apparent density was computed by dividing the apparent volume (including closed pores inaccessible by helium gas) of the sample with the mass.

### 4.3.4 Bulk density

The same set of samples used for apparent density measurement were quickly dipped in a melted paraffin wax in order to cover the surface openings of the samples and then allowed to cool at room temperature. Samples were then dropped into a liquid (water) displacement pycnometer and the displaced volume was recorded as the volume of the sample. Bulk volume of the fresh, unfried chicken nuggets samples was determined by quick freezing and wax application before the water displacement measurement. Bulk density was determined as the ratio of the mass of sample divided by the bulk volume. For unfried formulated batters, a graduated cylinder was used to determine the mass of
known volume of the samples. Porosity, $\varepsilon$ was then calculated as the ratio of bulk density, $\rho_b$ to apparent density, $\rho_{ap}$ subtracted from one, i.e. a ratio of the void/space volume present in the sample to its overall volume:

$$
\varepsilon = 1 - \frac{\rho_b}{\rho_{ap}}
$$

(4.2)

### 4.3.5 Moisture content

Moisture content (MC) of the chicken nuggets batter coatings was determined as the ratio of mass of moisture in the sample to the mass of the dry matter (dry basis, wb). Fried batter coating samples taken at different time intervals were freeze-dried in a freeze dryer (Modulyod-115, ThermoSavant, Holbrook, NY, USA) at 100 mbar for 36 hours and were allowed to equilibrate in a desiccator for 30 min. The difference in weight before and after drying was used for MC computation.

### 4.3.6 Fat content

Fried chicken nuggets batter coatings were freeze-dried and ground in a coffee grinder (Bodum 5679 C-Mill, NY, USA). Fat content was determined following the protocol of AOAC 960.39 (AOAC, 1990). Samples (3 to 5 g) were weighed into thimbles for fat extraction in a solvent extractor (SER 148, Velp Scientifica, Usmate, Italy) using petroleum ether. Fat content was determined as the ratio of the mass of extracted fat and dry matter of the sample.
4.3.7 Statistical analysis

All treatments were applied in triplicates and analysis of variance (ANOVA) was performed on SAS version 8.2 (SAS, Inst., Cary, USA) at 5% probability of error. Where treatment was statistically significant, mean separation was performed using Scheffe’s multiple comparison test.

4.4 Results and discussion

Figures 4.1a and 4.1b show the graphs of moisture content against frying time at different temperatures and for different batter formulations, respectively. Moisture content (db) for the preformed and formulated fried batter coatings obtained varied from 30 – 109% and 33 – 109%, respectively. The percentage of moisture content reported for the preformed batter coating is similar to the range reported by Adedeji et al. (2009). These authors have reported a range of 28.6 – 54.1% wet basis (40 – 118% dry basis). Moisture content generally decreased with frying time due to the evaporation of moisture induced by the elevated temperature of the frying medium. There was significant (P < 0.05) effect of frying temperature and batter formulation on the moisture content of batter coating. The initial moisture content of the preformed batter was lower compared to the formulated batters. This can be attributed to the differences in their formulations. Batter formulated with 100% wheat flour retained more moisture after frying for 240 s compared to the others substituted with rice flour, even though it had lower initial moisture content. This could be due to difference in composition and particle size of the samples (Adedeji and Ngadi, in press). For example, Shih and Daigle (1999) reported that the ratio of Amylose to Amylopectin content of the starch in a batter formulation influences its pasting and
Figure 4.1 Effect of frying temperature (a) and batter formulation (fried at 180°C) (b) on moisture loss in deep-fat fried chicken nuggets coating. W#R# - wheat:rice flour ratio in percentage.
Figure 4.2 Frying temperatures (a) and batter (fried at 180°C) formulation effect (b) on fat uptake of deep-fat fried chicken nuggets coating. W#R# - wheat:rice flour ratio in percentage.
film forming properties, thus the difference in their water holding capacity. Moisture content remained relatively constant after 60 - 90 s of frying and this was due to reduced amount of moisture left in the sample and this pattern is similar to that shown by porosity.

The effect of formulation and frying temperature on fat absorption during frying are shown in Figs. 4.2a and 4.2b. Fat content obtained for the preformed and formulated batter coatings were between 3.84 - 13.4% and 0.37 – 36.1%, respectively. ANOVA test showed that temperature and batter formulation significantly (P < 0.05) affected the variation shown in fat uptake. Batter formulated with 100% wheat flour absorbed the least amount of oil. This could be connected to the same reason for higher moisture retention by the same formulation discussed earlier. Fat content increased with frying time and became constant after 60 s. Adedeji et al. (2009) had reported similar trend for microwave precooked and unprecooked deep-fat fried chicken nuggets coatings.

### 4.4.1 Porosity

The plot of porosity as a function of frying time at different temperatures (170, 180, 190°C) for the preformed batter coating are shown in Fig. 4.3. Porosity range for the preformed fried batter was 2.2 - 47.9%. Adedeji and Ngadi (2009) reported porosity obtained by X-ray micro-computed tomography (CT), for a similar chicken nuggets batter coating, and fried under similar conditions to be in the range from 7 – 14%. The difference between the pycnometer result as reported in this study and that of X-ray micro-CT scanning could be attributed to sensitivity and range of pore measurement for the 2 different techniques. X-ray microCT used in their study can only measure pores
greater than or equal to 5 µm, while helium pycnometer would measure pores as small as 0.22 nm (Ayral et al., 1992, Krus et al., 1997, Skyscan, 2008). Datta et al. (2007) made similar presentation for porosity of bread obtained from processed scanned image and helium pycnometry. The authors presented porosity between 38 – 42% for the imaging method and 72 – 79% for helium pycnometry method. The reason they adduced on the difference was that most of the smaller pores are outside the range measurable by the
imaging technique they used. Thus, considering the results in this study, it is apparent that there were considerable small size pores in the fried coatings.

Analysis of variance (ANOVA) showed that porosity of the preformed batter was significantly ($P < 0.05$) affected by frying time and temperature. However there was no interaction effect of frying time and temperature on variation observed in porosity at a confidence interval of 95%. Mean separation showed that there was a significant difference in porosity obtained at 170°C and 180°C compared to 190°C. Porosity was generally higher at 190°C frying temperature compared to the lower temperatures. There was a general increase in porosity with frying time at every temperature. A gradual increase in porosity was observed for the first 60 to 120 s of frying, beyond this period, porosity remained relatively constant. Taiwo and Baik (2007) reported a gradual increase in porosity of deep-fat fried sweet potato with frying time. Kawas and Moreira (2001) also presented porosity data for pretreated tortilla chips that showed increase with frying time. Krokida et al. (2000) showed that porosity of fried potato increased with frying time but became constant after 5 min of frying. The increase in porosity with frying time was a result of changes in the structural configuration of the sample, which was induced by the heat and mass transfer process. In deep-fat frying, food is subjected to intense heating that leads to starch gelatinization (swelling of starch granules) and evaporation of moisture which forces its way out into the frying oil, leaving behind voids and creating path on its way out. The longer the frying the more pores are created until an equilibrium is reached when there is limited moisture left in the food. Other factors such as initial moisture content, the intensity of frying, pretreatments and product formulation
Figure 4.4 Effect of formulation on porosity of batter fried at 180°C. W#R# - wheat:rice flour ratio in percentage.


The effects of formulation on porosity in batters formulated using different combinations of wheat and rice flours, fried at 180°C for various time intervals, are shown in Fig. 4.4. For these samples, porosity ranged between 10 and 55% with frying time. The porosity after frying for 240 s varied between 42 – 46%. Adedeji and Ngadi (In
also presented a final porosity, for batter formulations similar to those used in this study, in the range of 18.2 – 32.2%. The variation observed between these two studies can be attributed to the difference in scale of measurement described above. Dogan et al. (2005) used gas pycnometry method and obtained final porosity in the slightly lower range of 33 – 37% for fried chicken nuggets batter coating formulated with different types of flours. Analysis of variance showed that there was significant (P < 0.01) effect of batter formulation and frying time on porosity. However, there was no significant difference between the mean of final porosities of the different batter formulations. Batter formulated with 100% rice flour tends to have higher porosity compared to the others during the initial period of frying but this reduced during the later stage to levels shown by others. The higher initial increase in porosity of batter formulated with 100% rice flour could be adduced to the fact that they had higher initial moisture content (62.4%) compared to the other formulations (Fig. 4.1b). Batter formulated with 100% wheat flour had the least porosity, however, it was not statistically different from the others. The addition of 30% rice flour into the formulation increased porosity when compared to 100% wheat flour, which then reduced with the addition of higher percentage rice flour and remained relatively constant up to 100% rice flour substitution. The same pattern was shown by Adedeji and Ngadi (In press) for similar formulation under the same frying conditions although they showed that porosity of batter with 100% wheat flour was significantly different from others. The low porosity displayed by batter with 100% wheat flour could be attributed to the high protein content of wheat compared to rice, which gave it a higher water binding capacity (Dogan et al., 2005; Senthil et al., 2002). Porosity increased gradually with frying time for all formulations up to about 60 s as was
discussed previously, after which it decreased slightly until frying was terminated. This pattern was shown in all the formulations. The increase in porosity up to the 60 s mark was also shown in the preformed batter (Fig. 4.3). However, the rate of increase in the porosity within the 60 s of frying of the preformed batter was lower compared with the formulated batter (Fig. 4.4). The higher rate of pore development observed in the formulated batter compared to the preformed batter could be attributed to the higher initial moisture content of the formulated batter (Figs 4.1a and 4.1b). Reduced initial moisture content would make less water available for evaporation at the surface and since pore formation is closely related to moisture removal, it is not surprising that the preformed batter showed a lower pore formation rate at the onset of frying because there was less moisture available for removal (Kassama and Ngadi, 2005; Lamberg et al., 1990, Mellema, 2003; Ziaifar, et al., 2009). Moreira et al. (1997) reported that higher initial moisture content is synonymous with higher water diffusion rate during frying of fried tortilla chips, which they reported resulted in formation of small capillary pores that caused increased fat uptake compared to product of lower initial moisture content. Also, the observed higher porosity of the formulated batter compared to preformed batter could be attributed to difference in composition. The formulated batter contained a leavening agent (NaHCO₃) that was absent from the preformed batter. Baking powder would normally lead to the production of air pockets within the network of the formulated batter, which is visually assessable. Dogan et al. (2005) had also reported a decreasing trend for porosity of fried chicken nuggets batter formulated with soy and rice flours and they observed a slight decrease in porosity after frying for about 9 min. They attributed the decrease in porosity to oil suctioned into the air pores created by evaporated moisture
propelled by capillary force. Pintthus et al. (1995a) in their research on restructured potato asserted that since porosity and oil uptake increase simultaneously, oil uptake at some point will begin to cause a decrease in porosity.

Apparent density for the preformed fried batter coating ranged between 0.9 – 2.6 g/cm³, while the bulk density was between 0.9 – 2.1 g/cm³. Apparent and bulk densities for the formulated fried batter coating varied between 0.09 – 2.6 g/cm³ and 0.71 – 1.3 g/cm³. Apparent density generally showed a gradual increase with frying time, which tends to reduce with increasing frying temperature in the preformed batter and in the formulated batter, it showed an increasing trend with increased addition of rice flour into the formulation. The increase in apparent density with frying time indicates that there was increase in mass of the sample per unit volume, modulated by mass transfer during frying (Krokida et al., 2000). Taiwo and Baik (2007) reported a slight increase in apparent density with frying time for deep-fat fried sweet potato. Kassama and Ngadi (2003) also reported an increase in apparent density for fried chicken meat with frying time. Increased apparent density with frying time was also reported by Krokida et al. (2000) in their study on fried potato. Bulk density for all the samples decreased gradually with frying duration and temperature, but increased with the addition of more rice flour into the formulation. Math et al. (2004) reported that bulk density of fried papad, an Indian snack, decreased with frying time and temperature. Kassama and Ngadi (2003) reported a decrease in bulk density with frying time for chicken meat fried between 170 – 190°C. They presented a bulk density in the range of 0.9 - 1.2 g/cm³. These authors also observed a decrease in bulk density with increasing frying temperature. Change in bulk density has been attributed to development of air pores, moisture loss and fat uptake.
during frying, which led to a much higher increase in volume relative to increase in mass observed with frying time (Math et al., 2004, Krokida et al., 2000; Taiwo and Baik, 2007).

The graph of porosity versus moisture content for the preformed and formulated batter coatings are shown in Figs. 4.5a and 4.5b. The relationship between moisture loss and porosity for the samples were modeled using a simple linear expression (eq. 4.3):

$$
e = a + b*VR$$  \hspace{1cm} (4.3)

where $a$ and $b$ are constants, $VR$ is a dependent variable which could be moisture or fat content. The relationship above expressed porosity as a function of moisture content. Analysis of variance (ANOVA) showed that the effect of the model was significant ($P<0.01$) on the variation in porosity at every temperature and was also significant with respect to the formulation effect. Correlation coefficient, $r$ varied between 0.89 - 0.95 and 0.75 – 0.92 for the preformed and formulated batter coatings, respectively. The $a$ and $b$ values of the model fitting are shown in Table 4.1. This parameter shows a negative correlation and also indicates that the data fit the model very well. It also implies that

<table>
<thead>
<tr>
<th>Treatments</th>
<th>a</th>
<th>b</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>170°C</td>
<td>103</td>
<td>17.8</td>
<td>0.90</td>
</tr>
<tr>
<td>180°C</td>
<td>85.4</td>
<td>18.6</td>
<td>0.88</td>
</tr>
<tr>
<td>190°C</td>
<td>94.5</td>
<td>19.1</td>
<td>0.96</td>
</tr>
<tr>
<td>W100R0</td>
<td>-1.52</td>
<td>110</td>
<td>0.75</td>
</tr>
<tr>
<td>W70R30</td>
<td>-1.06</td>
<td>86.3</td>
<td>0.86</td>
</tr>
<tr>
<td>W50R50</td>
<td>-0.77</td>
<td>66.5</td>
<td>0.92</td>
</tr>
<tr>
<td>W30R70</td>
<td>-0.99</td>
<td>77.2</td>
<td>0.88</td>
</tr>
<tr>
<td>W0R100</td>
<td>-0.96</td>
<td>76.3</td>
<td>0.89</td>
</tr>
</tbody>
</table>

$r$: correlation coefficient, $a$ and $b$ are parameters of the linear equation 4.3.
Porosity increased as moisture content decreased during the frying process. Kassama and Ngadi (2005) used a similar linear model to determine the correlation between porosity and moisture content of deep-fat fried chicken meat and obtained a good fit. Rahman and Prakash (1990) also predicted porosity of dried squid as a function of moisture ratio. They also used a linear model and obtained r of 0.97.

The plots of porosity against fat content are shown in Figs. 4.6a and 4.6b. Fat content of the samples generally increased with increasing porosity. In order to describe the relationship between porosity and fat content of the fried chicken nuggets batter coatings, several models (linear and nonlinear) were tried, and the best fit was obtained with simple linear regression equation similar to equation 4.3. The correlation coefficients, r ranged between 0.71 – 0.76 and 0.78 – 0.97 for the preformed and formulated batter coatings, respectively, at 5% probability of error (Table 4.2). The positive correlation shown between porosity and fat content signifies that both increased

<table>
<thead>
<tr>
<th>Treatment</th>
<th>a</th>
<th>b</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>170°C</td>
<td>-293</td>
<td>81.6</td>
<td>0.78</td>
</tr>
<tr>
<td>180°C</td>
<td>-311</td>
<td>82.5</td>
<td>0.85</td>
</tr>
<tr>
<td>190°C</td>
<td>-362</td>
<td>83.4</td>
<td>0.78</td>
</tr>
<tr>
<td>W100R0</td>
<td>1.16</td>
<td>15.3</td>
<td>0.78</td>
</tr>
<tr>
<td>W70R30</td>
<td>1.04</td>
<td>14.9</td>
<td>0.93</td>
</tr>
<tr>
<td>W50R50</td>
<td>0.84</td>
<td>15.5</td>
<td>0.87</td>
</tr>
<tr>
<td>W30R70</td>
<td>1.02</td>
<td>13.3</td>
<td>0.82</td>
</tr>
<tr>
<td>W0R100</td>
<td>1.37</td>
<td>9.13</td>
<td>0.97</td>
</tr>
</tbody>
</table>

r: correlation coefficient, a and b are parameters of the linear model.
Figure 4.5 The graph of porosity versus moisture content for preformed (a) and lab formulated (b) deep-fat fried (180°C) chicken nuggets batter coatings. W#R# - wheat:rice flour ratio in percentage.
Figure 4.6 A plot of porosity against fat content for (a) preformed and (b) lab formulated deep-fat fried (180°C) batters. W#R# - wheat:rice flour ratio in percentage.
concurrently during frying. Kassama and Ngadi (2004) had correlated porosity and fat uptake of fried chicken meat and obtained R as 0.79 at a confidence interval of 99%. Pinthus et al. (1995a) obtained higher R values (0.99 and 0.94) between fat content and porosity for control and treated restructured deep-fat fried potato, respectively. They ignored the effect of intruded fat, hence the higher correlation coefficients.

4.5 Conclusion

Porosity of deep-fat fried preformed and lab formulated chicken nuggets batter coatings were determined by helium pycnometry method. Porosity of the preformed batter varied between 2.2 - 47.9% within the three frying temperatures while that of formulated batter ranged from 10 – 55%. Batter formulation and frying temperature significantly influenced porosity. Apparent density demonstrated a gradual increase with frying time for the preformed batter coating while bulk density decreased. Apparent and bulk densities increased as the level of rice flour in the formulation increased. Moisture and fat content were significantly (P < 0.05) influenced by the batter formulation and frying temperature. The preformed and formulated fried batter coating porosity showed negative and positive correlation with moisture loss and fat uptake, respectively.

4.6 References


Porosity of deep-fat fried chicken nuggets batter coating was obtained using helium pycnometer. The effect of frying temperature and batter formulation were found to be significant (P < 0.05) on the variation observed in porosity. In chapter 5, an imaging technique, X-ray micro-computed tomography was introduced to obtain 3-D images of fried chicken nuggets coating and the chicken core. Various pore characteristics were also obtained.
V. MICROSTRUCTURAL CHARACTERIZATION OF DEEP-FAT FRIED BREADED CHICKEN NUGGETS USING X-RAY MICRO-COMPUTED TOMOGRAPHY

5.1 Abstract

The use of X-ray micro-computed tomography (CT) for food microstructural evaluation is relatively new and recent studies have shown that the technique has the potential to elucidate microstructural properties of food with greater details and resolution than some other imaging techniques such as microscopy. The technique was used to study the microstructural characteristics of chicken nuggets deep-fat fried at 180°C for various time intervals between 0 and 4 min. The results obtained showed a significant (P<0.05) influence of frying time on the microstructural properties of the deep-fat fried breaded chicken nuggets such as porosity, fragmentation index and pore count. Porosity of the batter coating varied from 7.0 – 14%, while that of the core was from 4.5 - 7.7%. The number of pores also increased significantly with frying time. The chicken nuggets coating and core pores showed a decreased interconnectivity after frying. The shapes of the coatings’ pores were between rod-like/cylindrical and spherical structure and those of the core were more rod-like. The pore size distribution for the coating and the core showed that there was increase in volume and number count for pores with diameter < 100 µm with frying time. There were some degree of correlations between the sample’s (coating and core) porosity, frying time, fat content and moisture loss.
5.2 Introduction

Recently, there has been an increased interest in the study of food microstructures. This is due to the fact that consumers’ preferences have shifted to foods that are healthier and have inviting aesthetic appeal, superior taste and convenience. It has been reported that most constituents that determine food qualities exist at micro-level. Food researchers and manufacturers are focusing more attention to understanding, and establishing the relationship food microstructures has with quality (Lim and Barigou 2004; Aguilera, 2005). Understanding how these elements are configured, related and interact to affect food quality, processing and process optimization has become very important in developing tailored and novel food items to meet the demand of today’s diverse and sophisticated consumers (Heertj, 1998; van Dalen et al., 2003; van Dalen et al., 2007). Several techniques have been used to study food microstructures and these include microscopy (Kalab et al., 1995; Ferrando and Spiess, 2000), magnetic resonance imaging (Maas and Line, 1995; Ramos-Cabrér et al., 2005), computer vision technique (cameras) (Hullberg and Ballerini, 2003; Du and Sun, 2006;), porosimetry (Rahman et al., 2002; Kassama and Ngadi, 2005a) and most recently X-ray computed tomography (CT) (Falcone et al., 2004; Trater et al., 2005; Leonard et al., 2008). X-ray CT has numerous advantages over the other techniques and these include a non-invasive method of imaging and a non-elaborate sample preparation procedure. In X-ray CT imaging, the components of the product are differentiated based on differences in densities, which also determine the degree of X-ray absorption. The technique also permits image acquisition under normal environmental conditions with minimal artifacts production. In addition, X-ray CT imaging has a high resolving power to the few microns, and has the advantage of
producing 3-D models which details internal morphology of samples unlike some of the other techniques. Thus, it has the capacity to increase the accuracy of obtained qualitative and quantitative data on internal structures of the products.

Frying is one of the oldest methods of food preparation that adds distinctive qualities such as flavor, palatability, unique texture, color and structure to food. During frying the foods undergo physico-chemical changes, such as starch gelatinization, protein denaturation, shrinkage, swelling and crust-hardening as a result of heat and mass transfer. These changes define food microstructural properties such as porosity, pore size, pore shape, pore interconnectivity and pore size distribution, which significantly influence some quality characteristics of food such as mechanical properties, sensory attributes and textural characteristics (Aguilera, 2005; Huang and Clayton, 1990; van Dalen et al., 2007). These microstructural properties are also useful in estimating some of the food’s physical properties including thermal diffusivity, thermal conductivity and mass transfer coefficient (Aguilera, 2005; Rahman, 2001; van Dalen et al., 2007). Therefore, a good knowledge of these microstructural characteristics is very useful in fried products’ quality assessment, frying process optimization and development of novel fried foods. Kassama and Ngadi (2005a, 2005b) have studied pore characteristics of fried chicken meat with mercury intrusion porosimeter and helium displacement pycnometer, and they presented elaborate results on porosity, pore size distribution, pore size, and pore shape. There has not been any attempt to use a non-invasive technique to examine pores in composite products like chicken nuggets with breaded coating and chicken core. The aim of this study was to evaluate microstructural characteristics of deep-fat fried breaded chicken nuggets using X-ray micro-CT.
5.3 Material and methods

5.3.1 Material

Battered and breaded chicken nuggets procured from a commercial manufacturer were used in this study (Olymel, Boucherville, QC, CA). The batter and breading coatings of the nuggets were prepared from wheat flour, wheat crumbs, spices, guar gum, salt and corn starch and the core part from minced and molded chicken breast. The samples were kept frozen at -18°C until used, and were refrigerated at 4°C for about 4 h and room temperature for 30 min prior to frying.

5.3.2 Frying

The chicken nuggets were fried at 180 ± 2°C at selected time intervals (0, 1, 3 and 4 min) using a programmable fryer (Henny Penny Computron 7000 pressure Fryer, Model 500C, HP Corporation, Eaton, OH, USA), with capacity of 30 L. The samples were drained and allowed to cool at ambient condition.

5.3.4 Moisture content

For the determination of the moisture content, samples were placed in an oven (Isotemp 700, Fisher Scientific, Pittsburgh, PA) at 105°C for at least 24 h. Weight before and after drying were recorded and used to calculate the moisture content on dry weight basis (db).

5.3.5 Fat content

The fat content was determined according to AOAC method 960.39 (AOAC 1990). Freeze-dried samples weighing 3 - 5 g were ground using a blender (Bodum 5679 C-Mill,
NY, USA), and placed in thimbles in a VELP SER 148 (Velp Scientifica, Usmate, Italy) solvent extraction unit and the oil was extracted with petroleum ether. The fat content was computed by dividing the mass of extracted oil by the mass of the freeze-dried sample.

### 5.3.6 X-ray micro-CT scanning

Chicken nuggets samples were cut into pieces of dimension 0.5 x 0.5 x 1 ± 0.1 cm with a sharp knife, from the coated surface through the chicken core. The cut samples were wrapped in parafilm to prevent drying during scanning. The samples were scanned in a X-ray micro-CT (SkyScan 1072, SkyScan, Kontich, Belgium) (Appendix 2.1) at the following setting regulated to obtain maximal differentiation in samples’ components in the images: X-ray energy of 100 keV, a current of 98 µA, exposure time of 6.0 s and a rotation step of 0.68° through 180°. No filter was used in order to increase the contrast of the images obtained. The magnification of the images was 20x and a cross-section pixel size of 9.38 µm was realized. Each scanning lasted approximately 69 min and the images obtained were reconstructed into series of 2-D images using a reconstruction software (Nrecon, SkyScan Belgium) that operates on modified filtered back-projection algorithm (Feldkamp et al., 1984; Sasov and van Dyck, 1998). A total of 916 slices of 2-D images were obtained in 8-bit bitmap format at a resolution of 1024 X 1024 pixels for each sample that projects through the sample from the surface coating to the chicken core. The images were then reduced to half sizes (512 X 512 pixels) by TConv software (SkyScan, Belgium) in order to reduce the quantitative data acquisition’s processing time.
5.3.7 Image processing and analysis

Various image processing steps and quantitative assessment were performed. The region of interest (ROI) was selected randomly at an area close to the surface (coating ≈ 2 mm thick) and within the length of the chicken core. Thirty slices were chosen in three replicates for both the coating and the chicken core. Gaussian filter was applied to smoothing the grayscale images to remove noise. Segmentation of the images was carried by automatic thresholding, using Otsu’s algorithm (Otsu, 1979) on the platform of an image processing software, ImageJ (National Institute of Health, MD, USA). The threshold points obtained for the fried coating and core images were 78 and 70, respectively, within the range of 0 and 255 for 8-bit images. The optimal threshold point was then applied to automatically delineate between the background, which in this case was the solid constituent of the coating, and the object, which represent the pores. Dilation and despeckling (Median filter) were carried out to smoothing the edges of the objects (pores) and further remove noise from the binarized images, respectively. The 3-D image analyses and 3-D model construction were performed using CTan and CTvol softwares (Skyscan, Belgium), respectively.

All treatments were applied in triplicates and analysis of variance was performed using SAS 8.2 version (SAS Inst. Inc., Cary, N.C., U.S.A.) to determine the effect of frying time on various parameters of the samples. Duncan multiple range test was used for mean separation at $P < 0.05$.

5.4 Results and discussion

Fig. 5.1 shows the images of the ROI of grayscale, binarized and 3-D models of fried
Figure 5.1 Two-d grayscale (a) and binarized (b) images of chicken nuggets coating fried at 180°C for 4 min; 3-D model from the same sample showing pore as solid network (c); and other components of the sample as solid matrix (d).

chicken nuggets coating. The grayscale image (Fig. 5.1a) included a 421 x 421 pixels image of a randomly selected ROI of the fried chicken nuggets coating and the binarized image (Fig. 5.1b) was obtained after performing series of morphological operations such
Figure 5.2 Reconstructed three-dimensional model of deep-fat fried chicken nuggets
meat core: pores appearing as transparent network (a) and showing pores as
solid portion of the coating (b).

as dilation and smoothing.

Three-dimensional model of the sample image was produced by consecutive
stacking of several 2-D slices with the aid of image processing software (CTan, Skyscan,
Belgium). Figs. 5.1c and 5.1d show two 3-D models of the same fried chicken nuggets’
coating. Fig. 5.1c shows the spatial distribution of the pores as well as the areas where
they formed a solid network within a transparent body of the other coating components.
Fig. 5.1d highlights the pores as a transparent entity, interconnected and scattered within
the coating network. Figs. 5.2a and 5.2b are reconstructed 3-D images of the fried
chicken core. Fig.5.2a shows the pores as scattered solid bodies within the food matrix,
while in Fig. 5.2b the pores were deselected as the object and were shown as transparent
network within the batter medium similar to the coating image in Fig. 5.1d. These 3-D
images of the core show that the pores were uniform in size, smaller and more evenly

Figure 5.3 The graph of pore volume distribution against pore size for chicken nuggets coating-control (unfried) and sample fried at 180°C for 4 min. distributed than in the coating.

5.4.1 Three-D image analysis

Quantitative data were calculated from the obtained volume of interest (VOI) which comprised of stack of chosen 2-D ROI of the samples. The pore size distributions in terms of volume frequency (volume occupied by pores within a range of pore size) for both the
coating and the chicken core parts are shown in Figs. 5.3 and 5.4, respectively. The pore size obtained ranged from 9 to 309 µm and from 9 to 159 µm for the coating and chicken core, respectively. The limit of measurement of the X-ray system used in this study was 5 µm (Skyscan, 2001), though the actual resolution of the images was 9.4 µm because of the optimized setting of X-ray system, for better contrast, used. The pore size distribution was found to be generally bimodal in the fried chicken batter coating with two peaks at the range 70-98 µm and 155-183 µm (Fig.5.3), but was unimodal in the core (Fig. 5.4). Other authors have reported similar bimodality for porous foods such as bread baked

![Figure 5.4 Graph of pore volume frequency versus pore size distribution for chicken nuggets core - control (unfried) and fried (180°C for 4 min) sample.](image-url)
under different heating modes (Datta et al. 2007) and formulated fried batter (Adedeji and Ngadi, 2009). The prevalence of unimodality in our work could be attributed to a wider range of pore size selection (automatically chosen by the software based on the diameter of the smallest pore present in the sample) in this study, which limited the probability of obtaining other peaks at close pore size range. The graph of the volume frequency against pore size for the coating indicated a decrease in the volume of pores with diameter <127 µm, increases of pores with sizes between 155-253 µm and the reduction of pores ranging between 253-309 µm (Fig. 5.3). However, frying resulted in an increase in the volume of pores with size less than 84 µm in the chicken core.
A substantial decline in volume of larger pores within the range of 84 to 141 µm in the core part was also observed.

Figs. 5.5 and 5.6 show the percent pore numbers (count) versus pore size distribution for the coating and chicken core parts, respectively. Control (unfried) and deep-fat fried chicken nuggets coating with pore size < 65 µm constituted over 87% percent of the total pore count. Frying caused the development of smaller pores with average diameter of < 20 µm and a significant increase in pore count for pore size less than 100 µm in the coating as a result of structural changes such as collapse and disconnection of pore walls due to shrinkage and mass transfer, caused by thermal effect.
of frying. Similar result was observed for the chicken core. The core portion showed lower percentage pore count for pores less than 100 µm compared to the coating, both for the control and fried samples (Fig. 5.6). Considering the 3-D images of the coating and the core, it was anticipated from the visual observation that the core would present higher number of small pore, but the reverse was the case. This could be due to the structuring of the chicken core, which upon closer look showed on the average smaller and almost evenly distributed pore size. There were fewer pores in the range of 100 – 300 µm for both the coating and the chicken core after frying and this indicated that the bigger pores probably disintegrated into smaller ones. Pan and Singh (2001) in their study on beef cooking had associated pore disintegration to structural collapse during intense heat processing. The pore count showed high percentage for small pores (< 60 µm) but low percentage for pore volume for pores of the same size range, both in the coating and chicken core (control and fried) samples. Generally, there were fewer pores with high percent volume for both the coating and chicken core, implying that larger pores constituted the most pore volume though they were fewer in number (Figs. 5.3, 5.4, 5.5 and 5.6).

Table 5.1 presents the quantitative data of pore characteristics obtained from the 3-D analysis of the deep-fat fried breaded chicken nuggets. The results showed that porosity ranged between 7 - 14% for the coating and 4.4 - 7.7% for the chicken core. Barutcu et al. (2009) presented similar porosity values ranging between 21.8 - 48.4% for deep-fat fried batter coating formulated with wheat, rice and soy flours and fried using microwave and conventional fryer. The high values reported by these authors could be
Table 5.1 Pore parameters of chicken nuggets coating and chicken core portion as measured using X-ray micro-CT and CTan software.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Frying Time, min</th>
<th>Porosity, %</th>
<th>Fragmentation Index 1/mm</th>
<th>Structure Model Index (shape)</th>
<th>No. of Pores</th>
</tr>
</thead>
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<tr>
<td>Coating</td>
<td>0</td>
<td>6.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>3,772&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5</td>
<td>1,951&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3</td>
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<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
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</tr>
<tr>
<td></td>
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</tr>
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<td>Core</td>
<td>0</td>
<td>4.45&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2</td>
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<td>2</td>
<td>729&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean with different letter superscript in the same column are significantly differently (P<0.05). Fragmentation index - degree of interconnectivity.

attributed to differences in the composition of the batter and the frying method. Akdeniz et al. (2005) using a fluid displacement method, reported a porosity range of 6 - 15% for formulated fried coating of carrot. Kassama and Ngadi (2005b) presented a porosity range between 0.05 and 27% for fried chicken meat. They had used fluid displacement technique to obtain porosity. This disparity could be due to the differences in the type of products used. In this study, minced chicken product blended with other products was used as compared to an intact chicken breast meat in their study. Frying time significantly (P < 0.05) affected the porosity of both the breading coating and the chicken core. Porosity of the coating increased significantly as frying progressed indicating changes in the structural formation initiated by intense heat from the frying oil leading to physicochemical changes such as gelatinization of starch and denaturation of protein components, and also moisture and fat migration from within and to the product, respectively (Huang and Clayton, 1990; Du and Sun, 2006). Similar observations have
been reported during deep-fat frying (Pinthus et al., 1995b; Kassama and Ngadi, 2005b; Taiwo and Baik, 2007). Kassama and Ngadi (2005a) asserted that changes in physicochemical properties of fried chicken meat might be responsible for the variation observed in the porosity with frying time. McDonald and Sun (2001) attributed changes in porosity of cooked meat to the initial moisture content of the meat and the cooling effect. There was no significant change in the porosity of the chicken core during the first 3 min of frying and this could be due to delayed heat flux caused by the thermal properties of the chicken core. Fragmentation index (FI)/degree of interconnectivity is a measure of concavity, which indicates connectivity, and convexity of a solid surface, which indicates disjoint structure. Low FI represents high connectivity between the pores and high FI indicates disconnection of the pores (Hahn et al., 1992; Skyscan, 2008). FI showed high values at high frying time but no particular trend is shown for both the coating and core portion of the sample with frying time. In general, the FI increased with frying time indicating some level of decrease in pore connectivity with frying. Pore connectivity is a very significant factor that affect oil uptake in fried product (Mellema, 2003). The structure model index (SMI) describes the shape of the object (pores) within the 3-D model. Ideal plate, cylinder and sphere have the SMI values of 0, 3, and 4, respectively (Hildebrand and Ruegsegger, 1997; Skyscan, 2008). Majority of the coating pores showed a cylindrical and spherical shape, while core pores were shown to have shapes that were more rod-like with SMI between 2 - 3. There was no significant effect of frying on the shape of the sample generally. Frying significantly (P < 0.05) affected the pore number with the coating showing an increase with frying while the core portion
Table 5.2 Pearson correlation between properties of chicken nuggets fried at 180°C for min and frying time.

<table>
<thead>
<tr>
<th></th>
<th>Frying time</th>
<th>Moisture content</th>
<th>Fat content</th>
<th>Coating Porosity</th>
<th>Core Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frying time</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture content</td>
<td>-0.97*</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat content</td>
<td>0.74</td>
<td>-0.89</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coating Porosity</td>
<td>0.88</td>
<td>-0.73</td>
<td>0.35</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Core Porosity</td>
<td>0.68</td>
<td>-0.63</td>
<td>0.47</td>
<td>0.69</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*Significant level = P < 0.05

remained relatively the same. The number of pores in the coating was higher than the chicken core part.

The results of correlation between the chicken nuggets properties are shown in Table 5.2. There was strong positive correlation between frying time, and the porosity of the coating and the core (r = +0.88 and r = +0.68, respectively). Kassama and Ngadi (2005b) reported similar result for fried chicken meat. Porosity and fat content (gained) showed slight positive correlation which further indicated that increase in pore volume influenced the amount of fat uptake during frying. There was a significant (P < 0.05) correlation between the moisture content (loss), and fat content versus frying time. Moisture content and fat content showed a strong negative correlation (r = - 0.89) indicating that substantial part of the moisture loss was replaced by the fat.
5.5 Conclusion

Overall, the use of X-ray micro-CT was effective in elucidating microstructural properties of breaded chicken nuggets. Porosity, pore size distribution, pore shape and pore connectivity were obtained from the analysis. Frying time significantly influenced porosity. The porosity of the coating was significantly different from that of the chicken core. The number and volume of small pores increased during deep-fat frying and there were more pores with less volume than there were with high volume for both the coating and chicken core. In terms of moisture loss, both the coating and chicken core porosity showed a good correlation with frying time.

5.6 Reference


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40(4), 661-668.

microtomography for characterizing microstructure of extruded biopolymer
foams. *Food Research International, 38*(6), 709-719.


Imaging and analysis of porous cereal products using X-ray microtomography.
*Image Analysis and Stereology, 26*, 169-177.
Porosity, pore size distribution, pore shape and pore connectivity were obtained from the image analysis of deep-fat fried chicken nuggets coating and core. It was established that the technique could be effective in studying pore properties of fried coated foods. In chapter 6, chicken nuggets with different batter formulations were studied using X-ray micro-computed tomography imaging. Rheological properties of the unfried batters were evaluated, and other characteristics of the fried batters such as texture and color were also obtained.
VI. MICROSTRUCTURAL PROPERTIES OF DEEP-FAT FRIED CHICKEN NUGGETS COATING – EFFECT OF BATTER FORMULATION

6.1 Abstract

A study on batter formulation effect on microstructural and physico-chemical properties of deep-fat fried chicken nuggets was carried out. Two flour (wheat and rice) mixes at 5 ratios (100:0, 70:30, 50:50, 30:70 and 0:100) and two carboxymethyl cellulose ratios (0 and 1%) were combined. Samples were fried at 180°C for 4 min. Fried samples were then scanned, and the obtained images were analyzed. The addition of hydrocolloid and rice flour significantly influenced the porosity, fragmentation index and pore count. Porosity ranged between 18.2 – 32.1%. Pore count, fragmentation index and structure model index (shape) of the fried coating increased with the addition of hydrocolloid while porosity decreased. Porosity was affected by the proportions of either rice or wheat flours in the batter. Viscosity, batter pickup, color, texture, moisture and fat content of the batter systems were significantly affected by the formulation. Correlations were found between the physico-chemical properties of the unfried and the fried coating, and porosity.
6.2 Introduction

Food coatings are applied for value addition especially in fried foods. They act as barrier to mass transfer during frying thereby reducing oil uptake and preventing moisture loss. They are also added to improve food texture, flavor, aesthetic appeal, weight and volume (Fiszman and Salvador, 2003; Mohamed et al., 1998; Pinthus et al., 1993; Xue and Ngadi, 2006, 2007). Food coatings exist either as a batter and/or breading. They are usually made from a combination of flours or starch, water, seasoning and with or without leavening agent to form batter or breading/batter coating on foods. Batters can either be adhesive semi-liquid into which food is dipped and subsequently coated with breading or tempura which usually contain leavening substance and applied solely to food surface. Batter formulation is extremely flexible, allowing for maximum adaptation to food product development. However, there has been some effort to relate their physical and chemical properties such as fat uptake, moisture loss, texture, structure, viscosity, thermal gelation to their composition and formulation (Akdeniz et al., 2005; Akdeniz et al., 2006; Lee and Inglett, 2007; Maskat and Kerr, 2004; Sahin et al., 2005; Xue and Ngadi, 2006).

The formulation, composition, physico-chemical properties and microstructural configuration of batter elements and their relationships are very significant in product development and quality definition (Maskat and Kerr, 2004). In order to meet today’s sophisticated consumers’ need for variety and convenient food items; meet the challenges of diversification and/proliferation of an automated food production system and the increased concern about fat content in fried foods, experimenting with formulation of batter system and relating their various properties is especially necessary.(Fiszman and
The cost effectiveness of combining two or more flours or the addition of other ingredients, such as hydrocolloids, to improve their functionality and quality is another major driving factor (Mukprasirt et al., 2000a; Xue and Ngadi, 2006, 2007). Wheat flour is the traditional flour of choice for baking and coating application. Substitution of wheat flour with other flours such as rice flour could lead to value addition, increased appeal from the Asian-American market that has grown significantly in the last decade and a possible reduction in production cost of batter system (Loewe, 1993; Mukprasirt et al., 2000a; Puspitowati and Driscoll, 2007). Rice flour based-batter could be alternative for individual with gluten allergies (Fiszman and Salvador, 2003). Rice flour has a better water holding capacity, therefore may reduce fat uptake than whole wheat flour batter system (Dogan et al., 2005; Shih and Daigle, 1999). Xue and Ngadi (2006) suggested that rice flour-based batters could be healthier because they contain fewer calories. Also, the inclusion of hydrocolloids/gums into batter formula has been reported to improve the functional properties of batter system (Llorca et al., 2001). Hydrocolloids are added to breading/batter system to control viscosity, batter pickup and the water holding capacity (Mukprasir et al., 2000b; Rimac-Brncic et al., 2004). They undergo recoverable gelation in an aqueous medium when heated and this unique property helps to increase batter pick-up and to promote the barrier-resistant effecting oil uptake and moisture loss (Dziezak, 1991; Mellema, 2003; Meyers, 1990). The most used hydrocolloids in food applications are of the cellulose derivates such as carboxymethyl cellulose (CMC), hydroxypropyl methylcellulose (HPMC), methylcellulose (MC). CMC is the least hydrophobic.
Recently, the study of food microstructural properties such as porosity, pore volume, pore size distribution and their relation to food properties has increased. This is owed to the fact that most elements that determine food quality exist at a level <100 µm and the knowledge of their properties would be a major contribution to the development of tailored foods and effective tool in food system modeling and process optimization (Aguilera, 2005; Boukouvalas et al., 2006). There is limited number of reports in the literature on the microstructural properties of deep-fat fried coating. Llorca et al. (2001) reported on the microstructures of fried battered squid rings. Microstructural properties such as porosity, pore shape and pore interconnectivity are very important in modeling mass transfer during processing of porous foods, in quality improvement and evaluation, in process optimization and development of novel fried products. The objective of this study was to evaluate the effect of batter formulation on microstructural and physico-chemical properties of fried chicken nuggets batter coating.

6.3 Material and methods

6.3.1 Materials

The chicken breast and a brand name wheat flour (Five Rose All Purpose Flour, Les Cuisines Five Roses kitchens, QC, Canada) used for the study were procured from a local grocery store in Montreal, Canada. Long grain rice flour, RL-100, used was supplied by Rivland Partnership (Riceland Foods, Arizona, USA). The compositions of the two flours used are presented in Table 6.1 and compared with values (in brackets) reported for standard flour by USDA (2008). Carboxymethyl cellulose (CMC) used was supplied by
Table 6.1 Composition and particle size of wheat and rice flour

<table>
<thead>
<tr>
<th>Flour type</th>
<th>Wheat (%w/w)</th>
<th>Rice (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>13.3 (10.33)</td>
<td>7.2 (5.95)</td>
</tr>
<tr>
<td>Moisture Content</td>
<td>11.6 (11.92)</td>
<td>11.0 (11.9)</td>
</tr>
<tr>
<td>Ash</td>
<td>0.43 (0.47)</td>
<td>0.6 (0.6)</td>
</tr>
<tr>
<td>Fiber</td>
<td>3.3 (2.7)</td>
<td>0.7 (2.4)</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>0.88 (0.98)</td>
<td>0.5 (0.3)</td>
</tr>
<tr>
<td>Particle size</td>
<td>24 µm</td>
<td>105 µm</td>
</tr>
</tbody>
</table>

Numbers in parenthesis are values provided by USDA (2008). Data were sourced from the manufacturers’ nutrition facts.

(TIC Gums Inc, Maryland, USA). The chicken breast were cut into 5 x 5 x 1.2 ± 0.1 cm and stored in Zip-lock bags at frozen conditions prior to use.

6.3.2 Batter formulations and pickup

Two flours were blended at five different ratios of wheat and rice flours namely 100:0; 70:30; 50:50; 30:70 and 0:100, respectively. A fixed quantity of leavening agent (Sodium hydrogen bicarbonate, NaHCO₃, H. Cantin Ltd, Quebec, Canada) and salt (NaCl) were added at 0.5% and 1%, respectively, to all the samples. Batter slurry was prepared by adding water to flour mix in the ratio of 1.5:1. The system was thoroughly mixed with the aid of a kitchen mixer (B-Speed Mixer, Black and Dekker, ON, CA). Weighed chicken samples were dipped into the batter system for about 30 s and allowed to drip for 25 s and immediately fried. The batter pickup was calculated as the difference in weight of the
batter before and after dipping, divided by the initial weight of the chicken multiplied by 100.

6.3.4 Flow behavior

The study of flow behavior was conducted using a strain/stress control Rheometer (Advanced Rheometer 2000, TA Instruments, Delaware, USA) equipped with a 4 cm diameter parallel plate. The gap between the plates was set to 1 mm, which was considered large enough with regard to the flour particle size. Approximately 1.2 ml of the sample was placed at the center of the Rheometer platform and the free surface of the sample edges was covered with silicone oil and a steel cover to minimize water loss during the measurements. The flow behavior (viscosity and the power index values) of the batter systems were determined over a shear rate range of 2 - 150 s⁻¹ at 25°C. Apparent viscosity was reported as the mean of three replicates for each sample.

6.3.5 Frying

The coated samples were fried at 180°C for 4 min in a programmable fryer (Henny Penny Computron 7000 pressure Fryer, Model 500C, HP Corporation, Eaton, OH, USA), with capacity of 30 L. They were drained, cooled down to room temperature (25°C±1), and stored in Zip-lock bags prior to further analysis.
6.3.6 Moisture content

The moisture content of the samples was determined on a dry weight basis by placing samples in an oven at 105°C for 24 h. Weight before and after heat treatment was taken for computation.

6.3.7 Fat content

Fat content was determined by following the modified AOAC method 960.39 (AOAC, 1990). Samples of 2-3 g were freeze-dried and ground in a blender (Bodum 5679 C-Mill, NY, USA), and placed in thimbles in a VELP SER 148 (Velp Scientifica, Usmate, Italy) solvent extraction unit and oil was extracted with petroleum ether. The oil content was computed on dry weight basis by dividing the mass of extracted oil with the mass of freeze-dried sample.

6.3.8 Color measurement

The surface color parameters of the coated fried samples were measured based on CIE system by a colorimeter (Minolta Spectrophotometer CM-3500d, Japan). The color parameters L* (lightness), a* (redness), b* (yellowness), chroma (C) and Hue angle (H) values of the samples were obtained.

6.3.9 Texture analysis

The textural properties of the fried coated samples were measured using an Instron Universal Testing machine (Instron Corporation, Canton, MA). Multiple probe of six heads with 3 mm diameter each were applied to the sample, few minutes after frying, at
cross head speed of 10 mm/min and a load of 50 kN. The penetration depth of the probes set was 7.5 mm. The load versus displacement graph was plotted and the maximum penetration load was obtained.

6.3.10 X-ray microCT scanning and image processing

The fried coatings were cut into 1 x 1 x 0.5 (±0.1) cm and wrapped in parafilm to prevent drying out during scanning. The samples were scanned in an X-ray micro-CT scanner (1076 Skyscan, Belgium) (Appendix 2.1) at the following settings: 100 keV, 98 µA, 6.0 s exposure time, 0.7° rotational step and a magnification of 30x was achieved. The obtained images were reconstructed into series of 2-D images using reconstruction software (Nrecon, Skyscan, Belgium) that operates based on filtered back-projection algorithm (Feldkamp et al., 1984; Sasov and van Dyck, 1998). A total of 916 slices of 2-D images were obtained in 8-bit bitmap (1024 X 1024 pixels) for each sample and analyzed (Nrecon and CTan, respectively, Skyscan, Belgium). The 3-D models were developed using CTvol software and some of the structural parameters such as porosity, fractal dimension, fragmentation and structure model index were determined. Porosity was obtained by dividing the digitally measured volume of pores by the volume of the solid structure of the sample. Structure model index (SMI) determination is based on dilation of the 3D voxel model developed, i.e., artificially adding one voxel thickness to all binarized object surfaces. SMI is defined by this equation (Hildebrand and Ruegsegger, 1997):

\[
SMI = 6 \left( \frac{S^*V}{S^2} \right)
\]  

(6.1)
where $S$ is the pore surface area before dilation, $S'$ is the change in surface area caused by dilation and $V$ is the initial, undilated pore volume. Structure model index (SMI) describes the shape of the pores within the fried batter network. Ideal plate, cylinder and sphere have SMI values of 0, 3, and 4, respectively (Hildebrand and Ruegsegger, 1997; Skyscan, 2003). Fragmentation index was developed by Han et al. (1992) to define connectivity between trabecular bones and the principle is defined by the following expression (eq. 6.2):

$$FI = \frac{P_1 - P_2}{A_1 - A_2}$$

(6.2)

where $A$ and $p$ are pore surface area and perimeter, respectively and the subscript numbers 1 and 2 indicate before and after image dilation. The structural connectedness results in enclosed sample spaces, followed by dilation of the sample surfaces which contract the perimeter, implying a disconnection, and when their ends experience perimeter expansion by dilation, signifying connection. To apply this expression to pore network in a 3D image, the pores are built as solid matrix with the transparent network of the solid components of the sample. Fragmentation index (FI) describes the degree of disconnection between pores. Low FI designates high connectivity between the pores and high FI indicates disconnection of the pores (Hahn et al., 1992; Skyscan, 2003). Fractal dimension, a measure surface roughness of the pores, is determined by using “box counting” method either in 2D and 3D. The surface/volume is divided into an array of equal squares or cubes, and the number of squares containing part of the object surface is counted. This is replicated over a range of box sizes such as 3 - 100 pixels. The number of boxes containing surface is plotted against box length in a log-log plot, and the fractal
dimension is obtained from the slope of the log-log regression (Chappard et al., 2001). Detailed information on these procedures are provided in the Skyscan manual for CTan and CTvol softwares (Skyscan, 2008).

6.3.11 Statistical Analysis

All treatments were applied in triplicates. Analysis of variance was performed using SAS (8.2 version, SAS Inst. Inc., Cary, N.C., U.S.A.) (SAS, 1999) in order to determine the effect of formulation on various properties of the samples. Duncan multiple range test was used for mean separation at $P<0.05$ where treatment effect was significant.

6.4 Results and discussion

The grayscale and binarized images of chicken nugget coating formulated by a mixture of 100% wheat flour and 1% CMC are shown in Figure 6.1. The image pre-processing step of segmentation by thresholding led to delineation between the pores and the rest of the

![Figure 6.1 Grayscale (a) and binarized (b) image of a formulated coating (100% wheat flour + 1% CMC) of deep-fat fried chicken nuggets.](image)
Figure 6.2 Three-dimensional images of formulated batter coating of fried chicken nuggets: (A) 100% wheat flour; (B) 100% wheat flour plus 1% carboxymethyl cellulose (CMC); (C) 70% wheat flour + 30% rice flour + 1% CMC; (D) 50% wheat and rice flours + 1% CMC; (E) 30% wheat flour + 70% rice + CMC; (F) 100% rice flour + 1% CMC.

constituent of the sample. The scale for thresholding was chosen by performing automatic thresholding on grayscale 2-D slices of the image using Otsu’s algorithm (Otsu, 1979) such that the optimal threshold was used to maximize the interclass variance between the dark and bright region of the grayscale image. Three-dimensional models were developed by digitally stacking binarized 2-D images together.

Figure 6.2 (A, B, C, D, E and F) are 3-D images of batter coating of deep-fat fried chicken nuggets. There is evidence of varied porosity attributable to batter formulation.
Figure 6.3 The bar chart of pore volume frequency over the pore size distribution.

Figure 6.2B showed that inclusion of CMC hydrocolloid into the 100% flour decreased the amount of pores developed during frying. However, inclusion of rice flour into the formulation reversed the decrease in pore development observed. More pores were noticeable in the coating that contained 30% rice flour (Figure 6.2C) compared to the later samples. Figures 6.2C, 6.2D, 6.2E and 6.2F showed a decreasing trend in pore formation during frying as a result of increased rice flour addition into the formulation. However, there seem to be formation of larger pores with increase in the proportion of rice flour in the coating formulation.
The fried batter pore size ranged from 19.0 – 582 µm. The pore size distribution of fried batters as affected by formulation is shown in Figure 6.3. The graph shows a bimodal pore size distribution; with two peaks around 94 to 169 µm and 544 to 582 µm. Datta et al. (2007) also reported a bimodal pore size distribution for bread baked using different heating modes. Karathanos and Saravacos (1993) reported a bimodal pore distribution pattern using mercury intrusion porosimetry for granular and unpressed Amioca starch that contained 11% moisture. The bimodal pore size distribution may be typical of most porous foods. There was a general increase in pore volume frequency for all the fried batter coating between 19 and 131 µm after which the volume percentage starts to decline. However, batter formulated with 100% rice and 1% CMC hydrocolloid showed an increase beyond 131 up to 206 µm after which a plateau was reached. Fried batter with 100% rice flour and 1% CMC tends to show an equal distribution of pore volume over the size range between 131 to 469 µm. Batter formulated with 100% wheat flour without CMC addition showed a very high percentage of volume (23.5%) for large pores (544 – 582 µm) compared to others (Figure 6.3). This could be as a result of the leavening effect of baking powder and the viscoelastic texture of the batter system which supports development of larger porous network before and during frying (Llorca et al., 2001). Overall, pores with size range between 19.0 to 244 µm constitute more than 60% of the pore volume for all the samples, implying majority of the pores for all the samples were within this range. Increased addition of rice flour into the batter mix resulted in decreased pore volume for pores between 19.0 and 169 µm and an increased pore volume from 244 to 431 µm.
The plot of cumulative pore volume as a function of pore size distribution is presented in Fig. 6.4. There was a gradual increase in cumulative pore volume as the pore size increased for all the samples, especially between 20 and 300 µm pore size. Beyond 300 µm pore size; there was minimal increase in cumulative pore volume indicating that there were fewer large pores in all the samples. The cumulative pore volume varied from 3.11 to 4.93 mm$^3$ for all the batter formulation. Fried batter system with 100% wheat flour and 1% CMC hydrocolloid addition developed the least pore volume. This would be significant in its mass transfer characteristics (Mellema, 2003).

The sample formulated with 50% wheat and rice flour plus 1% CMC showed the highest volume. There was no particular trend shown for the cumulative pore volume.
### Table 6.2 Pore parameters of deep-fat fried chicken nuggets coating formulated from wheat and rice flour

<table>
<thead>
<tr>
<th>Sample</th>
<th>Porosity</th>
<th>SMI</th>
<th>FI, 1/mm</th>
<th>PSA/PV, 1/mm</th>
<th>Fractal Dimension</th>
<th>Number of pores</th>
</tr>
</thead>
<tbody>
<tr>
<td>W100R0 + CMC0%</td>
<td>24.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2</td>
<td>9.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>291&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>W100R0 + CMC1%</td>
<td>18.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>13.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>341&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>W70R30 + CMC1%</td>
<td>32.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>9.47&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>181&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>W50R50 + CMC1%</td>
<td>31.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>10.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>196&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>W30R70 + CMC1%</td>
<td>28.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>9.94&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>234&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>W0R100 + CMC1%</td>
<td>27.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>7.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>127&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different letter superscript in the same column are significantly different. W = wheat; R = rice; CMC = carboxymethyl cellulose; SMI = structure model index; FI = Fragmentation index; PSA = Pore surface area; PV = Pore Volume.

The pore characteristics of the samples as measured using images obtained from X-ray micro-computed tomography (CT) are shown in Table 6.2. Analysis of variance (ANOVA) showed that batter formulation significantly (P<0.05) affected the porosity, fragmentation index (FI) and the total number of pores. Structure model index (shape), object surface to volume ratio (PSA/PV) and the fractal dimension of the pores were not significantly influenced by formulation. The addition of 1% CMC hydrocolloid into the batter system led to a substantial decrease of about 25% (Table 6.2). The addition of hydrocolloids has been reported to increase water holding capacity of batters and subsequently a reduced porosity (Ford, 1999; Mallikarjunan et al., 1997). However, when wheat flour was substituted with 30% rice flour, porosity increased significantly
Mohamed et al. (1998) reported high porosity for batter pre-gelatinized rice flour. Increasing the proportion of rice flour in the batter did not change porosity beyond 30%. SMI values for the samples range between 2 and 3. This shows that majority of the pores fall between a plate like and cylindrical/rod shapes. There was no significant effect of batter formulation on the shape of the pores developed. The addition of CMC hydrocolloid into the 100% wheat flour formulation led to significant increase in pore disconnection, but this decreased as rice flour was included into the formulation. The increased disconnection of the pores with inclusion of CMC might be due to the gelation of the gum within matrix of the batter system, forming in the pathways of the pores created during frying. The ratio of pore surface area to pore volume (PSA/PV) was not affected by either inclusion of CMC or substitution of rice flour. Fractal dimension describes the surface roughness of the pores which remained almost the same for all samples. The addition of CMC increased the pore count in the fried coating substantially (P < 0.05), which decreased when wheat flour was substituted with rice flour in the batter system.

The initial moisture content of the batter systems before frying ranged from 1.50 to 1.83 (g/g, db) and these are not significantly different (P < 0.05). The results of fried batter final moisture and fat content are shown in Figs. 6.5 and 6.6. Final moisture and fat content ranged from 0.49 to 0.816 (g/g, db) and 0.051 and 0.136 (g/g, db), respectively. The effect of composition was significant (P<0.05) on the variation observed in moisture and fat content of the fried batter systems. The addition of CMC caused an increase in
moisture content, which decreased as the level of rice flour inclusion in the formulation increased (Fig. 6.5). The increase recorded for moisture content after the addition of CMC was expected because of the better water holding capacity of the hydrocolloid (Davis, 1983; Mallikarjunan et al., 1997). However, the moisture content saw a significant decrease as rice flour was included into the formulation. The decrease was not significantly different from the batter system that contained 100% wheat flour with no hydrocolloid addition. This is indicative of decreasing water holding capacity with
increased rice flour added into the batter system contrary to expectation, which could be a result of increased flour particle size (Table 6.1). Maskat and Kerr (2002) recorded lower moisture content in chicken nuggets coating with large particle size compared to the one with smaller particle size.

The change in composition of the batter system due to the addition of 1% CMC and 30% rice flour led to a significant \( P < 0.05 \) decrease in fat content (Figure 6.6),

Figure 6.6  Final fat content of the coating (fried at 180°C for 4 min) plot against the batter formulation:  (A) 100% wheat flour; (B) 100% wheat flour plus 1% carboxymethyl cellulose (CMC); (C) 70% wheat flour + 30% rice flour + 1% CMC; (D) 50% wheat and rice flours + 1% CMC; (E) 30% wheat flour + 70% rice + CMC; (F) 100% rice flour + 1% CMC.
which could be due to gel forming properties of the hydrocolloid, which created a barrier film on the sample leading to a decrease in fat uptake. Other authors have reported the effectiveness of hydrocolloid addition and rice flour inclusion into batter formulation for fat reduction in fried foods (Dogan et al., 2005; Mallikarjunan et al., 1997; Mellema, 2003; Meyers, 1990; Sanz et al., 2004). A significant increase in fat absorption was shown when higher proportion of rice flour (≥ 50%) was added to the formulation, but compared to the whole wheat flour and no CMC batter, the change was insignificant. This increase could be a result of bigger particle size (lower damaged starch) and lower protein content of the flour mix when rice flour was added (Table 6.1) (Loewe, 1990; Olewnik and Kulp, 1990; Shih and Daigle, 1999). The bigger particle size implied less surface area that might have reduced the water holding capacity of the flour mix, which is evident in the moisture content, porosity and 3-D images (Figure 6.2) of the fried samples reported earlier. The impact of this is in reduced gelation and less barrier formation during frying leading to higher fat absorption. Mohamed et al. (1998) reported increased fat uptake with fried pre-gelatinized and glutinous rice flour batter compared to control. They attributed that the increase was due to reduced amylose content of the sample which would otherwise have helped to inhibit oil absorption. Dogan et al. (2005) however, reported a reduced fat absorption with inclusion of 5% rice flour into the batter system of fried chicken nuggets. Their result is comparable to this study at the level of rice flour used. Shih and Daigle (1999) also presented different oil uptake percentages based on varying chemical constituent (wheat/rice flour ratio, protein level and starch amylpectin and amylose ratio) of the chicken drumstick’s coating. The level of rice flour inclusion, particle size and chemical content determined the effect of rice flour on fat absorption.
Fig. 6.7 shows the effect of formulation on the viscosity of batter system. Addition of CMC into the formulation resulted in a significant increase in batter viscosity. Christianson et al. (1981) and Shi and BeMiller (2002) reported a significant increase in viscosity of wheat starch by the addition of a small amount of xanthan, guar, and cellulose hydrocolloids. Inclusion of rice flour beyond 30% level resulted in a decreased viscosity. This might be due to interaction between the constituent of the batter formulation. Obviously the water content of the samples began to decrease when 50% of wheat flour was substituted by rice flour (Fig. 6.5), which caused the reduction in
Table 6.3 Power law properties of batter with different composition of wheat and rice flour, and CMC content.

<table>
<thead>
<tr>
<th>Sample</th>
<th>K (Pa.s^n)</th>
<th>n</th>
<th>R²</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>W100R0 + CMC0%</td>
<td>14.08</td>
<td>0.40</td>
<td>0.99</td>
<td>2.75</td>
</tr>
<tr>
<td>W100R0 + CMC1%</td>
<td>26.59</td>
<td>0.49</td>
<td>0.99</td>
<td>9.64</td>
</tr>
<tr>
<td>W70R30 + CMC1%</td>
<td>32.88</td>
<td>0.45</td>
<td>1.00</td>
<td>3.56</td>
</tr>
<tr>
<td>W50R50 + CMC1%</td>
<td>26.79</td>
<td>0.43</td>
<td>1.00</td>
<td>2.26</td>
</tr>
<tr>
<td>W30R70 + CMC1%</td>
<td>21.81</td>
<td>0.39</td>
<td>1.00</td>
<td>2.26</td>
</tr>
<tr>
<td>W0R100 + CMC1%</td>
<td>19.21</td>
<td>0.45</td>
<td>0.99</td>
<td>3.46</td>
</tr>
</tbody>
</table>

r² coefficient of determination viscosity. The viscosity of all the batter systems decreased with increasing shear rate, indicating a pseudoplastic behaviour synonymous with non-newtonian shear thinning fluid that is very significant in processing and application of viscous materials. These observations can be modeled by the power law expression (eq. 6.3):

$$\tau = K\gamma^n$$  \hspace{2cm} (6.3)

where $\tau$ is the shear stress (N/m²), $K$ is the consistency index (Pa.s^n), $\gamma$ is the shear rate (1/s) and $n$ is the flow behaviour index. The power law model parameters for the batter formulations are shown in Table 6.3. The coefficient of determination $r^2$ and error values indicated a very good fit of the data by the power law model with values from 0.99 to 1.00 and 2.26 to 9.64, respectively. The value of consistency index ($K$) varied from 14.1 to 32.9 Pa.s^n. This is within the range of 0.46 and 69.2 Pa. s^n reported by Xue and Ngadi (2007) for batter system made from different combinations of flours and hydrocolloids.
Mukprasirt et al. (2000b) reported values ranging between 4.35 to 22.7 Pa.s$^n$ for rice flour based batters containing methylcellulose. The value of $K$ doubled with the addition of CMC hydrocolloid into the 100% wheat batter. The inclusion of 30% rice flour into the batter formulation caused further increase in $K$ value after which further addition of rice flour decreased the consistency index. This is synonymous with the viscosity pattern shown in Fig. 6.7. The trend of change in rheological properties of the batter system when rice flour was added could be explained by the fact that equal amount of water was added to the flour mix that contains increased amount of rice flour and which all weigh equally. The flow behaviour index, $n$, for all the samples are not significantly different ($P < 0.05$) and are all less than 1, confirming the pseudoplastic/shear thinning property of the batter systems. Shih and Daigle (1999) reported a similar pattern for batter viscosity formulated from a combination of phosphorylated rice starch, wheat and rice flours.

Table 6.4 presents the batter pickup values obtained after coating the chicken meat with the formulated batter systems and the maximum penetration load (indicating crispiness/hardness) obtained after frying. The batter pickup values varied from 45.1 to 72.2%. The composition of the batter significantly ($P < 0.05$) affected batter pickup. As expected, inclusion of hydrocolloid (CMC) and rice flour into the formulation led to increased batter pickup, which might be due to increased water holding capacity and viscosity as shown in Figs. 6.5 and 6.7. Akdeniz et al. (2005) reported increase in batter pickup for batter formulation when higher levels of pre-gelatinized tapioca starch were used. However, a gradual reduction in batter was observed after the level of rice flour in the batter system increased beyond 30%. This trend was observed for viscosity of the
Table 6.4 Batter pickup for the formulated coating and texture property of the fried.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Batter Pickup, %</th>
<th>Maximum Penetration Load (kN x 10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W100R0 + CMC0%</td>
<td>45.1ᵃ</td>
<td>6.73ᵃ</td>
</tr>
<tr>
<td>W100R0 + CMC1%</td>
<td>83.9ᶜ</td>
<td>7.53ᵇᵃ</td>
</tr>
<tr>
<td>W70R30 + CMC1%</td>
<td>68.4ᵇᶜ</td>
<td>9.15ᵇᵃ</td>
</tr>
<tr>
<td>W50R50 + CMC1%</td>
<td>72.2ᵇᶜ</td>
<td>9.77ᵃᵇ</td>
</tr>
<tr>
<td>W30R70 + CMC1%</td>
<td>55.7ᵃᵇ</td>
<td>10.1ᵇᵃ</td>
</tr>
<tr>
<td>W0R100 + CMC1%</td>
<td>37.9ᵃ</td>
<td>12.9ᵇ</td>
</tr>
</tbody>
</table>

Mean with different letter superscript in the same column are significantly different at P<0.05.

batter and final moisture content of the fried sample, which also showed in the amount of fat absorbed. Rice flour has been reported to have low thickening capability, thus forming thin layer when coated on food material (Dogan et al., 2005; Mukprasirt et al., 2000a; Shih and Daigle, 1999). This obviously was due to change in chemical constituents of the batter and the flour particle size especially when rice flour inclusion increased (Table 6.1). Decreasing protein content has been reported to reduce batter pickup, viscosity and water holding capacity (Christianson et al., 1981). The penetration maximum load of the fried batter saw a significant (P<0.05) increase when hydrocolloid was included and as the level of rice flour increased. This is an indication of increased crispiness as hydrocolloid was added and the wheat flour was substituted with rice flour in the formulation. The formation of fewer large pores with increased addition of rice flour,
Table 6.5 Color parameters as affected by coating compositions after frying.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Chroma value, C*</th>
<th>Hue value, H (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W100R0 + CMC0%</td>
<td>68.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>W100R0 + CMC1%</td>
<td>73.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>W70R30 + CMC1%</td>
<td>71.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>87.0&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>W50R50 + CMC1%</td>
<td>71.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>82.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>W30R70 + CMC1%</td>
<td>62.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.6&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>W0R100 + CMC1%</td>
<td>65.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>29.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>83.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean with different letter superscript in the same column are significantly different at P < 0.05.

As shown in the three-dimensional images and cumulative pore volume reported initially, could be the reason for increased load required for deformation when CMC and rice flour was added to the batter formulation. Shih and Daigle (1999) also reported increased hardness of fried batter as the level of rice flour inclusion increased.

The L*, a*, b*, C* and H (°) color indices of the samples are presented in Table 6.5. Inclusion of CMC significantly increased the lightness value (L*) for the fried sample, which gradually increased as wheat flour was substituted for by rice flour. Batter with 100% wheat flour and the batter with ratio 3:7 of wheat and rice showed a much lower L* value indicating higher Maillard and caramelization reactions of the sugar and amino acid constituent. There was no significant difference in the values of a* index though there was an increase in the samples redness with inclusion of hydrocolloid and rice flour. Yellowness (b*) of the samples also increased with the addition of hydrocolloid and rice flour into the formulation. Chroma value (C*) which is a measure...
Table 6.6 Pearson correlation between porosity and some physico-chemical properties of the fried batter coating.

<table>
<thead>
<tr>
<th></th>
<th>Moisture content</th>
<th>Fat content</th>
<th>Batter Pickup</th>
<th>Consistency index</th>
<th>Texture</th>
<th>Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat content</td>
<td>-0.97*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batter Pickup</td>
<td>0.79</td>
<td>-0.75</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consistency index</td>
<td>0.47</td>
<td>-0.56</td>
<td>0.76</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td>-0.62</td>
<td>0.62</td>
<td>-0.37</td>
<td>0.09</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Porosity</td>
<td>-0.62</td>
<td>0.46</td>
<td>-0.21</td>
<td>0.32</td>
<td>0.53</td>
<td>1</td>
</tr>
</tbody>
</table>

* Significant at P<0.001

of color saturation also increased with the same trend. Hue angle (\(H^\circ\)) remained almost the same for all the formulations.

Table 6.6 presents the Pearson correlation between the physico-chemical properties of the formulated batter system. The correlation between the moisture and fat content was negative and significant at 99.9% confidence interval, implying almost a simultaneous diffusion of moisture and fat in and out of the batter during frying, respectively (Gamble et al., 1987). There were positive correlations between the moisture content versus batter pickup and consistency index but not significant (P < 0.05), however texture and porosity showed equal negative correlation with moisture content. Fat content was negatively correlated with batter pick up and consistency index while it showed positive correlation with texture and porosity.
5.5 Conclusion

Pore characteristics of formulated deep-fat fried chicken nuggets coating were elucidated using X-ray micro-CT technique. Porosity, fragmentation index of the pores and pore number were significantly affected by batter formulation. The effect of batter formulation on pore SMI, PSA/PV ratio and fractal dimension were less pronounced. Batter pickup and viscosity varied with the addition of carboxymethyl cellulose (CMC) and rice flour into the formulation. The addition of CMC into the formulation caused an increase in moisture retention and a corresponding decrease in fat uptake. Addition of rice flour however, decreased moisture content and caused more fat to be absorbed. The addition of CMC and rice flour created a more crispy texture fried batter. There were some correlations shown among the physico-chemical properties of the unfried and the fried coating, and porosity. Fat and moisture content showed significant negative correlation.

6.6 References


In chapter 6, the effect of different constituent of batter on pore characteristics such as porosity, pore size distribution, pore interconnectivity and pore shape were studied. The relationship between pore characteristics, formulation and chemical constituent of the sample such as moisture and fat content were also presented. In chapter 7, confocal laser scanning (CLSM) imaging method was used to study pore characteristics and fat distribution in deep-fat fried chicken nuggets batter coating. Images were obtained at the excitation and reflection modes of the microscopes in order to show fat and pore distribution, respectively.
VII. MICROSTRUCTURAL EVALUATION OF DEEP-FAT FRIED CHICKEN NUGGET BATTER COATING USING CONFOCAL LASER SCANNING MICROSCOPY

7.1 Abstract

Porosity and pore size distribution are very important microstructural properties of fried foods needed in process optimization and product development. The objective of this study was to characterize the pore properties and quantify fat distribution in deep-fat fried chicken nuggets batter coating using confocal laser scanning microscopy. Samples were fried at three temperatures namely 170, 180 and 190°C. Detached batter coatings were stained non-covalently and 2-D images were obtained at fluorescence and reflection modes of the microscope. The images were analyzed for fat and pore distribution. Fat distribution obtained from image analysis was significantly (P < 0.05) affected by the frying temperature and time, and it decreased within the depth of the sample thickness. There was a strong correlation between fat distribution from the imaging technique and fat content obtained by the conventional method at two temperatures, 180 and 190°C. Porosity ranged between 4.97 and 32.7% and was significantly influenced by frying temperature. Pore size varied approximately between 1.20 and 523 µm. Frying process led to the formation of more micropores (pores < 42 µm) and bigger (pore ≥ 270 µm) pores while medium size pores diminished.
7.2  Introduction

The market for fried foods remains very large and it continues to increase in spite of the health concerns associated with foods high in calorie. The peculiar organoleptic properties of fried foods such as good mouth-feel, distinct flavor, unique taste and palatability 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 during the process. The significance of microstructural changes to oil absorption during frying has been discussed by Baumann and Escher (1995); Mallikarjunan et al., 1997 and Mellema (2003).

A number of novel and innovative approaches are being applied to study both surface and internal morphological details of foods. These include magnetic resonance
imaging; X-ray computed tomography and different microscopy techniques such as light microscopy (LM), atomic force microscopy (ATM) and scanning electron microscopy (SEM) (Kawas and Moreira, 2001; Kim et al., 2007; van Dalen et al., 2003, 2007; Wagner et al., 2008). Confocal laser scanning microscopy (CLSM) is an improvement over the traditional light microscopy in that it has the capability to scan product at different depths, reduce artifacts, and produce higher resolution images that can be rendered in three dimensions. Confocal microscopes (Appendix 3.1) uses laser, instead of light, as its source of illumination and the inclusion of pin holes, a device within the setup that eliminates out-of-focus images that would produce artifacts (Aguilera and Stanley, 1999; Dürrenberger et al., 2001; Subramanian and Hommerding, 2005). In preparing samples for CLSM imaging, they are stained with dyes/fluorophores/probes that fluoresce at various wavelengths when illuminated based on the component they are targeted to mark. This enables differentiation of components based on emission wavelength. Fat is marked by probes such as Nile Blue, Nile Red and Congo Red (Bouchon et al., 2003; Pedreschi and Aguilera, 2002; van Dalen, 2002); Proteins are marked by Rhodamine B, Fluorescein-Isothiocynate (FITC) and Safranin O (Blonk and van Aalst, 1993; Lee et al., 2003; van de Velde et al., 2003); carbohydrates are marked by FITC and Safranin O in the absence of protein (Dürrenberger et al., 2001; Funebo et al., 2000; Hans Tromp et al., 2001). CLSM is also used to obtain structural images of foods when used at the reflection/trans mode (Pedreschi et al., 2008).

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.

7.3 Materials and methods

7.3.1 Materials

Commercially produced chicken nuggets were procured from a local manufacturer (Olymel, Boucherville, QC, CA) and stored in the deep-freezer at -50°C prior to use. They were placed in a refrigerator at 4°C for 4 h and then transferred to room temperature for 30 min 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.

7.3.2 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. Fried samples were immediately removed from the oil and the surface blotted with tissue paper to remove surface oil and were allowed to cool at ambient temperature.
7.3.3 **Probe application and cryo-sectioning**

The following protocol was arrived at after some trials with the method of labeling, sample thickness and scanning settings. Solution of Nile Blue A (0.005%) was prepared by dissolving in demineralized water according to van Dalen (2002) method. It was non-covalently (one or two drops) added to the surface of fried product after frying. The stained samples were kept for 12 h at refrigeration temperature (4 - 8°C) for the dye to spread within the product matrix especially to areas where fat is located (van de Velde et al., 2003). Samples of the coating were taken by cutting cube shape (0.5 x 0.5 x 0.5 cm) out of the stained chicken nuggets. They were subsequently quick frozen in liquid nitrogen in preparation for cryo-sectioning and kept in a deep-freezer at -50°C prior to use. The cryo-sectioning of the samples was performed with a cryostat (Shandon Cryotome, Thermo Scientific, Waltham, MA, USA) (Appendix 4.1). The cryostat and samples were pre-conditioned to -20°C before the sectioning operation. The cryo-sectioning temperature was selected after several trials to the point where abrasion of the sample was prevented. Frozen samples were glued to the sample-plate (cryocassette) in the cryostat with the aid of frozen tissue embedding media (Histo Prep, FisherDiagnostic, NY, USA) and sectioning at preset depth of 60 µm was carried out. The first 5 cuts were discarded because of non-uniformity in cut due to surface irregularity of the sample. The sectioned sample was then placed on to a microscope slides (SuperFrost Fisherbrand, Fisher Scientific, ON, CA).
7.3.4 Microscopy Imaging

A confocal microscope (Bio-Rad Radiance 2100, Hemel Hempstead, UK) (Appendix 3.1), equipped with an argon(Ar) laser, installed on to a fluorescence microscope (Nikon Eclipse E800, Hertfordshire, UK) was used to acquire images from the samples. Images were obtained at fluorescence mode with excitation/emission wavelengths of 488/570 nm to show fat distribution, and reflection/trans mode to obtain grayscale images which show structure of the sample that include pore distribution. The speed of scanning was 0.14 Hz, line frequency was 500 Hz and zoom factor was 1x. The objective lens was set to obtain magnification of 10x with numerical aperture of 0.3 and to produce multiple (minimum of 12 images per sample) 8-bits 2-D images in TIF format with resolution of 1024 x 1024 pixels and pixel size of 1.197 µm. The 2-D stack of images was collected at 4 µm apart. The images were collected on a Windows 2000 PC work station running the latest BioRad LaserSharp 2000 software (Carl Zeiss, Oberkochen, Germany). The conversion of the images from stack to image sequence was performed on ImageJ version 1.42q (National Institute of Health, USA).

7.3.5 Image Analysis

The first step of image analysis was the use of binarization and morphological image processing operations to obtain fat distribution. These were carried using the ImageJ software (Nation Institute of Health, MA, USA). Stack of images from the same sample were read into the software. Two morphological operations namely smoothing and sharpening were applied to enhance the images (MATLAB, 2007). They use different filters to replace pixels by arithmetic computation from their neighborhood. The images
were then subjected to automatic thresholding using the Otsu’s algorithm (Otsu, 1979) on
the ImageJ platform. After the thresholding step, two steps of erosion and dilation were
applied to the binarized images to remove noise. 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) (MATLAB, 2007) workspace and the areas that
correspond to fat were delineated by segmentation. Fat distribution was obtained as a
percent of the ratio of the area corresponding to fat and the total area of the entire image
(See Appendix 1.1 for MATLAB code).

In order to delineate between the pores and the rest of the sample, the trans
images were loaded onto ImageJ software, the pore area were manually selected by
holding down the SHIFT key and using the mouse to trace out the lines around the
boundaries of the pores. 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 and 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 (See Appendix 1.2).

7.3.6 Fat content

Fat content was determined the conventional way by solvent extraction using petroleum
ether in a Soxhlet extraction system (SER 148, Velp Scientifica, Usmate, Italy) following
the protocol recommended by AOAC Method 960.39 (AOAC, 1990). Between 3 – 5 g of
ground freeze-dried fried batter coating were placed in thimbles 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.

7.3.7 Statistical Analysis

All images were acquired in a 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) (SAS, 1999).

7.4 Results and discussion

The images of unfried raw chicken nuggets coating are shown in Fig. 7.1A presents a grayscale image of the sample obtained at the reflection/trans mode of the microscope, showing a continuous phase of components within the sample with few visible pores. The binarized image of the unfried chicken nuggets coating in Fig. 7.1B shows that there are few pores present within the sample matrix prior to the frying process. The image obtained for the raw sample at the fluorescence mode of the microscope to show fat distribution is presented in Fig. 7.1C. The binarized image of unfried sample displaying fat distribution is shown in Fig. 7.1D. These images indicate that limited amount of fat was present in the batter system prior to frying and it was scarcely located within the batter matrix. Similar images for chicken nuggets fried at 180°C for 240 s are shown in Fig. 7.2. Pores are clearly seen in the grayscale image after frying for long period. Figs.
7.2B and 7.2D show the binarized images of the grayscale and fluorescence images. These binarized images were those further analyzed to obtain the quantitative data.

Figure 7.1 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 7.2 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 7.3 Grayscale images of chicken nuggets batter coating fried at 180°C obtained at the reflection/trans mode of the confocal laser scanning microscope.
presented below. The images in Fig. 7.3 are those of the chicken nuggets batter coating fried at 180°C for various time intervals. The images show an increased formation of bigger pores with frying time. These images also show that the degree of connectivity between adjacent pores tends to increase as frying progressed. 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; Kawas and Moreira, 2001; Krokida et al., 2000; Moreira, 2006). Gelatinization normally causes swelling of the starch granules while moisture loss leads to creation of voids, the combination of these changes led to the pattern of formation that persist in all the batter fried at the different temperatures. Figs. 7.4, 7.5 and 7.6 show montage of fluorescence images, at different frying times, obtained at the fluorescence mode of the microscope for batters fried at 170, 180 and 190°C, respectively. The images show that dye intensity increased with both frying time and temperature. This implies that fat absorption increased with frying time and temperature. The quantitative data of fat distribution as a function of frying time is shown in Fig. 7.7. The percent fat distribution within the frying temperature range was between 11.52 and 59.3%. Analysis of variance (ANOVA) shows
Figure 7.4 Confocal laser scanning images obtained in the fluorescence mode for samples fried at 170°C at different frying time.
Figure 7.5 Images obtained in the fluorescence mode for samples fried at 180°C at different frying time.
Figure 7.6 Two-dimensional images obtained in the fluorescence mode for samples fried at 190°C at different frying time.
that there is significant \( (P < 0.05) \) effect of frying temperature and time on the variation shown in fat distribution. Mean separation show 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 (Fig. 7.8). 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 goodness of fit obtained at 180 and 190°C indicates that fat content can be predicted, from the fluorescence (fat) distribution in the scanned images, with some degree of accuracy at these temperatures.

Fat distribution within the depth of the batter samples fried at 190°C for 180 s is
Figure 7.9 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 lateral view of the sample from its 3-D image.

shown in Fig. 7.9. These 2-D images taken at 4 μm apart show a diminishing intensity of the fluorophore/dye within the depth of a 60 μm thick sample. Fig. 7.9B shows the lateral view of the stack of the 2-D images, which also show the diminishing dye intensity. This signifies that fat content decreases from the surface towards the core of the sample. Some authors have asserted that fat absorption in fried foods is limited to the crust (Bouchon et al., 2003; Keller et al., 1986; Lamberg et al., 1990; Saguy et al., 1997). Bouchon and Aguilera (2001) showed a 3-D image of fried potato obtained using stack of 2-D images.
obtained from CLSM. They reported that oil is basically located at the crust region of fried potato especially around the cell region with few droplets showing in the area underneath, and that no oil was present in the interior of the fried sample. Pedreschi et al. (2008) reported similar information for oil distribution obtained in CLSM images of fried potato slices.

Porosity of the samples expressed as the ratio of area of pores to the area of the whole sample is shown in Fig. 7.10. Porosity ranged between 4.97 and 32.7% for all the samples fried over a period of 240 s and at three temperatures. Dogan et al. (2005)
obtained a porosity in the range of 23-40% for fried batter formulated with soy and rice flour using pycnometer. Adedeji and Ngadi (2009) also obtained porosity in the range of 7–14% for fried chicken nuggets batter using X-ray-micro-computed tomography (CT). The difference between these authors’ results and this study could be as a result of difference in sample composition and scale of measurement of the methods used. X-ray micro-CT would not measure pores below 5 micron and helium pycnometer used would penetrate pores as small as 0.22 nm (Ayral et al., 1992; Krus et al., 1997; Skyscan, 2008).

Porosity increased rapidly up to 60 – 90 s mark, after which it increased marginally or remained relatively constant until frying was terminated. Other authors such as Kawas and Moreira (2001), Taiwo and Baik (2007) and Krokida et al. (2000) have reported similar trend for porosity of different fried foods. The rapid increase in porosity could be associated with mass transfer phenomenon, which induced quick formation of pores at this period. Frying is a simultaneous heat and mass transfer process where frying oil acts as the medium of heat into the sample, causing the conversion of water in the food to vapor. The vapor generates high pressure under intense heat and thereby forces its way out of the food leaving behind voids and creating same on its path into the frying oil. This is usually seen as the rigorous bubbling during the early stage of frying. The reduced porosity after some seconds of frying is indicative of less moisture left in the sample. Other factors that may influence pore formation include food composition, initial moisture content, product’s surface area, pretreatment and frying conditions (Adedeji and Ngadi, In Press; Krokida et al., 2001; Ngadi et al., 2001; Taiwo and Baik, 2007). ANOVA showed that there is significant effect of frying temperature on the variation
seen in porosity with frying time. Porosity of batter fried at 170°C was significantly (P < 0.01) different from those at 180 and 190°C.

Pore size distribution frequency is shown in Figure 7.11 for chicken nuggets batter coating fried at 180°C for different frying times. The pore size in this graphs are presented as the number of pixel that correspond to a particular area of the pore from which the diameter is deduced. Each pixel equals 1.197 x 1.197 µm, as obtained from the image scanning in CLSM. The calculated area of each pixel equalled 1.43 µm². If a circular pore is assumed, the diameter of a pore that contains 1000 pixels will be equivalent to 42.7 µm and the one that contains 150,000 pixels will be equal to 523 µm. These were deduced from the area formula for a circle. The raw batter contained pores that were less than or equal to 204.8 µm (2.3 x 10⁴ pixels) in diameter. More than 70% of the unfried batter pores were between 1.2 and 95 µm (1 - 5 x 10³ pixels) in diameter. There were formation of smaller pore (< 42 µm) and bigger pores as frying progressed as seen after 120 to 240 s of frying. Pores as big as 270 - 523 µm (4.0 x 10⁴ – 14.0 x 10⁴ pixels) were formed in the later stage of frying, while medium size (191 – 270 µm = 2.0 x 10⁴ – 4.0 x 10⁴ pixels) pores diminished tremendously. The change in pore distribution during frying of chicken nuggets batter coating leading to greater formation of smaller and bigger pores as described could be associated with moisture migration and physicochemical transformation such as shrinkage induced by the intense frying heat.
Figure 7.11 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.
7.5 Conclusion

Fat distribution and pore characteristics of deep-fat fried chicken nuggets batter coating were obtained using confocal laser scanning microscopy. 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 with the conventional method at two frying temperatures, 180 and 190°C. Porosity ranged between 4.97 and 32.70% and was significantly influenced by frying temperature. Pore size ranged approximately between 1.20 – 523 µm. Frying led to the formation of smaller and bigger pores.

7.6 Reference:


microscopy (CSLM) and covalent labelling techniques. *Colloids and Surfaces B: Biointerfaces, 31*(1-4), 159-168.


From chapter 3 to 7, different techniques were used to evaluate the pore characteristics of deep-fat fried chicken nuggets coating. The results obtained from each technique differ and it is essential to reconcile these differences by comparative analysis of the results. In chapter 8, the results of pore properties from the different methods used in this study were compared and conclusions were made based on the analysis made.
VIII. COMPARISON OF PORE CHARACTERISTICS OF DEEP-FAT FRIED CHICKEN NUGGETS COATINGS OBTAINED BY DIFFERENT TECHNIQUES

8.1 Abstract

Comparative analysis of deep-fat fried chicken nuggets batter coating pore characteristics obtained from different techniques were made, considering the effect of processing conditions and batter formulation. All the methods used for pore characterization were effective within their various limits of measurement. Pycnometer porosity data would be more reliable because of the wider size range it could measure. X-ray microCT and confocal laser scanning microscopy would provide additional information on pore characteristics of the fried batter coating in terms of visual 3-D images, degree of pore connectivity, pore shape and size distribution information. Porosimetry method would require validation of its result by another method of measurement such as the pycnometer and would be more useful in analysis of dry samples. A combination of the different methods is useful in acquiring a more complete pore characteristic for deep-fat fried chicken nuggets coating.
8.2 Introduction

Microstructural properties of fried foods such as porosity and pore size distribution play an important role in their process optimization, novel food development and quality assessment (Aguilera, 2005; Datta et al., 2007). There are a number of techniques applied for determining these characteristics of food. For example, porosimetry was used by Kassama and Ngadi (2005a, b) and Ngadi et al. (2001) to study pore characteristics of fried chicken meat and chicken meat patties, respectively; Taiwo and Baik (2007) used volume displacement method to study the porosity of fried sweet potato; Adedeji and Ngadi (2009) used X-ray micro-computed tomography (CT) to study microstructural characteristics of chicken nugget coating. Microscopy technique was used by Bouchon and Aguilera (2001) to study fried potatoes and they were able to show that oil is located in the interior pockets or within the surrounding intact cells. The various techniques have limitations that influence their applications. The range of measurement in terms of pore size; ability to produce images for visual observation, qualitative and quantitative assessment are some of the specific reasons why a technique is preferred to another. Mercury intrusion porosimetry (MIP), for example, could provide pore characteristics in the range of 0.005 to 360 µm, however the samples must be bone-dry before measurement (Micromeritics, 1999). X-ray microCT (Skyscan 1072, Skyscan, Kontich, Belgium) imaging resolving power is only up to 5 µm (Skyscan 2001), produces three dimensional images and requires minimal sample preparation. Confocal laser scanning microscopy would produce 2-D images that can be reconstructed to form 3-D images, product constituents can be stained with different dyes for qualitative and quantitative evaluation and produces images in the micron scale (Aguilera and Stanley, 1999;
Dürrenberger et al., 2001; Subramanian and Hommerding, 2005). Some authors have suggested combining these techniques in studying the microstructural properties of porous materials such as food so as to have a more complete information about the sample being studied and to make comparison between techniques to see variation and the reason they occur (Hermansson et al., 2000; Kalab et al., 1995; Aguilera and Stanley, 1999; Datta et al., 2007). The objective of the study was to compare microstructural property of fried chicken nuggets coating obtained by various techniques.

8.3 Materials and method

8.3.1 Materials and Batter formulation

Two types of batter were used in this aspect of the study. The first type was a batter from chicken nuggets purchased from a local manufacturer (Olymel, Boucherville, QC, CA) in Quebec province of Canada. The nuggets were kept at sub-freezing temperature prior to use. They were randomly selected among the batch for frying. These samples were placed in the refrigerator to equilibrate before they were fried. Wheat flour (Five Rose All Purpose Flour, Les Cuisines Five Roses kitchens, QC, Canada) was procured from a local store in Montreal, Canada. Rice flour was supplied by Rivland Partnership (Riceland Foods, Arizona, USA). The second type of batter was laboratory formulated using wheat and rice flours at different ratios namely 100:0, 70:30, 50:50, 30:70 and 0:100. Set amount of salt, 1%, carboxymethyl cellulose CMC (TIC Gums Inc, Maryland, USA), 1% and NaHCO3 (baking powder) 0.5% were added to the wheat-rice flour mixture. Flour mixture and water were added at the ratio 1:1.5, respectively. The mixture was thoroughly mixed together using a kitchen mixer (B-Speed Mixer, Black and
Dekker, ON, CA). Cut chicken breasts were dusted with flour before dipping into the batter for better batter pick-up

8.3.2 Frying

Samples were fried at temperatures ranging between 170 - 190°C in a programmable fryer (Henny Penny Computron 7000 pressure Fryer, Model 500C, HP Corporation, Eaton, OH, USA), that has a capacity for 30 L, at a duration ranging between 0 and 240 s. The fried samples were blotted with paper tissue to remove surface oil and allowed to cool down to room temperature before the breaded coating was carefully removed.

8.3.3 Porosimetry technique (See 3.3.3)

Pore structure characteristics were determined following the methods described by Ngadi et al. (2001) and (Micromeritics, 1999). Freeze-dried samples were weighed into the penetrometer of the porosimeter (Auto Pore III series 9400, Micromeritics Inst Co., Norcross, GA, USA) (Appendix 6.1). The porosimeter has a pore measuring range down to 0.005 µm and operates at a maximum pressure of 228 MPa (33000 psi). The penetrometer is made of glass material in a cylindrical shape with an enlargement at the one end in bulb shape, which comes in various sizes for different sample structure (powder or solid). The particular one used for this study has a 15 cm³ bulb volume, a total stem volume of 1.131 cm³ and a maximum measurable volume of 1.057 cm³. The estimated pore volume was determined between 90 and 25% of the maximum measurable volume. The amount of pore volume equivalent to a pore size was obtained by Eq. 3.2 above. A plot of cumulative pore volume over the pore size range of the sample is
presented as the pore distribution graph. Porosity ($\varepsilon$) was obtained by subtracting the ratio of apparent and bulk volume from one (Eq. 3.4). The standard protocol for pore characteristics determination by porosimeter is available in Micromeritics (1999).

8.3.4 Pycnometer method (See 4.3.3 and 4.3.4)

In order to determine porosity, apparent and bulk densities of the sample are determined using fluid displacement methods in a pycnometer. Apparent density of the samples was measured in a helium pycnometer (Model 1305 Multivolume, Micromeritics Instrument Corporation, Norcross, GA) (Appendix 5.1). The detached coatings of the fried samples were weighed prior to analysis. Each sample was placed in the 35 cm$^3$ sample chamber of the pycnometer and was subjected to cyclic action (purging) by pressurizing and depressurizing with helium gas prior to analysis in order to expel all the air and vapors trapped in the pores and crevices. The analysis was conducted at ambient temperature with pressure of up to 135 kPa (19.5 psi). Detailed procedure is provided in the standard protocol manual of Micromeritics (1992). Each sample was measured thrice and three replicates were used. To determine bulk density, the same set of samples used for apparent density measurement were quickly dipped in a melted paraffin wax liquid and allowed to cool fill the surface openings on the samples. Samples were then dropped into a liquid (water) displacement pycnometer and the displaced volume was recorded as the volume of the sample. Bulk density was determined as the ratio of the mass of sample divided by the bulk volume.
8.3.5 X-ray microCT imaging (See 5.3.6 and 5.3.7)

Samples scanned in X-ray microCT scanners (SkyScan 1072, Skyscan, Kontich, Belgium) (Appendix 2.1) were prepared by the methods described in chapter 5.3.6. Fried samples were quick frozen in liquid nitrogen and kept in the deep-freezer for 24 h to prior scanning. The images were acquired at the following settings: 100 keV, 98 µA current, 6.0 s exposure time and a rotation step of 0.68° through 180°. No filter was used in order to increase the contrast of the images obtained. The magnifications of the images was 20x and a cross-section pixel size of 9.38 µm was realized. The duration of the scanning based on the rotation step was about 69 min. The obtained images were reconstructed into series of 2-D images using a reconstruction software (Nrecon, SkyScan Belgium) that operates based on modified filtered back-projection algorithm (Feldkamp, et al. 1984; Sasov and van Dyck, 1998). A total of 916 slices of 2-D images were obtained in 8-bit bitmap (1024 X 1024 pixels) for each sample, which project through the sample from the surface coating to the chicken core. These images were reduced to half the size (512 X 512 pixels) by TConv (SkyScan, Kontich, Belgium) in order to reduce the duration of processing for quantitative data acquisition. Various image processing steps were performed. The region of interest (ROI) was selected randomly; smoothing (Gaussian filter) of grayscale images was performed to remove noise. Thresholding was done to delineate between the object (pores) and the rest of the sample by automatic thresholding step, applying Otsu’s algorithm (Otsu 1979) to obtain a thresholding points of 78 and 70 for the fried coating and core, respectively, within the range of 0 and 255 for 8-bit images obtained in this study; dilation and despeckling (Median filter) were carried out to further remove noise from the binarized images. All 3-D image analysis and 3-D model
construction were performed using CTan and CTvol softwares (Skyscan, Kontich, Belgium), respectively. Pore diameter as presented is the average pore size within a pore. The ROI for the breading coating and the chicken nuggets core were selected at area close to the surface (coating ≈ 2 mm thick) and within the length of the chicken core, respectively. Thirty slices were chosen in three replicates for both the coating and the chicken core.

8.3.6 Confocal laser scanning microscopy (See 7.4.1 and 7.4.2)

Solution of Nile Blue A (0.005%) was prepared by dissolving in demineralized water according to van Dalen (2002) method. It was non-covalently (one or two drops) added to the surface of fried product after frying. The stained samples were kept for 12 h at refrigeration temperature (8°C) for the dye to spread within the product matrix especially to areas where fat is located (van de Velde et al., 2003). Samples of the coating were taken by cutting cube shape (0.5 x 0.5 x 0.5 cm) out of the stained chicken nuggets. They were subsequently quick frozen in liquid nitrogen in preparation for cryo-sectioning and kept in a deep-freezer at -50°C prior to use. The cryo-sectioning of the samples was done in a cryostat (Shandon Cryotome, Thermo Scientific, Waltham, MA, USA) (Appendix 4.1). The cryostat was pre-conditioned to -20°C before the sectioning operation. Frozen samples were glued to the sample-plate (cryocassette) in the cryostat with the aid of frozen tissue embedding media (Histo Prep, FisherDiagnostic, NY, USA) and sectioning at preset depth of 60 µm was carried out after five steps (300 µm thickness removed) of scrapping to remove unevenness on the surface of the sample. The sectioned sample was
then placed on to microscope slides (SuperFrost Fisherbrand, Fisher Scientific, ON, CA).

A confocal microscope (Bio-Rad Radiance 2100, Hemel Hempstead, UK), equipped with an argon/krypton laser, installed on to a fluorescence microscope (Nikon Eclipse E800, Hertfordshire, UK) was used to acquire images from the samples. Images were obtained at fluorescence mode with excitation/emission wavelengths of 488/570 nm to show fat distribution, and transmission mode to show structure of the sample. The objective lens was set to obtain magnification of x10 with numerical aperture of 0.3 and to produce multiple grayscale 8-bit 2-D images of TIF format with resolution of 1024 x 1024 pixels and pixel size of 1.197. The images were analyzed using a code developed on MATLAB (R2006a, Version 7.2.0.232) software. The image processing entail delineating between the pores and the rest of the sample, removing noise and calculating porosity as a ratio of the object area to the rest.

8.4 Results and discussion

Porosity values of deep-fat fried chicken nuggets breading/batter coating obtained by the four techniques are presented in Table 8.1. The final porosity obtained from the pycnometer measurement at 170, 180 and 190°C frying temperatures were 40.09, 42.71 and 47.92%, respectively, while the initial porosity was 3.78%. Final porosity from CLSM for the respective frying temperatures were 32.70, 25.44 and 31.43%, and the initial porosity was 4.97%. Porosimeter measurement gave porosity at the frying temperatures as 43.66, 40.56 and 43.89% from an initial value of 68.90%. X-ray microCT final porosity was 14.05% from an initial porosity of 6.99% for sample fried at
180°C. Porosity values obtained from pycnometer, CLSM and X-ray microCT increased with frying time while the values from porosimeter decreased. The decreasing trend shown in porosimeter data is attributable to the freeze drying of the samples prior to MIP measurement, which is a major requirement for MIP procedure (Giesche, 2006; Micromeritics, 1999). The decreasing trend shown in pycnometer, CLSM and X-ray microCT data was as a result of moisture loss, fat intrusion and other structural changes during frying (Adedeji and Ngadi, 2009; Dogan et al., 2005, Kassama and Ngadi, 2005a). Porosity values from pycnometer were higher compared to values from CLSM and X-ray microCT. The range of difference is between 17.86 – 40.44% less when CLSM values at various temperatures were compared with pycnometer values, 70.66 and 44.77% less when CLSM and pycnometer values were compared to X-ray microCT values obtained at 180°C frying temperature. The differences in the porosity obtained by the different techniques could be attributed to the limitations in each of the techniques. Pycnometer has capability to measure pores as small as 0.22 nm (Ayral et al., 1992; Krus et al., 1997) because of its small atomic size. It is not surprising that porosity values from pycnometer measurement were on the higher side, since it will measure the tiniest of the open and flow-through (free) pores. However, closed pores are not measurable by pycnometer methods. CLSM and X-ray microCT used captured images in their porosity computation and the extent of measurement is contingent on the spatial resolution of the techniques. In CLSM and X-ray microCT techniques, visual images were produced for qualitative assessment, which is not possible with pycnometer measurement and closed pores were included in the quantitative assessment of porosity. Spatial resolution of the images acquired in CLSM was 1.197 μm (See 7.4.2 and 8.3.6) and pixel size in X-ray microCT
was 5 μm (Skyscan, 2001). Even though CLSM and X-ray microCT would include closed pores in their porosity measurement, any pore less than 1.197 or 5 μm would be excluded. Assuming minimal amount of closed pores, the percentage reported above as the difference between porosity from pycnometer and the imaging methods (CLSM and X-ray microCT) would equal the amount of small pores present in the samples under various processing conditions. Porosity obtained from MIP method would have provided further confirmation to pycnometer data because of low pore size measuring point (0.005 μm), if the freeze drying step was not required.

However, Pearson correlation between porosity obtained by pycnometer versus CLSM and porosimeter showed strong positive and negative relationship with correlation coefficients, r of 0.85 and -0.89, respectively, at P < 0.01. Between CLSM and porosimeter data, there was also a strong negative R which equal to -0.72. These show a good predictability between the porosity obtained by these three techniques. Because of few data available for the X-ray microCT, they were not compared to the other data.

Table 8.2 shows the porosity data for chicken nuggets batter coating formulated with different flours (wheat and rice) after frying for 4 min at 180°C. There was no significant (P < 0.05) effect of formulation on the final porosity of batters where wheat flour was substituted for rice flour, except for the batter from 100% wheat flour that showed significant (P <0.05) difference with the other formulations. Porosity values obtained from pycnometer are also on the higher side compared to those from X-ray microCT imaging. This is attributable to the same reason for porosity determination under different frying conditions.
Table 8.1 Porosity data of deep-fat fried chicken nuggets batter coating obtained from different techniques.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Frying time, s</th>
<th>Pycnometer</th>
<th>CLSM</th>
<th>Porosimeter</th>
<th>X-ray µCT</th>
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<td></td>
<td>0</td>
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<td></td>
<td>15</td>
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<td>14.80(2.87)</td>
<td>70.13(3.49)</td>
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<td>170°C</td>
<td>30</td>
<td>16.92(4.07)</td>
<td>20.58(6.77)</td>
<td>62.94(11.21)</td>
<td>-</td>
</tr>
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<td>45</td>
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<td>20.98(1.58)</td>
<td>59.00(11.86)</td>
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<tr>
<td></td>
<td>60</td>
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<td>22.60(9.25)</td>
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<td>32.70(1.13)</td>
<td>43.66(8.88)</td>
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<td>4.97(0.66)</td>
<td>68.9(3.27)</td>
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<td>4.97(0.66)</td>
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<td>15.14(1.82)</td>
<td>51.82(7.36)</td>
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<td>90</td>
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<td>24.80(8.15)</td>
<td>55.65(5.08)</td>
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<td>29.58(2.03)</td>
<td>53.78(1.29)</td>
<td>-</td>
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<td>47.92(3.05)</td>
<td>31.43(2.21)</td>
<td>43.89(2.72)</td>
<td>-</td>
</tr>
</tbody>
</table>

Numbers in parenthesis are standard deviations. CLSM = Confocal laser scanning microscopy, X-ray µCT = X-ray micro-computed tomography.
Table 8.2 Porosity data obtained for different formulations of chicken nuggets batter coating fried at 180°C for 4 min.

<table>
<thead>
<tr>
<th>Batter Formulation</th>
<th>Pycnometer</th>
<th>X-ray µCT</th>
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<tr>
<td>W100R0</td>
<td>41.77\textsuperscript{a}</td>
<td>18.21\textsuperscript{a}</td>
</tr>
<tr>
<td>W70R30</td>
<td>46.63\textsuperscript{a}</td>
<td>32.18\textsuperscript{b}</td>
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<td>W50R50</td>
<td>41.64\textsuperscript{a}</td>
<td>31.77\textsuperscript{b}</td>
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<tr>
<td>W30R70</td>
<td>45.03\textsuperscript{a}</td>
<td>28.17\textsuperscript{b}</td>
</tr>
<tr>
<td>W0R100</td>
<td>46.43\textsuperscript{a}</td>
<td>27.99\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textit{W#R#} = wheat and rice flour percentage in the formulation. Mean with different letter superscript in the same column are significantly (P < 0.05) different.

Pore size distribution of the chicken nuggets batter coatings fried at 180°C obtained by X-ray microCT and CLSM imaging are presented in Figs. 5.3 and 7.11 (pages 99 and 169). The size distribution range obtained by X-ray microCT and CLSM were 9 – 309 µm and 1.2 – 523 µm, respectively. Over 70% of pores obtained from unfried coating in both methods were less than 100 µm. In the data presented by the two methods, there were formation of smaller pores (< 42 µm) with frying time and disintegration of the medium size pores around 155 - 239 µm and 199 – 270 µm for X-ray microCT and CLSM, respectively. CLSM imaging showed formation of few bigger pores.
8.5 Conclusion

All the methods used for pore characterization were effective within their various limits. Pycnometer porosity data would be more reliable because of the wider size range it could measure. X-ray microCT would provide additional information on pore characteristics of the fried batter coating in terms of visual 3-D images they produce, degree pore interconnectivity, pore shape and pore size distribution information. CLSM also provides visual images for quantitative and qualitative analysis, and pore size distribution information. Porosimetry method would require validation of its result by a more direct method of measurement such as the pycnometer and would be more useful in analysis of dry samples. All the methods would be useful in acquiring a more complete pore characteristic for deep-fat fried chicken nuggets coating.

8.6 References


IX. GENERAL SUMMARY

9.1 General conclusion

Deep-fat fried coated foods such as chicken nuggets have a very huge market, which keeps growing because of changes in the social lifestyle of consumers and their unique appealing qualities. The health concern about the amount of fat uptake during the frying process necessitates the development of several methods to ensure a reduction in fat content of fried foods. Application of film forming materials such as batter systems have been applied as barrier to control fat absorption in fried products, in addition to the fact that they contribute to improve the overall qualities of the product like volume, texture, juiciness and aesthetic appeal. It is still not very clear how batter systems structural transformation during frying process actually does the job of mass transfer control, though it has been asserted that changes in microstructural configuration and the constituents of the batter are closely related to the amount of fat absorbed. Detailed study of the microstructural changes in batter coating during frying are lacking.

In this study, pore characteristics of deep-fat fried batter/breading coatings were studied by different techniques namely mercury intrusion porosimetry (MIP), helium pycnometry, X-ray micro-computed tomography (CT) and confocal laser scanning microscopy (CLSM). The effect of processing conditions and batter formulation on pore properties were also evaluated. The different techniques were effective in characterizing the pore properties of deep-fat fried chicken nuggets coating within their various limit. MIP showed that porosity of fried freeze dried batter coating decreased with frying time and there was significant effect of frying temperature on the variation observed in
porosity. With MIP measurement, porosity showed significant correlation with moisture loss and fat absorption. There were two peaks shown in the pore size distribution and about 30% of the pores were capillary (≤ 1 µm) pores. The intrusion and extrusion volume of mercury under application of gradual pressure showed hysteresis phenomenon, indicating that there were geometries present in the samples aside cylindrical shape assumed. However the percentage of entrapped volume was below 10% in all the samples.

Porosity data obtained from helium pycnometry showed it increased with frying time. There was significant effect of frying temperature on the variability of porosity with frying time. The higher the frying temperature, the more pores were formed. Batter formulation significantly influenced porosity. Wheat flour batter had low porosity compared to others substituted with different levels of rice flour. Pearson correlation analysis showed that moisture and fat content significantly (P < 0.05) correlated with porosity.

Visual images of the chicken nuggets coating and core were produced using X-ray microCT imaging. Pore present in the coating presented various size range, while the chicken core images displayed evenly distributed pore size. Frying time effect was significant (P < 0.05) on the variation observed in the microstructural properties of the batter such as porosity, fragmentation index and pore count both in the coating and the core. Pore volume and total number of pores increased with frying time, and there was formation of micropores (pores < 100 µm). The shapes of the coatings’ pores were between rod-like/cylindrical and spherical structure, and those of the core were rod-like. Some degree of correlation were shown between samples’ (coating and core) porosity,
frying time, fat content and moisture content. Images of batter formulated with different levels of wheat and rice flours were also produced with X-ray microCT. The images showed that increased addition of rice flour in the batter formulation led to formation of more larger pores. Pore size distribution showed bimodality, indicative of prevalence of pores between 94 - 169 µm and 544 - 582 µm. Porosity, fragmentation index of the pores and pore number were significantly (P < 0.05) affected by batter formulation. Lower porosity was obtained for batter with 100% wheat flour compared to batter substituted with different levels of rice flours. The addition of rice flour into the formulation decreased fragmentation index, indicating increase in the degree of connectivity. Most of the pores were cylindrical in shape with structure model index ranging between 2 – 3. The degree of roughness or fractal dimension of the pores was not significantly affected by formulation. Pore count decreased with increase addition of rice flour into the batter formulation. Effect of formulation was significant on other physical properties of batter such as batter consistency, batter pick-up, fried batter color, texture, moisture and fat content.

CLSM method was used to evaluate the pore characteristics and fat distribution in fried chicken nuggets batter coating. Fluorescence and trans/reflective images of the samples showed how fat and pore were distributed within the samples’ matrix. The fluorescence images also showed that fat distribution diminished from the surface toward the core of the sample, up to few microns (about 1 mm) deep. Porosity, pore size distribution and fat distribution were quantitatively evaluated for the fried chicken nuggets batter coating. Porosity was significantly affected by frying temperature and time. Also, there was formation of smaller (pores ≤ 42 µm) and bigger (pore ≥ 270 µm)
pores, while medium size (191 – 270 µm) pore disintegrated during frying. Fat distribution was significantly (P < 0.05) affected by frying temperature and time. Mean separation showed that fat distribution at 170°C frying temperature was statistically different from those at 180 and 190°C. Fat distribution obtained with CLSM imaging and fat content obtained with the conventional solvent extraction method were strongly positively correlated at two temperatures 180 and 190°C, with correlation coefficients of 0.79 both. This shows a good predictive capacity of data from one method to the other.

All the techniques were effective in pore characterization of deep-fat fried chicken nuggets within their limit of measurement. However, their combination would give a more complete information for the pore properties, by providing information where others are lacking. For example, pycnometer can be combined with X-ray microCT imaging to obtain complete pore characteristics of a fried product. This is based on the fact that pycnometer will provide a more reliable porosity data because of its ability to measure pores into the nano-scale, while X-ray microCT will provide other microstructural properties such as pore count, pore shape and visual images.

9.2 Claims of contribution to knowledge

This study made original contributions to microstructural characterization of deep-fat chicken nuggets coatings, which would be useful in novel batter development and modeling of frying process for the product.

1. Microstructural characteristics (namely porosity, pore size, pore size distribution, pore shape, pore volume and pore area) of freeze dried deep-fat fried chicken
nuggets coating were determined under various frying conditions (frying time and temperature) using mercury intrusion porosimetry. Porosity of the coating were in the range of 40 to 69%. Average pore diameter of the fried coating was from 0.25 - 8.32 µm. It was shown that the freeze drying step led to a decreasing trend in porosity with frying time. The study equally established that not all the pores present in the samples are cylindrical as indicated by the hysteresis phenomenon.

2. Pore characteristics of deep-fat fried batters industrially preformed and lab formulated with different levels of wheat and rice flours were obtained using Helium pycnometry. Porosity range for the preformed fried batter coating was 2.15 - 47.9% and for the formulated batter, it was between 10 and 55%. It was established that batter formulation and frying temperatures significantly influenced pore development. The higher the frying temperature, the higher the porosity. The inclusion of rice flour at different percentage up to 100% led to increased porosity. This study showed that porosity of the batters obtained with pycnometer increased with frying time.

3. A protocol suitable for X-ray microCT imaging of fried batter system was developed. Three dimensional images acquired from X-ray microCT scanning were used to obtain microstructural characteristics (namely porosity, pore size distribution, pore count, pore shape, degree of pore interconnectivity and fractal dimension) of industrially preformed and lab formulated deep-fat fried chicken nuggets coating and core. Porosity of the preformed batter coating and core
ranged from 7.0 – 14.1% and 4.45 – 7.7%, respectively, that of the formulated batter ranged from 18.2 – 32.2%. The study showed that the addition of carboxymethyl cellulose into batter formulation with 100% wheat flour reduced porosity and subsequently fat uptake, while rice flour addition reversed the trend. Pore shapes for all the coatings studied were found to be between plate-like and cylindrical structure.

4. Methodology for preparation of deep-fat fried batter samples for confocal laser scanning under fluorescence microscope was developed. Two dimensional images showing fat distribution pattern and pore structures of deep-fat fried chicken nuggets coating were acquired for quantitative and qualitative assessments. The predictability of fat content from CLSM image analysis was also established at two frying temperatures namely 180 and 190°C. Porosity obtained varied between 4.97 and 32.7%.

5. This study established the possibilities and the limitations of each of the pore characterization techniques, and demonstrated that combination of more than one technique would provide a more comprehensive data on pore characteristics of fried batter coating. For examples: Helium pycnometry will only provide porosity data; X-ray microCT and CLSM would not measure porosity beyond the limit of their resolving power though other microstructural parameters such as pore size distribution and qualitative assessment of samples from image visuals are
possible. This study also exposed the fact that MIP would not provide reliable
data for hydrated samples that would need the pre-drying step. The study also
provided fried coating microstructural property data that will be useful in
modeling deep-fat frying process for optimization.

9.3 Recommendations for future work

There is a need for further investigation into how mercury intrusion porosimetry
technique can be used for hydrated material such as fried products or a way of correlating
data from other techniques, such as pycnometry, with MIP data be sought for the
development of predictive models.

X-ray computed tomography system with higher resolving power such as
nanotomography should be explored in characterizing pore properties of fried chicken
batter coating so as to capture data that might exist at this scale that were probably
excluded because of the limit of microtomography in terms of the resolving power.
X. GENERAL REFERENCES


APPENDICES
APPENDIX 1: MATLAB code used for pore characterization and fat distribution.

Appendix 1.1: Matlab procedure for multiple image computation of fat distribution and porosity.

```
path = 'path of the stored images in the directory';
D = dir(fullfile(path,'*.tif'));
Ratio = [];
for i=1:12  %%% i is the number of images in the folder desired for processing
    I = imread(fullfile(path,D(i).name));
    level = graythresh(I);
    BW = im2bw(I,level);
    a = sum(sum(BW));
    b = 1024*1024;
    ratio = a/b;
    Ratio = [Ratio;ratio];
end;
```

Appendix 1.2: Matlab code for pore size distribution histogram development.

```
I = imread('image path in the computer.tif');
[labeled,numObject] = bwlabel(I,4);  %%% To count the number of objects/pores in an image
coatingData = regionprops(labeled,'basic');  %%% Measures object properties
```
hist([coatingData.Area], 20) \texttt{%%% Constructs the pore count histogram as a function of pore size.}
Appendix 2.1: Skyscan 1072 X-ray micro computed tomography.

Figure A2.1 shows the image of the table-top X-ray micro CT produced by Skyscan, Kontich, Belgium that was used for this study. The equipment is located at the Center for Bone and Periodontal Research in McGill University downtown Montreal. The enclosed part houses the sample stand where the sample to be scanned is placed on a rotatable stand. The system is interphased with a PC for automatic data acquisition.

Figure A2.1. Tabletop X-ray micro-computed tomography, Skyscan 1072.
APPENDIX 3.1: Confocal laser scanning microscope.

Figure A3.1 Confocal laser scanning microscope

The confocal laser scanning microscope (Bio-Rad Radiance 2100, Hemel Hempstead, UK) used in this study is shown above. The equipment is found at the Institute of Parasitology, Macdonald Campus of McGill University. It comprises of Argon laser system mounted a Nikon fluorescence microscope (Nikon Eclipse E800, Hertfordshire, UK).
APPENDIX 4.1: Cryostat.

The Cryostat consists of the digital panel where the temperature and thickness control buttons are located. The Frozen chamber houses the sample stand and the microtome for frozen section.

Figure A4.1 Shandon Cryotome, Thermo Scientific, Waltham, MA, USA.
APPENDIX 5.1: Helium pycnometer.

The helium pycnometer (Model 1305 Multivolume, Micromeritics Instrument Corporation, Norcross, GA) used in this study is shown Figure A4.1. It comprises mainly of the sample chamber, the digital screen for pressure output, the Fill and Vent valves, and their rate control knobs, the four-way valve (used for altering the volume of expansion chamber) and the chamber setting knobs.

![Helium Pycnometer](image)

Figure A5.1 Helium Pycnometer.
APPENDIX 6.1: Mercury intrusion porosimeter.

The porosimeter (Auto Pore III series 9400, Micromeritics Inst Co., Norcross, GA, USA) used in this study is shown in Appendix 6.1 Figure 1. The porosimeter is capable of measuring more than 0.005 μm pore sizes and operates at maximum pressure of 228 MPa (33000 psi). There are two main sample ports, the low and high pressure ports.

Figure A6.1 Auto-Pore III Mercury Porosimeter image.