SELECTED PHYSICOCHEMICAL PROPERTIES OF SUPERCRITICAL FLUID EXTRUDATES

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Steam-based extrusion (SBX) is widely utilized for starch-based products but utilization of protein in SBX has been reported to be limited due to high temperature and high shear conditions. Supercritical fluid extrusion (SCFX) uses supercritical CO₂ (SC-CO₂) as a blowing agent, enabling utilization of heat and shear sensitive ingredients including whey protein. The objective of this study was to investigate the effects of whey protein and process parameters on the expansion and microstructure of SCFX extrudates using on-line slit die rheometer, X-ray microtomography, and video analysis. The potential of SCFX for healthy snack production with whey protein and with higher resistant starch (RS) content was also evaluated. Whey protein acted as a diluent leading to reduced melt viscosity that resulted in lower cell number density and overall expansion. Thermal analysis indicated only limited chemical interaction between whey protein and pregelatinized corn starch. SC-CO₂ increased the expansion of whey protein added starch-based extrudates but structural collapse was observed at 0.75 wt% SC-CO₂ level during post-extrusion drying at 85°C. Cell size from transverse cross-sections of SCFX extrudates decreased with radial distance from the center. In the longitudinal direction, the cells shapes were more elliptical than spherical. Not only piece density but also the ratio of cell wall thickness to cell diameter were observed to be a good predictor of mechanical properties. SCFX process showed 30-200 fold longer expansion time compared to SBX and can be utilized for making novel products. The pressure drop profile in the die was found to be critical in controlling not only overall expansion but also the rate of expansion.

SCFX extrudates showed 4 fold higher RS content than SBX extrudates while the product densities of both extrudates were comparable. Feed moisture content and processing temperature were found to be critical for RS formation in SCFX and SBX extrudates. A SCFX process has been successfully developed for production of novel healthy snack containing 40~60 wt% whey protein with unique porous structure and texture. The finding showed that an extrusion process at temperatures below protein denaturation temperature using SC-CO₂ can help prevent hard texture and create a uniform microstructure.

BIOGRAPHICAL SKETCH

Ki Yul Cho was born in Seoul, Korea, on October 30, 1972. He obtained his B.S. and M.S. degrees in Food Science and Technology at Korea University in 1995 and 1997, respectively. Upon graduation he worked for Pumluone Company Inc. as a product developer. In August 2002 he enrolled in the Ph. D. program at Cornell University with a major in Food Engineering, and minors in Food Chemistry and Biological and Environmental Engineering. This dissertation is dedicated to Hyeri, all of my family, and my Lord

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CHAPTER 1

INTRODCUTION

1. Steam-Based Extrusion and Its Limitations

Conventional steam-based extrusion (SBX) process is a commercially practiced technology to produce a large variety of expanded solid food products due to its versatility (Alavi, Gogoi, Khan, Bowman, & Rizvi, 1999). During SBX cooking, water acts as both a plasticizer for melt formation and a blowing agent for expansion (Wang, Ganjyal, Jones, Weller, & Hanna, 2005). Conventional SBX process usually requires low moisture (i.e., 18-28 wt%), high temperature (i.e., 120-180°C) and high shear conditions for good expansion (Moraru & Kokini, 2003). Although limited control of macro- and microstructure formations in SBX process can be achieved by manipulating operational parameters, such as in-barrel moisture content, die geometry, die temperature, screw rotation speed, and dry feed rate, steam-expanded products usually show non-uniform cellular structure and cell sizes (Barrett & Peleg, 1992). The polydispersity index (PDI), which indicates the uniformity of cell size distribution, of steam-based extrudates is approximately 0.29 suggesting heterogeneous cell distributions (Alavi et al., 1999).

The harsh operating conditions of the SBX process often prevent effective utilization of heat and shear sensitive ingredients, such as proteins, vitamins, and certain flavors. The general finding is that the addition of protein decreases the expansion resulting in undesirable hard texture (Matthey & Hanna, 1997). Researchers (e.g., Adamu & Jin, 2001; Kim, Tanhehco, & Ng, 2006; Unlu & Faller, 1998) have proposed the SBX process as a candidate for resistant starch production. However, several researchers (e.g., Faraj et al. 2004; Sajilata, Singhal, & Kulkarni, 2006) have reported a lack of RS formation during conventional SBX processing. In SBX

processing, the specific mechanical energy (SME), which is inversely related to inbarrel moisture, is the main factor that controls expansion. The typical low in-barrel moisture content of the SBX process results in higher SME, higher shear, and greater melt temperature, which results in higher expansion. However, it also leads to excessive dextrin formation. Such low in-barrel moisture conditions are detrimental for resistant starch formation due to limited starch mobility during starch retrogration (Longton & LeGrys, 1981).

2. Supercritical Fluid Extrusion and Its Applications

Supercritical fluid extrusion (SCFX) is a novel technology that uses supercritical carbon dioxide (SC- CO_2) as a blowing agent instead of steam, enabling the formation of an expanded structure at lower temperatures ($<100^{\circ}$ C) (Alavi et al., 1999; Rizvi, Mulvaney, & Sokhey, 1995). A higher moisture content (i.e., 30-45 wt%) in the extruder barrel is utilized to keep the product temperature low via reduction of viscous dissipation of energy and to maximize SC-CO₂ solubilization in the melt. In this process, expansion of the melt is achieved by first solubilizing SC-CO₂ in the melt, and then inducing nucleation due to pressure drop in the die. This is followed by cell growth caused by diffusion of CO_2 into the nucleated cells (Rizvi et al. 1995). In the final stage, further diffusion of CO₂ into the cells and their consequent expansion cause the cell walls to progressively decrease in thickness and to increase the rate at which CO₂ diffuses out of the surface of extrudates into the environment (Rizvi et al. 1995). Post-extrusion processes, such as baking and frying, not only impart desirable flavor and appearance to the SCFX product, but also generate further expansion. This is because SCFX extrudates experience a time-delayed expansion until the structure is set (Alavi et al., 1999). Figure 1.1 illustrates SCFX extrudates at the die exit with and without SC-CO₂ injection.



Figure 1.1. SCFX extrudates at the die exit (Left: control with no SC-CO₂ injection; right: expanded SCFX extrudate with 0.7 wt% SC-CO₂)

A greater thermodynamic instability due to a high drop in the pressure rate in the die compared to SBX results in high cell density, ranging from 2.3 x 10^6 to 6.8 x 10^6 cells/cm³ (Alavi et al., 1999). The SCFX process allows control of the average cell size of extrudates which can range from approximately 50 to 250 microns with a high polydispersity index of ~0.95, but also the volumetric expansion of final products by manipulating process parameters, such as die dimension, SC-CO₂ concentration, and SC-CO₂ residence time (Alavi & Rizvi, 2005; Winoto, 2005). Moreover, SC-CO₂ expanded extrudates exhibit predominantly homogeneous closed cell structures and nonporous surface, which facilitates flavor encapsulation and provides better textural control over steam-based products (Alavi et al., 1999). SC-CO₂ can be utilized to encapsulate flavors and potent micronutrients into expanded products because it is an excellent solvent at supercritical state.

Because the SCFX process is conducted at lower shear conditions and temperatures lower than 100°C, it enables the use of temperature and shear sensitive ingredients in product formulations (Winoto, 2005). Alavi et al. (1999) found that the

incorporation of thermosetting proteins (< 10 wt%), such as egg white and whey protein, increased the overall expansion of SCFX extrudates by reducing structure collapse due to protein gelation. Sokhey, Rizvi, & Mulvaney (1995) successfully utilized the SCFX process to produce ready-to-eat cereal with a longer bowl life. SC-CO₂ leavened bread dough can be produced using SCFX in ~2 minutes by eliminating fermentation (Hicsasmaz, Dogan, Chu, & Rizvi, 2003). Chen, Dogan and Rizvi (2002) developed SCFX masa-based chip products with unique microstructures. Although support exists for the utility of the SCFX process in the development of a number of novel and unique products which cannot be produced using the conventional SBX process, the potential benefits of the SCFX process have not been fully explored in areas, such as the production of high-protein (> 60 wt%) expanded snacks and the generation of resistant starch.

3. Utilization of Whey Protein in Extrusion Processing

A number of researchers have investigated the effect of proteins on expansion characteristics during the extrusion process, indicating an increased interest in generating healthy extruded foods (Onwulata, Smith, Konstance, & Holsinger, 2001). In particular, researchers have given more attention to whey protein due to its abundance and underutilization. Whey protein is a rich source of branched-chain amino acids and has a well-balanced amino acid profile (Onwulata et al., 2001). However, the utilization of whey protein in SBX processing has been often limited due to its detrimental effects on expansion and texture (Kim & Maga, 1987). Researchers have attributed the detrimental effect of whey protein to its heat-sensitive thermosetting property and interaction with starch during conventional SBX processing (Onwulata et al., 2001).

One of the many possible approaches to minimizing the detrimental effects of whey protein is extrusion processing at low temperatures. The challenge associated with the hard texture of high whey protein-based extruded products can be prevented by extrusion at lower temperatures (i.e., below 70°C). This is because the denaturation temperatures of whey proteins, including β -lactoglobulin, α -lactalbumin, bovine serum albumin, and immunoglobulin, range from 59°C to 82°C (Alavi et al., 1999). In addition, low processing temperatures can also minimize the chemical complexation between starch and protein, which is known to result in less expansion and to produce a harder texture. Some researchers have reported that whey protein-starch interaction is responsible for the observed decrease in expansion of steam-based extrudates (Allen, Carpenter & Walsh, 2007; Matthey & Hanna, 1997). Matthey et al. (1997) attributed amylose-whey protein complexation to a decrease in apparent amylose content, resulting in reduced expansion. Allen et al. (2007) reported that there was a significant degree of covalent bonding between whey protein and starch at extrusion temperatures between 158°C and 170°C.

Therefore, the low processing temperature associated with the SCFX process is beneficial because it allows for a more effective utilization of whey protein in extruded products. SCFX processing of extruded snacks with high whey protein containing formulations (>50 wt%) offers possibilities for producing healthy snacks with high nutrient densities and novel textures. The lower pH of the melt due to dissolved SC-CO₂ also inhibits the Maillard reaction, which would otherwise cause further loss of essential amino acids (Mulvaney & Rizvi, 1993).

4. Melt Rheology and Macro-/Microstructure Formation during Extrusion Processing

It is well known that the rheology of melt during extrusion processing governs the macro- and microstructure formations that result from nucleation, die-swell, cell growth, and cell collapse (Moraru et al., 2003). In general, starches show higher viscosities than proteins in solutions with the same concentration and at similar shear rates (Resch, Daubert, & Foegeding 2004; Sopade, Hardin, Fitzpatrick, Desmee, & Halley, 2006). Heat-induced pure protein gels typically show a higher degree of elasticity than pure starch gels, whereas a mixture of these two biopolymers shows lower elasticity compared to the original pure gels (Muhrbeck & Eliasson, 1991). Although there are a number of studies examining the effects of melt rheology on the foaming mechanisms of synthetic polymeric foams, there is lack of information on the role of melt rheology of biopolymeric foams on microstructure formation (Han & Han, 1988).

Figure 1.2 schematically illustrates the possible effects of melt viscosity on the microstructure formation of three different SCFX melts differing considerably in viscosity, but having negligible elastic property differences. In the die, nucleation occurs. It is well-established that the extent and the rate of nucleation are both functions of melt shear viscosity (Park et al., 1995). Because higher melt viscosity leads to greater pressure drop rate, resulting in more nucleated cells, the highest cell number density is expected with melt A because it has the highest viscosity. Once nucleation occurs, dissolved gas in the SCFX melt can diffuse into nuclei, resulting in cell growth, or diffuse out from the extrudate surface into the environment. Theoretically, nucleation is a one-step process. However, in reality, the pressure drop rate in the die does not occur instantly (Park, Baldwin, & Suh, 1995; Winoto, 2005). Higher melt viscosity reduces diffusion rates, resulting in more dissolved gas in melt.



SCFX extrudate C2

Figure 1.2. Schematic of microstructure formation in three melts with different viscosities

This leads to further nucleation, rather than cell growth or gas loss. The smaller cell size can be observed in melt A compared to melt C (Chen et al., 2006). During the cell growth stage, higher melt viscosity provides more resistance to cell wall extension, resulting in smaller cell size. Melt viscosity also affects the integrity of cells in the finished products. When the pressure inside exceeds the maximum melt strength, cell rupture occurs, resulting in extreme gas loss from cells. Melt A shows less gas loss and cell collapse compared to melt B. The low viscosity of melt C leads to either severe structure collapse (SCFX extrudate C1) or further expansion (SCFX extrudate C2), depending on post-extrusion drying conditions. Cell coalescence can also occur when melt strength is very low (Trater, Alavi, & Rizvi, 2005). Research focused on the effects of protein on the melt rheology would be useful for controlling the microstructure formation and textural properties of SCFX extrudates.

Recently, the current researcher observed that it takes approximately 3 to 20 seconds to attain maximum expansion in SCFX processes, compared to less than 0.5 seconds in SBX processes (Arhaliass, A., Bouvier, J. M., & Legrand, J., 2003). As shown in Figure 1.2, the time-dependent expansion can be explained by melt rheology. This unique time-delayed expansion behavior of SCFX processing allows researchers to utilize emerging technologies, such as convective microwave drying, to generate newly expanded products with novel textural properties.

5. 3D Microstructure and Mechanical Properties of Expanded Foams

Traditionally, researchers have examined the product density of expanded foams as a predictor of their mechanical properties due to the simplicity of measurement and the reasonable correlation with the mechanical properties. Gibson and Ashby's model (1997), which relates the relative mechanical properties (i.e., foam compressive modulus to unfoamed material compressive modulus) to the relative density (foam density to material density), can predict the mechanical properties of solid food foams, such as starch-based extrudates and bread (Liu & Scanlon, 2003). However, Gibson and Ashby's model has limitations, given that the extrudate microstructure also affects the mechanical properties of finished products (Cheng, Alavi, Pearson & Agbisit, 2007; Liu et al., 2003; Warburton, Donald, & Smith, 1992).

Figure 1.3 shows the possible effects of microstructure on the mechanical properties of three extrudates. First, the microstructural attributes of cellular solid foams with the same density can vary. Foam A and foam B have the same porosity and density, but different cell sizes. Based on Gibson and Ashby's (1997) model, the two foams should have the same mechanical properties.



Figure 1.3. Schematic of expanded foams with the same expansion ratio, but different microstructures

However, Barrett and Peleg (1992) showed that the breaking and plateau stresses of corn grits extrudates were inversely related to average cell size. The general finding is that the modulus of deformation and breaking stress of expanded foams decrease as

cell size increases. On the other hand, there are reports that cell size alone is not a good predictor of the mechanical properties (Gogoi, Alavi, & Rizvi, 2000). As shown in Figure 1.3, foam B and foam C have the same cell size, but different densities due to the differences in porosity. Foam B is expected to show a harder and more brittle texture than foam C due to its higher density. Gogoi et al. (2000) suggested that most mechanical properties of solid polymeric foams depend only weakly on cell size, whereas the struts of the cells (i.e., cell wall) act as load-bearing beams. This suggests that cell thickness governs the foam's mechanical properties.

Moreover, many cellular solids, including starch-based extrudates and bread crumbs, are anisotropic in nature. Anisotropy can be observed either in cell shape or in cell size distribution. Several researchers have reported that structural anisotropy affects the resultant mechanical properties of solid foams (Gibson et al. 1997; Warburton et al. 1992). Therefore, it can be concluded that the mechanical properties of cellular foams are governed jointly by their cell wall material properties and the cellular structure that can be characterized as the ratio of open to closed cells, average cell size and cell size distribution, cell wall thickness and shape, uniformity of the structure, and presence of skin or crust (Peleg 1997). Improved knowledge of the microstructure-mechanical property relationship would be beneficial for tailoring the textural properties of SC-CO₂ expanded products. In addition, in order to investigate the effect of microstructure on the mechanical properties of expanded foams, an accurate microstructural analysis tool is required.

A novel technology for non-invasive imaging of the 3D microstructure of cellular foams, X-ray microtomography was successfully utilized to evaluate the microstructures of expanded snack foods (Trater et al. 2005). X-ray microtomography overcomes the limitations of traditional 2D imaging techniques, like SEM and optical microscopy, and provides several accurate microstructural features, such as average

cell equivalent diameter, cell wall thickness, and void fraction (Trater et al., 2005). Figure 1.4 illustrates the advantages of X-ray microtomography over the SEM technique. The 2D images are destructive in nature and do not provide accurate information about cell size distribution because cells are generally sliced off-center. Further, conventional 2D images do not allow imaging of the same specimen at different depths. Drawbacks of the conventional imaging technologies can be eliminated by using non-invasive techniques that generate 3D maps of the internal structure of small samples with micrometer resolution (Agbisit et al., 2007).



2D microstructure by SEM

Figure 1.4. Comparison between 3D X-ray microtomography and 2D SEM images

Recently, X-ray microtomography was found to be useful when examining the correlation between the 3D microstructure and mechanical properties of cellular solid foods (Agbisit et al., 2007; Babin, Della Valle, Dendievel & Lourdin, 2007). 3D image analyses, including X-ray microtomography, provide a better understanding of the relationship between the mechanical properties and the microstructure of expanded SCFX extrudates than 2D image analyses (Trater et al., 2005).

6. Resistant Starch Formation during Extrusion Processing

Resistant starch (RS) is defined as the portion of starch that passes into the colon because it is not digested in the small intestine (Unlu & Faller, 1998). The results of a number of studies indicate that resistant starch has health benefits (Sajilata et al., 2006). Resistant starch reduces plasma glucose and insulin levels in the same manner as dietary fiber (Yue, Rayas-Duarte, & Elias, 1999). Resistant starch is generally classified into four categories: physically inaccessible starch (RS1), resistant starch granules (RS2), retrograded starch (RS3), and modified starch (RS4) (Sajilata et al. 2006; Unlu & Faller, 1998). In general, RS3 formation can be manipulated by controlling the factors which affect starch retrogradation, including the source of the starch, amylose to amylopectin ratio, polymer chain length, moisture content (or starch concentration), cooking and cooling temperature regime, storage temperature and duration, pH, and presence of additives, such as salts, sugars, and lipids (Sajilata et al., 2006).

Extrusion processing destroys RS1 and RS2, but can lead to RS3 formation (Unulu et al., 1998). Therefore, an effective approach to increasing the RS level in starch-based products is an optimized extrusion process. However, the amount of resistant starch generated by steam-based extrusion (SBX) is often limited, most likely due to its low in-barrel moisture content. Starch retrogradation is accelerated when the

starch concentration is approximately 50-60 wt% (Longton & LeGrys 1981). Minimum retrogradation is observed above 90 wt% starch concentration due to the reduced mobility of starch molecules. In addition, retrogradation is inhibited at lower than 10 wt% starch concentrations due to the dilution effect (Longton et al., 1981). It is possible to increase resistant starch content of SBX extrudates by increasing the inbarrel moisture content. However, a higher in-barrel moisture content is detrimental to the expansion of SBX extrudates, leading to denser products.

It is well-established that crystallization, including starch retrogradation and resistant starch formation, is a temperature-bound process (Figure 1.5). Nucleation is favored at temperatures far below the melting temperatures (Tm) of crystals, but above the glass transition temperature (Tg). However, propagation is limited under these conditions (Eerlingen et al., 1993). At temperatures far above Tg but below Tm, propagation is favored, whereas nucleation is limited. The overall crystallization rate primarily depends on the nucleation and propagation rates.



Figure 1.5. Effect of storage temperature on the crystallization rate of starch

It is generally at its maximum at the average temperature of Tg and Tm. Eerlingen et al. (1993) reported that the highest resistant starch concentration was initially obtained at 0°C. However, for long storage times, the highest yield of resistant starch was obtained at 100°C. Eerlingen et al. (1993) claimed that the propagation of amylose crystals was favored at 100°C, even though the nucleation rate was rather limited, resulting in more RS starch generation. Yue et al. (1999) found that drying pasta at high temperature causes increased resistant starch content in the final product. They compared three different drying conditions: LT cycle (18 hours at 25~40°C), HT cycle (12 hours at 30~70°C), and UHT cycle (6.5 hours at 30~80°C). After drying, the resistant starch contents were 1.68, 2.27, and 2.51 for the LT, HT, and UHT cycle, respectively. They attributed the increase in resistant starch to hydrothermal treatment, namely, annealing during drying. Eerlingen et al. (1993) reported that heating or storage of starchy materials at elevated temperature (~100°C) can lead to A-type rather than B-type polymorphs. A-type crystallites have lower enzymatic digestibility; therefore, they exhibit more RS formation compared to B-type crystallites.

In the SCFX process, high moisture conditions which enhance the mobility of starch molecules during the retrogradation process are beneficial to RS generation in SCFX extrudates. Moreover, the post-extrusion drying process which is necessary to set the structure of SCFX extrudates can be manipulated to increase the RS content. It is likely that SCFX processing is more suitable for the production of highly expanded starch-based foams with relatively high RS contents compared to SBX processing without any loss of textural properties.

7. The Scope of This Study

This work focuses on understanding the role of melt rheology on the expansion and 3D microstructure formation in whey protein added to SCFX

extrudates. The potential of a novel SCFX technology to produce healthy snacks containing high whey protein (> 50 wt%) and expanded food products containing increased resistant starch content is reported. The chapters are organized in the following manner:

- Chapter 1: Introduction: Previous findings on SBX and SCFX processes from the literature and the scope of this study are presented.
- Chapter 2: 3D Microstructure of Supercritical Fluid Extrudates I: Melt rheology and microstructure formation: The effects of rheological properties and process conditions on the expansion and 3D microstructure of starch/protein SCFX extrudates were investigated using an online slit die rheometer and X-ray microtomography.
- Chapter 3: 3D Microstructure of Supercritical Fluid Extrudates II: Cell anisotropy and the mechanical properties: 2D and 3D microstructure analysis was utilized to study the effects of the microstructural attributes, including cell anisotropy, on the mechanical properties of SCFX extrudates.
- Chapter 4: The Time-Delayed Expansion Profile of Supercritical Fluid Extrudates: The effects of the ratio of SC-CO₂ to feed and die dimension on SCFX expansion were studied using a visualization technique. Pressure drop rate through the die was calculated, and its effect on expansion behavior in SCFX extrusion was quantified.
- Chapter 5: Resistant Starch Formation in Supercritical Fluid Extrusion: The potential benefits of SCFX for the production of expanded extrudates with higher RS content compared to SBX was evaluated.
- Chapter 6: New Generation of Healthy Snack Food by Supercritical Fluid Extrusion: An effective process for expanded SCFX chips with high nutrient

density was developed. A comparison of SCFX products with commercial snack products was also performed.

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CHAPTER 2

3D MICROSTRUCTRE OF SUPERCRITICAL FLUID EXTRUDATES I: MELT RHEOLOGY AND MICROSTRUCTURE FORMATION

Abstract

The influence of melt rheology and processing conditions on the expansion and 3D microstructure of biopolymeric foams produced by supercritical fluid extrusion (SCFX) were investigated. Starch-based SCFX extrudates with five whey protein isolate (WPI) concentrations (0-18 wt%) and four SC-CO₂ levels (0-0.75 wt%) were produced. Melt rheology was studied with an online slit die rheometer. The 3D microstructure of foams was determined using X-ray microtomography. The starchbased melt showed shear-thinning behavior, with a lower consistency coefficient and higher flow behavior index with the addition of SC-CO₂. Whey protein acted as a diluent, which resulted in reduced melt viscosity. Thermal analysis indicated only limited chemical interaction between whey protein and pregelatinized corn starch. SC-CO₂ increased the expansion of whey protein added starch-based extrudates. However, structural collapse was observed at the 0.75 wt% SC-CO₂ level during post-extrusion drying at 85°C. A cross-sectional expansion ratio of SCFX extrudates decreased by 48.9% with the addition of 18 wt% WPI, indicating a suppression of expansion on whey protein incorporation. The cell number densities per solid volume and average cell size of SCFX extrudates were $1.3 \times 10^3 - 1.9 \times 10^4$ cells/cm³ and 310.0-724.4 µm, respectively, depending on WPI and SC-CO₂ levels. Cell wall thickness had the lowest values at the 0.75 wt% SC-CO₂ level, indicating extreme gas loss from the extrudate surface to the environment. A decrease in melt viscosity due to the addition of whey protein might be responsible for the lower cell number density and related decrease in expansion. Processing parameters and whey protein levels were critical to controlling the microstructure of starch-based SCFX extrudates.

1. Introduction

Extrudate expansion and its microstructure formation during the extrusion process are the consequences of nucleation, die-swell, cell growth, and cell collapse (Moraru & Kokini, 2003). The macro- and microstructure formation of extrudates is governed by the extrusion process parameters and material formulation, as well as the rheological properties of melt. In turn, the extrudate microstructure affects the mechanical properties of finished products (Cheng, Alavi, Pearson, & Agbisit, 2007).

Starch and protein are two major components of cereal-based extrudates. Greater attention has been paid to the role of starch, which is typically the base biopolymer, in the expansion of extruded snacks and breakfast cereals (Moraru et al., 2003). It is well-established that at least 60-70 wt% starch in formulations is required for reasonable expansion to occur (Conway, 1971). Moraru et al. (2003) reported that the type of starch (e.g., cereal, root, and tuber), degree of gelatinization, physical/chemical modification, and amylose/amylopectin ratio are the critical factors for the expansion during starch-based extrusion. However, a number of recent studies have been conducted to investigate the effect of proteins on the expansion characteristics, suggesting an increased interest in nutrient density of extruded products (Onwulata, Smith, Konstance, & Holsinger, 2001).

The general finding is that the addition of protein, especially high protein concentration ($> \sim 15 \text{ wt\%}$), decreases the expansion, resulting in an undesirable hard texture (Matthey & Hanna, 1997). The detrimental effect of proteins has been attributed to their heat-sensitive thermosetting property, interaction with starch, and limited hydration during conventional steam-based extrusion, which involves high

temperatures (>120°C) and short residence time (Onwulata et al., 2001). On the other hand, the results of several studies show that the protein incorporation at low concentrations (3-15 wt%) increase the expansion of the protein-fortified extrudates (Cheng et al. 2007; Fernandez-Gutierreza, Martín-Martínezb, Martínez-Bustosc, & Cruz-Oread, 2004). The contradictory results reported by different researchers might be related to melt rheology modification due to the complex starch/protein interaction during steam-based extrusion using different process conditions. Although there have been a number of studies examining the role of melt rheology during starch-based extrusion using online or off-line rheometers, there is lack of knowledge on the effect of protein addition on the melt rheology and the expansion characteristics (Bhattacharya & Padmanabhan, 1992). Moreover, the intermolecular interactions between the two biopolymers might be limited during extrusion processes at low temperatures (<100°C), such as supercritical fluid extrusion (SCFX).

SCFX has been used for the production of highly expanded biopolymeric foams in which supercritical carbon dioxide (SC-CO₂) is used as a blowing agent (Rizvi, Mulvaney, & Sokhey, 1995). A higher moisture content (30~45 wt%) in the extruder barrel is utilized to keep the product temperature low and to maximize SC-CO₂ solubilization in the melt. Because the SCFX process is conducted at low shear conditions and temperatures lower than 100°C, it enables the use of temperature- and shear-sensitive ingredients in product formulations (Winoto, 2005). The SCFX process for starch-based foams consists of the following major steps: (1) development of a melt with gas-holding rheological properties by the gelatinization and mixing of the feed in the extruder barrel, (2) injection of SC-CO₂ into the melt and mixing in the extruder barrel to create a melt-CO₂ solution, (3) pressure drop in the die leading to a thermodynamic instability and consequent nucleation of cells, and (4) expansion caused by diffusion of CO₂ into the nucleated cells as the extruder proceeds through

the die and immediately after its exit from the die. In the final stage, further diffusion of CO₂ into the cells and their consequent expansion cause the cell walls to progressively decrease in thickness and increase the rate at which CO₂ diffuses out of the surface of extrudates into the environment (Rizvi et al., 1995). In the SCFX process, the subtle phase change of injected CO₂ from supercritical phase to gas phase is exploited to obtain precisely controlled homogenous microcellular structures and unique product textures. A greater thermodynamic instability due to high pressure drop rate in the die results in high cell density, ranging from 2.3 x 10^6 to 6.8 x 10^6 cells/cm³ (Alavi, Gogoi, Khan, Bowman, & Rizvi, 1999). The SCFX process allows researchers to control not only the average cell size of extrudates which may range from about 50 to 250 micrometers with high polydispersity index of ~ 0.95 , but also the volumetric expansion of final products by manipulating process parameters, such as die dimension and SC-CO₂ residence time (Winoto, 2005). Alavi et al. (1999) found that the incorporation of thermosetting proteins, such as egg white and whey protein, increased the overall expansion of SCFX extrudates by reducing structure collapse due to protein gelatin. Additional studies examining the melt rheology of starch/protein mixtures would be beneficial the understanding of the role of protein in SCFX expansion and microstructure formation.

A novel technology for non-invasive imaging of the 3D microstructure of cellular foams, X-ray microtomography was successfully utilized to evaluate the microstructures of expanded snack foods (Trater, Alavi, & Rizvi, 2005). X-ray microtomography overcomes the limitations of traditional 2D imaging techniques, such as SEM and optical microscopy, and provides several accurate microstructural features, such as average cell diameter, cell wall thickness, and void fraction (Trater et al., 2005).

The purpose of this research was to examine the effects of rheological properties and process conditions on the expansion and 3D microstructure of starch/protein SCFX extrudates. In this study, we used blends of pregelatinized corn starch and whey protein isolate (WPI) as the model system to characterize the rheology of a starch-WPI-water-CO₂ mixture using an online slit die rheometer. The determined rheological properties were correlated with the expansion characteristics and 3D microstructural attributes to provide a better understanding on SCFX microstructure formation.

2. Materials and Methods

2.1. Materials

Pre-gelatinized corn starch and whey protein isolate (Bipro®) were purchased from Cargill, Inc. (Minneapolis, MN) and Davisco Food International, Inc. (Eden Prairie, MN), respectively. Five formulations with different WPI concentrations (0, 3, 6, 12, and 18 wt%) were prepared by mixing the two dry ingredients proportionally in a premixer for two hours.

2.2. SCFX extrusion and rheology measurement

Whey protein added starch-based SCFX extrudates were produced using a Wenger TX-57 twin screw extruder (Wenger Manufacturing, Sabetha, KS) with 4.5 heads, a barrel diameter of 52 mm, and an L/D ratio of 28.5 configured for supercritical fluid extrusion. SC-CO₂ was injected into the melt through four valves located around the extruder barrel at L/D = 24. The SC-CO₂ levels used were 0, 0.25, 0.5, and 0.75 wt%. During SCFX processing, the pressure in the extruder barrel prior to the die entrance was increased to 10.7 MPa for all experiments. The screw rotation

speed and dry feed rate were 120 rpm and 35 kg/hr, respectively. In-barrel moisture content was 43.8 wt% (w.b.).

The slit die rheometer used in this study had length (L) = 325 mm, width (W) = 50.8 mm, and height (H) = 3.175 mm. Two calibrated pressure transducers were inserted at two ports along the slit die rheometer at a distance of 200 and 300 mm from the die entrance. The drop in pressure between the two pressure transducers was recorded every two seconds. A bypass line with a valve was utilized to vary the rate of the main flow as was done in Winoto (2005). For each set of extrusion treatments, three to four different flow rates of the extrudate were used. The flow rates of the extrudate were measured by weighing the material outflow during a 20-second time interval at steady state extrusion conditions. The pressure and mass flow rate data were used to calculate shear stress and shear rate in the slit die rheometer and to subsequently obtain the melt apparent viscosity. The shear stress at the walls of the slit die was calculated from the pressure drop data across the two pressure transducers using the following equation (Chen & Rizvi, 2006; Winoto, 2005):

$$\tau_w = \frac{\Delta P H}{2\Delta L} \tag{1}$$

where, τ_w = shear stress (Pa) at the walls, ΔP = pressure drop (Pa), H = slit die height (m, 3.175 x 10⁻³ m in this study), and ΔL = distance between the two pressure transducers (m, 3.25 x 10⁻¹ m in this study).

The apparent shear rate was calculated from the mass flow rate and the melt density using the following equation:

$$\dot{\gamma}_{app} = \frac{6}{WH^2} \frac{\dot{m}}{\rho} \tag{2}$$

where, $\dot{\gamma}_{app}$ = apparent shear rate (1/s), W = slit die width (m, 5.08 x 10⁻² m in this study), \dot{m} = mass flow rate (kg/s), and ρ = melt density (kg/m³, 1250 kg/m³ in this study).

The density of the melt without $SC-CO_2$ injected was estimated from the density of its components based on literature values (Winoto 2005). When the melt was injected with $SC-CO_2$, this density value was adjusted by taking into account the density of CO_2 at the recorded pressures in the slit die.

A double log plot of shear stress versus shear rate showed a linear relationship with the slope of the regression line equal to n and the intercept equal to log K', according to the following power law model:

$$\tau_w = K' (\dot{\gamma}_{app})^n \tag{3}$$

where K' is the uncorrected consistency coefficient and n is the flow behavior index.

The wall shear rate ($\dot{\gamma}_w$,1/s) can be calculated by applying the Rabinowitsch correction for a slit die (Winoto 2005):

$$\dot{\gamma}_{w} = \frac{2n+1}{3n} \dot{\gamma}_{app} \tag{4}$$

The real consistency coefficient, K (Pa.sⁿ), was obtained using the following equation (Hicsasmaz, Dogan, Chu, & Rizvi, 2003):

$$K = K' \left(\frac{3n}{2n+1}\right)^n \tag{5}$$

Finally, the apparent viscosity (η , Pa.s) was calculated using the following equation:

$$\eta = K(\dot{\gamma}_w)^{n-1} \tag{6}$$

Product temperature at die exit was maintained at approximately 55°C for all experiments. Two identical cylindrical dies with 4.2 mm diameters were attached at the end of the slit die rheometer. The emerging extrudates from the die were collected on metal trays and dried at 85°C in a convection oven to obtain 5-6 wt% moisture content for further study.

2.3. Viscosity reduction factors calculation

To quantitatively determine the effects of $SC-CO_2$ and WPI added on the melt viscosity, the viscosity reduction factor (VRF) was calculated using the following equation (Chen et al. 2006):

The apparent viscosity of SCFX melt at 130 s⁻¹ was used for comparison. In addition, 0 wt% WPI (pregelatinized corn starch only) melt with no SC-CO₂ injection was used as reference.

2.4. Thermal analysis

The melting peak and glass transition temperature (Tg) of the extrudates were determined using a TA 2920 modulated differential scanning calorimeter (MDSC, TA instruments, NJ) with the procedures described by De-Meuter, Rahier & Van Mele (1999) and Shuck et al. (2005): Specifically, the test conditions included 10° C/min heating rate, $\pm 1^{\circ}$ C amplitude, 60-second period, and $10-100^{\circ}$ C scan range. An empty aluminum pan was used as the reference. Ten mg of ground extrudates were used for Tg determination. For melting peak evaluation, 5 mg of samples with 5 mg of distilled

water was used. The melting peak of the mixture of pregelatinized corn starch and WPI was also determined for comparison. Samples were hermetically sealed using a sample encapsulating press.

2.5. Expansion and physical property characterization

The cross-sectional expansion index (SEI) was calculated as the crosssectional area of the extrudate divided by the cross-sectional area of the die (Chen et al. 2006). The diameter of the extrudate was measured using a digital vernier caliper for use in the cross-sectional area calculation. Each value was an average of 30 readings.

Piece density (PD, kg/m³), defined as the ratio of the weight of the sample to its total volume including the voids, was measured using the geometrical method (Winoto, 2005). The volume of the extrudate was calculated by multiplying the crosssectional area and the length, assuming that the extrudate is a straight cylinder.

The volumetric expansion index (VEI) was calculated by taking the ratio of unexpanded extrudate piece density over expanded extrudate piece density using the following equation (Winoto 2005).

$$VEI = \frac{\sigma_u}{\sigma_p} \tag{8}$$

where σ_p = the piece density of the expanded sample (kg/m³) and σ_u = the density of the unexpanded material (1405 kg/m³ in this study).

Defined as the ratio of pore volume to that of the total volume of the sample, the void fraction (V_p) was calculated using the following equation (Alavi et al. 1999):

$$V_p = 1 - \frac{\sigma_p}{\sigma_u} \tag{9}$$

2.6. 3D image analysis

A desktop X-ray microtomography imaging system (Model 1072, 20-100 kV/0-250 µA, SkyScan, Aartselaar, Belgium) set at 40 kV/100 µA was used to scan the samples (Trater et al., 2005). A CCD camera was used to collect the X-ray data. Image reconstruction software was provided by Skyscan. For each treatment, a set of 2D images for the entire sample was obtained after reconstruction. This set was partitioned into a volume of interest (VOI), which consisted of 15 consecutive 2D slices separated by a constant distance. One-quarter of the cross-sectional area of each slice was utilized for image analysis. Calculations of microstructural parameters were based on measurements of cell perimeter, void, and solid areas obtained for each slice using image analysis software (Scion Image for Windows[®], Scion Corp., Frederick, MD) with a threshold value of 35. Computations for each microstructure parameter were provided by Trater et al. (2005). The measurements included average cell diameter (CD), cell wall thickness (CWT), image void fraction (Vi), polydispersity index (PDI), cell density per unit total extrudate volume (CNt), and cell number density per unit solid volume (CNs).

2.7. Statistical analysis

An analysis of variance (ANOVA) was conducted using the MINITAB statistical program (Minitab, Inc.). Unless otherwise indicated, all reported differences were statistically significant at $\alpha = 0.05$.

3. Results and Discussion

3.1. Flow behavior properties of SCFX melt

Table 2.1 shows the consistency index (K) and the flow behavior index (n) of starch/WPI/water/CO₂ mixtures determined in the shear rate range of 50 to 150 s⁻¹,

assuming that the yield stress was negligible. The consistency index decreased when the ratio of SC-CO₂ to feed was increased for all formulations possibly due to the dilution effect of SC-CO₂. This trend of K value is well-supported by Chen et al. (2006), Winoto (2005), and Hicsasmaz et al. (2003). Willett, Jasberg, & Swanson (1999) also showed that the K values of thermoplastic starch melts decreased with increased moisture content and the addition of other plasticizer-like low molecular weight additives.

WPI	SC-CO ₂	V	N	P ²
(wt%)	(wt%)	N	1N	К
	0.00	9690	0.30	0.88
0	0.25	7648	0.34	0.95
0	0.50	7207	0.33	0.91
	0.75	6061	0.36	0.78
	0.00	9957	0.28	0.85
2	0.25	8377	0.31	0.86
3	0.50	7297	0.33	0.94
	0.75	6960	0.33	0.88
	0.00	9812	0.28	0.92
6	0.25	6944	0.35	0.83
0	0.50	6382	0.35	0.79
	0.75	6100	0.35	0.91
	0.00	7762	0.32	0.83
10	0.25	7472	0.32	0.88
12	0.50	5796	0.35	0.97
	0.75	5939	0.34	0.87
	0.00	6633	0.33	0.83
10	0.25	5294	0.36	0.89
18	0.50	5100	0.36	0.95
	0.75	4842	0.36	0.94

Table 2.1. Flow behavior properties of SCFX melt

The flow behavior index increased with the addition of SC-CO₂ (Table 2.1). A higher flow behavior index was obtained for SCFX dough compared to the control without

 $SC-CO_2$ (Hicsasmaz et al. 2003). They reported that the gas phase present in SCFX-leavened dough deformed easily and acted like an energy absorber, compensating for the shear-thinning effect compared to the control dough.

Lee, Tzoganakis & Park (1998) also showed that the flow behavior index of polyethylene/polystyrene/CO₂ blends increased from 0.29 to 0.34 when the CO₂ concentration was increased from 0 to 2%. Chen et al. (2006) suggested that the presence of plasticizers affected polymer entanglement density in the mixture. As the amount of plasticizers increased the diluting the system, polymer entanglement density decreased, leading to larger n value and subsequently decreased shear thinning behavior. Della Valle, Colonna, Patria, & Vergnes (1997) reported that a decrease in the n value occurred with an increased amylose content in the formulation. They also suggested that linear amylose would more likely contribute to entanglement compared to the highly branched amylopectin. In this study, the lowest n value was observed for all formulations with no SC-CO₂ addition. This suggests that SC-CO₂ acted as a plasticizer by reducing amylose entanglement density. In general, when WPI concentration was increased from 0 to 18 wt%, the K value decreased, whereas the n value did not show such a trend. This finding suggests that whey protein is a less effective diluent compared to SC-CO₂.

3.2. Apparent viscosity of starch-whey protein-water mixtures

The apparent viscosity of the starch-WPI-water mixture was calculated with K and n values respective of each formulation and the corresponding shear rate (Figure 2.1). The apparent viscosity of starch-based SCFX extrudates decreased when additional free volume was added via SC-CO₂ injection. The SC-CO₂/polymer mixtures are generally regarded as the polymer diluted by a solvent (Chen et al. 2006).



Figure 2.1. Apparent viscosity of SCFX melt (Top: 0 wt% WPI, bottom: 18 wt% WPI)

Therefore, the viscosities of the SC-CO₂/polymer mixtures decrease due to the dilution of the concentration of entangled polymer chains and the increase in mixture free volume due to dissolved SC-CO₂. The differences in apparent viscosity between the SC-CO₂ injected melt and the control melt were more pronounced in the low shear rate region. On the contrary, the apparent viscosities were similar at high shear rates (Figure 2.1). The apparent viscosity of SCFX melt was decreased further when WPI concentration in the melt was increased.

When two biopolymers with different viscosities form a composite, the resultant viscosity can either increase or decrease depending on the original viscosities of the biopolymers and their chemical interaction. First, the viscosity reduction due to WPI addition in this study can be explained by the lower viscosity of whey protein compared to pregelatinized starch and the interaction between whey protein and starch. In dilute systems (i.e., lower than 10 wt% solid content), starch generally exhibits higher viscosity than whey protein in the solutions at the same concentration and shear rate. This is possibly due to the higher molecular weight of starch and the linear molecular shape of amylose compared to relatively smaller globular whey proteins. Resch, Daubert, & Foegeding (2004) reported that the derivatized whey protein solution showed approximately 200-fold lower apparent viscosity than the pregelatinized starch solution at the same concentration and shear rate. Sopade, Hardin, Fitzpatrick, Desmee, & Halley (2006) demonstrated that the peak viscosity of 10 wt% wheat starch/whey protein mixtures at a pH of 7.0 decreased seven-fold when they varied the whey protein-to-starch ratio from 0 to 1. In concentrated starch/whey protein systems (40-80 wt% solid content) like a melt in the extrusion process, the interaction between starch and protein might play a more important role in the rheology of the starch/protein mixture.

Starch/protein interactions can involve their direct chemical complexation and the competition in water affinity between starch and protein. Chemical complex formation can occur in two ways. First, anionic group in starch, such as the phosphate group in potato amylopectin molecules, can bind with proteins due to electrostatic forces (Zaleska, Ring, & Tomasik, 2001). Second, covalent bonding, including the Maillard reaction, might be responsible for starch/protein complexation. Several researchers have reported that the whey protein-starch interaction is responsible for the decrease in the expansion of steam-based extrudates. Matthey et al. (1997) attributed amylose-whey protein complexation to the decrease in the apparent amylose content resulting in reduced expansion. Allen, Carpenter, & Walsh. (2007) asserted that the total protein content (i.e., the percentage of protein content that was resoluble after extrusion) decreased due to possible amylose-whey protein complexation when the whey protein concentration was increased from 16 to 40%. Allen et al. (2007) claimed that there was a significant degree of covalent bonding between whey protein and starch at extrusion temperatures between 158°C and 170°C. On the other hand, the results of a number of studies demonstrated the thermodynamic incompatibility between starch and protein, resulting in phase separation during the extrusion process (Muhrbeck and Eliasson 1991; Tolstoguzov 1993). In this study, the glass transition temperatures of SCFX extrudates were measured to evaluate the degree of compatibility between starch and whey protein. Two separate glass transition temperatures of whey protein and starch in 18 wt% WPI added SCFX extrudates were determined (Figure 2.2). Results indicate their thermodynamic incompatibility and limited chemical complexation between starch and whey protein during the SCFX process. The determined glass transition temperatures of starch and whey protein were comparable to values reported in the literature (Schuck et al. 2005; Zeleznak, & Hoseney, 1987). Two different glass transition temperatures were detected when there

was no interaction between the two polymers (Mousia, Farhat, Blachot, & Mitchell, 2000). If starch and protein form a complex, a single glass transition temperature should appear in the middle of the original two-glass transition temperatures of the two polymers (Zhong & Sun, 2001).



Figure 2.2. Glass transition temperatures (Tg) of SCFX extrudates

Proteins also possess the ability to affect water distribution in the matrix (Moraru et al., 2003). Both starch and protein are hygroscopic and require water to undergo structural changes (i.e., gelatinization and denaturation, respectively). Therefore, competition exists between starch gelatinization and protein denaturation due to limited water in a concentrated system, such as a melt. For example, Wulansari, Mitchell, & Blanshard (1999) reported that the gelatinization of waxy corn starch was reduced by the presence of gelatin during extrusion. Muhrbeck et al. (1991) showed

that a system with two continuous networks was formed when a starch network was formed prior to bovine albumin protein gel formation.

On the other hand, when a protein network was formed first, rather than forming a continuous network, starch acted as a filler. Therefore, the structure and the resultant rheology of the starch/protein binary system was strongly dependent on the process conditions, including temperature, shear force, pH, and ionic strength (Muhrbeck et al. 1991). In this study, the mixtures of pregelatinized corn starch and WPI were extruded at 55°C, which is lower than the typical denaturation temperature of whey proteins, such as beta-lactoglobulin and alpha-lactalbumin. Therefore, pregelatinized starch readily formed a continuous matrix, whereas the gelation of whey protein was limited.



Figure 2.3. Thermograms of the unextruded material (pregelatinized corn starch : native WPI = 82 : 18) and 18 wt% WPI added SCFX extrudates

Figure 2.3 shows the thermograms of the blend of pregelatinized corn starch and native WPI, as well as 18 wt% WPI SCFX extrudate. A large portion of the whey protein in SCFX extrudate was still in the undenatured or partially denatured form following SCFX extrusion. In addition to the low extrusion temperature of the SCFX extrusion process, limited moisture in the melt might inhibit whey protein denaturation due to the strong moisture affinity of pregelatinized corn starch. Therefore, in this study, it was reasonable to conclude that pregelatinized corn starch, which has higher viscosity than whey protein, was primarily responsible for forming a continuous matrix, whereas whey protein acted as a filler reducing the melt viscosity.

To investigate the viscosity reduction effects of SC-CO₂ and whey protein quantitatively, viscosity reduction factors (VRF) at a shear rate of 130 s⁻¹ were calculated (Figure 2.4).



Figure 2.4. Viscosity reduction factor of SCFX extrudates

This approach was successfully utilized to quantify the plasticizing effect of SC-CO₂ on the melts of biopolymers and synthetic petrochemical polymers (Chen et al., 2006). The apparent viscosity of the 0 wt% WPI sample decreased by 16% with the addition of 0.75 wt% SC-CO₂. On the other hand, a 23% viscosity reduction at the 0 wt% SC-CO₂ level was observed when WPI concentration was increased from 0 to 18 wt%. These results suggest that both SC-CO₂ and WPI act as diluents by reducing melt viscosity. However, SC-CO₂ is a more effective plasticizer.

3.3. Expansion characteristics of SCFX extrudates and melt rheology

Figure 2.5 illustrates the cross-sectional expansion index (SEI) of SCFX extrudates with respect to the ratio of SC-CO₂ to feed and WPI levels. The SEI increased when SC-CO₂ was injected up to the 0.5 wt% SC-CO₂ level for all formulations, whereas significant structure collapse was observed at a ration of 0.75 wt% SC-CO₂ to feed. Further, 0 wt% WPI SCFX extrudates, which showed the highest viscosity and die swell, exhibited the greatest level of expansion, indicating that higher viscosity and elasticity favored higher SEI in the SCFX process. The volumetric expansion index (VEI) showed the same trend as SEI, except that the degree of structure collapse at the 0.75 wt% SC-CO₂ level decreased. This highlights the role of longitudinal expansion on overall expansion (Figure 2.6).

The effects of melt viscosity on SCFX expansion might be fourfold. First, melt shear viscosity governs the nucleation in the die. It is well-established that both the extent and the rate of nucleation depend on the degree of thermodynamic instability caused by the rapid pressure drop (Winoto, 2005). The nucleation rate is a function of melt shear viscosity when the die dimension and volumetric melt flow rate are kept constant. Second, melt viscosity determines the gas-holding capacity of melt (Chen et al., 2006).



Figure 2.5. Cross-sectional expansion of SCFX extrudates



Figure 2.6. Volumetric expansion of SCFX extrudates

Once nucleation occurs, dissolved gas in SCFX melt can diffuse into nuclei, resulting in cell growth, or diffuse out from the extrudate surface into the environment. The diffusion rates in both directions would be dependent on melt viscosity. Higher melt viscosity reduces diffusion rates from the matrix to the nucleated cells or environment. This results in more dissolved gas in the melt, leading to further nucleation, rather than cell growth or gas loss. As a result, a smaller cell size can be observed (Chen et al., 2006). Therefore, the lower melt viscosity of the WPI added extrudates can be attributed to their lower SEI and VEI. It was observed that 0.75 wt% ratio of SC-CO₂ to feed for all formulations exceeded the melt gas holding capacity. This resulted in severe gas loss and significant structure collapse. Third, melt viscosity resists cell growth (Moraru et al., 2003). As dissolved gas in melt diffuses into nuclei, cell growth occurs. This growth leads to the extension of cell wall material. Higher melt viscosity provides greater resistance to cell wall extension, resulting in smaller cell size. In this case, the extensional or elongation viscosity plays a more important role than the shear viscosity; however, those two viscosities of starch-based melt are positively correlated (Moraru et al., 2003). Finally, melt viscosity influences the integrity of cells during the extrusion process (Chen et al., 2006). During the cell growth stage, cells grow until either gas depletion in melt occurs due to cell growth and gas loss or the pressure inside the cell exceeds what the cell wall material can withstand. When the pressure inside exceeds the maximum melt strength, cell rupture occurs, resulting in severe gas loss of cells. Cell coalescence also tends to occur when cell number density is very high and/or melt strength is very low (Trater et al., 2005). Higher viscosity of 0 wt% WPI SCFX extrudates can provide higher melt strength, leading to reduced structure collapse during SCFX extrusion and post-extrusion drying.

The effect of melt elasticity on SCFX expansion can be inferred from die swell phenomena in this study. The degree of die swell can be defined as SEI at a ratio

of 0 wt% ratio of SC-CO₂ to feed. Although the degree of die swell of the SCFX extrudates did not differ significantly (p>0.05), 0 wt% WPI SCFX extrudates with the highest die swell also had the highest cross-sectional expansion (Figure 2.5). It is wellestablished that higher melt elasticity favors higher die swell and cross-sectional expansion (Moraru et al., 2003). Researchers have investigated the elastic property of starch/protein gel using universal texture analyzers and rheometers. However, the results vary greatly depending on the types of starch and protein, formulations, gel preparation conditions, and analysis methods (Muhrbeck et al., 1991). In general, heatinduced pure protein gel possesses a higher degree of elasticity than pure starch gel, whereas the mixture of two biopolymers shows lower elasticity compared to original pure gels. This indicates the importance of the microstructure of mixed gel (Muhrbeck et al., 1991). In this study, undenatured or partially denatured whey protein due to low process temperature (55° C) acted as a diluent leading to lower elasticity inferred by the lower extent of die swell. VEI showed the same trend as SEI, implying that melt elasticity also affects overall expansion (Figure 2.6). Future research examining the role of melt elasticity on SCFX extrusion is warranted.

In summary, the addition of WPI suppressed the cross-sectional and volumetric expansion of SCFX extrudates. On the other hand, Alavi et al. (2003) reported that thermosetting additives, such as whey protein concentrate (WPC-34), can be used to increase the yield stress of the starch/moisture matrix to reduce the driving force for cell collapse in SCFX. In steam-based extrusion, most researchers reported a significant decrease in expansion due to whey protein addition, whereas Cheng et al. (2007) found that whey protein led to increased final expansion. These results suggested that both the source of the protein (e.g., whey, soy, or wheat) and the difference in the protein manufacturing methods are critical for controlling the expansion of whey protein-fortified extrudates. Kinsela (1995) reported that both the

composition and the degree of protein denaturation of WPI and WPC might vary depending on manufacturing methods.

3.4. 3D microstructure of SCFX extrudates

Figure 2.7 depicts typical cross-sections obtained by X-ray microtomography. Further, Table 2.2. shows 3D imaging measurements of microstructural parameters, obtained from integrating 2D data over the VOI. Coefficients of correlation between various parameters are listed in Tables 2.3 (at a constant ratio of SC-CO₂ to feed, 0.25 wt% SC-CO₂ level) and 2.4 (for the same formulation, 6 wt% WPI).

Based on 3D X-ray microtomography analysis, average cell diameter (CD) increased when the WPI concentration was increased from 0 to 18 wt% for each ratio of SC-CO₂ to feed, whereas cell number density decreased, except for 18 wt% WPI extrudate at a 0.75 wt% ratio of SC-CO₂ to feed (Table 2.2). A negative correlation between WPI (whey protein concentration) and η (apparent viscosity at shear rate of 130 s⁻¹) confirmed that whey protein reduced melt viscosity (Table 2.3). There was also a strong negative correlation found between η and CD, indicating that reduced resistance to cell growth led to larger cell size at a constant SC-CO₂ level (Table 2.3). On the other hand, Table 2.3 shows that η correlated positively with cell number density (CNt and CNs) and expansion characteristics including SEI, Vi (image void fraction), and Vp (physical void fraction) at a constant ratio of SC-CO₂ to feed. These findings indicate that higher melt viscosity results in higher cell density due to a higher pressure drop rate in the die, leading to higher expansion despite smaller cell size. Moreover, the lower gas diffusivity in higher viscosity melt, thus higher gasholding capacity enhance nucleation, rather than cell growth as discussed earlier (Chen et al., 2006). Therefore, it can be concluded that whey protein suppresses expansion in SCFX extrusion by reducing cell number density due to its viscosity

reduction effect. In addition, the phase-separated protein disrupts the continuous starch-based matrix during the expansion process. This leads to poor gas-holding properties of the extrudates, thus decreased expansion.



0 wt% WPI 0.75 wt% SC-CO₂

18 wt% WPI 0.75 wt% SC-CO₂

Figure 2.7. Reconstructed X-ray microtomography cross-sectional images of SCFX extrudates

CNs (x10 ⁴ cells/cm ³)	3.2	3.8	4.9	2.6	3.1	4.2	2.3	3.0	3.6	1.4	1.9	2.5	0.6	1.6	6.2
CNt (x10 ³ cells/cm ³)	3.1	3.4	7.0	3.1	1.8	5.0	2.8	2.5	4.5	3.2	2.8	2.7	1.4	2.9	18.7
ICI	0.83	0.76	0.71	0.80	0.78	0.77	0.77	0.79	0.69	0.83	0.82	0.76	0.75	0.73	0.84
Vp	0.82	0.84	0.81	0.78	0.81	0.80	0.75	0.81	0.80	0.70	0.76	0.72	0.56	0.74	0.49
Vi	0.76	0.80	0.78	0.76	0.78	0.76	0.73	0.79	0.75	0.71	0.76	0.75	0.70	0.77	0.66
CWT (µm)	67.4	59.9	61.8	80.6	72.5	71.9	84.1	74.4	78.8	101.0	84.9	86.1	110.8	95.8	80.5
CD (µm)	650.0	643.2	550.2	640.3	641.0	565.4	631.6	650.0	599.0	677.4	682.8	580.6	724.4	703.7	310.0
SC-CO ₂ (wt%)	0.25	0.50	0.75	0.25	0.50	0.75	0.25	0.50	0.75	0.25	0.50	0.75	0.25	0.50	0.75
WPI (wt%)		0			3			9			12			18	

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Table 2.2. 3D morphological parameters of SCFX extrudates

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	Ň	0.0	0.0	0.6	1.0								
0 14110 O	Vi	0.87	0.72	0.46	0.89	1.00							
1M CZ.U 1	CWT	-0.63	-0.30	-0.04	-0.52	-0.86	1.00						
rameters a	9	-0.79	-0.98	-0.38	-0.91	-0.77	0.44	1.00					
various pa	SEI	0.91	0.85	0.46	0.95	0.98	-0.75	-0.89	1.00				
IO SHOULS	۴	0.93	0.94	0.56	0.99	0.88	-0.52	-0.93	0.95	1.00			
2.3. COITE	u	-0.04	-0.43	-0.68	0.48	-0.41	-0.17	0.40	-0.39	-0.34	1.00		
I aDIC	K	0.57	0.81	0.75	0.88	0.79	0.45	-0.79	0.81	0.79	-0.84	1.00	
	WPI	-0.94	-0.87	-0.55	-0.98	-0.96	0.68	0.88	-0.99	-0.98	0.37	-0.81	1.00
		CNs	CNt	ICI	Vp	vi	CWT	8	SEI	۲	đ	K	WPI

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$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	μ	00 0-	-0-Y	0.78	-0.58	-0.91	1.00	0.66	-0.27	1.00			
$\begin{array}{c ccccc} & SC-CO_2 & K \\ \hline CNs & 0.99 & -1.00 \\ CNt & 0.76 & -0.62 \\ PDI & -0.92 & 0.84 \\ Vi & 0.33 & -0.50 \\ Vi & 0.76 & -0.87 \\ Vi & 0.76 & -0.87 \\ CWT & -0.98 & 1.00 \\ CD & -0.84 & 0.72 \\ SEI & -0.53 & 0.36 \\ \eta & -0.96 & 1.00 \\ R & -0.98 & 1.00 \\ R & -0.98 & 1.00 \\ R & -0.98 & 1.00 \\ \end{array}$	u	000	000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00		
SC-CO2 SC-CO2 CNt 0.99 CNt 0.76 PDI -0.92 Vi 0.76 Vi 0.76 CWT -0.92 CWT -0.92 SEI -0.53 η -0.96 n 0.00 K -0.96 O.00 -0.96	×	-100	-0.67	0.84	-0.50	-0.87	1.00	0.72	0.36	1.00	0.00	1.00	
CNs CNt CNt CNt CNT CWT CWT CNT CD CD CD CD CD CD CD CD CD CNS CNS CNS CNS CNS CNS CNS CNS CNS CNS	SC-CO ₂	0000	0.76	-0.92	0.33	0.76	-0.98	-0.84	-0.53	-0.96	0.00	-0.98	1.00
		CNe		ICId	dγ	Vi	CWT	8	SEI	Ľ	đ	ĸ	sc-co ₂

Table 2.4. Correlations of various parameters at 6 wt% WPI concentration

SCFX extrudates exhibited the largest average cell size at a 0.25 or 0.5 wt% ratio of SC-CO₂ to feed, whereas the smallest cell size was obtained at the 0.75 wt% SC-CO₂ level for all formulations (Table 2.2). This might be due to either higher cell number density (CNt and CNs) or enhanced gas loss at the extrudate surface resulting in cell collapse at the 0.75 wt% SC-CO₂ level. A strong positive correlation was found between cell number density (CNt and CNs) and SC-CO₂ level due to the increased number of nucleating sites (Table 2.4). CWT decreased as the ratio of SC-CO₂ levels (Table 2.2). Contrary to the strong positive correlation between apparent viscosity (η) and expansion characteristics at the same SC-CO₂ level, a negative correlation was found between η and Vi and Vp for the same WPI concentration. This is because the viscosity reduction effect of SC-CO₂ is detrimental to generation of higher pressure drop rate generation. However, it provides more nucleating sites and gas molecules for cell growth. These results support the notion that both melt rheology and the amount of blowing agent is critical for controlling the expansion of SCFX extrudates.

A positive correlation was found between CWT and CD. However, negative correlations were found between CWT and SEI, Vi, Vp, CNt and CNs, indicating that lower cell number density leads to a thicker cell wall (Tables 2.3 and 2.4). This finding clearly shows that the combination of CD, CWT, and CN governs the final expansion of SCFX extrudates. The positive correlation between CWT and CD was also observed in steam-based extrudates (Trater et al., 2005). Trater et al. (2005) attributed this finding to significant cell coalescence and/or low cell number density. The highest void fractions determined from 3D image analysis were obtained at the 0.5 wt% SC-CO₂ level for all formulations; the same trend was found for SEI and VEI (Table 2.2). The imaging void fraction (Vi) was consistent with the physical void fraction (Vp).

In general, the polydispersity index (PDI), a measure of the degree of uniformity of cell diameter distribution, decreased when the SC-CO₂ level was increased from 0.25 to 0.75 wt% (Table 2.2 and Figure 2.8).



Figure 2.8. Cell diameter distribution of 0 and 18 wt% WPI SCFX extrudates

The increased heterogeneity of cell size distribution at higher SC-CO₂ levels might be explained by faster nucleation in the die (i.e., premature cell growth). Cells which nucleated earlier continue to grow, resulting in larger cell size in final foams compared to that of cells generated later (Xu & Park, 2003). As a result, less uniform cell size distribution is expected. Han and Han (1988) reported that nucleation occurred earlier in the molding process when the amount of blowing agent was increased. Therefore, a greater number of prematurely generated cells at higher SC-CO₂ levels was responsible for less uniform cell size distribution in this study. This finding also supported the notion that more premature cell generation results in severe gas loss from the extrudate surface into the environment (Xu et al., 2003) (see Figures 2.5 and 2.6). No trend emerged in term of PDI with respect to WPI concentration. However, the PDI of 18 wt% WPI SCFX extrudates at a 0.75 wt% ratio of SC-CO₂ to feed was high (PDI = 0.84). This suggests that both homogenous nucleation and heterogeneous nucleation can occur with 18 wt% WPI SCFX extrudates. As previously discussed, due to their thermodynamic incompatibility, whey protein and starch exist as two separate phases. Moraru et al. (2003) reported that dispersed protein phases in a continuous starch matrix can act as nucleating agents by inducing heterogeneous nucleation.

As a result, the cell number density increases, whereas the average cell size decreases. More uniform cell size distribution is expected with heterogeneous nucleation (Sahagun, Gonza'lez-nuez, & Rodrigue, 2006). It was unclear whether heterogeneous nucleation occurred with a $3\sim12$ wt% WPI concentration and $0.25\sim0.5$ wt% ratios of SC-CO₂ to feed based on PDI results. Sahagun et al. (2006) reported that the interaction between the concentration of the minor phase and the amount of blowing agent influences the type of nucleation in synthetic plastic foam. It was found

that the amount and the size of the dispersed phase have a positive effect on foam nucleation and can be used to control the cell size.

Whey protein can also affect the foaming properties of SCFX expansion and microstructure formation. The behavior of proteins at air-water interfaces can influence foaming properties. Proteins are surface active agents capable of reducing surface tension and enhancing foam stabilization (Kinsella, 1995). Whey proteins can stabilize the air in foams before post-extrusion drying sets the foam structure.

4. Conclusions

Online viscosity measurement revealed that partially denatured whey protein acted as a diluent by reducing melt viscosity. The viscosity reduction factors indicated that the free volume added due to SC-CO₂ is an effective mechanism for viscosity reduction. Lower viscosity of WPI added SCFX melts resulted in lower cell number density and decreased gas-holding capacity, as well as less cross-sectional and volumetric expansion. Although SC-CO₂ had the melt viscosity reduction effect, SCFX expansion increased when SC-CO₂ was injected up to a 0.5 wt% ratio to feed. However, significant structure collapse was observed at the 0.75 wt% SC-CO₂ level due to gas loss from the extrudate surface into the environment. An exceptionally smaller average cell size and a relatively higher polydispersity index of 18 wt% WPI SCFX extrudates at the 0.75 wt% SC-CO₂ level indicated that heterogeneous nucleation can occur depending on the formulation and the amount of blowing agent. Processing parameters and whey protein levels were found to be critical to controlling the microstructure of starch-based SCFX extrudates. Results from this study indicate that X-ray microtomography is a useful tool for determining the microstructure of food foams. X-ray microtomography was able to accurately capture several features of cellular structure, which was not possible with methods employed in the past. This

imaging technology has the potential to promote better understanding of SCFX expansion and microstructure formation.
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CHAPTER 3

3D MICROSTRUCTURE OF SUPERCRITICAL FLUID EXTRUDATES. II: CELL ANISOTROPY AND THE MECHANICAL PROPERTIES

Abstract

The mechanical properties of biopolymeric cellular foams are often governed by their microstructure. 2D and 3D microstructural data of supercritical fluid extrudates were obtained with X-ray microtomography and correlated with the mechanical properties determined using compression and three-point bending tests. Cell size from transverse cross-sections of SCFX extrudates decreased with radial distance from the center. In the longitudinal direction, the cell shapes were more elliptical than spherical and were aligned along the extrusion direction. These findings indicated the presence of a certain degree of anisotropy in SCFX extrudates in both directions. Both piece density and the ratio of cell wall thickness to cell diameter were observed to be good predictors of compressive and flexural mechanical properties, including jaggedness parameters. Compressive modulus data suggested that cell shape anisotropy due to cell elongation in the longitudinal direction actually affected the mechanical properties of SCFX extrudates. X-ray microtomography was found to be useful to investigate 2D and 3D morphology of SCFX extrudates, including cell shape and cell size anisotropy.

1. Introduction

In the previous chapter, the effects of supercritical fluid extrusion (SCFX) process conditions and formulations on the melt rheology, as well as the expansion characteristics and 3D microstructure formation of SCFX extrudates were described. In this study, the mechanical properties of SCFX extrudates are presented in relation

to their microstructure because the quality attributes of expanded foods are often judged from their texture (Peleg, 1997).

The mechanical properties of cellular foams are governed jointly by their cell wall material property and the cellular structure that can be characterized as the ratio of open to closed cell, average cell size and cell size distribution, cell wall thickness and cell shape, uniformity of the structure, and presence of skin or crust (Peleg, 1997). However, regardless of their microstructure, the product density of expanded foams (i.e., either piece or bulk density) has been widely used as a predictor of their mechanical properties due to the simplicity of measurement and the reasonable correlation with the textural properties. In general, more expanded products with lower product density exhibit low breaking strength and compressive modulus (Sokhey, Rizvi & Mulvaney, 1996). Fang and Hanna (2000) proposed a simple power law relationship between the mechanical properties and the density of starch-based extruded foams. Moreover, Gibson and Ashby's (1997) model has been successfully utilized for the mechanical property modeling of synthetic petrochemical foams. The cellular solid approach of Gibson and Ashby's model was also found to be useful to predict the mechanical properties of solid food foams, such as starch-based extrudates and bread (Liu & Scanlon, 2003).

However, discrepancies with regard to the exponent value of Gibson and Ashby's model and a lack of fit of experimental data to the power law for cellular food products are often reported (Liu et al., 2003; Warburton, Donald, & Smith, 1990). There are several potential reasons for this. First, it might be due to the fact that Gibson and Ashby's model utilizes the mechanical properties of the solid matrix, which are difficult to determine experimentally with precision (Gibson et al. 1997; Hutchinson & Mantle, 1987). Second, the microstructure attributes of cellular solid foams with the same density can vary. Destrumaux, Bouvier, & Burri, (1998) have

proposed that corn grits with the same density, two cellular structures (coarse or fine) can be obtained depending on extrusion conditions. This suggests that microstructure affects the texture of corn grits. Barrett and Peleg (1992) also showed that the breaking and plateau stresses of corn grits extrudates were negatively correlated with the average cell size. Finally, many cellular solids, including starch-based extrudates and bread crumb, are anisotropic in nature. Anisotropy can be observed either in cell shape or in cell size distribution. Several researchers (e.g., ; Gibson et al. 1997; Warburton et al. 1992) have reported that structural anisotropy affects the resultant mechanical properties of solid foams. Therefore, it the correlation between the mechanical properties and the density of extrudates might be improved when the microstructural attributes are taken into account. Liu et al. (2003) suggested improving this correlation by integrating the fractal dimension of the solid foam in order to take into account the macroscopic length of the foam sample. Recently, Bureau and Gendron (2003) proposed that researchers should utilize the ratio of foam density to average cell diameter as a single parameter for relating to the mechanical properties of polyolefin foams, rather than relative density.

The extrusion process has been widely utilized in expanded cellular solid foods (Alavi et al., 1999). Expanded products using conventional steam-based extrusion usually exhibit non-uniform cellular structure. Typically, steam-based extrudates have an average cell size of 1-3 mm, whereas individual pore diameters can be as large as 6-8 mm (Barrett et al., 1992). The polydispersity index (PDI), which indicates the uniformity of cell size distribution, of steam-based extrudates is approximately 0.29, suggesting heterogeneous cell distributions (Alavi et al., 1999). A novel technology, supercritical fluid extrusion (SCFX) utilizes supercritical CO_2 (SC- CO_2) as a blowing agent instead of steam and allows researchers to produce expanded products with a polydispersity index as high as 0.96 (Alavi et al., 1999). It is also possible to control the macro-and microstructure of SCFX extrudates, such as piece density, cell size, and cell size distribution, by manipulating process conditions, such as pressure drop rate in the die, the ratio of SC-CO₂ to the feed, and SC-CO₂ residence time (Winoto, 2005). Research leading to improved knowledge about the microstructure-mechanical property relationship would be beneficial for tailoring textural properties of SC-CO₂ expanded products. Although SCFX extrudates have a relatively uniform cell size distribution, they are expected to show a certain degree of anisotropy in microstructure because SCFX extrudates expand more in the cross-sectional direction compared to the longitudinal direction (Sokhey et al., 1996).

In addition, 3D image analyses like X-ray microtomography provide a better understanding on the relationship between the mechanical properties and the microstructure of expanded cellular foams than 2D image analyses, such as scanning electron microscopy (SEM) and light microscopy (Trater, Alavi & Rizvi, 2005). The 2D images are destructive in nature and do not provide accurate information on cell size distribution because cells are generally sliced off-center. Further, conventional 2D images do not allow imaging of the same specimen at different depths. Drawbacks of the conventional imaging technologies can be overcome by using non-invasive techniques that generate 3D maps of the internal structure of small samples with micrometer resolution (Agbisit, Alavi, Cheng, Herald & Trater, 2007). Due to its advantages over traditional 2D image techniques, X-ray microtomography is more frequently utilized for microstructure analysis of cellular solid foods. Recently, X-ray microtomography was found to be useful for determining the correlation between 3D microstructure and the mechanical properties of cellular solid foods (Agbisit et al., 2007; Babin, Della Valle, Dendievel & Lourdin, 2007). The authors reported that 3D microstructural attributes, such as average cell size and average cell wall thickness, were positively correlated with the mechanical properties.

The purpose of this work was to (1) determine the 2D and 3D microstructures of starch-based SCFX extrudates focusing on cell anisotropy with X-ray microtomography and (2) investigate the effects of the microstructural attributes on the mechanical properties of SCFX extrudates.

2. Materials and Methods

2.1. Sample preparation

Full experimental details for SCFX extrudates production were described in the previous chapter. Whey protein added starch-based SCFX extrudates were produced using a Wenger TX-57 twin screw extruder (Wenger Manufacturing, Sabetha, KS) for 2D and 3D image analysis and mechanical property measurement. The ratios of pregelatinized corn starch to whey protein isolate (WPI) were 0, 3, 6, 12, and 18 wt%, whereas the ratios of SC-CO₂ to feed were 0.0, 0.25, 0.5, and 0.75 wt%. Emerging extrudates from the die were collected on metal trays and dried at 85°C in a convection oven to obtain 5-6 wt% moisture content for further analysis.

2.2. Piece density measurement

Piece density (PD) (kg/m³), defined as the ratio of the mass of the sample to its total volume including the voids, was measured using the geometrical method (Winoto, 2005). The volume of the extrudate was calculated by multiplying the crosssectional area by the length, assuming the extrudate is a straight cylinder. The procedure was repeated 10 times for each set of samples.

2.3. X-ray microtomography

As described in the previous chapter, SCFX extrudates were scanned using a desktop X-ray microtomography imaging system (Model 1072, 20-100 kV/0-250 μ A,

SkyScan, Aartselaar, Belgium) set at 40 kV/100 μ A. A microtomographic scan was performed by rotating the specimen at small angular increments. The radiographs were then reconstructed into 3D images using Cone-Rec software (Skyscan, Belgium). Longitudinal images were obtained using Dataview software (Skyscan, Belgium) from reconstructed cross-sectional images. Both cross-sectional and longitudinal images of SCFX extrudates were used for further 2D and 3D image analysis.

2.4. 2D image analysis

Sigma Pro version 5.0 software (SPSS, Inc.) was utilized for 2D image analysis. Prior to the analysis, the thresholding process was conducted with a value of 45. The 2D cell area (CA) was equivalent to the sum of calibrated pixels units. In this study, the 2D cell diameter (D_i) was obtained from the 2D cell area, assuming that each cell is circular, and the average values in cross-sectional (CD_{cross}) and longitudinal (CD_{long}) directions were presented.

The 2D radial variation of microstructural characteristics was evaluated by dividing each 2D cross-section into eight shells. The 2D cell diameter (D_i) was obtained from cells in each shell. The average value was presented as a function of the distance from the center. The cell number density in each shell (CN_{shell}) was determined by counting the cell number and expressed as the cell number per shell unit area.

The anisotropy of cell shape in cross-sectional and longitudinal views was determined with shape factor (SF), a measure of an object's circularity. It was determined using Sigma Pro software (SPSS, Inc.) for each cell on cross-sectional and longitudinal images, and the average values were presented. The shape factor for each cell was defined as:

$$SF = 4\pi \frac{CA}{(CP)^2}$$
(1)

where the 2D cell perimeter (CP) was equivalent to the sum of all distances measured from edge pixels for each specified cell.

A perfect circle has a shape factor of 1.

Cell elongation in longitudinal directions was characterized by the anisotropy ratio (AR) that was defined as the average cell diameter ratio of cross-sectional to longitudinal directions.

The uniformity of cell sizes in cross-sectional and longitudinal directions was assessed on the basis of polydispersity index (PDI) defined as:

$$PDI = \frac{\overline{D_{N}}}{\overline{D_{W}}} = \frac{\sum (D_{i}N_{i})}{\sum (D_{i}^{2}N_{i})}$$
(2)

where, $\overline{D_N}$ is the number average cell diameter, $\overline{D_W}$ is the weighted average cell diameter, and N_i is the number of cells having D_i.

PDI varies between 0 and 1, and a value closer to 1 signifies a more uniform cell size distribution.

2.5. 3D image analysis

3D microstructure analysis was conducted using Skyscan software (CT-Analyser, version 1.5.0.2, Aartselaar, Belgium). A volume of interest (VOI) function, for which the regions of interest (ROI) were interpolated across slices, was used and then segmented into white and black regions. The lower and upper grey threshold settings were 45 and 255, respectively. First, the total volume of SCFX extrudates sample (TV), total volume of cells (objectives, TCV), and total surface area of cells

(TCS) were determined and utilized for further analysis. The void fraction (V_f) was calculated by dividing total cell volume (TCV) by total volume (TV). The 3D average cell diameter, 3D cell wall thickness, and 3D cell number density were obtained using three different approaches and compared with the data obtained by manual 3D morphology analysis from the previous chapter (CD_{manual} , CWT_{manual} , and CN_{manua}) (see Table 2.3).

2.5.1. Trabecular bone analysis analogy

First, cell diameter ($CD_{trabecular}$) and cell wall thickness ($CWT_{trabecular}$) were measured directly by means of their analogues in trabecular bone analysis using CT-Analyzer software. Bellido, Scalon, Page & Hallgrimsson (2006) successfully utilized trabecular parameters, such as trabecular thickness and trabecular separation, to determine bubble diameter and bubble separation in dough, respectively. In this study, structure thickness and structure separation in trabecular bone analysis were utilized as cell diameter ($CD_{trabecular}$) and cell wall thickness ($CWT_{trabecular}$).

2.5.2. Cubic model

Cell diameter (CD_{cubic}) and cell wall thickness (CWT_{cubic}) were calculated using a modified cubic model (Olurin, Arnold, Korner, & Singer (2002). The authors assumed that closed cell foams are comprised of many cuboids joined together to form the cellular architectures. In this study, it was assumed that the length of each side of the inner cube, which represents the void occupied by gas, is CD_{cubic} and the length of each side of the outer cube, which represents the entire cell, including the cell wall, is CD_{cubic} + CWT_{cubic} . Therefore, the total volume of SCFX extrudates sample (TV), total volume of void cells (objectives, TCV), and total surface area of cells (TCS) can be defined as follows:

$$TV = CN_{cubic} \times (CD_{cubic} + CWT_{cubic})^3$$
(3)

$$TCV = CN_{cubic} \times CD_{cubic}^{3}$$
(4)

$$TCS = CN_{cubic} \times 6 \times (CD_{cubic})^2$$
(5)

where CN_{cubic} is the number of cells in the total sample volume (TV).

From equations 4 and 5, $CD_{cubic} = 6 \times TCV / TCS$ (6)

From equations 3 and 4,
$$CWT_{cubic} = CD_{cubic} \times [(TV/TCV)^{1/3}-1]$$
 (7)

2.5.3. Spherical model

A spherical model was utilized to calculate cell diameter ($CD_{spherical}$) and cell wall thickness ($CWT_{spherical}$). The cell wall thickness ($CWT_{spherical}$) was defined as the ratio of total volume (TV) to total cell surface (TCS). Cell number per sample volume (CN_{CT}) was determined directly using CT-Analyzer. It was assumed that total cell volume (TCV) was occupied by a number of cells (N_{CT}). Cell diameter ($CD_{spherical}$) was obtained from equivalent cell volume ($CV_{spherical}$) using the following equations:

$$CV_{spherical} = TCV / N_{CT}$$
(8)

$$CD_{spherical} = 2 \times (3CV_{spherical} / 4\pi)^{1/3}$$
(9)

2.5.4. Degree of anisotropy and fragment index

The degree of anisotropy (DA) was obtained using CT-Analyzer software. A value of 0 would correspond to total 3D isotropy, whereas a value of 1 would indicate total 3D anisotropy (Bellido et al, 2006). Mean intercept length (MIL) and Eigen analysis were used to calculate DA using CT-Analyzer. Mean intercept length was obtained by sending a line through a 3D image volume containing binary objects and dividing the length of the test line through the analyzed volume by the number of times the line passed through or intercepted part of the solid phase (Bellido et al., 2006). The fragment index (FI), a measure of connectivity of cellular structure, was

determined by CT-Analyzer software. The FI can be calculated in 3D using the volume and the surface of a binary solid before and after image dilation (Bellido et al., 2006). Lower FI values indicate a higher degree of connectivity. Negative FI values represent closed and concave surface of cells, whereas positive values indicate convex cell surfaces.

2.6. *Texture analysis*

2.6.1. Compression test

Ten samples for each treatment were analyzed using Instron Universal Machine, model 1124 (Instron Corporation, Canton, MA), for compression (Sokhey et al., 2000). Cross-head speed was 10 mm/s. The cylindrical samples with 12 mm length were compressed up to 70% in axial directions. The diameter was measured at right angles to each other at five locations on the sample to calculate the average cross-sectional area. The stress-strain curve was based on the force-deformation data and sample dimensions. The compressive modulus (Ec) was calculated as the slope of the linear region. The fracture stress (Fr) was obtained at the first fracture or rupture, whereas the crushing stress (Cr) was calculated as the mean stress from the point of first rupture (Abigist et al., 2007).

Jaggedness parameters were also determined using a compression test. The number of peaks (N), integral of the curve (S) (i.e., area below the curve from 0 to 70% strain) and distance of puncture (d) were derived from the force-deformation curves. Using N, S, and d values, parameters, such as number of spatial ruptures (Ns), average specific force structure ruptures (AFR), and crispness work (CW) were calculated (van Hecke, Allaf & Bouvier, 1998):

Number of spatial ruptures =
$$\frac{N}{d}$$
 (10)

Average specific force of structural ruptures = $\frac{S}{d}$ (11)

Crispness work =
$$\frac{Average \ specific \ force \ of \ structural \ ruptures}{Number \ of \ spatial \ ruptures} = \frac{S/d}{N/d} = \frac{S}{N}$$
 (12)

2.6.2. Three point bending test

The three point bending test was conducted using Instron Universal Machine, model 1124 (Instron Corporation, Canton, MA), as was done in van Hecke, Allaf & Bouvier (1995). The flexural modulus (Ef) and the breaking stress (Sb) were determined using Instron and calculated using the following equations:

$$Ef = (dF/dt) \times (L^{3}/4veh^{3})$$
(13)

$$Sb = F_{at break} x (3L/2eh^2)$$
(14)

where $F_{at break}$ and dF/dt are the breaking force and the slope of the force time curve, respectively, e and h are the average extrudate thickness and the average cross-sectional diameter of the extrudate, respectively, L is the distance between supports of the bending cell (i.e., 20 mm), and v is the speed of deformation (i.e., 10 mm/min).

2.7. Statistical analysis

An analysis of variance (ANOVA) was conducted using the MINITAB statistical program (Minitab, Inc.). Unless otherwise indicated, all reported differences were statistically significant at $\alpha = 0.05$.

3. Results and Discussion

3.1. 2D image analysis

Figure 3.1 shows the original scanned image and representative reconstructed 2D slices of a typical SCFX extrudate in cross-sectional and longitudinal directions. One of distinctive characteristics of SCFX extrudates, an outside non-porous, skin-like layer can be seen in the cross-sectional and longitudinal views.



Figure 3.1. X-ray microtomography images of 0 wt% WPI SCFX extrudates at the 0.25 wt% SC-CO₂ level

SCFX extrudates displayed a variation in microstructure in the cross-sectional direction. Cell size from transverse cross-sections of SCFX extrudates decreased with radial distance from the center, indicating the presence of a relatively non-porous skin. The variation was characterized by a large number of small cells toward the surface, as well as a relatively small number of large cells toward the center (Figure 3.2). The findings can be explained by four ways. First, CO₂ gas loss at the surface of the SCFX extrudates leads to a reduction in cell size. This phenomenon tends to occur at the surface of the extrudates because nucleated cells inside the extrudates act as barriers by preventing gas diffusion from the center of extrudates into the environment (Park, Baldwin & Suh, 1995).



Figure 3.2. (Top) 2D radial variation in average cell diameter and (bottom) number of cells per unit area in each shell (0 wt% WPI SCFX extrudates at the 0.25% SC-CO₂ level)

Second, faster nucleation at the center in the die compared to the position close to die wall result in larger cells at the center of extrudates. Pontente Ernst and

Obloski (2006) reported that nucleation initially occurs at the center in the crosssectional direction, resulting in a parabolic-shaped nucleation line. The nucleated cells at the center then have more time to grow with diffused gas from the matrix compared to cells near the die. This results in variations in cell size in the cross-sectional direction. Third, shear-induced nucleation can be enhanced at the die wall, leading to high cell density close to the extrudate surface. Han and Han (1998) reported that both rapid pressure drop and shear force can induce nucleation. Finally, the structural setting of the extrudate matrix during post-extrusion drying limits cell growth at the surface. Moisture loss due to drying resulting in an increase in the glass transition temperature leads to greater resistance to expansion. During the drying process, mass and heat transfer at the extrudate surface occur faster than at the center, which leads to a more rapid setting of cell structure and less expansive force closer to the extrudate surface.

In the longitudinal direction, the cell shapes were more elliptical than spherical. In addition, they were aligned along the extrusion direction (Figure 3.1). The shear field in the die during the extrusion process is responsible for the elongation and orientation of nucleated cells. This results in the morphological anisotropy of SCFX extrudates in a longitudinal direction (Han et al., 1988). This finding was supported by the determined anisotropy ratio (AR), the ratio of the average cell diameter in the longitudinal direction to that of cross-sectional direction (Table 3.1). The AR was greater than 1 for all samples, suggesting that the cells were elliptical, rather than circular (Table 3.1). In addition, the shape factors (SF) in the longitudinal direction were lower than those in the cross-sectional direction. This finding supports the notion that the cells in SCFX extrudates were elongated in extrusion direction.

S SC	-CO2 vt%)	Sample code	Cross- sectional diameter (um)	Cross- sectional SF	Cross- sectional PDI	Longitudinal diameter (µm)	Longitudinal SF	Longitudinal PDI	AR
Γ	.25	A1	569.1	0.38	0.84	655.0	0.33	0.75	1.15
0	0.50	A2	577.8	0.36	0.76	816.7	0.29	0.74	1.41
0	.75	A3	428.3	0.32	0.71	482.6	0.30	0.66	1.13
Ľ	0.25	B1	616.0	0.52	0.78	746.3	0.48	0.82	1.21
Ŭ	0.50	B2	621.6	0.40	0.80	701.4	0.42	0.84	1.13
Ŭ	0.75	B3	469.8	0.47	0.80	626.5	0.44	0.80	1.33
	0.25	C1	518.2	0.45	0.77	618.0	0.44	0.77	1.19
Ŭ	0.50	5 C	527.1	0.37	0.79	545.1	0.43	0.80	1.03
-	0.75	с С	451.6	0.37	0.69	543.0	0.35	0.74	1.20
	0.25	D1	614.4	0.30	0.84	618.2	0.22	0.87	1.01
-	0.50	D2	682.8	0.38	0.76	762.7	0.33	0.82	1.12
-	0.75	D3	580.6	0.32	0.86	635.8	0.30	0.87	1.10
	0.25	E1	614.5	0.54	0.70	659.0	0.44	0.81	1.07
	0.50	E2	608.9	0.51	0.73	650.0	0.40	0.86	1.07
-	0.75	E3	302.0	0.42	0.75	341.7	0.40	0.63	1.13

4 -XHUN F ÷ 4 ÷ 4 4 4 ÷ = Ę . С Table

Ellipsoidal cell shape in the longitudinal direction also has been observed in conventional steam-based extrudates (Warburton et al., 1992). Warburton et al. (1992) reported that the extent of cell elongation was dependent upon the in-barrel moisture content and formulations. This suggests that cell elongation, which is governed by melt rheology, is a universal phenomenon during the extrusion process. Further, cell shape anisotropy in different foaming directions was reported in polyolefin foams (Rodriguez-Perez, Velasco, Arenco, Almanza & De Saja, 2000).

Determined polydispersity indices (PDI), which are the indicators of cell size distribution uniformity, varied from 0.63 to 0.87 in cross-sectional and longitudinal directions. There was no clear directional trend with PDI. The values were much higher than that of steam-based extrudates (~0.29), but lower than the values (~0.95) reported by Alavi et al. (1999. This finding suggests that SCFX extrudates exhibit more uniform cell size distribution than steam-based extrusion; however, the PDI of SCFX extrudates depends on formulations.

3.2. 3D image analysis

X-ray microtomography is a powerful tool for 3D cellular solid foam morphology research. It has a number of advantages over conventional 2D image analysis techniques. However, the accuracy of 3D morphology results obtained by Xray microtomography greatly depends on the methodology and analysis employed. As previously stated, manual 3D morphology analysis has been successfully utilized for steam-based biopolymeric extrudates (Trater et al., 2005). Therefore, many researchers use the commercial 3D microstructure analysis software or develop the new programs (Bellido et al., 2006). In this study, 3D microstructure analysis was conduced using CT-Analyzer, the commercial software provided by Skyscan. The generated data were compared with those obtained by manual 3D analysis in the previous chapter.

First, analysis using CT-Analyzer provided useful 3D microstructure parameters, such as the degree of anisotropy (DA), fragment index (FI), and void fraction (Vf). DA is a measure of the presence of preferential alignment of structures along a particular directional axis in 3D. DA values ranged from 0.31 to 0.61. This finding confirms that cells in SCFX extrudates were slightly elongated as was found in the 2D analysis (Table 3.2).

Sample code	DA	Fragment index	Piece density (PD, kg/m ³)	Vf
A1	0.41	-0.28	263.8	0.74
A2	0.54	-0.49	224.9	0.79
A3	0.61	-0.42	266.6	0.72
B1	0.45	-0.16	314.0	0.71
B2	0.46	-0.20	266.0	0.78
B3	0.61	-0.42	279.3	0.75
C1	0.42	-0.68	355.8	0.74
C2	0.49	-0.66	268.2	0.82
C3	0.55	-0.47	287.9	0.76
D1	0.37	-0.59	423.5	0.68
D2	0.36	-0.66	342.5	0.73
D3	0.55	-0.47	391.7	0.70
E1	0.31	-0.63	350.0	0.76
E2	0.57	-0.59	365.7	0.74
E3	0.65	-0.13	714.5	0.51

Table 3.2. Piece density and 3D image measures of SCFX extrudates using CT-Analyzer

For all formulations, DA was lower at the $0.25 \text{ wt}\% \text{ SC-CO}_2$ level, indicating that cells were closer to sphere shape. On the contrary, anisotropy was more evident at the $0.75 \text{ wt}\% \text{ SC-CO}_2$ level, possibly due to the increased melt volumetric flow rate.

Although the DA value did not show the direction of elongation, it would be reasonable to believe that the increase in DA was due to cell elongation along extrusion direction. The FI was originally developed to measure the connectivity of the trabecular bone (Bellido et al., 2006). Low FI values signify a better connected structure. On the contrary, a prevalence of enclosed cells can result in negative FI values. The results in Table 3.2 show that SCFX extrudates contained predominately closed cells. In general, FI was higher with higher SC-CO₂ levels, indicating that more open cells were generated with an increased ratio of SC-CO₂ to feed. Gogoi et al. (2000) reported that the ratio of closed to open cells is one of the critical structural parameters that govern the mechanical properties of cellular solid foams. The piece density (PD) and void fraction (Vf) determined by X-ray microtomography showed strong negative correlation (r = -0.92), confirming the validity of the 3D image analysis using CT-Analyzer software (Table 3.2).

In general, the cell diameter and cell wall thickness data obtained using CT-Analyzer software replicated the trends observed using 3D measurements (CD_{manual}) and CWT_{manual}) presented in the previous chapter. For example, smaller cell sizes and thinner cell walls were observed when the ratio of SC-CO₂ to feed was increased from 0.25 to 0.75 wt% (Table 3.3). However, significant differences in cell diameter and cell wall thickness among different analytical approaches were observed. The cell diameters ($CD_{trabecular}$) obtained directly by means of the analogue in the trabecular bone analysis using CT-Analyzer software were significantly smaller than the cell diameters obtained using the other three methods. On the other hand, $CWT_{trabecular}$ showed approximately four- to seven fold higher values than CWT_{manual} . The data obtained based on cubic and spherical models were comparable to the data obtained based on the manual 3D analysis (Table 3.3). Interestingly, CD_{cubic} and CWT_{cubic} were closer to CD_{manual} and CWT_{manual} than $CD_{spherical}$ and $CWT_{spherical}$.

	I	T				I						I I			I		1						
	CNCT	(x 10 ² cm ⁻²)	5.9	3.4	20.7	3.1	1.2	11.5	17.0	11.1	17.1	6.6	6.8	12.2	1.7	23.1	98.5						
	CN _{manual}	$(x10^{-} cm^{-2})$	3.1	3.4	7.0	3.1	1.8	5.0	2.8	2.5	4.5	3.2	2.8	2.7	1.4	2.9	18.7						
	CWT _{spherical}	(mm)	149.9	123.9	77.2	155.3	160.5	114.6	6.99	107.6	97.3	118.5	116.3	116.5	142.9	95.7	60.8		one analvsis				
	CD _{spherical}	(mm)	621.7	764.2	404.1	753.7	1087.7	645.1	654.8	789.9	591.6	603.9	736.5	603.9	774.1	393.8	215.2		ı trabecular b		odel		
ı	CWT _{cubic}	(mm)	70.6	47.6	39.1	81.3	65.8	57.0	48.5	40.5	43.6	61.2	56.7	57.1	72.9	45.1	46.6	aner su	e analogue in	model	spherical mo		
	CD _{cubic}	(mm)	664.1	588.5	331.6	656.7	747.6	498.9	436.0	519.7	439.9	582.6	588.4	479.2	620.5	423.8	187.2	o nevio	using the	the cubic	ising the	I C	ftware
	CWT _{trabecular}	(mn)	204.2	147.5	109.9	244.4	201.9	150.2	186.5	156.7	150.9	233.6	194.3	197.2	308.1	157.3	117.5	adanted from tl	ere determined	termined using	re determined u	e previous pape	T-Analyser so
	CD ^{trabecular}	(mm)	414.5	577.3	329.1	535.8	553.3	465.7	281.6	323.5	274.4	437.2	440.8	373.0	428.9	355.0	163.5	T	VT frahecular W(cubic were det	VT _{spherical} WC1	pted from th	nined using C
	CWT _{manual}	(mm)	67.4	59.9	61.8	80.6	72.5	71.9	84.1	74.4	78.8	101.0	84.9	86.1	110.8	95.8	80.5	W) pue i	rahemiar and CV	ubic and CWT	pherical and CW	nanual was adaj	CT was detern
	CD _{manual}	(mm)	650.0	643.2	550.2	640.3	641.0	565.4	631.6	650.0	599.0	677.4	682.8	580.6	724.4	703.7	310.0	where CD		ទី	g	N.	čŇ
	Sample	code	A1	A2	A3	B1	B2	B3	C1	3	C	DI	D2	D3	E1	E2	E3	-					

Table 3.3. 3D image measures of SCFX extrudates using manual analysis and CT-Analyser software

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This finding supports the use of Ashby and Gibson's (1997) model, based on a cubic model consisting of beams and struts, for the mechanical property analysis of cellular solid foams, including SCFX extrudates. Cell number density (CN_{CT}) directly determined using CT-Analyzer software was approximately one- to seven folds higher than cell number density (CN_{manual}) obtained from manual analysis. This finding suggests that a greater number of small cells, which were not analyzed in manual 3D analysis, were included in the CT-Analyzer measurement.

In summary, CT-Analyzer software was useful to evaluate the 3D anisotropy and to determine the 3D morphological attributes of SCFX extrudates, such as cell diameter and cell wall thickness. However, results varied depending on the model utilized. Therefore, additional research on the standardization and validation of X-ray microtomography for 3D microstructure analysis of cellular solid foods is recommended in the future.

3.3. Relationship between macrostructure and mechanical properties

The determined values of the mechanical properties and the correlation with the structural parameters are listed in Table 3.4. When the piece density and the mechanical properties of SCFX extrudates were plotted in double log scale, no clear trends were observed in relation to WPI concentration. This indicates that WPI had little effect on the mechanical properties (Figure 3.3). On the other hand, the piece density was strongly positively correlated with the mechanical properties in both the compression and the bending tests.

Interestingly, 18 wt% WPI with 0.75 wt% SC-CO₂ (i.e., sample E3) had the highest piece density and the smallest cell size (Tables 3.2 and 3.3) with very high Ec, Fr, Ef and Sb compared to other samples (Table 3.4).

	ig test	Breaking stress (Sb, MPa)	0.81	0.55	0.89	0.89	0.84	0.89	0.98	0.79	0.77	1.03	0.70	0.96	0.95	0.72	1.66
	Bendi	Flexural, modulus (Ef, MPa)	0.61	0.36	0.57	0.74	0.56	0.63	0.46	0.46	0.48	0.78	0.56	0.51	0.56	0.48	1.22
		Crispness work (CW, N- mm)	219.7	122.1	128.9	236.7	174.5	193.7	245.2	247.2	149.2	366.7	180.0	208.2	265.1	271.0	481.8
		Average specific force of structural ruptures (AFR, N)	421.8	341.9	323.5	355.0	464.1	430.0	409.5	437.5	358.0	550.0	428.4	458.0	395.0	420.0	159.0
	Compression test	Number of spatial ruptures (Ns,mm ⁻¹)	1.92	2.80	2.51	1.50	2.66	2.22	1.67	1.77	2.40	1.50	2.38	2.20	1.49	1.55	0.33
		Crushing stress (Cr, MPa)	0.50	0.69	0.49	0.39	0.36	0.49	0.44	0.35	0.59	0.53	0.73	0.54	0.35	0.36	0.10
		Fracture stress (Fr, MPa)	1.08	0.62	2.09	1.38	0.79	0.92	1.47	0.65	0.78	1.82	1.26	1.42	1.15	1.77	13.34
		Compressive modulus (Ec, MPa)	13.5	11.8	14.1	17.9	12.7	22.9	26.7	14.9	21.5	22.1	15.9	18.7	20.4	19.3	169.2
		Sample code	A1	A2	A3	B1	B2	B3	C1	5	C	D1	D2	D3	E1	E2	E3

Table 3.4. Compression textural properties of SCFX extrudates with different WPI and SC-CO₂ levels



Figure 3.3. The mechanical properties of SCFX extrudates versus their piece density (PD) (WPI concentration: A: 0 wt%, B: 3 wt%, C: 6 wt%, D: 12 wt%, and E: 18 wt%)

Because sample E3 acted as an outlier, compressive and bending moduli, fracture stress, and breaking stress were plotted against piece density with sample E (dotted regression line) and without sample E (solid regression line) for comparison (Figure 3.3). Whereas strong correlations between the piece density and the compressive modulus and fracture stress under compression were observed, the crushing stress showed no clear correlation with the piece density (r = 0 without sample E). Abigist et al. (2007) and Gibson et al. (1997) also reported that the crushing stress was relatively less sensitive to the foam density compared to the compressive modulus. Sample E3 demonstrated that extremely high compressive modulus and exhibited very low

crushing stress. This is because sample E3 fractured into several pieces at a relatively small travel distance from the probe due to its hardness.

The mechanical properties of unexpanded foam that served as a reference were not incorporated in this study due to the difficulty of obtaining the mechanical properties of the solid matrix with precision. On the other hand mechanical propertypiece density without reference showed reasonably high correlation coefficients. The complexity of the determination of starchy cell material properties has been observed in many previous studies because the properties for unexpanded material vary with different processing conditions (Abigist et al., 2007; Hutchinson et al. 1987; Gibson et al., 1997). Chanvrier, Della Valle & Lourdin (2006) suggested that the formation of protein aggregates under thermo-mechanical treatment led to the phase separation of molded corn starch/zein blends resulted in an increased brittleness of corn-based glassy materials. Although a strong correlation (r > 0.8) between the mechanical properties and piece density without the reference was noted in this study, further research on the accurate measurement of the cell wall material of glassy biopolymeric foams is recommended. In addition, the presence of an outer skin with different properties than those of internal cell walls might result in difference in the mechanical parameters obtained from the compression and bending tests. Gogoi et al. (2000) reported that the puncture strength of skin was higher than that of internal cell walls. These authors reported that the peak puncture force was decreased when 4-10 wt% whey protein concentrate (WPC-34) was incorporated into starch-based model formulation SCFX extrudates. In their study, Gorgoi et al. (2000) did not measure the thickness of either the skin or the cell wall. Chang, Cheah & Seow (2000) reported that the compression test is less sensitive to the presence of skin layers than the bending or indentation test.

Table 3.4 shows the jaggedness of parameters, such as number of spatial ruptures (Ns), average specific force structure ruptures (AFR), and crispness work (CW). Abigist et al. (2007) showed that the jaggedness of the force-deformation curves was the key characteristic that correlated with crispiness. An increase in PD resulted in a significant decrease in the average number of peaks (Figure 3.4). Further, crispiness work increased as the piece density was increased.

Overall, the results demonstrated that the piece density is a good predictor of the mechanical properties of SCFX extrudates. However, samples A1 and A3, which had similar piece densities (~0.25), exhibited significantly different mechanical properties, such as compressive modulus and fracture stress. This indicates that the microstructural attributes, such as cell size and cell wall thickness, affect the mechanical properties of SCFX extrudates (Table 3.4).

3.4. Microstructure-mechanical property relationships

When compressive and flexural moduli, including the data of sample E3 which acted as an outlier, were plotted against cell diameter (CD_{manual}), a strong negative relationship was observed (Figure 3.5). Other mechanical parameters, such as fracture stress and breaking stress, also showed a general weakening of the foam structure with greater expansion of the cells (Figure 3.5). Some previous researchers have observed similar relationships between the mechanical properties of expanded starch-based extrudates and their average cell diameter (e.g., Abigist et al. 2007; Barrett and Peleg 1994; Barrett, Normand, Peleg & Ross, 1994; Van Hecke et al. 1995). However, when the mechanical properties were plotted against cell diameter (CD_{manual}) without sample E3, no clear trend was observed.



Figure 3.4. The jaggedness parameters of SCFX extrudates versus their piece density (PD) (WPI concentration: A: 0 wt%, B: 3 wt%, C: 6 wt%, D: 12 wt%, and E: 18 wt%)



Figure 3.5. The mechanical properties of SCFX extrudates and their cell diameter (CD_{manual}) (WPI concentration: A: 0 wt%, B: 3 wt%, C: 6 wt%, D: 12 wt%, and E: 18 wt%)

This finding suggests that other morphological properties, such as cell wall thickness, cell number density, cell size distribution, and degree of anisotropy are also critical to the mechanical properties. Gogoi et al. (2000) investigated the relationship between the cell size and the mechanical properties of starch-based SCFX extrudates and concluded that their mechanical properties were independent of cell size. They suggested that most mechanical properties of solid polymeric foams depended only weakly on cell size.

In this study, moderate positive correlations between cell wall thickness (CWT_{manual}) and the mechanical properties were observed, confirming the critical role of cell wall thickness (Table 3.4). Further, CD/CWT was highly correlated with the mechanical properties of SCFX extrudates (Figure 3.6).



Figure 3.6. The mechanical properties of SCFX extrudates versus their ratio of cell wall thickness (CWT_{manual}) to cell diameter (CD_{manual}) (WPI concentration; A: 0 wt%, B: 3 wt%, C: 6 wt%, D: 12 wt%, and E: 18 wt%)

This finding demonstrates the combined effect of cell diameter and cell wall thickness on the mechanical properties of the starch-based brittle foams. It is clear from these results that foams with a higher CD/CWT (i.e., thicker cell walls and smaller cell diameters) have higher compression and bending moduli compared to foams with a lower CD/CWT (i.e., thinner cell walls and larger cell diameters). This relationship was also observed in steam-based extrudates (Abigist et al., 2007) and non-food cellular solid foams (Gibson et al., 1997).

Warburton et al. (1992) reported that cell elongation in the longitudinal direction leads to the mechanical anisotropy of cellular solid foams in those two directions. In general, the compressive modulus and fracture stress of starch-based extrudates in cross-sectional direction result in higher values than in the longitudinal direction. In this study, compressive modulus was 10-fold higher than the flexural modulus (Table 3.4). Direct comparison of the data from two different test methods was not appropriate. However, cell shape anisotropy due to cell elongation directions might be partially responsible for this finding. In this study, accurate compressive measurements in the longitudinal direction were difficult to obtain, due to the cylindrical shape of the sample. Therefore, the effect of cell shape anisotropy (AR) on the mechanical properties in this study was estimated according to the cubic model proposed by Gibson et al. (1997). It was assumed that an idealized open cell in an axisymmetric foam was elongated in the longitudinal direction, which resulted in cell shape anisotropy (AR).

Originally, the model was:

$$E_{c} = C \times E_{s} \times (t/l)^{4} \times (h/l)$$
(15)

where, E_c and E_s are compressive modulus parallel to elongated direction and compressive modulus of solid material, respectively, C is a constant of proportionality, t is cell wall thickness, and I and h are length of beams in cross-sectional and longitudinal directions, respectively.

In this study, it was assumed that C and E_s were constant for all samples, whereas cross-sectional cell diameter (CD_{cross}), longitudinal cell diameter (CD_{long}), and

cell wall thickness (CWT_{cubic}) were represented by l, h, and t, respectively. If the cells of the SCFX extrudates were isotropic, compressive modulus (E_c) would be directly related to (CWT/CD_{cross})⁴. On the other hand, if the cells were elongated in the longitudinal direction, the anisotropy ratio (AR) (i.e., CD_{long}/CD_{cross}) should be greater than 1, and Ec/AR would be related to (CWT/CD_{cross})⁴. When E_c was plotted against (CWT/CD_{cross})⁴, the correlation coefficient was 0.54 without sample E3. However, the correlation coefficient between Ec/AR and (CWT/CD_{cross})⁴ was 0.65, indicating the presence of a shape anisotropy effect due to cell elongation (Figure 3.7).



Figure 3.7. The effect of anisotropy ratio (AR) on compressive modulus

A number of researchers have reported that cell shape anisotropy in the crosssectional direction and cell size distribution affect the mechanical properties of cellular solid foam (Warburton et al., 1992) . In this study, the results of 2D cell shape factors (SF) and 3D degree of anisotropy (DA) showed that the cell shape of SCFX extrudates was close to a sphere. However, there is some cell shape anisotropy or irregularity. In addition, the polydispersity index (PDI), which represents the extent of cell size spread, in the cross-sectional and the longitudinal directions varied from 0.63 to 0.86. Therefore, variations in cell shape and cell size influenced the mechanical properties of the SCFX extrudates. However, it was difficult to investigate the effects of such variations on the product texture due to the structural complexity of SCFX extrudates. For example, although their piece densities were similar, sample A3 had a higher compressive modulus (E_c), cell shape factor (SF), and polydispersity index (PDI) compared to sample A1. It was not clear if the higher SF and PDI resulted in higher E_c because sample A3 had a smaller average cell size and a thicker cell wall that increased the product stiffness. It would be challenging to manipulate one morphological parameter of cellular solid foams while keeping other parameters constant. As a result, several studies on the relationship between cell anisotropy and the mechanical properties have been conducted using numerical modeling. Recently, Li, Gao, & Subhash (2006) investigated the effects of cell shape and strut crosssectional area variations on the elastic properties of 3D open cell foams. They reported that the elastic moduli increased as cell shapes became more irregular, but decreased as strut cross-sectional areas became less uniform.

Generally, it is expected that the number of spatial ruptures (Ns) during deformation would increase with higher cell number density (Agibist et al., 2007). A very weak positive correlation (r = 0.1) between Ns and CN_{manual} was noted without the sample E3. On the contrary, a negative correlation was observed between Ns and CN_{manual} when the sample E3 was considered (Figure 3.8).



Figure 3.8. Number of spatial ruptures versus cell number density (top) and the ratio of cell wall thickness (CWT_{manual}) to cell diameter (CD_{manual}) (bottom) (WPI concentration; A: 0 wt%, B: 3 wt%, C: 6 wt%, D: 12 wt%, and E: 18 wt%)
Although each peak on the force–deformation curve theoretically represents one cell being broken, it could be an indication that a group of cells is being fractured at the same time and/or breaking of off the whole sample into two or more pieces.

Therefore, cell number density alone was not a good predictor of Ns. On the other hand, when Ns was plotted against the ratio of cell wall thickness (CWT) to cell diameter (CD), a strong negative correlation was observed. This indicates that the jaggedness property of SCFX extrudates can be explained by the combined effect of cell size and cell wall thickness.

4. Conclusions

The 2D and 3D morphological parameters of SCFX extrudates were determined using X-ray microtomography and correlated with their mechanical properties. In the axial direction, a larger number of cells with smaller diameters was observed close to the surface of the SCFX extrudates, indicating the presence of a relatively non-porous skin. In the longitudinal direction, the cells shapes were more elliptical than spherical and were aligned along the extrusion direction, indicating that some anisotropy exists in SCFX extrudates. CT-Analyzer software was found to be useful for the evaluation of the 3D morphological parameters of SCFX extrudates. Cell size was not a good predictor of the mechanical properties SCFX extrudates. On the other hand, the ratio of cell wall thickness to cell diameter was highly correlated with not only compressive and flexural moduli, but also jagged parameters, such as number of spatial ruptures and crispiness work. This study contributed to the literature examining the effects of the 2D and 3D morphological properties on the deformation mechanism of starch-based brittle foams. Further research using noninvasive imaging techniques, such as X-ray microtomography, are warranted. The results of such studies will be beneficial for tailoring the texture of expanded food products.

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CHAPTER 4

THE TIME-DELAYED EXPANSION PROFILE OF SUPERCRITICAL FLUID EXTURDATES*

Abstract

The typical time-delayed expansion behavior of supercritical fluid extrusion (SCFX) extrudates was investigated via video analysis. Cross-sectional, longitudinal and volumetric expansion characteristics were evaluated varying SCFX process conditions such as ratio of SC-CO₂ to feed and die size. The result showed that the initial cross-sectional expansion of SCFX extrudate was time, SC-CO₂ concentration, and pressure drop rate dependent. The growth time of SCFX extrudates was approximately 30-200 fold longer compared to a typical growth time of steam-based extrudates. Die-swell in SCFX extrusion accounted for 32-45% of the maximum cross-sectional expansion but had insignificant effect on volumetric expansion. The pressure drop profile in the die was found to be critical in controlling not only the extent of volumetric expansion of SCFX extrudates but also the rate of SCFX expansion via cell nucleation and cell growth. Rapid initial cross-sectional and volumetric expansion of SCFX extrudates promoted faster structure collapse and strategies to enhance the volumetric expansion, which determines the textural properties of expanded products, were discussed. The retarded SCFX expansion is very unique and can be utilized for development of novel SC-CO₂ expanded products with tailored texture.

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1. Introduction

The extrusion cooking process is a commercially practiced technology to produce a large variety of expanded food products and the final texture and quality is closely related to the morphology and cell structure of the extrudate. It is now well established that macro/microstructure formation in extrusion processes is the consequence of several overlapping events including biopolymer structural transformations (starch gelatinization and/or protein denaturation), nucleation, dieswell, cell growth, and cell collapse (Moraru & Kokini, 2003).

During conventional steam-based extrusion cooking, water acts both as a plasticizer for melt formation and as a blowing agent for expansion (Wang, Ganjyal, Jones, Weller, & Hanna, 2005). When the melt passes through the extruder die, it undergoes a sudden pressure drop resulting in water vapor nuclei generation. These cells grow in size as additional water vapor diffuses into the nuclei. Furthermore, thermal expansion of water vapor causes further expansion. At the maximum level of extrudate expansion, however, it starts to experience some collapse due to elastic forces in the cell wall (Arhaliass, Bouvier, & Legrand, 2003). Conventional steambased extrusion usually requires low moisture (18~28 wt%), high temperature (120~180°C) and high shear conditions for good expansion. These harsh operating conditions often lead to excessive dextrin formation and prevent effective utilization of heat and shear sensitive ingredients such as proteins, vitamins, and certain flavors. Although limited control of the extrudate expansion and the microstructure in conventional steam-based extrusion can be obtained by manipulating parameters such as moisture content, die geometry, die temperature, screw rotation speed, and feed rate, steam-expanded products usually show non-uniform cellular structure and cell sizes in the range of 1~3 mm (Barrett & Peleg, 1992). In addition, extrudate growth and collapse occur on a very short time scale resulting in little control during postextrusion processes. The expansion time in steam-based extrusion determined via video analysis was observed to vary between 44 and 87 ms, depending on the dimension of the die used (Arhaliass et al., 2003).

On the other hand, use of supercritical carbon dioxide (SC-CO₂) as a blowing agent instead of steam, uncouples the dual role of water as a blowing agent and a plasticizer during supercritical fluid extrusion (SCFX). In this process, expansion of the melt is achieved by first solubilizing $SC-CO_2$ in the melt, and then inducing nucleation due to pressure drop in the die, which is followed by cell growth caused by diffusion of CO₂ into the nucleated cells (Rizvi, Mulvaney, & Sokhey, 1995). A higher moisture content (30~45 wt%) in the extruder barrel is utilized to keep the product temperature low via reduction of viscous dissipation of energy and to maximize SC-CO₂ solubilization in the melt. Since SCFX extrusion is conducted at temperatures lower than 100°C as well as at lower shear, it enables the use of temperature and shear sensitive ingredients in product formulations. A greater thermodynamic instability due to high-pressure drop rate results in high cell density in the range of 2.3 x 10^6 and 6.8 x 10⁶ cells/cm³. (Alavi, Gogoi, Khan, Bowman & Rizvi, 1999). The SCFX process allows control of not only the average cell size of extrudates which may range from about 50 to 250 microns with high polydispersity index of ~0.95 but also the volumetric expansion of final products by manipulating process parameters such as die dimension, SC-CO₂ concentration, and SC-CO₂ residence time (Alavi & Rizvi, 2005; Winoto, 2005). SCFX extrudates show nonporous surface and predominantly closed cell structures. In addition, SC-CO₂ can be utilized to encapsulate flavors and potent micronutrients into expanded products since it is an excellent solvent in nature at supercritical state. Interestingly, it was also observed in previous work in our laboratory that SCFX extrudates show a unique, time-delayed expansion behavior. Whereas typical time for extrudate expansion in conventional steam-based extrusion is

less than 0.5 seconds, in SCFX processes it takes almost up to 3-20 seconds to attain maximum expansion. High moisture content in the melt reduces glass transition temperature of the product and the SCFX extrudates are still in rubbery state upon exiting the die and have low enough viscosity to cause cell growth or shrinkage. As a result, SCFX extrudates tend to expand further until their structure is set during postextrusion processes such as drying and frying (Alavi et al., 1999; Chen, Dogan & Rizvi, 2002). This unique time-delayed expansion behavior would allow us to utilize newly emerging technologies such as convective microwave drying to tailor the mechanical and textural properties of SCFX extrudates. On the other hand, poorly designed process conditions would result in collapsed and harder extrudates. Thus, a fundamental understanding of the mechanics of time dependent SCFX expansion would be useful in creating products of novel morphology and optimizing the postextrusion drying processes. Knowledge on the pressure profile through the die and its effect on SCFX expansion may be utilized to increase the overall expansion and minimize structure collapse due to the gas loss from the surface. Moreover, it should be possible to increase the blowing agent efficiency resulting in reduced operating cost. In this study, the effects of SC-CO₂ injection rate and die dimension on the time dependent expansion behavior of SCFX extrudates were investigated using a visualization technique. Pressure drop rate through the die was calculated and its effect on expansion behavior in SCFX extrusion was quantified. New strategies to minimize gas loss and to enhance overall expansion including nucleation time manipulation and surface to volume ratio control have been proposed. Cross-sectional, longitudinal and volumetric expansion indices were used to describe SCFX expansion characteristics since it has been shown that melt expansion in most cases is notably non-isotropic (Arhaliass et al., 2003).

2. Materials and Methods

2.1. Feed formulation

The feed formulation consisted of 49.5 wt% pre-gelatinized corn starch (Cargill Food and Pharma Specialties), 24 wt% pre-gelatinized potato starch (Staley), 24 wt% sugar, 1 wt% salt, and 1.5 wt% distilled monoglyceride as a dough conditioner (Davisco). 7 wt% whey protein concentrate (WPC-34, Main Street Ingredients) was added to the above mix to improve the structural stability of the extrudates (Winoto, 2005). In-barrel moisture content was 43.8 wt%.

2.2. SCFX extrusion

A co-rotating twin screw Wenger TX-57 Magnum extruder (Wenger Manufacturing, Sabetha, KS) with 4.5 heads, barrel diameter of 52 mm, and L/D ratio of 28.5 was configured for supercritical fluid extrusion. SC-CO₂ was injected into the starch melt through four valves located around the extruder barrel at L/D = 24. This increased the mixing effect and reduced residence time for mixing. A flow restrictor plate was installed on the exit end of the last barrel and before the die assembly to maintain and regulate pressure as described by Rizvi et al. (1995). During SCFX processing, pressure in the extruder barrel prior to the die entrance was built up to 10.7 MPa.

Screw rotation speed and dry feed rate were 120 rpm and 35 kg/hr, respectively. Product temperature at die exit was maintained at approximately 55°C for all experiments. The die used in the experiment was circular in cross section and had a straight section following a taper. The entrance diameter, total length and the length of straight section were constant at 17 mm, 33.7 mm, and 18 mm, respectively, while the die diameter was 2.9, 4.2, or 5.9 mm. Two identical circular dies were mounted on the die plate for each diameter used.

2.3. Experimental design

For each experiment, pressure drop rate was varied using dies of three different diameters (2.9, 4.2 and 5.9 mm) but the same straight-section length of 18 mm. The SC-CO₂ injection rates of 0.0, 0.25, 0.5, and 0.75 wt% were utilized.

2.4. Pressure drop rate and average melt flow rate

For a non-Newtonian power law fluid such as a starch melt, the pressure drop across a capillary (cylindrical die) is given by the following equation:

$$-\Delta P = \frac{2K\Delta L}{R} \left[\frac{Q\left(3 + \frac{1}{n}\right)}{\pi R^3} \right]^n \tag{1}$$

where: ΔP = pressure drop (Pa), K = melt consistency coefficient (Pa.sⁿ), ΔL = length of capillary or die (m), R = capillary radius (m), Q = volumetric flow rate (m³/s), n = melt flow behavior index (Park, Baldwin & Suh, 1995).

The average residence time in the extruder die is

$$\Delta t \approx \frac{\Delta L}{\langle v \rangle} = \frac{\Delta L}{Q / \pi R^2} = \frac{\pi R^2 \Delta L}{Q}$$
(2)

where: Δt = average residence time (s) and $\langle v \rangle$ = average velocity (m/s). Therefore the pressure drop rate ($\Delta P/\Delta t$) is:

$$-\frac{\Delta P}{\Delta t_{1}} = 2K \left(\frac{Q}{R^{3}\pi}\right)^{n+1} \left(3 + \frac{1}{n}\right)^{n}$$
(3)

The viscosity of the starch/water/ CO_2 mixture was described using the power law model and the experimentally determined rheological parameters for the same extrudates are listed in Table 4.1 (Winoto, 2005).

SC-CO ₂ concentration (wt%)	Ν	K (Pa.s ⁿ)	R^2
0.00	0.36	978	0.87
0.25	0.39	845	0.77
0.50	0.39	784	0.81
0.75	0.47	529	0.95

Table 4.1. Effect of SC-CO₂ concentration on melt rheological parameters (Adapted from Winoto, 2005)

The pressure drop rates through the three different dies used in this study were calculated using a numerical approach. Pressure was assumed to be atmospheric at the die exit and the die was discretized into 30 segments of equal length. The Reynolds number, calculated based on SCFX experimental conditions in this research, showed that the flow was laminar. The pressure drop rate was calculated using Matlab programming software (Mathworks, Inc.). The pressure drop rate calculations were based on simplifying assumptions, the most significant of which was the modeling of the starch melt rheology as a power law fluid but neglects any extensional behavior of the starch melt. Although the pressure drop rate calculations are accurate within the limits afforded by the power law model, in actuality the pressure profile could be affected by entrance and exit pressure drops, and two phase-melt rheology (Winoto, 2005).

Based on the calculated pressure drop rate, the total volume of the melt including gaseous CO_2 for each discrete segment of the die was calculated. The change in CO_2 was calculated using the pressure-volume data provided by Nist Chemistry Webbook (http://webbook.nist.gov/chemistry/). The average velocity of the

melt through the die was determined by dividing the volumetric flow rate by the crosssectional area of the die.

2.5. Expansion characterization

A visualization technique was developed to determine expansion profile as a function of time as shown in Figure 4.1. The expansion behavior of SCFX extrudates on a speed controlled conveyor belt was recorded using a CCD camera (JVC GR-DVL-805) and transferred to a computer as a digital file. The following equations were used to define product expansion including the cross-sectional expansion index (SEI), the longitudinal expansion index (LEI), and the volume expansion index (VEI).

$$SEI = \frac{S_e}{S_d},\tag{4}$$

$$LEI = \frac{V_e}{V_d},\tag{5}$$

$$VEI = SEI * LEI, \tag{6}$$

where, S_e and S_d are the cross sections of extrudate and die, respectively; V_e and V_d are the velocity of extrudate and the velocity of melt in the die, respectively.

Using captured images, the diameter of the SCFX extrudates at each time frame was obtained and used for calculation of the cross-sectional area of extrudates. The ratio of the cross-sectional area of the extrudate to that of the die was expressed as the cross-sectional expansion index (SEI). The velocity of extrudate was determined



Figure 4.1. Schematic of supercritical fluid extrusion and visual analysis set up

using a speed controlled conveyor belt assuming that it is constant after exiting from the die due to a free-surface flow as shown by Wang et al, (2005) for conventional steam-based extrusion. The velocity of extrudate was divided by the calculated velocity of extrudate at the die exit to obtain the longitudinal expansion index (LEI). The volumetric expansion index (VEI) was defined as the combination of SEI and LEI (Alavrez-Martinez, Kondury, & Harper, 1988).

The cross-sectional expansion that was caused by $SC-CO_2$ only was modeled using Matlab programming software (Mathwork, Inc.) for the first 5 seconds. To discount the die swell effect, SEI of expanded SCFX extrudate was divided by SEI of control sample with 0.0 wt% SC-CO₂.

2.6. Blowing agent efficiency

The blowing agent efficiency (BAE) representing the actual amount of SC-CO₂ that was utilized for expansion can be evaluated using following equations:

Theoretical maximum VEI =
$$\frac{Volume_{melt} + Volme_{gas}}{Volume_{melt}} = 1 + \frac{m_{CO_2}}{m_{melt}} \times \frac{V_{CO_2}}{V_{melt}}$$
(7)

$$BAE (\%) = 100 x Actual maximum VEI / Theoretical maximum VEI (8)$$

where, m_{co2} and m_{melt} are mass flow rate of CO₂ and melt, respectively. V_{CO2} and V_{melt} are specific volume of CO₂ and melt at 55°C at 1 atm, respectively.

Theoretical maximum VEI is the volumetric expansion ratio when all dissolved gas was used for expansion without any loss. The theoretical maximum VEI was 4.1, 7.2 and 10.3 at 0.25, 0.5 and 0.75 wt% SC-CO₂ level, respectively.

2.7. Surface to volume ratio

The surface to volume ratio (ψ) of cylindrical extrudate can be calculated using the following equation:

$$\psi = \frac{\pi dl}{\pi \left(\frac{d}{2}\right)^2 l} = \frac{4}{d} \tag{9}$$

where, d and l are the diameter and the length of cylindrical extrudate, respectively.

3. Results and Discussion

3.1 Pressure drop rate through the die

The pressure drop rates due to extrudates containing different amounts of SC-CO₂ and flowing through dies of different diameter were calculated using rheological parameters reported by Winoto (2005) for the same extrudates (Table 4.1). In the case of SCFX process, the volumetric flow rate of melt would be affected by the amount of dissolved SC-CO₂ since its volume continually increases as the pressure along the die decreases and CO₂ begins to come out of solution, resulting in faster volumetric melt flow toward die opening.

Figures 4.2a and 4.2b show the pressure drop in the die versus time. In this study, the calculated pressure drop rate was increased by approximately by 11-18 folds indicating the expected contribution of die dimensions to pressure profile in the die when the die diameter was decreased from 5.9 to 2.9 mm for each SC-CO₂ concentration (Table 4.2). It can be seen from equation 3 that pressure drop rate can be varied using die radius alone (Park et al., 1995). The pressure drop rate in the straight section of each die increased by approximately 3 to 7 times on average when SC-CO₂ was present, depending on the injection rate. The result is contradictory to the findings of Han, Koelling, Tomasko, & James (2002). The pressure profile simulation of polystyrene/CO₂ mixture in the die showed that pressure drop rate decreased with the increasing CO₂ concentration under same flow rate. This is because CO₂ had significant viscosity lowering effect on polystyrene melt. The authors claimed that a higher CO₂ concentration creates larger supersaturation ratios for nucleation, however, a larger pressure drop rate cannot be developed. On the other hand, experimentally determined pressure drop rate in the die was independent of CO₂ concentration. The discrepancy between two studies can be attributed to their different extent of melt viscosity reduction effect of CO_2 and experimental settings. In this study, only 0.25-0.75 wt% SC-CO₂ was utilized whereas Han et al. (2002) used up to 8 wt% CO₂ in their experiments. Starch-based melt did not hold more CO₂ than 0.75 wt% during SCFX processing.



Figure 4.2. Pressure profiles through 4.2 mm die with different SC-CO₂ levels (a) and in three different dies at 0.25 wt% SC-CO₂ level (b)

Die size	SC-CO ₂	Average pressure drop	Pressure at die
(mm)	(wt%)	rate (MPa/s)	entrance (MPa)
· · · ·	0.00	23.6	0.65
2.0	0.25	52.2	0.81
2.9	0.50	75.1	0.85
	0.75	121.7	1.24
	0.00	5.3	0.37
4.2	0.25	13.8	0.45
4.2	0.50	21.4	0.48
	0.75	34.5	0.59
5.9	0.00	1.3	0.24
	0.25	4.0	0.29
	0.50	6.6	0.31
	0.75	10.7	0.40

Table 4.2. Calculated pressure at die entrance and pressure drop rate through the die

In addition, the viscosity reduction effect of SC-CO₂ on the starch-based melt was relatively lower than polystyrene melt based on the experimentally determined rheological parameters (Table 4.1). The finding suggests that the increased volumetric flow rate due to additional gas volume could govern the pressure drop rate in starch-based SCFX processing. Moreover, the pressure at the die entrance in this study should be higher with 0.75 wt% SC-CO₂ since the pressure at the end of barrel was maintained at 10.7 MPa using a flow restrictor (Figure 4.1). This is because the pressure drop between the end of barrel and the die entrance would be smaller with higher SC-CO₂ level. As a result, higher pressure drop in the die with higher ratio of SC-CO₂ to feed was expected.

Scanning electron microscope (SEM) images of SCFX extrudates produced using the same formulation and process conditions in this study clearly show the effects of SC-CO₂ and pressure drop rate on the expansion and the morphology (Winoto, 2005). As the die diameter was decreased from 5.9 to 2.9 mm, the crosssectional expansion and the number of cells increased whereas the average cell size decreased (Figure 4.3).



Figure 4.3. Effects of SC-CO₂ and die size on product morphology (21X magnification) Left: 5.9 mm die, right: 2.9 mm die. Top: 0.5 wt% SC-CO₂, bottom: 0.75 wt% SC-CO₂ (Adapted from Winoto, 2005)

When the ratio of SC-CO₂ to feed was increase from 0.5 to 0.75 wt% SC-CO₂, the expansion and the cell number density further increased for each die. During SCFX process, dissolved gas and starch/moisture matrix exist as a single phase above

the gas solubility pressure at the temperature of interest. When, however, the pressure of melt drops below the solubility pressure, the gas/starch/moisture mixture becomes supersaturation, which is unstable stage. Thus the system would seek more stable stage with lower free energy resulting in nucleation. Diffusion of gas molecules into the nucleated cells would reduce free energy further. In classic homogenous nucleation theory (Park et al., 1995), the cell nucleation rate is given by

$$N_{\text{nucl}} = f_0 C_0 \left(-\Delta G_{\text{nucl}} / kT \right)$$
(10)

$$\Delta G_{\text{nucl}} = (16\Pi \pi \gamma^3 \text{bp}) / (3\Delta P^2) \tag{11}$$

where, N_{nucl} is the nucleation rate and ΔP is the pressure drop of the gas/starch/moisture mixture.

It can be seen from equations 10 and 11 that there could be less free energy change with higher pressure change resulting in faster nucleation. Since pressure drop in real foaming systems does not occur instantaneously, higher pressure drop rate would cause a greater degree of thermodynamic instability. Therefore, based on this theory, SCFX extrudate with 2.9 mm die using 0.75 wt% SC-CO₂ would show the faster nucleation and the highest cell number density (Figure 4.3).

Interestingly, more large voids or cells can be observed with 5.9 mm die and 0.75 wt% SC-CO₂ level compared to 2.9 mm die and 0.25 wt% SC-CO₂ level (Figure 4.3). Winoto (2005) provided two likely explanations for this finding: cell coalescence and presence of undissolved gas pockets. Another possible explanation could be generation of premature cells. Once nucleation occurs, the remaining gas molecules in starch/moisture matrix could either nucleate further or diffuse into the existing nucleated cells. If pressure drop rate through a die is high enough, nucleation would be dominant over cell growth. Still, however, the nascent cells which are nucleated at the very early stage of foaming process would keep growing due to gas diffusion into them resulting in larger cell size compared to that of cells generated later. Xu & Park

(2003) called the cells nucleated at the early stage of petrochemical-based foaming process as premature cells. The authors suggested that the amount of premature cells is a function of the cell number density, dissolved gas concentration, gas diffusivity and nucleation time. As the nucleation occurs earlier during foaming process, more large cells in final expanded foam would be obtained with less uniform cell size distribution. Han & Han (1988) also showed that the nucleation position through a visible slit die during polystyrene foam extrusion was a function of the blowing agent concentration and feeding rate. They observed that the cell nucleated earlier when more blowing agent was injected into the system. Recently, Potente, Ernest, & Oblotzki (2006) investigated the nucleation in steam based-extrusion of starchy materials using a slit die with transparent inserts. The nucleation length (NL) defined as the nucleation position at the center that is the farthest nucleation position from the die exit was found to be negatively correlated with pressure drop rate. In other words, the nucleation occurred later when they increased the pressure drop rate at the nucleation position. In this study, figure 4.2a and 4.2b show that nucleation would occur earlier in the die with higher SC-CO₂ level and larger die size suggesting that less uniform cell size distribution could be obtained with 5.9 mm die at 0.75 wt% SC-CO₂.

3.2. Cross-sectional expansion of SCFX extrudate

The initial cross-sectional expansion for the first 45 seconds was measured and plotted against time (Figures 4.4a and 4.4b). The result showed that the initial cross-sectional expansion of SCFX extrudate was time and SC-CO₂ concentration dependent. Figure 4.4a illustrates that the SCFX extrudate at 0.75 wt% SC-CO₂ level expanded up



Figure 4.4. Effects of SC-CO₂ level using 4.2 mm die (a) and die size at 0.5 wt% SC-CO₂ level (b) on the cross-sectional expansion of SCFX extrudates

to 10 times of the original cross-sectional area whereas SCFX extrudate at 0.25 wt% SC-CO₂ level expanded up to a maximum SEI of 6.1. Higher initial cross-sectional expansion with more blowing agent was expected since it provides more nucleation sites and more gas for cell growth. However, interestingly, not only the maximum cross-sectional expansion but also the expansion rate was increased with higher ratio of SC-CO₂ to feed. It was observed that the SCFX extrudate with 0.75 wt% SC-CO₂ injection rate reached its maximum cross-sectional expansion in 5 seconds whereas it took 20 seconds for the SCFX extrudates with 0.25 wt% SC-CO₂ level.

Three possible physical explanations for this finding could be suggested. First, the diffusion distance between nucleated cells and gas molecules would become shorter as the number of cells increases. As shown in Figure 4.5, more cells would be generated with higher blowing agent concentration.



Figure 4.5. Schematic of cell growth model during SCFX expansion (where, empty circle: nucleated cell, solid circle: CO₂ gas molecule, and the length of arrow: diffusion distance)

At the same time, there are more available gas molecules for cell growth in starch/CO₂/moisture matrix at higher SC-CO₂ level. Consequently, the average diffusion distance from each gas molecule to each nucleated cell becomes shorter resulting in faster cell growth, thus faster cross-sectional expansion at 0.75 wt% SC-CO₂ level compared to 0.25 wt% SC-CO₂ level. Second, a higher concentration gradient at the cell interface may lead to a rapid diffusion of SC-CO₂ into nuclei (Taki, Nakayama, Yatsuzuka, & Ohshima, 2003). Since the cell growth is a mass transfer controlled process, high SC-CO₂ concentration would help the mass transfer of CO₂ gas from starch/moisture matrix to nucleated cells. Finally, the viscosity reduction effect of SC-CO₂ may also be critical. The higher viscosity of the melt acts as resistance to extensional cell growth which is caused by the increasing pressure difference between the inside cell and surrounding starch/moisture matrix. Winoto (2005) reported that the consistency coefficient for the same SCFX extrudates decreased from 978 to 529 Pa.sⁿ possibly due to dilution effect when SC-CO₂ concentration was increased from 0.0 to 0.75 wt% (Table 4.1). The reduced viscosity of gas/starch/moisture matrix would be favorable for faster cell growth resulting in faster cross-sectional expansion.

Figure 4.4b shows that cross-sectional expansion increased more than twofold when the die diameter was decreased from 5.9 to 2.9 mm at 0.5 wt% SC-CO₂ level. This finding can be partially attributed to the die-swell effect of the elastic property of starch-based SCFX melt. The melt in a smaller die is subjected to larger normal stress and is constricted in the die for a shorter amount of time compared to those of the larger dies (Winoto, 2005). The larger normal stress resulted in more longitudinal contraction to relax the tension, and the shorter time in the die caused the relaxation to occur mostly outside the die, resulting in larger sectional expansion. Determined die-swell value in 20 seconds after exiting from 4.2 mm die accounted for 45, 36, and 32% of cross-sectional expansion at 0.25, 0.5 and 0.75 wt% SC-CO₂ level, respectively (Figure 4.4a). For comparison, Faller, Huff & Hsieh (1995) reported that die-swell of steam-based corn meal extrudates, which was calculated using a theoretical method based on an assumption that the cross-sectional and longitudinal expansions and contractions are equal in magnitude and direction, accounted for approximately 18-38% of the extrudate cross-sectional area.

However, it was clearly seen that higher SEI and faster expansion in smaller die was not only due to larger die-swell but also caused by higher pressure drop rate when cross-sectional expansion due to SC-CO₂ only (SEI_{SC-CO2}) was obtained by discounting the die-swell effect (Figures 4.6a and 4.6b). It was assumed that die-swell effect was constant for each SC-CO₂ injection level and initial SEI_{SC-CO2} could be modeled using the following power law model.

$$SEI_{SC-CO2} = 1.00 + A \times (Conc.)^{B} \times t^{[-C \times (Conc.) + D]}$$
(12)

where, SEI_{SC-CO2} is cross-sectional expansion index due to $SC-CO_2$ expansion only, Conc. is the $SC-CO_2$ concentration (wt%), t is elapsed time after emerging from the die and A, B, C, and D are power law constants. The constants of the power law equation for SCFX extrudates using three dies were obtained by iteration method using Matlab programming software (Mathwork Inc.) to obtain values with less than 5% errors.

This empirical model provided useful information on SCFX expansion. It can be seen from equation 12 that the rate of cross-sectional expansion due to SC-CO₂ only (SEI_{SC-CO2}) is a function of SC-CO₂ concentration. The rates of SEI_{SC-CO2} in 0.2 second was 0.4, 1.96 and 5.58 s⁻¹ at 0.25, 0.5 and 0.75 wt% SC-CO₂ level with 4.2 mm die, respectively (Figure 4.6a). This finding indicated that SCFX extrudate with 0.75



Figure 4.6. The effects of SC-CO₂ level using 4.2 mm die (a) and die size at 0.5 wt% SC-CO₂ level (b) on the cross-sectional expansion of SCFX extrudates due to SC-CO₂ expansion only

wt% SC-CO₂ level expanded most rapidly in cross-sectional direction possibly due to higher cell number density and available gas molecule concentration (Figure 4.5). The rates of SEI_{SC-CO2} started to decrease after approximately 0.2 second for all SC-CO₂ concentrations as indicated in equation 12 as a negative sign in power law index. This finding can be attributed to gas depletion in the melt. The dissolved gas in starch/moisture matrix would be depleted due to gas diffusion into nucleated cells and gas loss from the surface of extrudate to the environment. SCFX extrudates expanded until they reached maximum SEI and then did not grow in cross-sectional direction anymore since cell growth could be limited by gas availability in the melt matrix at long times (Figures 4.6a and 4.6b). The time to reach the maximum cross-sectional expansion, in turn the time for gas depletion was shorter with high ratio of SC-CO₂ to feed indicating that more dissolved gas would be utilized in shorter time due to higher pressure drop rate resulting in high cell number density. A similar trend was observed when SEI_{SC-CO2} was plotted against time varying die size at a constant SC-CO₂ concentration (Figure 4.6b). The proportionality constant (A) in equation 12 that is associated to the cross-sectional expansion rate at the very early stage (< -0.2 second) was higher with 2.9 mm die indicating that higher thermodynamic instability induced by smaller die enhanced the initial cross-sectional expansion. Based on the findings, it is would be reasonable to conclude that higher pressure drop favors faster and higher cross-sectional expansion in SCFX extrusion. In addition, it is observed that crosssectional expansion can be modeled using a power law equation because there is a decrease in cross-sectional expansion rate as the amount of dissolved gas in the matrix decreases.

Slight decrease in cross-sectional expansion of SCFX extrudates with 0.75 wt% SC-CO₂ injection rate in 20 seconds after exiting the die was observed suggesting there are two distinct phases, a growth phase followed by a shrinkage phase in SCFX

expansion, similar to conventional steam-based extrusion (Figure 4.4a). However, the growth time of SCFX extrudates was approximately 30-200 fold longer compared to a typical growth time of steam-based corn meal extrudates reported by Arhaliass et al. (2003). The unique time-delayed expansion of SCFX extrudates could be attributed to the relatively lower concentration of blowing agent compared to that of steam-based extrusion. In fact, SCFX extrusion shows higher cell density ($\sim 10^6$ cells/cm³), which would favor faster cross-sectional expansion, than steam-based extrusion does (~200 cells/cm³) (Alavi et al., 1999; Wang et al., 2005). However, the theoretically available blowing agent concentration in steam-based extrusion, which is in-barrel moisture content, reaches 18-28 wt% whereas only 0.25-0.75 wt% of SC-CO₂ was utilized as a blowing agent in this study. As discussed earlier, a greater concentration gradient between starch/moisture matrix and nucleated cells results in faster diffusion, thus faster cross-sectional expansion. According to Fick's laws, the mass flow rate of blowing agent molecules would be proportional to the gas concentration difference between the matrix and nucleated cells. Higher mass flow rate into nucleated cells would be beneficial for faster cell growth.

3.3. Longitudinal expansion of SCFX extrudate

The average SCFX extrudate velocity exiting the die increased by approximately 3-4 fold when die size was decreased from 5.9 to 2.9 mm (Table 4.3). It also increased further with SC-CO₂ injection possibly due to additional volume of the added SC-CO₂. The determined LEI varied from 0.27 to 0.41 for SCFX extrudates depending on SCFX process conditions. Compared to the maximum SEI, which varied from 6 to 10, LEI was significantly lower showing that SCFX expansion was nonisotropic. This finding concurred with the previous result that cross-sectional expansion was 2-8 fold higher than longitudinal expansion in corn meal-based SCFX extrusion (Sokhey, Rizvi, & Mulvaney, 1996).

	Die size					
	2.9 mm		4.2 mm		5.9 mm	
SC- CO ₂ (wt%)	Ave. extrudate velocity (m/s)	LEI	Ave. extrudate velocity (m/s)	LEI	Ave. extrudate velocity (m/s)	LEI
0.00	0.91	0.32	0.44	0.36	0.22	0.41
0.25	1.08	0.30	0.54	0.35	0.30	0.36
0.50	1.23	0.29	0.64	0.31	0.37	0.35
0.75	1.37	0.27	0.73	0.29	0.44	0.35

Table 4.3. Average extrudate velocity and longitudinal expansion index (LEI)

Interestingly, LEI was smallest with 2.9 mm die and 0.75 wt% SC-CO₂ injection rate suggesting that SCFX extrudates expanded more in cross-sectional direction at the direct expense of longitudinal expansion as die-swell effect. This showed a negative correlation between SEI and LEI. Trends similar to SCFX extrusion have been observed in conventional steam-based extrusion, which means higher SEI than LEI is a universal characteristic in both extrusion processes possibly due to the elastic property of starch-based formulation (Launay & Lisch, 1983).

It has been reported that there is a structural anisotropy of biomaterial based foams due to the different degree of expansion in cross-sectional and longitudinal directions (Guy & Horne, 1988). The authors suggested the different cell orientation could be responsible for the different mechanical properties of the extrudates in crosssectional and longitudinal directions. Online LEI measurement in this study would be useful not only for volumetric expansion calculation but also for correlation of the expansion characteristics and the textural properties of SCFX extrudates in the future research.

3.4. Volumetric expansion in SCFX extrudate

The volumetric expansion, which is the combination of cross-sectional and longitudinal expansion, showed the same trend as cross-sectional expansion because cross-sectional expansion was dominant in SCFX extrusion (Figures 4.7a and 4.7b). However, the difference in VEI among 0.25, 0.50 and 0.75 wt% SC-CO₂ SCFX extrudates was smaller than that in SEI due to reduced LEI particularly at higher SC-CO₂ concentration. VEI of control samples without SC-CO₂ injection varied from 0.95 to 1.03 indicating that there was insignificant effect of die-swell on VEI in SCFX extrusion. Guy et al. (1988) correlated the extrudate length-to-diameter ratio and the cell number per sample weight, and suggested that die swell controls the overall expansion of the extrudate in steam-based extrusion, especially at small number density of gas cells (approx. 600 cells/g). However, the length-to-diameter ratio analysis of extrudate might not be suitable for analysis of die swell because they could not decouple the effects of die swell and cell growth in cross-sectional expansion. Considering the mass conservation argument, it would be more reasonable to conclude that die swell has a minimal effect on the volumetric expansion of SCFX and steambased extrusion whereas cell nucleation and cell growth due to gas diffusion are mainly responsible for the volumetric expansion in both extrusion processes.

Once SCFX extrudates with 0.25 and 0.50 wt% SC-CO₂ levels reached their maximum volumetric expansion, they maintained the structure for up to 45 seconds. However, SCFX extrudates with 0.75 wt% SC-CO₂ level showed structure collapse in time (Figure 4.7a). Structure collapse was more evident particularly in SCFX extrudates using 2.9 and 5.9 mm die (Figure 4.7b). It has been reported that



Figure 4.7. The effects of SC-CO₂ level using 4.2 mm die (a) and die size at 0.5 wt% SC-CO₂ level (b) on the volumetric expansion of SCFX extrudates

extrudate collapse during foaming process occurs when the wall of cells becomes very thin and can no longer sustain the pressure inside cell (Wang et al, 2005; Moraru et al., 2003). This phenomenon is closely related to the rheological properties of starch/moisture matrix. Typically, low melt viscosities would facilitate cell collapse. Since SC-CO₂ has viscosity-lowering effect, SCFX extrudates with 0.75 wt% SC-CO₂ level could be susceptible to cell collapse. However, Alavi, Rizvi, & Harriott (2003) reported that thermosetting additives such as whey protein concentrate (WPC) can be used to increase the yield stress of starch/moisture matrix to reduce the driving force for cell collapse. In this study, 7 wt% WPC-34 was utilized to minimize cell collapse. For comparison, the degree of collapse of steam-based extrudates was found to be strongly related to in-barrel moisture content which is the blowing agent and plasticizer (Della Valle, Vergnes, Colonna, & Patria, 1997). When moisture content of the melt was increased, the initial maximum SEI of steam-based extrudates increased but the final SEI decreased due to extrudate collapse caused by the lower viscosity.

Structure collapse of SCFX extrudates can also occur when gas loss from cell to the environment becomes significant (Alavi et al., 2005; Lee, Ramesh & Campbell, 1996). As gas molecules in starch/moisture matrix are depleted, each cell ceases to grow but gas would escape from cell to the environment resulting cell collapse unless the structure is set. Since SCFX extrudates contained approximately 40 wt% moisture after extrusion, the structure was still pliable and susceptible to gas loss. In this case the thickness of cell wall would be important, since gas would diffuse out through cell-to-cell. In addition, if initial cell growth is very rapid, gas loss would be facilitated through the pliable thin cell walls. Since the initial volumetric expansion was faster in SCFX extrudate with 0.75 wt% SC-CO₂ (Figure 4.7a) and SCFX extrudate using 2.9 mm (Figure 4.7b), significant gas loss was expected through their thinner cell walls than other SCFX extrudates.

Another possible explanation for more structure collapse in SCFX extrudates with 0.75 wt% SC-CO₂ and 5.9 mm die is the generation of premature cell growth. It was found that premature cells have detrimental effects on the volumetric expansion of final foam product (Xu et al., 2003). When the amount of premature cell growth exceeds a critical level, significant decrease in volumetric expansion in polystyrene/CO₂ system was observed. The structure collapse due to premature cell growth was attributed to the rapid gas escape from premature cells to the environment. Based on the pressure profile calculation, the nucleation onset positions of SCFX extrudates with 0.75 wt% SC-CO₂ and SCFX extrudates using 5.9 mm die would be located far from die exit, which implies more premature cells growth (Figure 4.2). As a result, more irregularly large cells were observed (Figure 4.3). The results concur with the fact that the significant structure collapse of SCFX extrudate with 0.75 wt% and SCFX extrudate using 5.9 mm die occurred in 20 seconds after exiting the die (Figures 4.7a and 4.7b).

The relationship between the volumetric expansion of SCFX extrudates and gas loss can be clearly seen in Table 4.4. Although it was possible to obtain the most expanded product using 0.75 wt% SC-CO₂ injection rate, blowing agent efficiency (BAE) was lowest. This finding suggested that gas loss from SCFX foam surface was more severe at higher SC-CO₂ level through thin cell walls at early foaming stage. Low BAE in SCFX extrudates using 5.9 mm die could be attributed not only to lower pressure drop rate compared to 2.9 and 4.2 mm die resulting in less volumetric expansion, but also to significant premature cell generation due to early nucleation. It would be useful to take account of nucleation position in terms of die design for SCFX extrusion process to maximize volumetric expansion.

In addition, surface area of SCFX extrudates could be limiting factors in SCFX expansion due to gas loss from the surface. 2.9 mm die SCFX extrudate had higher

SC-CO ₂	BAE (%)		
(wt%)	2.9 mm die	4.2 mm die	5.9 mm die
0.25	41.9	55.2	30.2
0.50	36.3	34.4	25.0
0.74	26.1	24.7	19.2

Table 4.4. Blowing agent efficiency (BAE) of SCFX extrudates after 20 second exiting the die

surface to volume ratio than 4.2 and 5.9 mm die SCFX product (Figure 4.8). Although the difference decreased in time, 2.9 mm die SCFX extrudate showed 30-60% higher surface to volume ratio than those of 4.2 and 5.9 mm die SCFX extrudate. Since gas loss occurs mainly from the surface of SCFX extrudates, a larger surface area could be responsible for the promoted gas loss in 2.9 mm die SCFX extrudate. Lee et al. (1996) showed that the LDPE foam sheet thickness, which was associated with surface to volume ratio, had a strong inverse effect upon gas escape during sheet foaming process. Therefore, controlling surface to volume ratio by die dimension can be another strategy to enhance SCFX expansion.

Based on the findings regarding volumetric expansion and structure collapse, it is worth noting that the optimization of post-extrusion processes would be critical to increase volumetric expansion and blowing agent efficiency. The structure of SCFX extrudates with higher SC-CO₂ level and using smaller die should be set immediately, i.e. within 20 seconds after exiting the die, by drying, baking or frying to obtain highly expanded product having crispy texture. Newly emerging technologies such as convective microwave drying could be utilized to develop novel SCFX products with tailored texture. On the other hand, it would be possible to impart crunchy texture by equilibrating SC-CO₂ expanded products at ambient temperature or by baking at relatively lower temperature prior to the next post-extrusion operations. Chen et al. (2002) developed a SCFX extrusion process for masa-based SCFX chips and SC-CO₂ expanded chips were baked at 80°C prior to frying at 180°C to produce baked halfproduct. They were able to manipulate final expansion, thus texture and oil-uptake with pre-baking process. Moreover the delayed expansion characteristics would enable us to manipulate cell morphology, thus texture by stretching SCFX extrudates at different speeds since SCFX extrudates keep expanding and pliable for a quite long time after extrusion compared to steam-based extrudates. The findings in this study could be useful to optimize a SCFX extrusion and post-extrusion processes.



Figure 4.8. The effect of die size on the surface to volume ratio of SCFX extrudates at 0.5 wt% SC-CO₂

4. Conclusions

The effects of the ratio of SC-CO₂ to feed and pressure drop profile in the die were investigated and were found to be critical in controlling volumetric expansion of SCFX extrudates. Pressure drop rate could be varied using SC-CO₂ injection rate and
die diameter. It was observed that a high pressure drop rate is favorable for achieving a larger expansion ratio for the starch-based SCFX formulation. SCFX expansion showed a typical time-delayed behavior that can be utilized to produce novel products. Time to reach maximum sectional expansion varied from 3 to 20 seconds depending on SC-CO₂ injection rate. LEI varied from 0.27 to 0.41 and was inversely correlated with SEI indicating sectional expansion dominated SCFX expansion. Die-swell in SCFX extrusion accounted for 32-45% of sectional expansion while showed insignificant effect on volumetric expansion. SCFX extrudates with 0.75 wt% SC-CO₂ level and using 5.9 mm die showed significant structure collapse implying that the prevention of gas loss would be essential to increase the final volumetric expansion. In addition, the control over the surface to volume ratio of SCFX extrudates could be useful to achieve the maximum volumetric expansion, particularly of SCFX products using small die size. Based on the findings, strategies to enhance SCFX expansion such as to make nucleation take place as close as possible to die exit and to reduce time for structure solidification were proposed.

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CHAPTER 5 RESISTANT STARCH FORMATION IN SUPERCRITICAL FLUID EXTRUSION

Abstract

The formation of resistant starch (RS) during supercritical fluid extrusion (SCFX) was evaluated and compared to steam based-extrusion (SBX) for different formulations and processing conditions, such as the ratio of supercritical CO₂ to feed, in-barrel moisture content, and post-extrusion drying temperature. In general, corn starch-based SCFX extrudates showed a fourfold higher RS content than SBX extrudates, whereas the product densities of SBX and SCFX extrudates were comparable. The total RS content in both WPI added SCFX and SBX extrudates decreased due to a dilution effect. However, the RS content in the starch portion increased, indicating that the interaction between the whey protein and the lipid possibly led to additional double helix formations. This was supported by the results obtained from DSC and X-ray diffraction. Higher in-barrel moisture content during SCFX processing reduced the expansion of extrudates, but increased their RS contents. Limited amylopectin retrogradation of SCFX extrudates was observed at postextrusion drying temperatures of 85°C and 100°C compared to 25°C. On the other hand, SCFX extrudates dried at higher temperatures showed A-type polymorph, whereas SCFX extrudates dried at 25°C showed traces of B-type polymorph, which has a higher enzymatic digestibility than A-type. In-barrel moisture content and processing temperatures were found to be critical for RS formation in both the SCFX and SBX extrudates. The SCFX process demonstrated the potential to produce cerealbased foods with higher RS contents with expansion and texture comparable to SBX extrudates.

1. Introduction

Resistant starch (RS) is defined as the portion of starch that is not digested in the small intestine and passes into the colon (Sajilata, Singhal, & Kulkarni, 2006). Resistant starch, which is known to have a number of health benefits, is generally classified into four categories: physically inaccessible starch (RS1; i.e., partly milled grains and seeds), resistant starch granules (RS2; i.e., ungelatinized starch), retrograded starch (RS3), and modified starch (RS4) (Sajilata et al. 2006; Unlu & Faller, 1998). Extrusion cooking is widely used in the food industry for the production of breakfast cereals and snacks (Alavi, Gogoio, Khan, Bowman, & Rizvi, et al. 1999). Several researchers have reported that RS3 can be produced using steam-based extrusion (SBX) processing (Unlu et al, 1998; Adamu & Jin, 2001; Kim, Tanhehco, & Ng, 2006). Therefore, an effective approach to increasing the RS level in starchbased products is an optimized extrusion process. However, other researchers have reported a lack of RS formation during conventional SBX processing (Faraj, Vasanthan, & Hoover, 2004; Siljestrom et al. 1986), implying that the complex interactions among the operational parameters and the compositional variability preclude understanding of RS formation mechanisms. Low in-barrel moisture content (IBMC) (18~28% w.b.), which is detrimental for RS formation, is utilized in SBX to obtain desirable expansion (Moraru & Kokini, 2003).

Supercritical fluid extrusion (SCFX) uses supercritical CO_2 (SC-CO₂) as a blowing agent instead of steam, uncoupling the dual role of water as a blowing agent and a plasticizer (Rizvi, Mulvaney, & Sokhey, 1995). In SCFX process, high IBMC (40~60% d.b.) is used to increase SC-CO₂ solubility into the melt and to keep the product temperature below 100°C (Winoto, 2005). Such high moisture conditions, which enhance the mobility of starch molecules during the retrogradation process, are beneficial for RS generation in SCFX extrudates. Moreover, the post-extrusion drying process which is necessary to set the structure of SCFX extrudates can increase the RS content. It has been reported that heating or storage of starchy materials at elevated temperature (~100°C) can lead to A-type polymorph, rather than B-type polymorph (Eerlingen, Crombez, & Delcour, 1993). A-type crystallites have lower enzymatic digestibility, possibly resulting in greater RS formation compared to B-type crystallites (Farhat et al., 2001). Another advantage of utilizing the SCFX process for RS generation is its low operational temperature that enables the effective use of heatsensitive ingredients, such whey protein (Alavi et al., 1999). Recently, Lundqvist and Eliasson (2005) reported that the whey protein-lipid interaction reduces amylose-lipid complexation, whereas it enhances the amylose-amylose interaction, thus and starch retrogradation. Therefore, the use of whey protein in SCFX extrusion might increase not only nutrient density, but also RS content in the final products. In this is study, the potential of SCFX for the production of expanded extrudates with higher RS content was evaluated and compared to SBX. In addition, the effects of the ratio of supercritical CO₂ (SC-CO₂) to feed, whey protein isolate (WPI) concentration, IBMC and post-extrusion drying temperature (PDT) on RS formation were examined.

2. Materials and Methods

2.1. Materials

For the SBX process, unmodified corn starch (Gel 034570) and WPI (INPRO 90) were obtained from Cargill, Inc. (Minneapolis, MN) and Vitalus Nutrition, Inc. (Bellingham, W.A., UK), respectively. For the SCFX process, pregelatinized corn starch (Instant Gel) and WPI (Bipro®) were purchased from Cargill, Inc. (Minneapolis, MN) and Davisco Food International, Inc. (Eden Prairie, MN), respectively. Four formulations with different WPI concentrations (0, 6, 12, and 18 wt%) were prepared by mixing the two dry ingredients together in a premixer. Pregelatinized wheat starch

(Pregel, MGP Ingredients, Inc., Atchinson, KS) was also utilized for the SCFX process to investigate the effects of different starch type, IBMC, and PDT on RS formation.

2.2. Extrusion processing

SBX processing was performed at Kansas State University using a pilot scale twin-screw extruder (Model TX-52, Wenger Manufacturing, Sabetha, KS) with screw diameters of 52 mm and an L/D ratio of 16:1. A circular die opening of 3.3 mm was used. The feed rate of raw material was 60 kg/hr, and the screw speed was 200 rpm. Water flow into the extruder was adjusted to achieve the 22.7% and 26.8% (w.b.) IBMC. The temperature at the die was 140°C. The product was cut immediately after exiting the extruder die and dried at 100°C with a double-pass dryer/cooler (Wenger Manufacturing, Inc., Sabetha, KS) for 15 minutes.

SCFX processing was conducted at Cornell University using a pilot scale twin-screw extruder (TX-57 Magnum, Wenger Manufacturing, Sabetha, KS) with a barrel diameter of 52 mm and an L/D ratio of 28.5. SC-CO₂ was injected through valves located around the extruder barrel at L/D = 24. Two identical circular dies, each with a diameter of 4.2 mm, were utilized. During SCFX processing, pressure in the extruder barrel prior to the die entrance was built up to 10.3 MPa. The screw speed and dry feed rate were 120 rpm and 35 kg/hr, respectively. Water was injected into the preconditioning cylinder at a rate of 1.75 kg/hr. Additional water was injected into the barrel to vary IBMC from 33.1 to 41.4% (w.b.). The product temperature at die exit was maintained at approximately 55°C for all experiments. The ratios of SC-CO₂ to feed were 0.0, 0.25, 0.5, and 0.75 wt%. A convection oven was utilized to dry the extrudates at 85°C and 100°C for up to five hours. The final moisture content of the extrudates was approximately 5% (w.b.) after drying.

2.3. Experimental design

To investigate the effects of IBMC and WPI concentration on RS generation in native corn starch-based SBX extrudates, a 2 x 4 factorial design was utilized with two IBMC levels (22.7 and 26.8% w.b.) and four WPI concentrations (0, 6, 12, and 18 wt%). To determine the effects of the ratio of SC-CO₂ to feed and WPI concentration on RS generation in pregelatinized corn starch-based SCFX extrudates, a 4 x 4 factorial design was utilized with four ratios of SC-CO₂ to feed (0, 0.25, 0.5, and 0.75 wt%) and four WPI concentrations (0, 6, 12, and 18 wt%). IBMC and PDT were kept constant at 41.4% (w.b.) and 85°C, respectively. To evaluate the effect of IBMC on RS formation in pregelatinized wheat starch-based SCFX extrudates, a 4 x 3 factorial design was utilized with four ratios of SC-CO₂ to feed (0, 0.25, 0.5, and 0.75 wt%) and three IBMC (33.1, 37.5, and 41.4% w.b.) while keeping PDT constant at 85°C. Finally, to study the effect of PDT on RS formation in pregelatinized wheat starchbased SCFX extrudates, a 4 x 3 factorial design was utilized with four ratios of SC-CO₂ to feed (0, 0.25, 0.5, and 0.75 wt%) and three PDT (25, 85, and 100°C) while IBMC was kept constant at 41.4% (w.b.). In this study, native starch was utilized as the base material for the SBX process, whereas pregelatinized starches were utilized for the SCFX process. Sajilata et al. (2006) reported that the initial degree of gelatinization affects RS generation. It was assumed that the native and pregelatinized starches were fully cooked prior to the drying process under the process conditions utilized in this study. This would result in the negligible effect of degree of starch gelatinization on RS formation.

2.4. Piece density determination

Piece density (PD, kg/m³), defined as the ratio of the mass of the sample to its total volume including the voids, was determined using the geometrical measurements

(Winoto, 2005). The volume of the extrudate was calculated by multiplying the crosssectional area and the length, assuming that the extrudate is a straight cylinder.

2.5. Resistant starch determination

The Resistant starch (RS) content of SCFX and SBX extrudates was measured according to the procedure provided by described in the Megazyme resistant starch assay kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland) (Kim et al,. 2006). This method involved the incubation of ground extrudates in a solution of pancreatic α -amylase and amyloglucosidase at 37°C for 16 hours. After digestion, the residue was recovered by centrifugation, and the RS content was determined with spectrophotometry.

2.6. *Differential scanning calorimetry*

The thermal characteristics of SBX and SCFX extrudates were analyzed using a differential scanning calorimeter (TA2923, TA Instruments, NJ). Four mg samples were weighed in aluminum pans, and 4 mg of distilled water was added. A 1:1 ratio of starch to water was utilized to observe larger melting peaks compared to a 1:4 ratio of starch to water. The sample pans were hermetically sealed, and a sealed empty pan was used as a reference. Samples were heated from 20°C to 120°C at the rate of 10°C/min. Transition peak temperature (Tp) and transition enthalpy (dH) were recorded.

2.7. X-ray diffractometry

The crystalline pattern of the samples was studied by wide-angle X-ray diffraction (Scintag, Inc.) at operating conditions of 45 kV and 40 mA. The samples were scanned from 5° to 30° using a scan rate of 2° /min.

2.8. Statistical analysis

An analysis of variance (ANOVA) was conducted using the MINITAB statistical program (Minitab, Inc.). Unless otherwise indicated, all reported differences were statistically significant at $\alpha = 0.05$.

3. Results and Discussion

3.1. Piece densities of SCFX and SBX extrudates

Figure 5.1 shows the piece densities (PD) of WPI added to corn starch-based SBX and SCFX extrudates. The PD of SBX extrudates increased for all formulations when IBMC was increased from 22.7% to 26.8% (w.b.), indicating that lower IBMC promotes greater expansion during SBX processing. Higher IBMC lowers the viscous forces that resist expansion and lead to higher initial expansion, as well as greater collapse resulting in reduced expansion (Moraru et al., 2003). In general, the PD of WPI added to corn starch-based SCFX extrudates decreased under constant IBMC condition when SC-CO₂ was injected up to 0.5 wt%. This is because SC-CO₂ provided more nucleating sites and gas for cell growth, resulting in higher expansion. However, an increase in PD at the 0.75 wt% SC-CO₂ level compared to the 0.5 wt% SC-CO₂ level was observed for all formulations, possibly due to enhanced gas loss from the extrudate surface into the environment. The PD also increased as the WPI concentration was increased in the SCFX extrudates, indicating that whey protein suppresses extrudate expansion.



Figure 5.1. Piece densities of WPI added corn starch-based extrudates (Top: SBX, different IBMC, p < 0.05; bottom: SCFX, different ratio of SC-CO₂ feed, 41.4% IBMC dried at 85°C, p < 0.05)

It has been reported that proteins tend to reduce the expansion of SBX extrudates, resulting in denser and harder products in most cases (Allen, Carpenter, & Walsh, 2007). In this study, however, the piece density of the SBX extrudates decreased when

the WPI concentration was increased. This suggests that protein incorporation can increase expansion depending on process conditions (Cheng, Alavi, Pearson, & Agbisit, 2007).

Wheat starch-based SCFX extrudates at the same SC-CO₂ level (0.5 wt%), IBMC (41.4% w.b.), and PDT (85°C) had 30% higher PD compared to corn starchbased SCFX extrudates. This indicates that different types of starches yield different degrees of expansion (Figure 5.2). In general, the PD of wheat starch-based SCFX extrudates with 33.1% IBMC was lower than the PD of samples with 37.5% and 41.4% IBMC. This suggests that IBMC affects melt rheology and subsequent expansion. Lower IBMC leads to higher melt strength and reduced collapse, resulting in higher expansion at the same ratio of SC-CO₂ to feed. As expected, drying at 25° C did not lead to further expansion, whereas drying at 100°C resulted in higher expansion compared to drying at 85°C (Figure 5.2). It was possible to produce expanded products with various PD using SCFX and SBX processes by manipulating process parameters, such as ratio of SC-CO₂ to feed, IBMC, WPI concentration, and PDT. The PD of SBX and SCFX extrudates were comparable. This indicates that the resultant texture of both extrudates produced by the two different extrusion processes is similar. The PD of expanded foods is often used as a predictor of their mechanical properties due to the simplicity of measurement and the reasonable correlation with the textural properties.



Figure 5.2. Piece densities of wheat starch-based SCFX extrudates (Top: different IBMC, dried at 85° C, p < 0.05; bottom: different PDT, 41.4% IBMC, p < 0.05)

3.2. Resistant Starch

3.2.1. RS formation in SBX process

The total RS content (%, w.b.) of corn-based SBX extrudates was 0.64-0.76 wt% depending on IBMC and WPI concentration (Figure 5.3). The RS content of the SBX extrudates did not increase in relation to IBMC. This suggests that the difference in IBMC between 22.7% and 26.8% (w.b.) was not large enough to lead to significant difference in RS content. The total RS content decreased from 0.76 to 0.64 wt% at 22.7% (w.b.) IBMC level when the WPI concentration was increased from 0 to 18 wt%. This is likely because the whey protein addition diluted starch content and reduced the amount of available starch molecules per unit mass of extrudate needed for the generation of RS. Interestingly, however, the RS content in starch portion alone (RS content per starch weight) increased.

Recently, Kim et al. (2006) extruded pastry wheat flour under various conditions of feed moisture (20%, 40%, and 60% d.b.) and screw speed (150, 200, and 250 rpm) at 120°C. These conditions are similar to the processing conditions used in this study. Kim et al. reported that RS contents, determined using the same method as this study, of wheat flour-based extrudates with 20% (d.b.) feed moisture were 0.48-0.52% (d.b.). Although the extrusion process has been found to be a promising candidate for RS production due to its versatility, it has been reported that the concentration of RS generated using conventional steam-based extrusion (SBX) is lower than those from other RS manufacturing methods, such as autoclaving (Parchure & and Kulkarni, 1997). The relatively low RS content in SBX extrudates can be explained by the typical process conditions of SBX processing, such as low IBMC (14-28% w.b.) and high processing temperature (120°C-180°C). Starch retrogradation is accelerated when the starch concentration is approximately 50-60 wt% (Longton & LeGrys, 1981).



Figure 5.3. The effect of WPI concentration on RS contents in WPI added corn starchbased SBX extrudates (top, p < 0.05) and SCFX extrudates (bottom, 41.4% IBMC dried at 85°C, p < 0.05) where filled bars represent total RS content and empty bars represent RS content in starch portion

Minimum retrogradation is observed above 90 wt% starch concentration due to reduced mobility of starch molecules. On the other hand, retrogradation is also inhibited at lower than 10 wt% starch concentration due to dilution effects (Longton et al., 1981). High processing temperature (120°C-180°C) in SBX is required not only for starch gelatinization and protein denaturation, but also for the generation of steam that acts as a blowing agent (Moraru et al., 2003). In most cases, rapid steam flash off occurs during SBX due to high temperatures at the die, resulting in a decrease in moisture up to 5~10% in the final extrudates (Moraru et al., 2003). This rapid moisture loss does not allow enough time for starch molecules to re-associate and leads to a low RS content.

3.2.2. RS formation in SCFX process

The total RS contents of corn starch-based SCFX extrudates at 41.4% (w.b.) IBMC varied from 2.53 to 3.08 wt% depending on the ratio of SC-CO₂ to feed and WPI concentration (Figure 5.3). A decrease in total RS contents was observed when the ratio of SC-CO₂ to feed was increased from 0 to 0.75 wt% for all formulations. Overall, it can be clearly seen that SCFX extrudates showed approximately four times higher RS content than SBX extrudates. Because there is difference in processing conditions between SBX and SCFX, direct comparison is not appropriate. However, the higher IBMC of SCFX, which would increase starch molecule mobility, could be partially responsible for higher RS contents in SCFX due to low product temperature (55°C) leading to more available moisture content for RS generation. To investigate the effects of starch type and IBMC on RS generation in SCFX, wheat starch-based SCFX extrudates were produced using three different IBMC and dried at 85°C (Figure 5.4).



Figure 5.4. The effects of in-barrel moisture content (top, dried at 85° C, p < 0.05) and post-extrusion drying temperature (bottom, 41.4% IBMC, p < 0.05) on RS content in wheat starch-based SCFX extrudates

At the same IBMC (41.4% w.b.) and PDT (85°C), wheat starch-based SCFX extrudates showed approximately 40% lower RS content than corn starch-based SCFX extrudates, indicating that RS generation is dependent on starch type. The difference in the extent of retrogradation and RS content between wheat and corn starch might be due to differences in amylose content, molecular sizes of amylose and amylopectin, and lipid content and its composition (Sajilata et al., 2006). In general, RS content increased when IBMC was increased from 33.1% to 41.4% (w.b.), indicating that higher IBMC leads to higher RS content. For this reason, IBMC is critical for controlling RS generation.

Kim et al. (2006) reported that RS content increased from 0.5 to 2.59 wt% (d.b.) when IBMC was increased from 20 to 60% (d.b.). The findings showed that it would be possible to increase RS content of SBX extrudates by increasing IBMC. However, higher IBMC content is detrimental to the expansion of SBX extrudates, leading to denser products as shown in Figure 5.1. The results in this study show that SCFX processing is suitable for the production of highly expanded starch-based foams with relatively high RS content compared to SBX processing without any loss of textural properties.

The total RS content of WPI added corn starch-based SCFX extrudates was decreased when WPI concentration was increased from 0 to 18 wt% due to dilution effects (Figure 5.3). RS content in starch portion of SCFX extrudates was higher than control samples without WPI addition for each ratio of SC-CO₂ to feed showing that whey protein incorporation could increase RS generation by approximately 5%-20%. The effect of protein on starch retrogradation has been studied, and the results have been variable and dependent on the protein type, formulation, and processing conditions employed. Escaparp, Gonzalez, Morales, & Saura-Calixto (1997) reported that RS yields in autoclaved and retrograded mixtures of starch/albumin decreased

compared to control (without protein) when the albumin concentration was increased. Escaparp et al. attributed this to a reduction in available amylose content for retrogradation via starch/protein interaction. Ottenhof and Farhat (2004) investigated the retrogradation of amylopectin in a wheat starch/gluten (10:1) blend prepared by extrusion and concluded that there was no evidence of any significant effect of the presence of gluten on starch retrogradation under the experimental conditions used. On the other hand, Lundgvist et al. (2005) showed that the addition of whey protein enhanced starch retrogradation due to the whey protein-lipid interaction. Lipids, especially free fatty acids, affect starch granule swelling, gelatinization, and retrogradation by complexation with amylose. Although amylose-lipid complex can be digested more slowly than free amylose, and even shows partial resistance to enzymatic hydrolysis, it is known that such a complex hinders double helix associations, thus inhibiting RS formation (Chung, Jeong, & Lim, 2003). Chung et al. (2003) investigated the effect of defatting high amylose corn starch on RS content in freeze-thawed starch and found that defatting increases RS content. It is known that the monomer form of beta-lactoglobulin, one of the major whey proteins, is capable of binding hydrophobic components, such as fatty acids (Lundqvist et al., 2005). When beta-lactoglobulin is added to a starch solution containing endogenous lipids, there might be a competition over the lipids between beta-lactoglobulin and starch leading to an increased amount of available amylose for retrogradation and RS generation (Lundqvist et al., 2005; Zhang & and Hamaker, 2004). Therefore, increases in RS content in the starch portion due to WPI incorporation in this study could be due to the whey protein-lipid interaction and increased amylose-amylose double helix formation. The increase in RS content was limited up to 20% because the amount of endogenous lipid in corn starch was less than 1 wt%. However, incorporation of whey protein in

starch-based extrudates would be a useful approach to increase not only nutrient density, but also the amount of RS in lipid-rich starch-based formulations.

Wheat starch-based SCFX extrudates did not show any typical trend with regard to RS content when PDT was increased from 25°C to 100°C (Figure 5.4). The effect of PDT on RS generation in SCFX can be twofold. First, PDT governs the postextrusion drying rate. SCFX extrudates dried at higher temperature lose moisture faster. Because starch recrystallization can only occur above glass transition temperatures, the lower PDT (25°C) that results in longer time for amylose reassociation is beneficial for starch retrogradation in SCFX extrudates. Second, PDT determines the starch recrystallization rate and polymorphism of starch crystallites in SCFX extrudates. Nucleation is promoted at temperatures far below the melting temperature (Tm) of crystals, but above glass transition temperature (Tg). On the contrary, propagation is limited under these conditions (Eerlingen et al., 1993). At temperatures far above Tg but below Tm, propagation is favored and nucleation is limited. The overall crystallization rate depends primarily on the nucleation and propagation rates and is generally at its maximum at the average of Tg and Tm. Eerlingen et al. (1993) investigated the effect of heating temperature on the formation of RS. They found that initially the highest RS concentration was obtained at 0°C, whereas the highest yield of RS was obtained at 100°C for long storage times. They concluded that the propagation of amylose crystals was favored at 100°C, even though the nucleation rate was rather limited, resulting in more RS starch generation. Shamai, Bianco-Peledb, & Eyal Shimoni (2003) examined the effects of storage temperature (40°C and 95°C) on the polymorphism of RS3 of high amylose corn starch, corn flour, and wheat starch. Eerlingen et al. (1993) found that retrogradation at 40°C for 24 hours led to the formation of B-type polymorph that formed faster and included more water molecules. A mixture of V-type and A-type, which has denser unit cells

compared to B-type, was obtained with storage at 95°C. Farhat et al. (2001) also reported that potato starch extrudate stored at 22°C exhibited B-type polymorph while the samples retrograded at 60°C showed A-type polymorph. They stated that the enzymatic digestibility of potato extrudates stored at a higher temperature was lower, indicating that A-type polymorph leads to a higher RS content. The general trend in the literature is that recrystallization in high water content and/or low temperature conditions leads to B-type polymorph, whereas low water content and/or high temperatures yield the A type. In this study, higher RS contents were acquired at 85°C or 100°C compared to 25°C depending on the ratio of SC-CO₂ to feed (Figure 5.4). It was found that PDT (85°C -100°C) can be utilized for increasing RS content in SCFX extrudates.

3.3. Thermal properties

It has been reported that melting peaks of approximately 40°C-60°C represents amylopectin recrystallization, whereas the melting peaks of amylose-lipid complex and amylose-amylose complex appear at 85-110°C and 130-170°C, respectively (Kim et al., 2006). DSC results indicated that there was a limited amount of amylopectin retrogradation in corn starch-based SBX and SCFX extrudates (Figure 5.5). Moreover, amylopectin retrogradation was reduced in corn starch-based SBX and SCFX extrudates when 18 wt% WPI was incorporated due to the dilution effect. A melting peak between 75°C and 88°C in 18 wt% WPI SCFX extrudates was observed. This peak might represent the melting of partially denatured whey protein, indicating that whey protein in SCFX extrudates is not fully denatured under the processing conditions used in this study (Alavi et al, 1999; Erdogadu, Czuchajowska, & Pomeranz, 1995). Interestingly, the amylose-lipid complex formation was reduced with the addition of WPI in both extrusion processes.



Figure 5.5. DSC thermograms of WPI added corn starch extrudates (Top: SBX, 22.7% IBMC; bottom: SCFX, 41.4% IBMC, 0.5 wt% SC-CO₂ level, dried at 85°C)

The melting enthalpy of the amylose-lipid complex was decreased from 0.33 to 0.18 J/g and from 0.30 to 0.07 J/g for SBX and SCFX extrudates, respectively, when 18 wt% WPI was incorporated. As previously discussed, this can be explained by the dilution effect due to the addition of whey protein, as well as the whey protein-lipid and/or amylose-whey protein-lipid complexation (Zhang et al., 2004). Erdogdu et al. (1995) also reported that the enthalpy of the amylose-lipid complex of starch solution was eliminated or reduced by the addition of whey protein. DSC results supported the presence of competition between amylose-lipid complexation and whey protein-lipid complexation, resulting in increased RS content. The melting enthalpy of amylopectin retrogradation decreased from 0.19 to 0.03 J/g when IBMC was decreased from 41.4% to 33.1% (w.b.), suggesting that higher IBMC and higher moisture in extrudates favored amylopectin retrogradation (Figure 5.6).

The melting enthalpy of amylopectin retrogradation of SCFX extrudate dried at 25°C was approximately six times higher than those of SCFX extrudates dried at 85°C and 100°C. This suggests that amylopectin retrogradation is limited in SCFX extrudates dried above 85°C (Figure 5.6). This is likely because drying at lower temperature increases not only the time required for retrogradation due to a slower drying rate, but also the recrystallization rate of amylopectin, rather than amylose. First, unlike amylase, the crystallization of amylopectin is a slow process that continues over a period of several hours to days (Eerlingen et al., 1993). In this study, the available time period for starch molecule reassociation was relatively short at 85°C and 100°C (i.e., less than five hours). Second, the lower thermal stability of amylopectin crystallites compared to amylopectin crystallites favors faster amylopectin retrogradation at 25°C. Although amylopectin crystallites melt in the temperature range of 40°C to 60°C, amylose crystallites require much higher temperatures to melt (i.e., 120°C to 170°C) (Kim et al., 2006).



Figure 5.6. DSC thermograms of wheat starch-based SCFX extrudates at the 0.5 wt% SC-CO₂ level (Top: different IBMC, dried at 85°C; bottom: different PDT, 41.4% IBMC)

As previously discussed, maximum recrystallization occurs at mid-temperature between Tg and Tm of starch molecules. For example, B-type starch gels containing more than 27% (w.b.) water have a Tg of approximately -5°C (Eerlingen et al., 1993). The Tm of amylopectin and amylose crystallites are 60°C and 150°C, respectively, indicating that maximum retrogradation would occur at approximately 27.5°C and 72.5°C for amylopectin and amylose, respectively. This suggests that amylopectin recrystallization would be limited or prevented at drying temperatures above 70°C, whereas amylose recrystallization would still occur. The findings in this study demonstrate that amylopectin retrogradation is limited at 85°C and 100°C, whereas amylose retrogradation can be responsible for significant RS content in SCFX extrudates dried at 85°C and 100°C.

3.4. X-ray diffraction

Corn starch-based SBX and SCFX extrudates exhibited a combination of V and A polymorphs (Figure 5.7). V-type patterns that can be characterized by strong peaks at $2\theta = 13^{\circ}$ and 20° represent amylose-lipid complexes with a single helix. Atype patterns, which have reflections at $2\theta = 15^{\circ}$, 17° , and 23° , indicate the presence of double helical crystallites. Zhang et al. (2004) reported an increase in X-ray diffraction intensity of starch/whey protein/fatty acid solution at $2\theta = 20^{\circ}$ due to three components complexation. Zhang et al. concluded that whey protein reduced the amount of amylose-lipid complex, but stabilized the amylose-lipid complex, which leads to a more organized structure. In this study, a decrease in the peak intensity of WPI added SBX extrudates at $2\theta = 20^{\circ}$ was observed, whereas no significant change was seen in WPI added SCFX extrudates. The addition of 18 wt% WPI did not lead to a significant difference in the peak intensities of SBX and SCFX extrudates at $2\theta =$ 17° and 23° .



Figure 5.7. X-ray diffractograms of WPI added corn starch-based SCFX extrudates (top: SBX, 22.7% IBMC; bottom: SCFX, 41.4% IBMC, 0.5 wt% SC-CO₂ level, dried at 85°C)

The peak intensities at $2\theta = 17^{\circ}$ and 23° have been utilized as the indicators of double helical structure (Farhat et al., 2001).

However, a higher crystallinity in the starch portion due to the enhanced double helix formation was expected because of the dilution effect due to the addition of the whey protein. These results indicated a higher RS content of whey protein in the SBX and SCFX extrudates. Corn starch-based SCFX extrudates showed higher intensities at $2\theta = 17^{\circ}$ and 23° than corn starch-based SBX extrudates, suggesting that SCFX extrudates showed more double helix formation compared to SBX extrudates. Wheat starch-based SCFX extrudates dried at 85°C also had a mixture of V and A polymorphs showing strong peaks at $2\theta = 15^{\circ}$, 17° , and 23° (Figure 5.8). On the other hand, there were not only V and A polymorphs, but also traces of B polymorph represented by additional shoulders at $2\theta = 5.5^{\circ}$ and 24° were observed in the wheat starch-based SCFX extrudates dried at 25°C. This result is consistent with the finding in the literature that A polymorph was formed at a higher temperature, whereas B polymorph was obtained at a lower temperature (Farhat et al., 2001). A higher extent of amylopectin retrogradation in wheat-based SCFX extrudates dried at 25°C might be partially responsible for stronger peak at $2\theta = 17^{\circ}$ compared to SCFX extrudates dried at 85°C.

The comparable RS contents of wheat starch-based SCFX extrudates dried at 25° C and 85° C can be attributed to A-type crystallites which have lower enzymatic digestibility formed at higher PDT. Although the X-ray diffraction technique was useful for the identification of polymorphs of SBX and SCFX extrudates in this study, crystallinity quantification was limited due to the relatively small portion of RS content in the samples (< 3 wt%). Future studies should include the use of C¹³ solid NMR or FTIR because they are able to detect minor difference in crystallites. The results of such studies would be beneficial for understanding RS formation in SBX

and SCFX extrudates.



Figure 5.8. X-ray diffractograms of wheat starch-based SCFX extrudates with 41.4% IMBC at the 0.5 wt% SC-CO₂ level (different PDT)

4. Conclusions

The potential benefits of the SCFX process for the generation of resistant starch (RS) compared to conventional steam-based extrusion (SBX) were evaluated. In general, corn starch-based SCFX extrudates showed RS contents that were approximately four times greater than the SBX extrudates, whereas the piece densities of SBX and SCFX extrudates were comparable. It was found that the RS content in the starch portion of SBX and SCFX extrudates increased as much as 20% when WPI was added. This indicates that whey protein incorporation is useful not only for nutrient fortification, but also for RS generation during the extrusion processing. A higher in-barrel moisture content during SCFX processing reduced the expansion of SCFX extrudates, but increased their RS contents. The drying of the SCFX extrudates at higher temperature (i.e., 85°C and 100°C) limited amylopectin retrogradation, but resulted in similar or even higher RS contents compared to the 25°C drying temperature condition. This suggests that enhanced amylose recrystallization occurred at higher temperatures. The results indicated that in-barrel moisture content and post-extrusion drying temperatures were both important factors for the formation of RS during SCFX processing. In this study, SCFX processing was found to be useful for the production of highly expanded cereal-based foods with higher RS contents compared to SBX-based extrudates.

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CHAPTER 6

NEW GENERATION OF HEALTHY SNACK FOOD BY SUPERCRITICAL FLUID EXTRUSION

Abstract

Supercritical fluid extrusion (SCFX) process has been successfully developed for production of novel healthy snack containing 40~60 wt% protein with unique porous structure and texture. SC-CO₂ injection rate and product temperature at the die were found to be critical to control the expansion and texture of the final product. Maximum cross-sectional expansion was obtained at 0.3 wt% added SC-CO₂ whereas more uniform internal structure was achieved at 0.7 wt% SC-CO₂ level. As WPC-80 concentration was increased from 52.8 to 78.2 wt% in the formulation, the crosssectional expansion of baked and fried products increased by 65.8% and 44.4%, respectively. It was observed that lower viscosity of whey protein compared to starch decreased expansion but helped enhance further expansion during post-extrusion drying. The finding showed that an extrusion process at the temperature below protein denaturation temperature using SC-CO₂ can help to prevent hard texture due to the thermosetting property of whey protein and to create a uniformly expanded structure. The textural properties were comparable to commercial extruded or fried chip products.

1. Introduction

Fried starch-based snacks are frequently portrayed as less than desirable foods due to their high caloric content and low nutrient density. On the other hand, proteinenriched snacks are gaining in popularity as healthier alternatives. Numerous attempts have been made to incorporate more protein in starch-based snack formulations, not
only to improve their nutritional quality but also to utilize protein-rich byproducts such as shellfish, minced fish, spent hen, bovine lung, cheese whey (Gilbert & Rakshit, 2005; Jaya Shankarar et al., 2005; Jaya Shankar & Bandyopahyay, 2005; Rhee et al., 2004; Lee et al., 2003; Chavez-Jauregui et al., 2003; Onwulata et al. 2001). Among these protein sources, more attention has been paid to cheese whey due to its abundance and nutritional benefits. Cheese whey utilization within the United States is still low and most of cheese whey is used as animal feed (Onwulata et al., 2001). Whey protein, however, is a rich source of branched chain amino acids and has well balanced amino acid profile. It is reported that whey protein offers benefits in dealing with weight management, cardiovascular health and even cancer prevention (Layman, 2003; Miller, 2000; Bounous, 2000). Whey protein concentrates also contain significant amounts of calcium that can be an added advantage when fortifying food products.

Extrusion cooking is widely practiced in cereal-based snack production due to its versatility (Martinez-Serna & Villota, 1992). Several research groups have tried to fortify starch-based extruded products with whey protein (Onwulata et al., 2003; Matthey & Hanna, 1997; Martinez-Serna & Villota, 1992; Kim & Maga, 1987). In general, the previous findings suggest that whey protein incorporation into conventional steam-based extrudates create adverse effects such as reduced expansion and increased bulk density, resulting in hard and brittle texture especially when whey protein concentration is increased above 35 wt%. The detrimental effect of whey protein has been attributed to its heat sensitive thermosetting property, interaction with starch, and limited hydration during conventional steam-based extrusion, which involves high temperature (>120°C) and short residence time. As a result, the amount of incorporated whey protein in extruded snack without any chemical modifications has been limited to about 35 wt% (Onwulata et al., 2003). Recently, researchers at Utah State University developed a process for texturizing whey protein concentrate (~80 wt% whey protein) with corn starch using a steam-based extrusion system (Taylor & Walsh, 2002). This extruded product blend containing 20-40 wt% starch and 60-80 wt% whey protein concentrate can be utilized as puffed snacks or meat extenders. This technology involves steam-based expansion at high temperature (~150°C) and pH modification using HCl and NaOH.

Supercritical fluid extrusion (SCFX) is an extrusion technology that uses supercritical carbon dioxide (SC- CO_2) as a blowing agent enabling formation of expanded structure at lower temperature ($<100^{\circ}$ C). In the SCFX process, expansion of the melt is achieved by first solubilizing $SC-CO_2$ in the melt, and then inducing nucleation due to pressure drop in the die, which is followed by cell growth caused by diffusion of CO₂ into the nucleated cell (Rizvi & Mulvaney, 1995). Since SCFX extrusion is conducted at low temperature (~60-80°C) and low shear conditions compared to conventional steam based extrusion, nutritional loss of heat sensitive ingredients is minimized. The lower pH of the melt due to dissolved SC-CO₂ inhibits Maillard reaction, which otherwise would cause further loss of essential amino acids (Mulvaney & Rizvi, 1993). Moreover, SC-CO₂ expanded extrudates show predominantly homogeneous closed cell structures and nonporous surface, which not only facilitate flavor encapsulation but also provide better textural control over steambased products (Alavi et al., 1999). Previous studies on uses of SCFX in our laboratory have shown that the physical properties of starch-based SCFX extrudates are governed by both extrusion and post-extrusion parameters, including die geometry, pressure drop rate, residence time, ingredient composition, and drying temperature (Alavi et al. 1999). The SCFX process has also been successfully utilized to produce a unique masa-based chip product (Chen, Dogan & Rizvi, 2002).

Based on our previous findings, SCFX processing of extruded snacks with high whey protein containing formulations (>50 wt%) would offer possibilities for producing new generations of healthy snacks with high nutrient density and novel texture. The challenging problem of hard texture of high whey protein-based extruded product could be prevented by extrusion at low temperature (below 70°C) without any chemical modifications since the denaturation temperatures of whey proteins including β -lactoglobulin, α -lactalbumin, bovine serum albumin, and immunoglobulin range from 59 to 82°C. The effects of SC-CO₂ injection rate and starch gelatinization of whey protein-based extrudate would be particularly critical to the structural and textural development of SC-CO₂ expanded snacks. Post-extrusion processes such as baking and frying would impart not only desirable flavor and appearance to SCFX product but also generate further expansion since SCFX extrudates experience a timedelayed expansion until the structure is set (Alavi et al., 1999; Chen et al., 2002).

In addition, understanding on the rheological properties of protein-based dough would be beneficial for SCFX process design and product quality improvement. It has been reported in the literature that not only process parameters but also melt rheology would determine the expansion characteristics and the microstructure, thus textural properties of extruded products. Whereas the viscosity of starch-based melt has been extensively investigated and correlated to expansion process, there is lack of information on the viscosity of concentrated protein melt or dough. The viscosity data of whey protein-based dough could be utilized, for example, for selecting SC-CO₂ injection rate during SCFX processing and post-extrusion baking temperature.

The objectives of this study were to measure the apparent viscosity of whey protein-based dough, to use the rheological parameters to develop an effective process for expanded SCFX chips with high nutrient density and to evaluate physical and textural properties of the final products. Comparison of SCFX products with commercial snack products was also performed to evaluate product acceptability.

2. Materials and methods

2.1. Materials

Whey protein concentrate (WPC-80, ~80 wt% protein) was obtained from commercial sources and its moisture content was adjusted to 10 wt% prior to SCFX process to prevent hydration problem due to short residence time during extrusion process. Pregelatinized corn starch and masa flour were obtained from Midwest Grain Products (Atchison, KS) and Lifeline foods (Saint Joseph, MO), respectively. Masa flour was chosen not only to improve the texture of SCFX expanded snack but also to impart a unique flavor to final product. Oat fiber that was added to improve structural stability of high whey protein samples was provided from Grain Millers (Saint Ansgar, IA). A water-dispersible fluid lecithin was provided by Central Soya (Fort Wayne, IN) and mixed with vegetable shortening before direct injection. Lecithin and vegetable shortening were added to provide better mixing and a softer texture, particularly in baked SCFX products. Powdered ingredients including WPC-80, masa flour, pregelatinized corn starch, oat fiber and salt were mixed in a mixer for one hour. Formulations, which were used in this study, are summarized in Table 6.1.

2.2. Dough viscosity measurement

The apparent viscosity of whey protein-based dough was determined using shear rate vs. shear stress (flow) tests at 65°C, with ARES rheometer (TA Instruments, New Castle, DE). Four whey protein dough (S1, S2, S3 and W4 in Table 6.1 except oat fiber addition) was prepared by mixing with 36 wt% water in a Horbart mixer

(Kitchen Aid) for 10 min. A cone and plate geometry (anodized aluminum cone, 6 cm diameter, 28 angle) was utilized.

Formulation labeling	S 1	S2	S3	W1	W2	W3	W4
WPC-80	52.5	52.5	52.5	52.8	60.6	70.3	78.2
Masa flour	12.6	18.5	24.3	16.6	12.7	7.8	3.9
Pregelatinized starch	24.3	18.5	12.6	16.6	12.7	7.8	3.9
Oat fiber	0.0	0.0	0.0	3.3	3.3	3.3	3.3
Salt	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Lecithin	3.0	3.0	3.0	3.1	3.1	3.1	3.1
Vegetable shortening	4.1	4.1	4.1	4.1	4.1	4.1	4.1

Table 6.1. Feed Formulations (wt%, wet basis)

2.3. Extrusion

A Wenger TX-57 (Wenger Manufacturing, Sabetha, KS) co-rotating twinscrew extruder with 4.5 heads, barrel diameter of 52 mm, and L/D ratio of 28.5 was configured for supercritical fluid extrusion. CO₂ gas (99.9% purity, Airgas, NY) was siphoned off from a cylinder and completely liquefied by a cooling bath. Then is pressurized by a liquid pump up to 20.7 MPa and the temperature was adjusted to 40°C in a vessel. Generated SC-CO₂ was pumped by a positive displacement pump for injection in the barrel. A SC-CO₂ generation and metering system with a PID control loop was used to stabilize and maintain the mass flow to the set point at high operating pressure range between 10 and 20 MPa and target temperature (Chen & Rizvi, 2006). A back-pressure type regulator was used to correct any changes in the extruder conditions rapidly and smoothly. SC-CO₂ was injected into the whey protein based melt through four valves located around the extruder barrel at L/D = 24. SC-CO₂ fluid was injected at constant flow rates (0.3, 0.5, and 0.7 wt% wet basis) into the dough melt. A flow restrictor plate was installed on the exit end of the last barrel and before the die assembly to maintain and regulate pressure. During SCFX processing, pressure in the extruder barrel prior to the die entrance was built up to 12 MPa. The pressure profile in the barrel is shown in Figure 6.1. SC-CO₂ injection pressure was automatically maintained higher than pressure inside the barrel for a continuous SC-CO₂ into the starch melt, at the desired rate and pressure. A slight drop in pressure was achieved at the SC-CO₂ injection point by placing a reverse screw element followed by a cut-flight screw element. This was done to allow the SC-CO₂ to move only in the forward direction. A reverse screw element at the end of the barrel provided a restriction for building up pressure for enhanced gas solubility near the die. The pressure was mainly controlled by the pressure restrictor located before the die.



Figure 6.1. The pressure profile in the barrel and SC-CO₂ injection point

The screw speed and dry feed rate were 130 rpm and 35 kg/h, respectively. Feed moisture content was maintained as 36 wt% by injecting water directly into the preconditioner and extruder. The mixture of lecithin and vegetable shortening was directly injected into the barrel. A teflon slit die (1.4 mm long X 35 mm wide) was used. Two product temperatures at the die, 65°C and 75°C were utilized to investigate the effect of melt temperature on the physical and textural properties of SCFX extrudates.

2.4. Post-extrusion process

The extrudates emerging from the die were collected on metal trays and immediately cut to approximately 35 mm in length. For baked products, the extrudates were baked in an impinger oven (Lincoln Foodservice Products, Inc. Fort Wayne, IN) at 175°C for 4 min to set the structure. It was followed by further drying at 110°C for 8-12 min to reduce the moisture content to approximately 5-6 wt%. For fried products, the extrudates were air-cooled for structure setting for 1-2 hours prior to frying. Air-cooled extrudates containing 30-33 wt% moisture content were submerged in the fryer at 175°C for 2 min. The baked products and the fried products were stored at 11.3% relative humidity for 48 hours for moisture equilibrium and kept in polyvinyl bags until they were analyzed. The moisture content of all finished product ranged from 4 to 5 wt%.

2.5. Experimental plan

In this study, two sets of experimental design were utilized. To investigate the effects of SCFX processing parameters such as $SC-CO_2$ injection rate, product temperature, and the degree of starch gelatinization on the physical and textural properties of SCFX whey protein-based snack, a 3 x 3 x 2 factorial design was

employed. Three SC-CO₂ injection rates (0.3, 0.4, and 0.7 wt%) and three different formulations (S1, S2 and S3 in table 1) having 2:1, 1:1 and 1:2 pregelatinized corn starch to masa flour ratio at two product temperatures (60°C and 75°C) were used. By varying the ratio of pregelatinized corn starch to masa flour, it would be possible to manipulate not only the degree of gelatinization of whey-protein-based dough but also the dough viscoelasticity which would play critical roles in cell nucleation and cell growth. Two different product temperatures would allow us to see the effect of temperature on the melt viscosity, thus SCFX expansion since protein denaturation and melt viscosity are dependent on melt temperature. SC-CO₂, the blowing agent in this study also has viscosity reduction effect (Chen et al., 2006). To determine the effect of total whey protein concentration, WPC-80 concentration was varied from 52.8 to 78.2 wt% (W1, W2, W3 and W4 in table 1) whereas SC-CO₂ injection rate, product temperature (PT), and the pregelatinized corn starch to masa flour ratio were fixed at 0.7 wt%, 65°C, and 1:1, respectively. Increasing whey protein concentration would impart nutritional benefits on SCFX chips. At the same time, higher whey protein concentration would affect the expansion during SCFX process and postextrusion processes such as baking and frying since it is generally known that whey protein leads to lower viscosity compared to gelatinized starch.

2.6. Product characterization

The cross-sectional expansion ratio (ER) was calculated as the cross-sectional area of the extrudate divided by the cross-sectional area of the slit die. The cross sectional areas of just extruded, baked, and fried products were calculated by multiplying their width and height, which were measured using a digital vernier caliper. For just extruded sample, cross-sectional expansion measurement was performed approximately 10 seconds after extrusion to minimize experimental

variations. Each reported value was an average 30 measurements. Bulk density (g/cm³), defined as the ratio of the mass of the sample to its total volume including the voids, was measured using the mustard seed displacement method (Alavi et al., 1999). The procedure was repeated 5 times for each set of samples. The representative microstructures of SCFX extrudates were examined with a scanning electron microscope (SEM Model 430, Leica Microsystems, Wetzlar, Germany) for cross-section morphology, using gold-palladium coating (Alavi et al., 1999).

2.7. *Texture analysis*

Breaking strength was determined using an Instron Universal Testing Machine (Model 1122, Instron Corp., Canton, MA) equipped with a 5000 N load cell and Kramer shear compression cell. For each run, a 5 g sample was placed randomly in the cell (Chen et al., 2002). The crosshead speed was 50 mm/min and the maximum force required to compress the sample was recorded. Breaking strength (N/g) was calculated by dividing the maximum shear force by sample weight. The reported values were averages of five determinations. The breaking strength of commercial extruded or fried products was also determined for comparison.

Compression and flexure moduli were determined with a dynamic mechanical analyzer (DMA 7e, Perkin Elmer, Norwalk, CT) (Chen et al., 2002). For compression moduli measurement, both SCFX and commercial samples were cut into a defined circular dimension and placed on a disc holder. The samples were then compressed by a disc probe at a rate of 500 mN/min. The ratio of static stress and percent strain was calculated to determine the modulus value. For flexure moduli (E_f), samples were cut into a defined rectangular dimension and place on a three-point bending set up. The sample was then pressed by a probe at a rate of 500 mN/min. The distance between two supports was 15 mm. Flexure modulus was calculated as

$$E_f = (dF/dl)(12L^3/48 w D^3)$$
(1)

where, dF/dl = initial slope of force-distance curve, L=distance between two supports (15mm), w = width of extrudate, and D = depth of extrudate.

2.8. Statistical analysis

For all measurements, duplicate analysis was performed, and the statistical analysis (ANOVA) was done using MinitabTM.

3. Results and discussion

3.1. The apparent viscosity of whey protein-based dough

Figure 6.2 illustrates that whey protein-based dough shows shear thinning behavior for all four formulations. Determined flow behavior index varied from 0.23 to 0.30. W4 formulation containing 78.2 wt% WPC-80 showed lower apparent viscosity than S1, S2, and S3 formulation containing 52.8 wt% WPC-80 but the difference became smaller at higher shear rate (80 s⁻¹). This finding concurs with the previous reports that whey protein has much lower viscosity than gelatinized starch and is responsible for the lower viscosity in whey protein/starch mixture. In dilute system, whey protein solution showed approximately 200-fold lower apparent viscosity than pregelatinized starch solution at the same concentration and shear rate (Resch et al., 2004). Recently, Sopade et al. (2006) investigated macromolecular interactions during gelatinization and retrogradation in starch/whey systems with Rapid Visco-Analyser (RVA). They observed that the peak viscosity reduced from 1400 to 200 RVA units when they varied whey protein to starch ratio from 0 to 1. As the ratio of pregelatinized starch to masa flour was increased from 1:2 to 2:1 in whey

protein-based dough containing 52.8 wt% WPC-80, the apparent viscosity increased slightly for all shear rates. This is because S1 has more pregelatinized starch content in formulation resulting higher degree of gelatinization. The viscosity of starch-based melt is known as a function of the degree of gelatinization (Chen et al., 2006).



Figure 6.2. Apparent viscosity of whey protein-based dough (S1, S2, S3 and W4)) determined at 65°C

Expansion during extrusion process is the consequence of several events including biopolymer structural transformations (starch gelatinization and/or protein denaturation), nucleation, die-swell, cell growth, and cell collapse. It is well established that the expansion is governed by process parameters and the rheological properties of the melt. However, the role of melt rheology, especially shear viscosity is not still clear yet. It has been reported that melt shear viscosity could either enhance or reduce extrudate expansion (Sopade et al., 2006; Chen et al., 2006). High shear

viscosity favors high pressure drop rate in the die resulting in high cell density. It reduces the loss of blowing agents from extrudate surface to the environment, cell coalescence during cell growth stage, cell collapse during stabilizing stage and provides higher gas holding capacity resulting in enhanced expansion. On the other hand, high viscosity could act as resistance to cell growth resulting in less expansion. It could inhibit further expansion during post-extrusion drying process. In fact, Kokini et al. (1992) reported that the overall expansion of waxy corn starch-based extrudates was increased as the shear viscosity decreased.

The discrepancy regarding the role of shear viscosity during extrusion could be not only due to the complexity of cell nucleation and cell growth but also due to different process conditions such as types of blowing agent (steam or supercritical fluids), temperature, and shear force in different studies. Typically, starch or proteinbased melt shows highly shear thinning behavior indicating that the effect of shear viscosity on extrudate expansion could also depend on shear rate in each step of foaming process. Therefore, the viscosity of whey protein dough which was used in this study could play different roles during SCFX extrusion and post-extrusion processes. Based on the findings on whey protein-based dough viscosity, two suggestions for process parameters were made. First, lower SC-CO₂ injection rate for whey protein-based SCFX chips compared to that for masa-based SCFX chips (up to 1.4 wt%) would be desirable since much lower melt strength was expected due to the low viscosity of whey protein dough (Chen et al., 2002). Second, fast structure setting at relatively high temperature would be necessary to prevent the structure collapse of whey protein-based chips. If post-extrusion baking is operated at low temperature for long time, (for example, at 80°C for 3 hours), the weak matrix of whey protein melt due to its low melt viscosity, thus melt strength would not be able to hold the expanded structure since it would take considerable time for structure setting. On the

other hand, if the SCFX extrudates are baked at high temperature, 175°C in this study, structure collapse could be minimized. Moreover, the lower viscosity of whey protein melt could be beneficial for further expansion during post-extrusion baking and frying.

3.2. Effects of SC-CO₂ injection rate and post-extrusion processes on the physical and textural properties of SCFX extrudates

Figure 6.3 shows that the cross-sectional expansion ratio (ER) of just extruded extrudates at 65°C prior to baking or frying was highest for all formulations when 0.3 wt% SC-CO₂ was injected. However, as SC-CO₂ injection rate was increased to 0.5 wt% level, the SCFX extrudates showed considerable initial expansion followed by immediate structure collapse resulting in reduced cross-sectional expansion. Further decrease in ER with 0.7 wt% SC-CO₂ injection was observed although there was no statistically significant difference between 0.5 and 0.7 wt% SC-CO₂ levels (p > 0.05). Additional SC-CO₂ injection more than 0.7 wt% level did not lead to any difference in cross-sectional expansion compared to SCFX extrudate with 0.7 wt% SC-CO₂ level.

In general, more dissolved blowing agent in the melt would provide more nucleating sites and gas molecules for cell growth resulting in higher cell density and volumetric expansion in petrochemical-based foaming process (Baldwin et al. 1998). Chen et al. (2002) showed that the cross-sectional expansion of just extruded masa-based SCFX chips was increased from 1.5 to 1.9 when SC-CO₂ injection rate was increased from 0.6 to 1.4 wt% indicating more blowing agent favors cross-sectional expansion in starch-based SCFX process.



Figure 6.3. The effects of SC-CO₂ and formulation (S1, S2 and S3) on the crosssectional expansion of just extruded whey protein-based SCFX extrudates at 65° C prior to baking or frying

However, it has also been reported that a higher blowing agent injection rate above a certain extent does not lead to additional increase in cell density and volumetric expansion. Moreover, volumetric expansion could be reduced with a high amount of injected blowing agent depending on process conditions. Park et al. (2000) observed that the volumetric expansion of biodegradable polyester foam using filament dies with 9.5 wt% CO₂ was higher than that of foam with 7.4 wt% CO₂ at the melt temperature of below 105°C. However, the volumetric expansion was decreased with higher CO₂ level above 105°C. The finding could be attributed to the lowered melt viscosity at high temperature resulting in enhanced gas loss. In the previous study from our lab, Chen et al. (2006) showed that the extent of starch-based SCFX extrudate expansion was governed by the gas holding capability which correlated well with the degree of gelatinization, thus apparent viscosity of melt. Therefore, decrease in cross-sectional expansion with higher SC-CO₂ level in this study could be attributed to significant gas loss from extrudate surface to the environment since the amount of gas exceeded the gas holding capacity of whey protein-based extrudates.

Another possible explanation for the finding could be the enhanced longitudinal expansion due to injected SC-CO₂, which can lead to increased overall expansion. It is well known that expansion during extrusion process is notably anisotropic (Arhaliass et al., 2003). Emerging melt from a die can expand in both cross-sectional and longitudinal directions. The overall or volumetric expansion is the combination of cross-sectional and longitudinal expansion. As discussed earlier, whey protein-based SCFX extrudates showed less cross-sectional expansion at 0.5 and 0.7 wt% SC-CO₂ levels compared to SCFX extrudates at 0.3 wt% possibly due to gas loss from the surface but it is possible for them to have higher overall expansion provided longitudinal expansion ratio is larger enough at higher SC-CO₂ concentrations. Longitudinal expansion defined as the ratio of extrudate velocity to melt velocity in the die would be greater than 1 when the velocity of extrudate is higher than that of melt. Dissolved CO₂ in the melt starts to nucleate due to pressure drop in the die at gas saturation pressure at which phase separation occurs. As the pressure decreases further, the volume of melt would increase due to the additional volume of CO₂ gas; in turn the volumetric flow rates of melt and extrudate would increase possibly resulting in enhanced longitudinal expansion. Moreover, it has been reported that lower melt viscosity favors longitudinal expansion whereas higher melt elasticity leads to higher cross-sectional expansion. (Launay & Lisch, 1983). Chen et al. (2006) showed that SC-CO₂ has viscosity reduction effect on biopolymeric melts since it acts as not only a blowing agent but also a plasticizer.

Cross-sectional expansion was further increased by post-extrusion baking and frying (Figure 6.4). Baked SCFX chips expanded approximately 2-fold with



Figure 6.4. The effects of SCFX process conditions (SC-CO₂ level and product temperature) and formulation (S1, S2 and S3) on the cross-sectional expansion of whey protein-based SCFX extrudates (a: baked, b: fried)

uniform microstructure and smooth surface compared to just extruded products whereas baked unexpanded product (no $SC-CO_2$ added) showed an irregularly swollen shape without any microstructure. Just extruded SCFX products had to be equilibrated for 1-2 hours prior to frying since some of products showed uneven swelling and blistering when they were fried right after extrusion. After equilibration, fried SCFX extrudates attained a lighter, smooth, uniform appearance compared with fried unexpanded control without SC-CO₂ injection. The surface of fried control samples were unevenly swollen and/or blistered even after longer equilibration process. Figure 6.3 shows that most expanded baked and fried products in cross-sectional direction were obtained with 0.3 wt% SC-CO₂ level. But some of baked and fried SCFX products with 0.3 wt% SC-CO₂ level showed uneven expansion and/or surface blistering as control samples without SC-CO₂ injection. This undesirable appearance of whey protein chips with 0.3 wt% SC-CO₂ level was attributed to thicker skin compared to products with 0.5 and 0.7 wt% SC-CO₂ levels. SCFX extrudates attain a unique smooth and shiny skin having relatively low cell density and its thickness decreases as SC-CO₂ injection rate increases (Alavi et al., 1999). The typical skin is a desirable attribute for breakfast cereal products since it increases the bowl-life. In this study, it was found that the thicker skin of 0.3 wt% SCFX extrudate acts as a moisture barrier which causes irregular expansion during baking or frying resulting in pillow shape products without ordered internal microstructure. Unlike expansion during SCFX process due to cell nucleation and gas diffusion, not only CO₂ inside the each cell but also the moisture in the matrix contributes to the thermal expansion of SCFX extrudates during baking and frying processes.

The moisture inside of 0.3 wt% SCFX extrudate would cause sudden expansion at baking and frying temperature resulting in undesirable huge holes whereas the moisture inside of 0.5 and 0.7 wt% products would diffuse more easily through thinner skins to the environment. Kawas & Moreira (2001) observed similar trend when they pre-baked tortillas chips prior deep fat frying. When they fried the steam-baked tortillas chips, the oil uptake decreased compared to control but a strong barrier, that is, the crust that was formed during steaming and baking that did not allow the water vapor to escape with ease. As the vapor tried to escape from the chip's interior, puffs or pockets were formed. Therefore, 0.3~0.7 wt% SC-CO₂ levels would be the optimum injection rate for whey protein-based SCFX chip production taking account of product appearance and its internal structure.

In general, the breaking strength of baked and fried products expanded with more than 0.5 wt% SC-CO₂ was observed to increase due to less expansion resulting in higher bulk density (Figure 6.5). The bulk density and the breaking strength of baked and fried products were found to be linearly correlated as reported in the literature (Figure 6.6) (Onwulata et al., 2001; Matthey & Hanna, 1997). Fried products were denser with higher breaking strength than baked products. It was possible to control the expansion and thus texture of whey protein-based SCFX products simply by manipulating SC-CO₂ injection rate.

Figure 6.7 shows that the cross-sectional expansion of baked whey proteinbased chips with 78.2 wt% WPC-80 decreased by 33% when just extruded SCFX extrudate was cooled down for 30 minutes at ambient temperature prior to baking whereas the breaking strength increased by 60%. Decrease in cross-sectional expansion leading to harder texture was mainly attributed to structural collapse due gas loss from the extrudate skin to the environment (Alavi et al., 1999). SCFX extrudates are in rubbery state right after extrusion due to high in-barrel moisture content (~36 wt%). Unless the structure is set by quick thermosetting and/or moisture removal, extrudate collapse would occur.



Figure 6.5. The effects of SCFX process conditions (SC-CO₂ level and product temperature) and formulation (S1, S2 and S3) on the breaking strength whey protein-based SCFX extrudates (a: baked, b: fried)



Figure 6.6. The effect of the bulk density on the breaking strength of SC-CO₂ expanded whey protein-based chips

It has been reported that structure collapse could be reduced by increasing melt viscosity, in turn melt strength (Alavi et al., 1999; Chen et al., 2006).

Although whey protein-based SCFX chips became harder after equilibration at ambient temperature due to slight structure collapse, the hardness was still acceptable. Therefore, if the post-extrusion process can be carefully designed with humidity-temperature control, it is possible to tailor the textural properties of SCFX chips using newly emerging technologies such as microwave oven baking. For comparison, Arhaliass et al. (2003) reported that vacuum shrinkage of closed steam cells is mainly responsible for steam-based extrudate structure collapse over seconds or longer periods after extrusion. As the melt temperature decreases due to heat transfer between the hot melt and the surrounding air, small amount of water vapor could condense which should tend to decrease the pressure inside the bubbles, and consequently create a light vacuum in bubbles leading to extrudate collapse. But, such phenomenon would not occur in SCFX process which is operated at lower than 100°C.



Figure 6.7. The effect of equilibration time at ambient temperature prior to baking on the cross-sectional expansion, bulk density and breaking strength of SC-CO₂ expanded whey protein-based chips with 78.2 wt% WPC-80 at 65° C

3.3. Effects of the degree of starch gelatinization and the product temperature at the die on the physical and textural properties of SCFX extrudates

As shown in Figure 6.3, the cross-sectional expansion of just extruded product at 65° C was highest with formulation S1 for each SC-CO₂ injection rate. Although whey protein-based SCFX extrudates did not show significant die-swell (0.9-1.1), which is the cross-sectional expansion without SC-CO₂ injection, higher pregelatinized starch formulation (S1) indicated higher die-swell compared to higher masa flour formulation (S3). The finding could be attributed to higher degree of gelatinization thus higher elasticity in formulation S1. Although masa flour could be partially gelatinized during the nixtamalization process, which consists of cooking the corn in a boiling lime solution for 35~50 min and steeping overnight, it has been reported that the degree of gelatinization of masa flour is less than 15% (Gomez et al., 1992). Since SCFX extrusion was conducted at the temperature at 65 and 75°C, additional gelatinization in the barrel would be limited during SCFX process. Chen et al. (2006) also showed that die-swell of starch-based SCFX extrudates increased as the degree of gelatinization increased. A viscoelastic melt, on emerging from a die, shows increase in diameter in cross-sectional way compared to the die diameter. The phenomenon is referred to as die-swell and results from the sudden removal of a constraining force on the melt. The degree of die-swell is dependent on mainly elastic properties of a melt and it is known that biopolymers or synthetic polymers having higher die-swell give higher cross-sectional expansion and generally higher volumetric expansion. As seen in Figure 6.2, formulation S1 having higher degree of gelatinization and die-swell ratio expanded more with SC-CO₂ injection. In addition, the higher viscosity of formulation S1 was more favorable for higher cross-sectional expansion because it viscosity helps to reduces gas loss and to maintain cell structure during SC-CO₂ foaming process (Figure 6.2).

Interestingly, however, high masa flour sample (S3) expanded more than high pregelatinized corn starch sample (S1) during baking and frying processes compared to controls prior to baking and frying (Figure 6.4). This finding could be explained by the lower viscosity of high masa flour melt (Figure 6.2). Expansion of SCFX extrudates during baking and frying would be driven by thermal expansion of CO_2 and moisture inside cell and whey protein/starch/moisture matrix. The baking and frying treatments were conducted at 175°C which leads to fast structure setting not only due

to thermosetting of biopolymers but also fast moisture removal. As moisture is removed from the product by baking or frying, the glass transition temperature increases. Once the product temperature reaches $\sim 30^{\circ}$ C above its glass transition temperature, structure collapse could be prevented. In this situation, the rigidity of cell wall could be the limiting factor to thermal expansion. Therefore, the lower viscosity of formulation S3 could be beneficial for expansion during baking and frying. Overall, however, formulation S1 showed the highest cross-sectional expansion in finished baked and fried products.

Another interesting finding in this study is that formulation S1 showed similar or higher breaking strength than formulation S3 in spite of its higher cross-sectional expansion implying that higher degree of gelatinization would induce stiffer cell wall structure (Figure 6.4). Kawsa et al. (2001) reported that heat treatment before frying increased the hardness of pre-baked tortillas chips. Therefore, the degree of starch gelatinization in whey protein-based formulation could be useful to design the textural properties of SCFX expanded chips.

Furthermore, it was found that product temperature at the die is critical to control the texture of final products. Extrusion at 75°C resulted in slightly higher cross-sectional expansion in baked products whereas did not lead to significant difference in fried products (Figure 6.3). Slight increase in the cross-sectional expansion of baked products at 75°C product temperature would result from enhanced starch gelatinization and protein denaturation leading to higher viscosity and elasticity. However, the breaking strengths of baked and fried products extruded at 75°C were significantly higher than those of SCFX extrudates at 65°C (Figure 6.5). As discussed earlier, higher degree of gelatinization could impart harder texture to finished products. At the same time, enhanced protein denaturation could be responsible for the finding. Numerous studies have been conducted on the effect of heat denaturation on the

viscosity of whey protein solution and the strength of whey protein gel. Whey protein forms viscoelastic gels upon heating, and the thermal gelation involves an initial denaturation-unfolding step followed by aggregation into a protein network by hydrophobic and sulfhydryl-disulfide interactions (Spodae et al., 2006). Bryant & McClements (2000) reported that the apparent viscosity at 100 s⁻¹ of 10% WPI solution at pH 7 was increased approximately 30 folds as heating temperature increased from 65 to 75°C indicating extensive heat protein denaturation. Boye et al. (1997) showed that the gel strength of commercial whey protein concentrate containing (WPC-75) 30% solid gel was heating temperature dependent. Below 70°C, the WPC gels formed were very soft and gave values less than 1 N. Heating above 70°C resulted in an increase in gel strength from 15.5 N at 75°C to 22.4 N at 90°C which suggests that increasing the temperature increased the gel strength. These findings suggest that heat denaturation leading to harder gel occurs around 70°C. Therefore, SCFX extrusion below 70°C would minimize the thermal denaturation of whey protein.

3.4. Effect of WPC-80 concentration on the physical and textural properties of SCFX extrudates

Addition of oat fiber resulted in less expansion and harder texture with 52.8 wt% whey protein products (Figures 6.4 and 6.8) corresponding with previous findings in the literature (Onwulata et al., 2001). When WPC-80 concentration was increased up to 78.2 wt%, the cross-sectional expansion of baked product increased from 1.61 to 2.67. This finding is very unique since it is well known that the incorporation of whey protein without chemical modification in large quantity causes less expansion in conventional steam-based extrusion (Onwulata et al., 2001).



Figure 6.8. The effect WPC-80 concentration on the cross-sectional expansion, bulk density and breaking strength of whey protein-based SCFX extrudates (a: baked, b: fried)

It is believed that whey protein denaturation and its interaction with starch in steambased extrusion are responsible for limited expansion and hard texture.

Another reason for less expansion during steam-based extrusion would be the lower viscosity of whey protein compared to starch as discussed earlier. To obtain reasonable expansion certain degree of gas holding capacity that is correlated with melt viscosity. High protein content in melt would have less gas holding capacity results in poor expansion in steam-based extrusion.

In fact, the less gas holding capacity of high protein melt is also detrimental for SCFX expansion. The maximum SC-CO₂ concentration which whey protein-based SCFX dough can hold was 0.7 wt% whereas it was 1.4 wt% in masa-based SCFX dough (Chen et al., 2002). Therefore, the cross-sectional expansion of high WPC-80 (78.6 wt%) right after extrusion was limited. However, there was significant expansion during post-extrusion processes just like formulation S3 (high masa flour) was expanded more during post-extrusion processes than formulation S1 (high pregelatinized starch). For higher WPC-80 concentration formulation, the resistance to thermal expansion during baking was decreased due to low viscosity resulting in more expansion in final products. SCFX chips showed not only desirable expansion but also uniform cellular structure which cannot be obtained using steam-based extrusion or conventional chip production methods. The texture of baked and fried products became softer and crispier as WPC-80 concentration was increased due to increased cross-sectional expansion.

3.5. Comparison with commercial products

To validate product acceptability, comparison of SCFX products with commercial snack products was performed in terms of textural properties (Figure 6.9). Commercial puffed corn meal-based snack showed the lowest breaking strengths whereas baked wheat crackers had the highest breaking strength among commercial products which were tested.



Figure 6.9. Breaking strength analysis of commercial products versus whey protein-based SCFX chips.

SC-CO₂ expanded chips showed a broad range of breaking strengths which are close to those of commercial products. Baked and fried products containing 52.8 wt% WPC-80 exhibited crunchier texture compared to others. Fried SCFX chip containing 78.2 wt% WPC-80 showed similar breaking strength with fried tortillas chip, potato chip, and corn chip. Compression and flexure moduli were measured using DMA and shown in Table 6.2. The results suggested that baked SCFX products have similar texture with commercial expanded corn meal snack whereas fried SCFX products are close to fried tortillas chips (no statistical difference, P > 0.05). Figure 6.10 illustrates the pictures of baked and fried whey protein-based chips (52.5 wt% WPC-80 at 0.7 wt% SC-CO₂ level). The baked whey protein-based chips showed a very shiny surface which is one of unique properties of SCFX extrudates (Alavi et al., 1999).

	Compression	Flexure
	modulus (MPa)	modulus (MPa)
Commercial tortillas chip	0.4 ± 0.05	0.8 ± 0.05
Commercial extruded snack	0.7 ± 0.04	1.2 ± 0.07
Baked SCFX chip (52.4% WPC-80)	1.1 ± 0.05	1.8 ± 0.07
Baked SCFX chip (78.2% WPC-80)	0.9 ± 0.04	1.2 ± 0.05
Fried SCFX chip (52.4% WPC-80)	0.8 ± 0.04	1.1 ± 0.04
Fried SCFX chip (78.2% WPC-80)	0.6 ± 0.02	1.0 ± 0.03

Table 6.2. Compression and flexure moduli of SC-CO₂ expanded products and commercial snack products

Fried products also had smooth surface compared to commercial tortillas chips which usually have rough surface due to blistering. Whey protein-based SCFX chips showed golden brown color especially after frying due to Maillard reaction which brought appealing flavor. Scanning electronic microscopy (SEM) pictures of representative fried whey protein-based SCFX chips (52.5 wt% WPC-80) shows typical more homogenous microcellular structure with higher cell density compared to commercial fried tortillas chips which had a flat dimension with irregular hole formation (Figure 6.11) (Chen et al., 2002; Kawsa et al., 2001). It was seen that SCFX chips produced at 0.7 wt% SC-CO₂ injection rate had higher cell density and more uniform cell structure than products extruded at 0.3 wt% SC-CO₂ level.

Overall, the simple manipulations of SCFX process parameters and formulation could be used to produce a variety of expanded snack products having high nutrient density with different microstructure and textural properties.



Figure 6.10. The pictures of whey protein-based SCFX chips (52.5 wt% WPC-80 at 0.7 wt% SC-CO₂ injection rate, a: baked, b: fried)



Figure 6.11. The SEM pictures of fried whey protein-based SCFX chips (52.5 wt% WPC-80, a): 0.3 wt% SC-CO₂, b: 0.7 wt% SC-CO₂; X56 magnitude)

4. Conclusions

Whey protein is an excellent ingredient for fortifying food products due to its nutritional benefits but it is still under utilized in the U.S. Fortification with whey protein in extruded products has been limited due to its thermosetting property. In this study, a novel technology, SCFX process has been successfully utilized to produce healthy snack chips containing up to 60 wt% protein content without any chemical modifications. It was found that it is possible to create uniformly expanded cellular structure when expansion operation was performed below whey protein denaturation temperature. The textural properties of baked and fried products were comparable to commercial extruded or fried chip products. SC-CO₂ level that is the blowing agent concentration was found to be critical to control the expansion and texture of final product. The degree of starch gelatinization was another key factor to manipulate product morphology. Not only SCFX operation parameters but also post-extrusion process conditions affected the quality of whey protein-based expanded products. In addition, since SC-CO₂ is an efficient solvent for certain flavors and nutrients and SCFX process can be utilized for flavor encapsulation, a novel expanded healthy snack with high nutrient density, and unique structure, texture and flavor could be produced using SCFX process.

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