# EDIBLE FILMS AND COATINGS

# FROM CALCIUM CASEINATE AND THEIR APPLICATIONS

by

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## ABSTRACT OF THE THESIS

Edible Films and Coatings from Calcium Caseinate and Their Applications

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Calcium caseinate (CaCas), isolated from nonfat dry milk (NFDM), is a milk ingredient for the production of protein-based edible films and coatings. When the supply of NFDM exceeds the demand, the conversion of CaCas to alternative value-added products through processes such as coating may help utilize and prevent future surpluses of NFDM. Two studies are examined in this project.

The motivation of the first study is to improve the mechanical properties of calcium caseinate-based films. Glycerol (Gly), a plasticizer, is currently used in film solutions to overcome the brittleness of CaCas films. However, Gly reduces the mechanical strength of the films (Tomasula et al. 1998). The addition of hydrophobic compounds or modifications of polymer network is a common approach to improve the mechanical properties of CaCas/Gly films through crosslinks. In this study, high methoxyl pectin (CP) was used in CaCas/Gly film solutions to make the edible films, and

its effect on elastic modulus (E), elongation at break (EAB), and tensile strength (TS) of the films were evaluated. The magnitude of the tensile properties showed that edible CaCas/Gly films was affected by film thickness, relative humidity (RH), and CP content (Bonnaillie et al. 2014).

The motivation of the second study is to improve the nutrient profiles, extend the bowl-life, and enhance the textures of RTE breakfast cereals by using CaCas-based coating materials. In the coating process of RTE breakfast cereals, high sugar concentrates or slurries are used to provide moisture barrier properties, preserve texture, and extend bowl-life of the cereals. However, this leads to health concerns such as childhood obesity and dental problems. In this study, glucose, NFDM, CaCas, and CaCas in blends with Gly, CP, and NFDM at constant 15% total protein concentration in coating solutions were applied on Wheaties® breakfast cereals by spraying the solutions on the surface of flakes with a drying process. The coatings provided an increased protein source, longer bowl life in milk, and crunchier and crispier texture by forming a uniform, sheen, and protective coating layer on the surface of the flakes.

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## ABBREVATION

| | |
|---|---|
| NFDM | Nonfat Dry Milk |
| CaCas | Calcium Caseinate |
| RTE | Ready-to-eat |
| Gly | Glycerol |
| CP | Citric Pectin or 3% Citric Pectin Solution |
| DI | Deionized Water |
| RH | Relative Humidity |
| E | Elastic Modulus, MPa |
| EAB | Elongation At Break,% |
| TS | Tensile Strength, MPa |
| CaCas/Gly | Calcium Caseinate and Glycerol solution or films |
| Ca+2 | Calcium ions |
| Ca-P | Calcium Phosphate |
| CPI | Canola Protein Isolates |
| NaCas | Sodium Caseinate |
| TMPs | Total Milk Proteins |
| UF-TMP | Total Milk Proteins via Ultrafiltration |
| EER | Ethanol Extraction Retentate |
| LDPE | Low Density Poly Ethylene |
| PVDC | Polyvinylidene Chloride |
| WVP | Water Vapor Permeability |
| DE | Degrees of Methyl Esterification |
| HM | High Methoxyl Pectin |
| LM | Low Methoxyl Pectin |
| CaCl2 | Calcium Chloride |

| | |
|---|---|
| CaCas/Gly/CP | Calcium Caseinate, Glycerol and Citric Pectin solutions of films |
| ICP-OES | Inductely Coupled Plasma-optical Emission Spectrometer |
| AOAC | Official Methods of Analysis, Association of Analytical Chemists |
| CaCas/CP | 15% (w/w) Calcium Caseinate and 0.3% of Citric Pectin Solution and Coating |
| CaCas/NFDM (1:2) | 10% (w/w) Calcium Caseinate and 5% (w/w) Nonfat Dry Milk coating solution |
| CaCas/NFDM (2:1) | 5% (w/w) Calcium Caseinate and 10% (w/w) Nonfat Dry Milk coating solution |
| CaCas/NFDM (1:1) | 7.5% (w/w) Calcium Caseinate and 7.5% (w/w) Nonfat Dry Milk coating solution |

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# 1. BACKGROUND

## 1.1. Milk proteins-based films

Nonfat dry milk (NFDM) is an important food ingredient made by removing the water from fluid skim milk to preserve it for future use. It accumulates in a dried form, and is stored because of its high amount of production, more than 9 billion pounds in 2011 (Botros et al. 2013). NFDM is used in several applications to increase the nutritious value of foods such as increasing protein and the solid content of yogurt (Tamime et al. 1984; Guinee et al. 1995; Remeuf et al. 2003). When the supply of NFDM exceeds the demand, its conversion to alternative value-added products may help utilize and prevent future surpluses.

Current research focuses on creating a new functional caseinates product in food applications including food packaging films and coatings for food products (Avena-Bustillos et al. 1993). Caseinates have a great potential as edible film-forming and coating materials (Tomasula 2009) in the food applications due to its high nutritional quality, excellent sensory properties, and potential to protect food products from their surrounding environment (Chen 2002).

### 1.1.1. Casein and Caseinate Structure

The forms of casein are differentiated by their calcium sensitivity and their charge distribution (Varnam 2001; Fox and Kelly 2006; Dickinson 2006). Casein protein is a

phosphoprotein consisting of $\alpha_{s1}$-casein, $\alpha_{s2}$-casein, and β-casein, which are calcium sensitive, and κ-casein, which is calcium insensitive (Uniacke-Lowe 2010). In milk, κ-caseins stabilize calcium-sensitive caseins avoiding precipitation with calcium ions ($Ca^{+2}$) (Swaisgood 1996; Horne 2006). The calcium-sensitive caseins are able to bind $Ca^{+2}$ by their phosphate groups located on the hydroxyl groups of serine. Phosphoserine residues are responsible for the existence of hydrophilic areas with a strong negative charge (Swaisgood 1996; Horne 2006). One model used to envision the structure of the casein micelle in milk is that of Schmidt (1982) in which the micelles is composed of sub-micelles bound via $Ca_9(PO_4)_6$, shown in Figure 1.

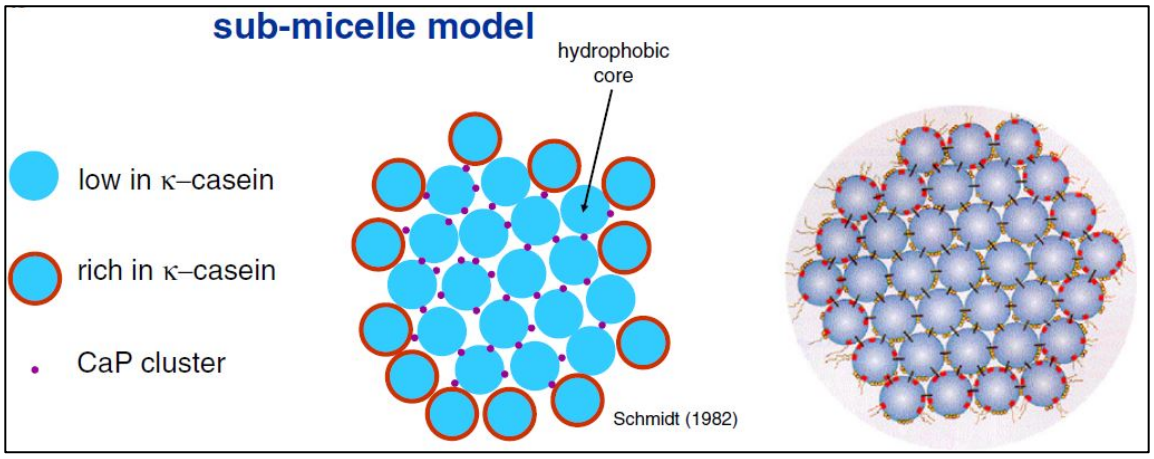


Figure 1: The formation of casein micelles in an aqueous medium (Schmidt 1982).

CaCas is made from NFDM by using acid to dissolve the Ca-P linkages to form acid casein precipitate and react the precipitate with a $Ca(OH)_2$ to form CaCas. The micellar structure no longer exists and the caseinate possesses a random coil structure, which is non-ordinate protein with a low level of α-helical and β-sheet structures (Chambi and Grosso 2006).

### 1.1.2. Caseinate and Glycerol Films

Due to the random coil structure and amino acid compositions of caseinate, the interactions —hydrogen, hydrophobic, and electrostatic bonds— play an important role to form caseinate films (McHugh and Krochta 1994a; Lacroix et al. 1998). The films need to incorporate a plasticizer such as Gly to reduce the brittleness and increases flexibility of the edible caseinate films for handling and testing (Tomasula et al. 1998; Janjarasskul and Krochta 2010). The plasticizer reduces the protein chain-to-chain interactions by placing into three-dimensional network, which increases the free-volume and eases the mobility of the polymer chain (Vieira et al. 2011). However, it weakens TS (a measure of film resistance to tension) and induces the EAB (a measure of film elasticity) of edible caseinate films (Tomasula et al. 1998; Chen 2002). An additive molecule with a long molecular chain is also required to provide a better EAB and mechanical integrity by incorporating a small amount of flexible crosslink (Bonnaillie et al. 2014).

For example, a research related to the effect of Gly on the mechanical properties of protein films showed that the molecular mobility (of canola protein isolates-CPI) in the film matrix was restricted leading to very brittle CPI film due to the interactions between side chains of partially denatured CPI (Chang and Nickerson 2013). However, the amount of added Gly played an important role because it caused the film to be too soft and sticky to be removed from its casting mold (Chang and Nickerson 2013). Gly

interferes with the CPI aggregates by forming more volume in the film matrix (Chang and Nickerson 2013). It was also showed that the films became weaker and more flexible with increasing Gly amount (Chang and Nickerson 2013). The results are in agreement with other studies, which showed that increasing plasticizer content (Gly) disrupted protein-protein interactions in the film matrix and decreased TS and E (a measure of film stiffness), and increased EAB (Tomasula et al. 1998; Chen 2002; Tomasula et al. 2003; Dangaran and Tomasula 2009).

Tomasula et al. (1998) reported that TS decreased with increasing Gly amount. At 20% (w/w) Gly content, TS decreased in the CaCas films. The values of EAB decreased in CaCas films by 40% (w/w) Gly (Tomasula et al. 1998). Since, Gly reduces the intermolecular forces in the films by inserting itself between the protein chains. Schou et al. (2005) reported TS and E of the sodium caseinate (NaCas) films incorporated with Gly decreased with increasing Gly content. The flexibility of NaCas films increased greatly with 0.24 and 0.32 ratios of Gly to protein (Schou et al. 2005). However, increasing Gly amount enables NaCas films more stretchable and malleable (Schou et al. 2005).

The presence of Gly also lowers the gas barrier properties and the mechanical strength of caseinate films (Tomasula 2009; Vieira et al. 2011). Glycerol reduces the protein chain-to-chain interactions and lowers the protein glass transition temperature, which is defined as a temperature of crystalline state (rigidity) of product (Hutchinson et al. 1989), to increase the flexibility of films (Hong and Krochta 2006). Some

modification is required to overcome the weaknesses of CaCas/Gly films by broadening the tensile properties of films for wide range applications. Therefore, the caseinates films are still at the developmental stage and requires more research to examine the tensile properties and characteristics of caseinate in the polymer network due to the caseinate films susceptibility to moisture which limits the use of the films in applications (Gennadios et al. 1996; Lacroix et al. 1998; Falguera et al. 2011).

### 1.1.3. Nonfat dry milk (NFDM) Films

Maynes and Krochta (1994) evaluated five total milk proteins (TMPs) films by comparing their TS, E, and EAB values. Two of them obtained from NFDM by producing via both ultrafiltration (UF-TMP) and ethanol extraction retentate (EER). The other three were obtained from commercial TMPs. In the study, UF-TMP showed the highest E, and highest TS at break. However, commercial TMP films had similar value of TS at break to low density polyethylene (LDPE) and similar EAB to polyvinylidene chloride (PVDC) and cellophane (Maynes and Krochta 1994). Therefore, milk protein-based edible films can be improved to be compatible with current packaging materials.

Individual proteins which are caseinates, and total milk proteins were derived from the proteins of NFDM to make the edible films instead of making an edible film directly from NFDM powder (Avena-Bustillos et al. 1993; McHugh et al. 1994) because the

lactose content of NFDM might adversely affect the edible film properties (Maynes and Krochta 1994).

There are also other studies based on sodium caseinate (NaCas) and calcium caseinate (CaCas) derived from NFDM milk by acid treatments, some of which are mentioned in the sections 1.1.2 and 1.3.3.

### 1.1.4. Cross-linking Methods for Caseinate-based Films

Most of the research on the edible caseinate films is dedicated to improving their physical properties by applying either the incorporation of hydrophobic compounds or modification of polymer network through the crosslinks of polymer chains. Chemical, enzymatic, and physical treatments make the cross-linking process possible due to the presence of charged functional groups in the amino acids of proteins (Chambi and Grosso 2006).

Avena-Bustillos et al. (1993) reported a chemical cross-linking method on the pre-formed NaCas and CaCas films. By using calcium chloride ($CaCl_2$) solution, the films were soaked in the solution for 1 min to induce calcium mediated cross-linking in casein, which forms ionic cross-links. The result showed that the cross-links reduced water vapor permeability (WVP) of the films by reducing the protein chain mobility and flexibility (Avesta-Bustillos et al. 1993).

Gelatin was also used to enhance the mechanical strength as well as the water vapor barrier properties via enzymatic treatment, reported by Chambi and Grosso (2006).

In the study specific case of gelatin-casein films, transglutaminase enzyme was used to catalyze the crosslink reactions between the reactive functional groups of gelatin and caseinates (Chambi and Grosso 2006). After the enzyme treatment, a decrease in WVP and increase of elasticity were observed (Chambi and Grosso 2006). Since, the crosslink turns the random structure of caseinate (in a solution) into a more organized chain structure, which result in optimizing molecular packing structure correlated to the improvement of mechanical and barrier properties (Chambi and Grosso 2006).

Cross-linking induced via physical treatment with UV or γ-irradiation was also investigated with some positive results (Ouattara et al. 2002) because the proteins were affected by γ-irradiation, which triggered the conformational changes, oxidation, free radical formation, and polymerization reactions of proteins. The study reported that the films were produced with improved mechanical properties because hydroxyl radicals were generated by irradiation of protein solutions and reacted with aromatic residues to form covalent bonds with more cohesion between polypeptide chains, which produce the films with better mechanical properties (Ouattara et al. 2002).

## 1.2. Edible Coatings on Food Products

The mechanical and barrier properties of casein and whey protein films have been taken advantages of in food applications to improve the quality of foods and extend shelf-life of dry products (Tomasula 2009) such as dry bakery products (Bravin et al. 2006). Also, Khwaldia et al. (2004) and Vargas et al. (2008) tested some other potential

applications of casein and whey films and coatings to provide moisture and gas barrier properties for fresh fruits and vegetables, meats, cereals, nuts and frozen foods. Whey protein-based films and coatings that applied on nuts, peanuts, eggs, confectionary products, meat products, fruits, and vegetables were reported by Dangaran and Krochta (2009). Another research was carried out based on the transglutaminase-crosslinked whey protein isolate films as a wrap to prevent quality changes in products such as meat pies (Yildirim and Hettiarachchy 1998).

Edible films and coatings provide an extension for the shelf life of the products by maintaining the sensory qualities and enhancing nutritional quality of fresh, minimally processed fruits and vegetables (Falguera et al. 2011). An example would be that research has been done on the effects of polyvinyl chloride incorporated with edible maize starch-based coatings, with glycerol as a plasticizer, applied on Brussels sprouts to preserve the quality parameters such as weight, firmness, commercial acceptability and nutritional quality (Falguera et al. 2011).

Edible films and coatings would also reduce the fat uptake and decrease water loss in deep-fat fried products by applying composite films made of soy protein isolate and whey protein isolate (Albert and Mittal 2002). Freitas et al. (2009) studied the effect of edible coatings from pectin, whey protein and soy protein isolate on the deep-fat-frying of products made from cassava flour and its puree. Whey protein provided 27% reduction in fat absorption (Freitas et al. 2009).

## 1.3. Citric Pectin

Pectin usually comes from most of the plant tissues, but apple pomace and citrus peels currently are the main sources of pectin as commercially available. Citric pectin (CP) is produced by extracting from raw materials (citrus peels) right after juice extractions to avoid the activity of pectin methylesterase enzyme, which produces blocks of deesterified material resulting in destroying pectin structure (May 1990). It can then be stored for many months and used in value-added applications.

### 1.3.1. Structure and Properties

Pectin is one of the natural water-soluble polysaccharides. It is used as a highly effective additive to improve product and processing properties in fields of foodstuffs.

The basic structure of pectin is a linear chain of poly-α-(1-4)-D-galacturonic acid with varying degrees of methyl esterification (DE) (Kurita et al. 2008). Depending on DE, pectins are divided between high methoxyl (HM) and low methoxyl (LM) pectin. HM pectins form gels mainly via hydrophobic interactions and hydrogen bonds in the presence of more than 55% sugar, while LM pectins can gel with only a small amount of sugar in the presence of calcium ions, $Ca^{2+}$ (Löfgren and Hermansson 2007).

The behavior of HM pectin in a solution is the formation of crosslinks, which forms a tangled and interconnected network in a three dimensional helix via hydrogen bonds with water molecules, known junction zones (Oakenfull 1984). Intermolecular forces, which are hydrophobic interactions and hydrogen bonding, play an important role

to stabilize the junction zones shown in Figure 2. Hydrophobic interactions occurred between the ester methyl groups of pectin molecules stabilize the threefold helical conformation in an aqueous medium by avoiding to contact with the water (Ben-Naim 1980; Oakenfull 1991). These interactions are driven by three dimensional hydrogen bonded water molecules, which adjacent to a hydrophobic surface of pectin chains (Ben-Naim 1980).

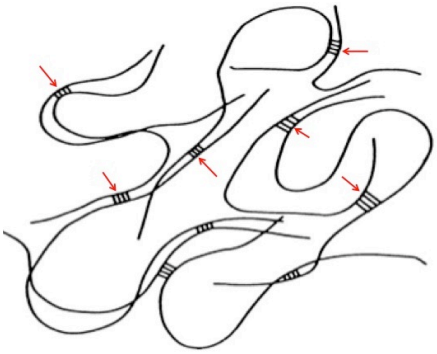

Figure 2: The gel networking of HM pectin in a liquid medium (Oakenfull 1991).

### 1.3.2. Interactions between Pectin and Caseinate

Pectin can provide rigid and stiff tensile properties to the films. The caseinates are known to have a random coil structure, which is non-ordinate proteins with a low level of α-helical and β-sheet structures (Chambi and Grosso 2006) and compose of polar and nonpolar amino acids (Chen 2002). CaCas has $Ca^{+2}$, which is responsible for electrostatic interactions with the methoxyl and carboxyl functional groups of pectin molecules. The motivation of adding pectin is the crosslinks between the functional groups of pectin and

calcium, called egg-box model in Figure 3, to increase tensile strength of the edible films (Rees et al. 1975; Morris et al. 1982; Flutti 2003).

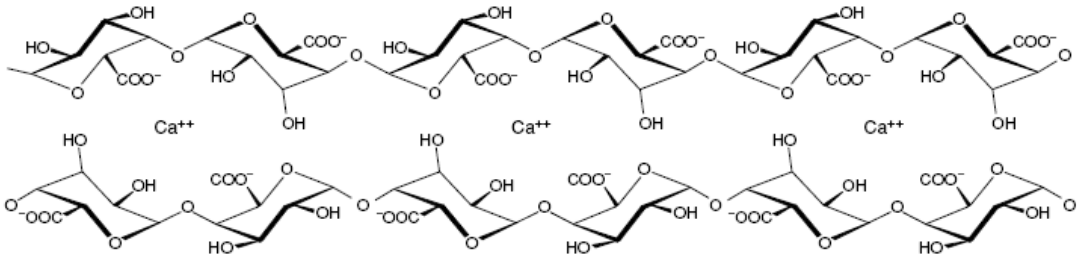


Figure 3: Egg-box model (Flutti 2003)

Other interactions such as hydrogen bonding and hydrophobic interactions occur to form the caseinate films (McHugh and Krochta 1994a; Lacroix et al. 1998). CaCas has both polar and nonpolar sites that possibly take a place between water and nonpolar pectin sites, which results in the films with more structured and compact network in molecular level (Chen 2002).

The various interactions between pectin and caseinate are complex and are greatly affected by the stoichiometric caseinate:pectin ratio, the pH, the presence of salts, etc (Pedersen and Jorgensen 1991; Maroziene and de Kruif 2000).

The casein films has several problems to be solved because they are affected by relative humidity and temperature changes, processing (e.g. casting), drying rate and type, storage conditions, plasticizers, thickness, addition of pectin, mixing sequences, etc. (Bonnaillie et al. 2014). In this case, films require more work to be done for broad commercial application.

### 1.3.3. Caseinate/Pectin Films and Coatings

Although the combination of other milk protein-based films and coating (ex. whey, total milk proteins, etc.) with pectin or other carbohydrates (ex. agar, carrageenan, etc.) were broadly studied (Bonnaillie et al. 2014). Due to the limited studies of caseinates, current research focuses on creating a new functional caseinates product with some modifications to use in food applications including food packaging films and coatings for food products (Avena-Bustillos et al. 1993).

Lacroix et al. (1998) studied on the effect of irradiation dose on the mechanical properties of cross-linked CaCas films. With the irradiation treatment and addition of $CaCl_2$ in the film solutions, the results showed that the cohesion of protein was improved due to the formation of electrostatic bonds. $CaCl_2$ made synergistic effect, and γ-irradiation increased the number of cross-links in molecular level (Lacroix et al. 1998). These improvements would be responsible for the increased mechanical strength of the films (Lacroix et al. 1998).

Letendre et al. (2002) evaluated the effect of polysaccharides, pectin and agar on the physicochemical properties of CaCas and WPI films. By treating pectin and agar with autoclaving, the cross-links occurred between protein and polysaccharides, which results in enhancing mechanical properties (Letendre et al. 2002). The possibility of cross-links was increased due to the disordered and disassociated polysaccharide chains by autoclaving (Letendre et al. 2002).

Bonnaillie et al. (2014) reported the addition of CP affected on the structure and mechanical properties of CaCas films plasticizing with Gly. The incorporation of CP with the range from 0.05% to 1% (w/w) into CaCas/Gly films increase the E of the films at ~35% RH, which results in the film with fragile properties. The films with ~0.4% CP, the EAB decreased than the films without CP (Control CaCas/Gly films). Beside CP amounts, the molecular structure of films and the interactions of materials, which are directly effective on the mechanical properties of the films were highly dependent on RH flocculation, and the preparation of film solutions (Bonnaillie et al. 2014).

Regarding to these studies, with different modifications such as crosslinking methods, incorporation with other compounds, CaCas can be improved and applicable for food applications to take benefits of its mechanical and nutritional properties.

### 1.4. Ready-to-eat breakfast cereals

Breakfast cereal is a RTE product consumed with milk, milk-based products, or without milk. RTE breakfast cereals are made of one or more of the cereal grains or their milled fractions as a major constituent. Other constituents, sweeteners, nutrients, flavoring and texturing ingredients are also used in the production of RTE cereals (Caldwell et al. 2000; Brennan et al. 2013). There are several types of RTE breakfast cereals categorized on its manufacturing processes such as flaking, puffing or shredding (Fast 2000; Nowakowski and Green 2012). For flaking, soft red wheat kernels are mainly

used and the addition of malt and sugars for flavor enhancement provide the desired brown color (Caldwell et al. 2000).

### 1.4.1. Flow of RTE Flakes Production

The flow of production is differentiated based on both the type of RTE breakfast cereals and source of cereal grains. Wheat flakes are produced based on several numbers of steps: 1) preprocessing, which provides a complete gelatinization of starch, an even distribution of flavor, and crushing kernels, 2) formulation, which represents the amounts of ingredients for production, 3) cooking, 4) lump breaking, 5) drying, 6) cooling and tempering, and 7) flaking, which improves the texture of final product (Fast 2000; Nowakowski and Green 2012).

### 1.4.2. Coatings

There is a coating process, which usually applies after drying process in the manufacture of RTE breakfast cereals (flakes). Coating with a sugar slurry solution is the last step to sweeten the pieces of the cereals to meet consumer demands linked to the several properties of the cereals. Sensory, textural, and bow-life properties are some of these properties, and the sensory properties, which are related to the appearance, flavor, and taste of the RTE flakes, and textural properties, which are linked to the hardness and crispness of the flakes (Caldwell 2000; Ricardo et al. 2012). The hardness is defined as maximum force applied to crush the RTE flakes (Anderson and Singh 2003), and the

crispness represents the work done on the flakes, which mimics the sensation of crispness of the flakes between teeth (Vincent 1998; Hofsetz and Lopes 2005). For instance, if the product is brittle, it requires little work to fracture the flakes, and it occurs quickly (Vincent 1998; Saklar et al. 1999). The bowl-life of RTE flakes is a measure of how long RTE flakes stay crunchy and crispy before absorbing milk, and it is always a concern for consumers (Long and Chatel 2006; Ricardo et al. 2012). The high sugar concentrates or slurries, of which are honey, sugars, or syrups are used in the coating process (Fast 2000) to enhance the texture and extend the bowl-life of the cereals. Therefore, the coating with a sugar solution provides moisture barrier properties, preserve the texture, and extend the bowl-life of the RTE flakes (Calandro and Murray 1992). However, most RTE breakfast cereals contain high sugar with the range of 1-56% concentration (Albertson et al. 2013), and the consumers have several health concerns, especially obesity and dental problems (Ruxton et al. 1999; Johnson and Frary 2001) in response to the consumption of RTE flakes.

The high consumption of RTE breakfast cereals in US, which accounts for over 80% RTE products retail sale in the US (Brennan et al. 2013), is another reason emphasizing the importance of health concern to modify the coating materials. Therefore, reducing the sugar content of RTE cereal flakes has become an important issue.

Current research and some companies focus on the reduction of sugar coating and the enhancement of nutrient quality by investigating alternative coating materials in order

to provide similar qualities as sugar coating. Long and Chatel (2006) reported that the coating with artificial sweeteners, but this results in lack of the textural and bowl life properties of original flakes.

Thomas et al. (2013) reported an examination based on the data in the USDA National Nutrient Database for Standard Reference (SR) to track trends in RTE breakfast cereals in response to health concerns. The top-selling companies, Kellogg and General Mills, which were determined based on unit sales from August 2010 to August 2011, reformulated their products in 6 years from 2005 to 2011. They reduced their sugar content from 27.5 to 24.8/100 g product in 6 years, but it was not a significant reduction (Thomas et al. 2013).

Luckett and Wang (2012) reported the investigation of debranched corn starches (Common corn, Waxy corn and Hylon II) with varying amylose content coating on Wheaties ® breakfast cereals. All three debranched starches provided better barrier properties than glucose coating as well as reducing milk absorption, which were correlated with their texture. Hylon II, containing high amylose content had an ability to form a coating layer and contributed a higher dietary fiber value. However, consumers prefer that breakfast cereals contain less sugar, more nutrition, and the desired texture all at the same time.

Cereal flakes need to be improved to enhance the texture and extend the bowl-life of the flakes by replacing of the sugar-based coating materials with alternative materials (Bone et al. 1986; Carpenter et al. 1989; Long and Chatel 2006; Luckett and Wang 2012).

## 1.5. Thesis Objectives

The long-term goal is to increase the utilization of NFDM by using its protein derivate, CaCas. Two studies were conducted towards achieving this goal: (1) by making edible CaCas-based films for packaging application, and (2) by producing edible CaCas-based coatings to apply on breakfast cereals to enhance their textural and nutritional properties.

The objective of the first project is to study the effects of ambient humidity, film thickness, and the addition of citric pectin on the tensile properties of edible CaCas/Gly films as described in Chapter 2.

The objective of the second project is to develop the coating solutions made from NFDM, CaCas, and CaCas in blends with Gly, CP, and NFDM to improve the nutrient profile, extend the bowl-life, and enhance the texture of RTE breakfast cereals as described in Chapter 3.

# 2. EDIBLE FILMS APPLICATION

## 2.1. Experimental Design

### 2.1.1. Overview

CaCas/Gly with CP solutions were prepared to produce CaCas/Gly and CaCas/Gly/CP edible films. The tensile properties of the films were tested at different humidity, film thickness, and amounts of CP. Also, the microscopy images of the films were obtained.

### 2.1.2. Materials and Methods

CaCas powder was obtained from the American Casein Co. (Burlington, NJ, US). Thermo Fisher Scientific Company (Madison, WI) provided its mineral analysis run with an ICP-OES (inductively coupled plasma-optical emission spectrometer), model iCAP 6300 Duo. CaCas contained (as measured over 3 to 6 replicates) 3.0% minerals, consisting of 47.7% calcium, 29.6% phosphorus, 18.2% sodium, 3.8% potassium, 0.5% magnesium, 0.1% zinc and iron, and trace amounts of copper and manganese (Bonnaillie et al. 2014). Glycerol was ACS grade and obtained from Sigma-Aldrich (St Louis, MO, US). DI water was produced by a Milli-Q Synthesis water purification system (Millipore, Billerica, MA, US). Pectin 1400, CP, with a high degree of methoxylation of 60±1% and a molecular weight of 236,000 g/mol (236KDa) was obtained from Danisco USA Inc.

(Madison, WI, US). Food colorant was purchased from a local store (McCormick &Co Inc., Hunt Valley, MD, US) (Bonnaillie et al. 2014).

### 2.1.3. Compositional Analysis of CaCas Powder

#### 2.1.3.1. Moisture Content

The moisture content of CaCas was measured in triplicate according to the method of AOAC (1990). Three dried round aluminum dishes were weighed, and approximately two grams of the sample were put into each dish and weighed. The samples were placed into an oven at 130 °C, heated for 75 min, and then placed in a desiccator and weighed. The percentage moisture was calculated as a percentage difference in the original sample.

$$\%\, Moisture = \frac{W_{C1} - W_{C2}}{W_{C1} - W_D} * 100$$

Where the initial weight of empty dishes is $W_D$, weight of dish and un-dried CaCas sample is $W_{C1}$, and weight of dish and dried CaCas sample is $W_{C2}$.

CaCas contained approximately 3% moisture (Bonnaillie et al. 2014).

#### 2.1.3.2. Ash Content

The ash content of CaCas was measured in triplicate according to the method of AOAC (1990). Three crucibles were weighed, and approximately 4-5 g of CaCas was put into each. The crucibles with CaCas samples were heated in a furnace at 550 °C for at least 6h until it turned white and free of carbon. The crucibles with the samples were then

removed from the furnace, cooled in the desiccator to a room temperature, and then weighed. The weight of ash was calculated by the equation below.

$$\%\ Ash\ Content = \frac{W_{ash} - W_{crucible}}{W_{cru+sample} - W_{crucible}} * 100$$

Where the weight of ash is $W_{ash}$, the weight of empty crucible is $W_{crucible}$, and weight of the crucible with CaCas sample is $W_{cru+sample}$.

CaCas contained approximately 3% ash (Bonnaillie et al. 2014).

### 2.1.4. pH Analysis

The pH of CaCas powder was determined in duplicate by using a pH/ion meter (Mettler Toledo, Schwerzenbach, Switzerland) at room temperature. 12% CaCas solution was prepared with DI water and stirred at 600 rpm until achieving a complete dissolving. Before performing the analysis, the instrument was calibrated with three standard buffer solutions. Its probe (a glass electrode) was merged into the solution and stayed there until a constant value was appeared on the screen. The probe was cleaned with DI water and slightly wiped between each test. The pH value of CaCas was 7.08 at room temperature.

### 2.1.5. Preparation of Edible Film Solution

#### 2.1.5.1. CaCas film solutions with Gly

CaCas/Gly film solutions were prepared with DI water as a control sample to a total solids concentration of 15% (w/w). The ratio of CaCas and Gly was kept constant at 3:1, mixing of CaCas, Gly, and DI water, their concentrations were 11.25, 3.75, and

85% (w/w) in a 100 mL film solution, respectively. After mixing the solution for 2 hours, McCormick Assorted food colorants (two drops/ 40 mL solution), which were blue (Brilliant blue−FD&C Blue 1), green (Allura red AC−FD&C Red 40), yellow (Tartrazine−FD&C Yellow 5), and red (Erythrosine−FD&C Red 3), were added into each film solution to observe the appearance and structure of the dried films because the films without colorant were quite transparent (Tomasula et al. 1998; Bonnaillie et al. 2014).

2.1.5.2. Calcium caseinate/glycerol/citric pectin (CaCas/Gly/CP)

Citric pectin solution (CP)

3% (w/w) CP solution was prepared with DI water by stirring at 1200 rpm for 2 hours because the pectin is hard to dissolve or mix well in the solutions (Rolin 1993). After preparing CP solution, it was added at various amounts in the constant ratio (3:1) of (CaCas/Gly) solution by keeping the same total solids concentration of solution at 15% (w/w). The compounds were added into the DI water one by one, giving an hour to mix for each. After all components were added, the solution was mixed for 2 h followed by adding two drops of food colorant. The film solutions were placed to vacuum filtration flasks to remove air bubbles in the solutions by the laboratory vacuum system (Bonnaillie et al. 2014). In this study, the compounds of film solutions mixed in different sequences to observe the effect of mixing sequences on the tensile properties of the films. Nine film solutions were prepared based on the different formulations with the same compounds at a constant amount of each compound for each solution shown in Table 1.

Table 1: Different formulations used for CaCas/Gly/CP film solutions (mixing sequences) (Bonnaillie et al. 2014)

| Formulation | Components 1+2 | Component 3 | Component 4 |
|---|---|---|---|
| Control A | Water + CaCas | Gly | - |
| A | Water + CaCas | Gly | CPsol |
| B | Water + CaCas | CPsol | Gly |
| E | Water + Gly | CaCas | CPsol |
| Control F | Water + Gly | CaCas | - |
| F | Water + Gly | CPsol | CaCas |
| G | Water + CPsol | CaCas | Gly |
| H | Water + CPsol | Gly | CaCas |
| K | CPsol + Gly | Water | CaCas |

Controls A and F film solutions were prepared without CP. The solutions with CP were prepared with two different concentrations of CP taken from 3% CP solution, 0.3 and 1%, respectively, at the constant of 15% total solid concentration (Bonnaillie et al. 2014).

Table 2: The amount of each material in a 100mL of the film solution w/ and w/o CP (Bonnaillie et al. 2014).

| Film names | 3% CP sol (g) | CaCas (g) | Gly (g) | DI water (g) |
|---|---|---|---|---|
| **Control films w/o CP** | 0 | 11.25 | 3.75 | 85 |
| **A, B, E, F, G, H, and K with 0.3% (w/w) CP** | 0.3 | 11.025 | 3.675 | 85 |
| **A, B, E, F, G, H, and K with 1% (w/w) CP** | 1 | 10.5 | 3.5 | 85 |

### 2.1.6. Preparation of Edible Films for Testing

#### 2.1.6.1. Film casting

Silicone baking mats (Weston Products LLC, Strongsville, OH, US) were used for casting the films. The mats provided easy peeling of dried films. After each use, silicone baking mats were cleaned with diluted Contrex detergent, wiped, and applied 70% ethanol solution, which was prepared with DI water, to remove any detergent residue (Bonnaillie et al. 2014).

Using a K-101 Control Coater apparatus (RK Print-Coat Instruments Ltd., UK) with speed setting #3, each film was casted on the silicone mats placed on Control Coater shown in Figure 4, by using different spreading bars at a controlled thicknesses from 100 to 500μm thick (Bars #100, 150, 200, 300 and 500μm, RK Print-Coat Instruments Ltd., UK) (Bonnaillie et al. 2014). Due to the various scales of the bars, the weight of film solution changed from 3 to 40g for casting the films on the silicone mats (Bonnaillie et al. 2014). Rectangular films with dimensions of approximately 15cm×25cm were produced in Figure 5.

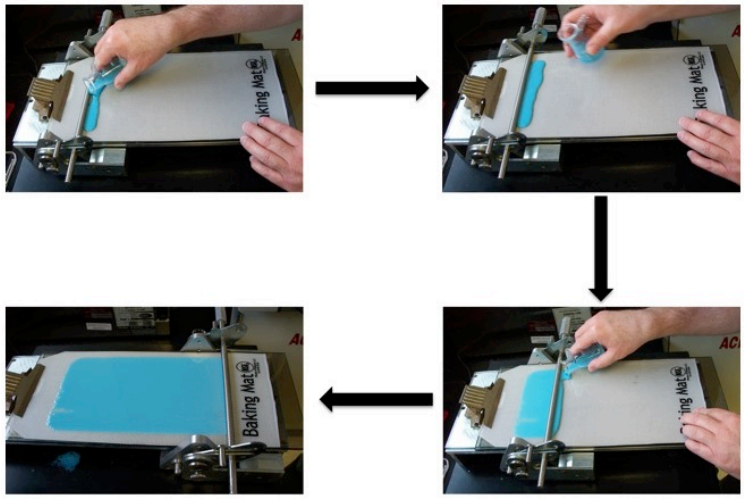

Figure 4: The casting of CaCas/Gly/CP film solution on a silicone mat with different spreading bars by using the Control Coater.

2.1.6.2. Drying and storing

The silicone mats with the casted film solutions were placed on the flat lab benches to allow the film soluions dry for 1-2 days at 20˚C depending on the current humidity. If humidity is high in the air, drying time lasts longer because humidity keeps the casted films wet. Relative humidity (RH) in the lab (Eastern Regional Research Center Wyndmoor, PA, US) varied from 22 to 70% during the year (Bonnaillie et al. 2014). Therefore, the drying of the film solutions can take either longer or shorter than 1-2 days.

After drying, the films were peeled off the mats, placed on a white A4 paper, and stored in an environmental chamber maintained at 50% RH and 20˚C (Model 6020-1, Caron, Marietta, OH, USA) for at least 2 days before testing. To maintain a stable RH in the chamber, DI water line inside the lab was used to supply the chamber with the needed water. Each film was cast at least 3 times (Bonnaillie et al. 2014).

Figure 5: The dried edible films on a white A4 paper.

2.1.6.3. Preparation of strips and thickness measurement

The films were cut into 5 mm×35 mm strips with a razor blade after storing them at 50% RH for 2 days. The filmstrips were subjected to ambient humidity (22 to 70% RH) overnight to reach an equilibrium state before testing the tensile properties of the films. The thickness of each filmstrip was measured using a thickness gauge with 1 μm precision (Mitutoyo Corp., Kanagawa, Japan) on five random locations on the strip. The average value was calculated as the thickness of the films.

2.1.7. Analysis of Edible Films

2.1.7.1. Tensile Properties Analysis

The E, TS, and EAB of the films were determined according to the ASTM D882-98 method (ASTM 1998) by using a TA.XTPlus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, US) with a cross-head speed of 0.2 mm/sec (Figure 6). The filmstrips were mounted vertically onto a tensile grips clamp with 25 mm gauge length, and stress/strain curves were recorded 5-6 strips of each film tested. The various

tensile properties were obtained by using the stress-strain curves. The E (MPa) is defined as the initial slope of the curve; TS (MPa) is the value from the curve which corresponds to the point of maximum stress; and EAB (%) is the value of the Strain (%) at which the strain rapidly decreases upon reaching the maximum stress, TS, shown in Figure 6.

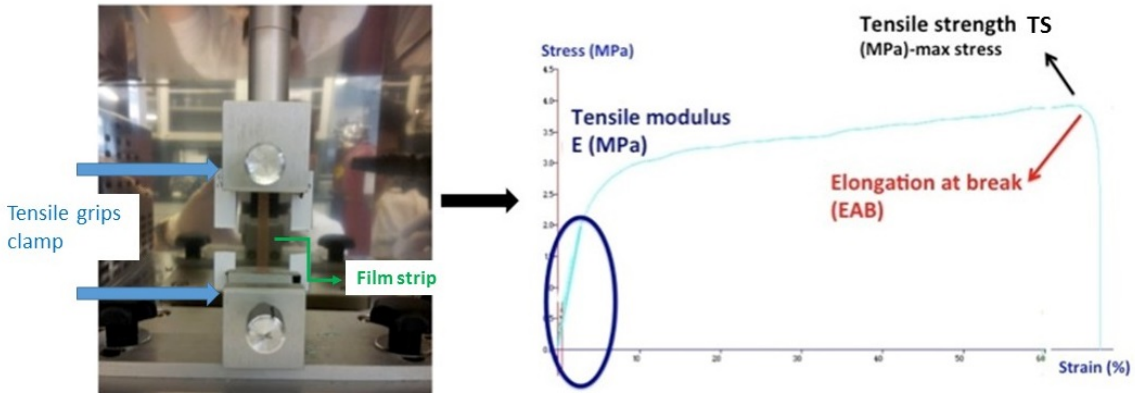


Figure 6: The filmstrip mounted on Texture Analyzer, and data obtained from stress-strain curve.

2.1.7.2. Microscopy Imaging

Dried films were quite transparent and easy to recognize the structural differences by only looking at them through the light. A Nikon Eclipse E800 microscope assembled with a CoolSnap CF digital camera (Nikon Corporation Instruments Company, Japan) was performed to have the appearance of the films. The films were cut with the areas of 2 mm × 3 mm and magnified to 4×. To capture digital images of the films, automatic exposure was used with the conditions that were intensity target of 3000 and 20 KHz of camera speed (Bonnaillie et al. 2014).

2.1.7.3. Statistical Analysis

Differences among E, EAB, and TS of the films based on the film thickness, RH, and CP content were evaluated by analysis of variance with means separation (One-way ANOVA), performed by using SPSS Version 21 (IBM Corp., London, UK) (provided by The State University of New Jersey, Rutgers Software System).

## 2.2. Results and Discussion

### 2.2.1. Tensile Properties

As mentioned in section 2.1.7.1, the various tensile properties of the films were obtained from the stress-strain curves. The E, TS, and EAB of the CaCas/Gly films were analyzed as a function of humidity, film thickness and CP content in this section.

#### 2.2.1.1. The Effects of Thickness and Humidity on the Tensile Properties of Controls films

Control A Films

Control A films were prepared by first mixing CaCas with DI water and then adding Gly with a constant 3:1 ratio of CaCas to Gly, which was a 15% (w/w) total solid concentration. Due to the random coil structure of CaCas (Chen 2002; Chambi and Grosso 2006) and the polar and nonpolar amino acid compositions of casein (Swaisgood 1992), CaCas completely hydrated in DI water. Both hydrogen bonds, which are between polar amino acids and water molecules, and the hydrophobic interactions, which are between the nonpolar amino acids chains, play an important role to form the molecular structure of the films. In the film solutions, Gly is used as a plasticizer, which may slip between the protein network chains to facilitate chain movement (Tomasula et al. 2003). Therefore, it provides flexibility and stretchability properties to the films.

At 58-65% RH and 67-70% RH

Figures 7, 8, and 9 represent the values of E, EAB, and TS for the Control A films as a function of film thickness, which were obtained from the stress-strain curves at 58-65% and 67-70% RH (Bonnaillie et al. 2014).

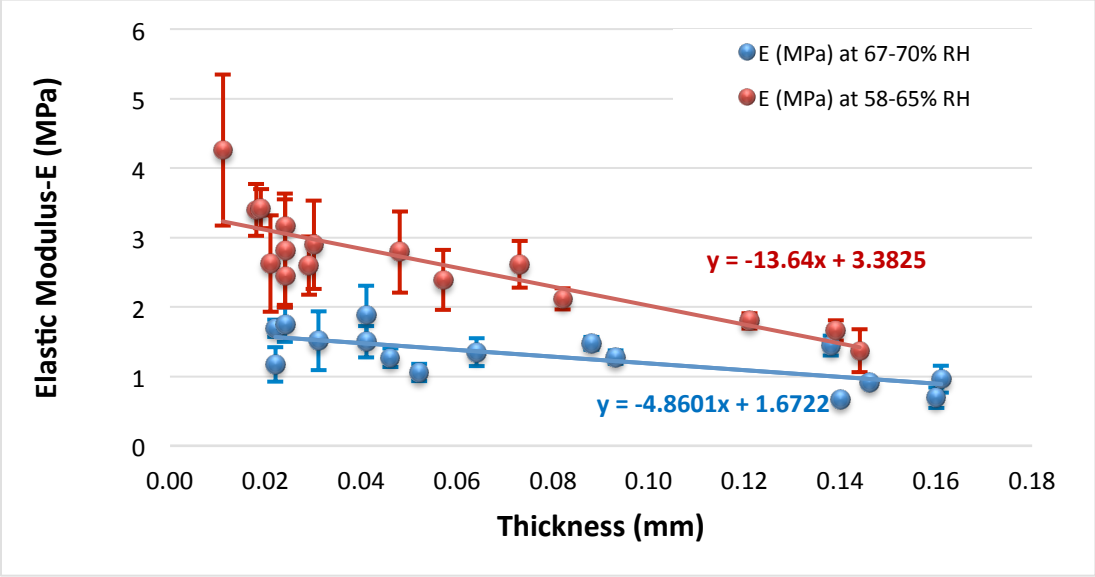


Figure 7: The effect of thickness on the E of Control A films at two different ranges of RH, 58-65% and 67-70% ($p>0.05$).

Figure 7 shows that E of Control A films was not affected by film thickness at 67-70% RH. There was a small change but not statistically significant ($p>0.05$). E decreased from 1.9 to 0.7 MPa at 67-70% RH, whereas E decreased from 4.3-1.4 MPa over the 0.011-0.144mm range of film thicknesses at 58-65% RH.

The results showed that the E values of Control A films at a high RH (67-70%) were less than those at 58-65% RH. The high humidity condition caused the films to become swollen with water molecules because the water molecule had a plasticizing effect in the protein films that increased the effect of the plasticizer (Gly) (Chen 2002). E

was adversely affected by the presence of the plasticizer because the plasticizer increased the mobility of protein chains by inserting itself in three-dimensional polymer network (Siew et al. 1999). Therefore, the interactions were interfered and then loosened the stiff and compact structure of film matrix.

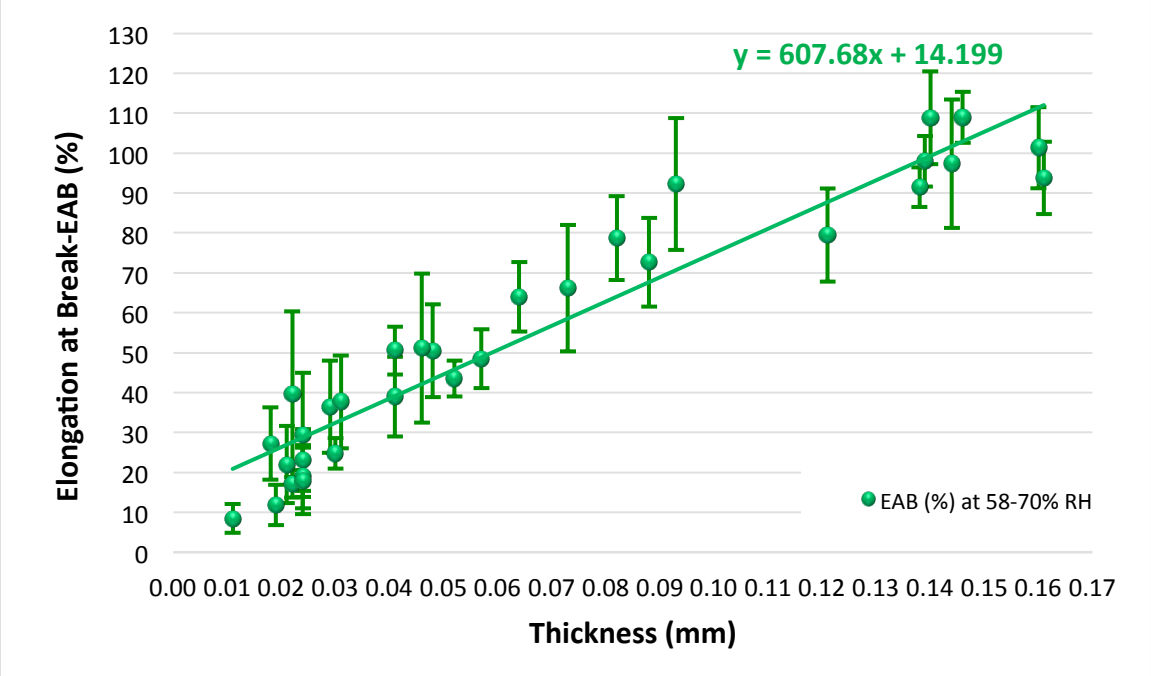


Figure 8: The effect of thickness on EAB of Control A films at 58-70% RH ($p<0.05$).

The effects of the thickness on the EAB of Control A films at 58-70% RH showed in Figure 8. At 58-70% RH, the EAB of the films significantly ranged from 8.45 to 109% with increasing the film thickness from 0.011 to 0.161mm ($p<0.05$). The EAB varied and increased as the films became thicker at a high RH because the high RH favored the EAB of the films to increase because water molecules acts as a plasticizer in protein films, which interfered with the protein-protein interactions and increased the flexibility of

protein chains (Tomasula et al. 1998; Chen 2002; Tomasula et al. 2003). Therefore, the films with the plasticizer became more elastic and stretchable.

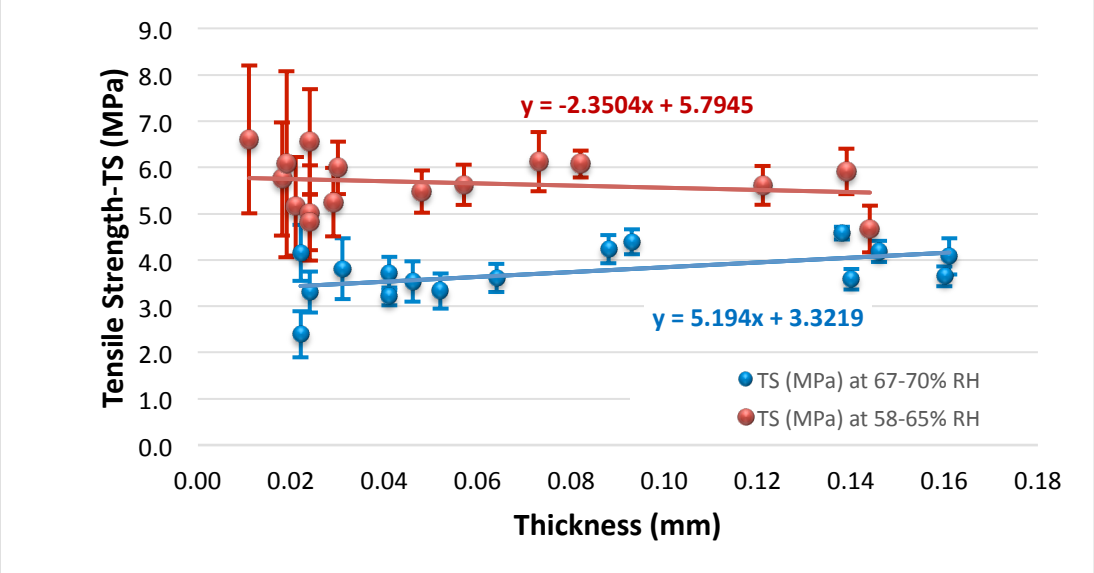


Figure 9: The effect on thickness on TS of Control A films at two different ranges of RH, 58-65% and 67-70%.

The effect of thickness on TS of Control A films at 58-65% and 67-70%RH is shown in Figure 9. The TS of the films slightly increased while increasing the film thickness at 67-70% RH compared to the ones at 58-65% RH ($p>0.05$). Higher ratios of protein to plasticizer in the film solutions resulted in higher values of TS of the films (Chick and Ustunol 1998; Tomasula et al. 1998). However, a high RH favored Gly to decrease the ratio of protein to plasticizer due to the plasticizing effect of water molecules in the protein films (Chen 2002). Therefore, the interactions were adversely affected and then reduced the TS of the films.

Control F Films

Control F films were prepared by first mixing Gly with DI water and then adding CaCas with a constant 3:1 ratio of CaCas to Gly as the same compositions of Control A films, which was a 15% (w/w) total solid concentration.

At 59-69% RH:

Figures 10, 11, and 12 show the E, EAB, and TS of Control F films with a range of film thicknesses. There was a significant change in the E, EAB, and TS of the films, which either increased or decreased, while increasing film thickness ranged from 0.025 to 0.134mm at 59-69% RH ($p<0.05$).

E of Control F films varied and decreased from 4.81 to 0.78 MPa with increasing film thickness from 0.025 to 0.134mm at 59-69% RH in Figure 10.

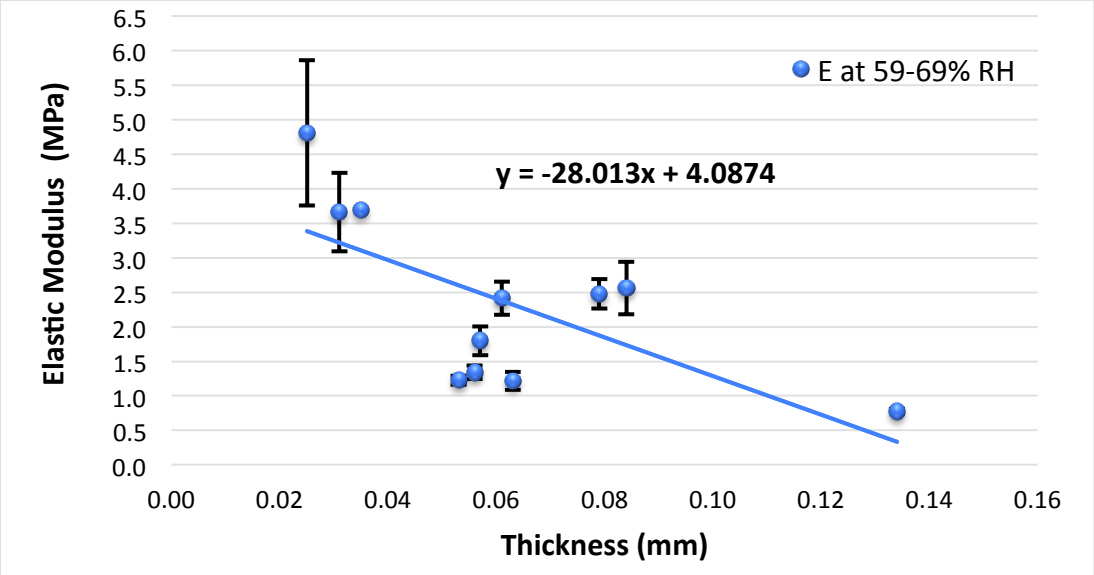


Figure 10: The effect of thickness on E of Control F films at 59-69% RH in 0.025-0.134mm film thickness ($p<0.05$).

EAB values were greatly affected by even small increases of film thickness at a high RH in Figure 11. The EAB of Control F films ranges from 12.2% to 106.1% with 0.025 to 0.134mm film thicknesses at 59-69% RH. Water molecules act as a plasticizer in the protein-based films and make the protein film network become more open and loose structure (Bonnaillie et al. 2014). Therefore, the EAB of the films increases with increasing film thickness at the high RH.

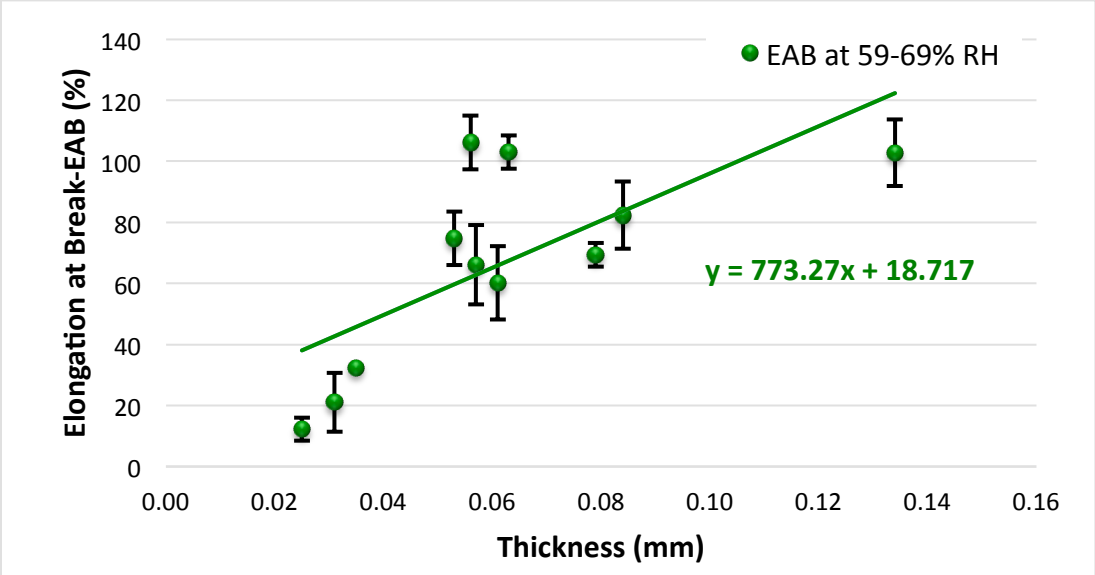


Figure 11: The effect of thickness on EAB of Control F films at 59-69% RH ($p<0.05$).

TS values of Control F films changed based on the increase of thickness at 59-69% RH as shown in Figure 12. TS of the films decreased from 7.4 to 3.8 MPa with increasing film thickness from 0.025 to 0.134mm at 59-69% RH ($p<0.05$).

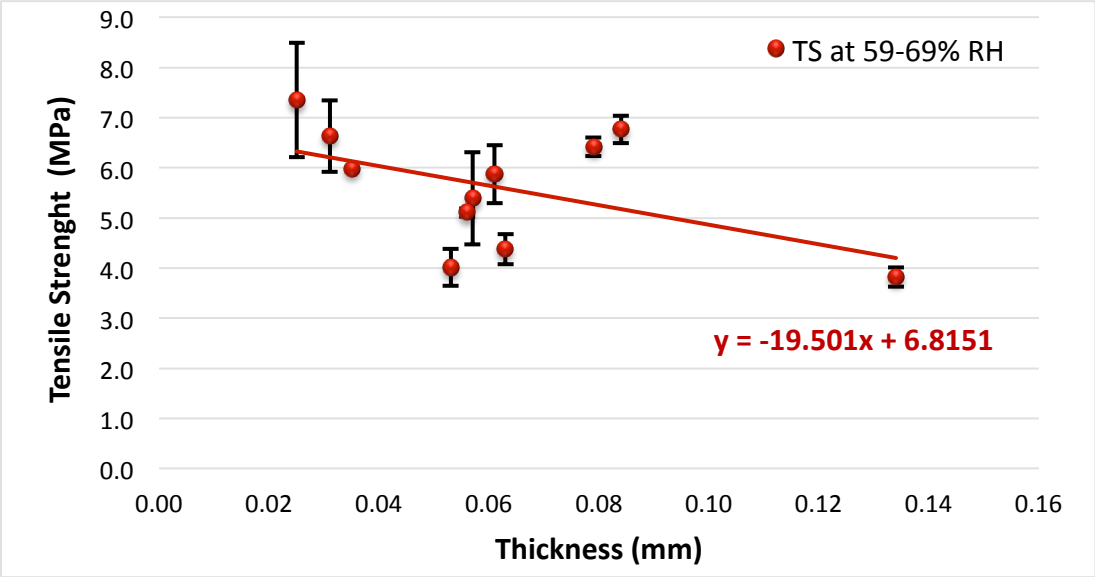


Figure 12: The effect of thickness on TS of Control F films at 59-69% RH ($p<0.05$).

The tensile property results of the Controls A and F films as a function of RH and film thickness are encouraging to use the films in either the dry foods that require little elasticity, such as coatings for RTE breakfast cereals, or soluble applications, such as single-serve soup pouches (Bonnaillie et al. 2014). These films may also be used as stock lids, which do not require flexible and stronger films, unlike plastic bags used for carrying the food products.

The average E, EAB, and TS of Controls A and F films at two different film thicknesses, which were 0.03 and 0.09mm is shown in Table 3.

Table 3: The average tensile properties of Control films (w/o CP) at 59-69% RH

| Control films (w/o CP) | Average film thickness (mm) | E (MPa) | EAB (%) | TS (MPa) |
|---|---|---|---|---|
| **Control A** (Water+CaCas+Gly) | 0.03 | 2.38±1.04 | 30.64±13.17 | 4.71±1.34 |
| | 0.09 | 2.11±1.01 | 73.64±10.21 | 5.49±1.45 |
| **Control F** (Water+Gly+CaCas) | 0.03 | 3.35±1.51 | 35.08±27.72 | 5.99±1.43 |
| | 0.09 | 2.04±0.73 | 82.50±17.32 | 5.95±1.48 |

EAB was affected by the film thickness. EAB values approximately doubled with the increase of the film thickness. The addition of Gly reduced polymer chain interactions that reduced the strength of polymer network and increased the susceptibility of the films to swell water vapor molecules (Tomasula et al. 1998; Dangaran et al. 2006; Bonnaillie et al. 2014). This change in molecular structure enabled the film to become more elastic and resistant to the force applied. Due to the plasticizing effects of water molecules, the movement of polymer chains in the protein network was facilitated that was related to the increase of film flexibility (Chen 2002). However, the values of tensile properties of CaCas/Gly films in our study did not confirm the previous study results in Tomasula et al. (1998). Tomasula and others (1998) found that the TS of CaCas/Gly with 7:3 ratio was 1.9 MPa in 0.15mm film thickness, which was lower than our value of 5.95 MPa in 0.09mm thickness, and the EAB was 76% in 0.15mm thickness, which was also lower than our value of 82.5% in 0.09mm thickness. The reasons for the differences are probably both the different film thicknesses and the ratio of CaCas to Gly. They used 0.15mm film thickness, which was thicker than our film thickness of 0.09mm, and their ratio of CaCas to Gly was 7:3, which is lower than the 3:1 ratio of CaCas to Gly in our study. Tomasula and others (1998) used 30% (w/w) Gly in CaCas film solutions at the constant of 6% (w/w) total solid concentration, whereas 5% Gly in CaCas solutions at the constant of 15% (w/w) total solid concentration was used in our study. Therefore, TS decreased and EAB increased with increasing plasticizer content that resulted in the film to become less stiff and more stretchable (Chick and Ustunol 1998). Also, the cross-head

speed of 5mm/min, which was lower than our value of 12mm/min was used for the tensile analysis in their study (Bonnaillie et al. 2014).

2.2.1.2. The Effect of 0.3 and 1% (w/w) CP concentrations on A, B, E, F, G, H, and K Films

The mixing sequences are shown in Table 4 while preparing the CaCas-based edible film solutions with 0.3 and 1% (w/w) CP.

Table 4: Different formulation used for CaCas/Gly/CP film solutions (mixing sequences) (Bonnaillie et al. 2014)

| **Formulation** | **Components 1+2** | **Component 3** | **Component 4** |
|---|---|---|---|
| A | Water + CaCas | Gly | CPsol |
| B | Water + CaCas | CPsol | Gly |
| E | Water + Gly | CaCas | CPsol |
| F | Water + Gly | CPsol | CaCas |
| G | Water + CPsol | CaCas | Gly |
| H | Water + CPsol | Gly | CaCas |
| K | CPsol + Gly | Water | CaCas |

Blends of CaCas/Gly films with CP and the change of mixing sequences affected the tensile properties of the films. The addition of 0.3% CP in the film solutions increased EAB, and reduced E and TS of the A, B, E, and F films while providing a higher TS, and lower E and EAB of the G, H, and K films as shown in Figure 13. The A and B films had minor different results with 0.3% CP. Adding a small CP after the addition and mixing of CaCas in the film solutions interrupted the interactions among CaCas, Gly, and DI water

by crosslinking with $Ca^{+2}$, which caused an uneven distribution of the interactions among the molecules (Thakur et al. 1997; Maroziene and Kruif 2002; Bonnaillie et al. 2014).

1% CP provided an increase in E, decrease in EAB, and increase in TS of the A, B, E, and F films. These films became stiffer, stronger, and less elastic, unlike the G, H, and K films.

With increasing pectin content in the film solutions, the G, H, and K films were stiffer, more stretchable, and stronger than the same films made from the film solutions with 0.3% CP concentration. E films with 0.3% CP showed a higher elasticity, less stiff, and less strong qualities, while the one with 1% CP was stiffer, stronger, and less elastic (Bonnaillie et al. 2014).

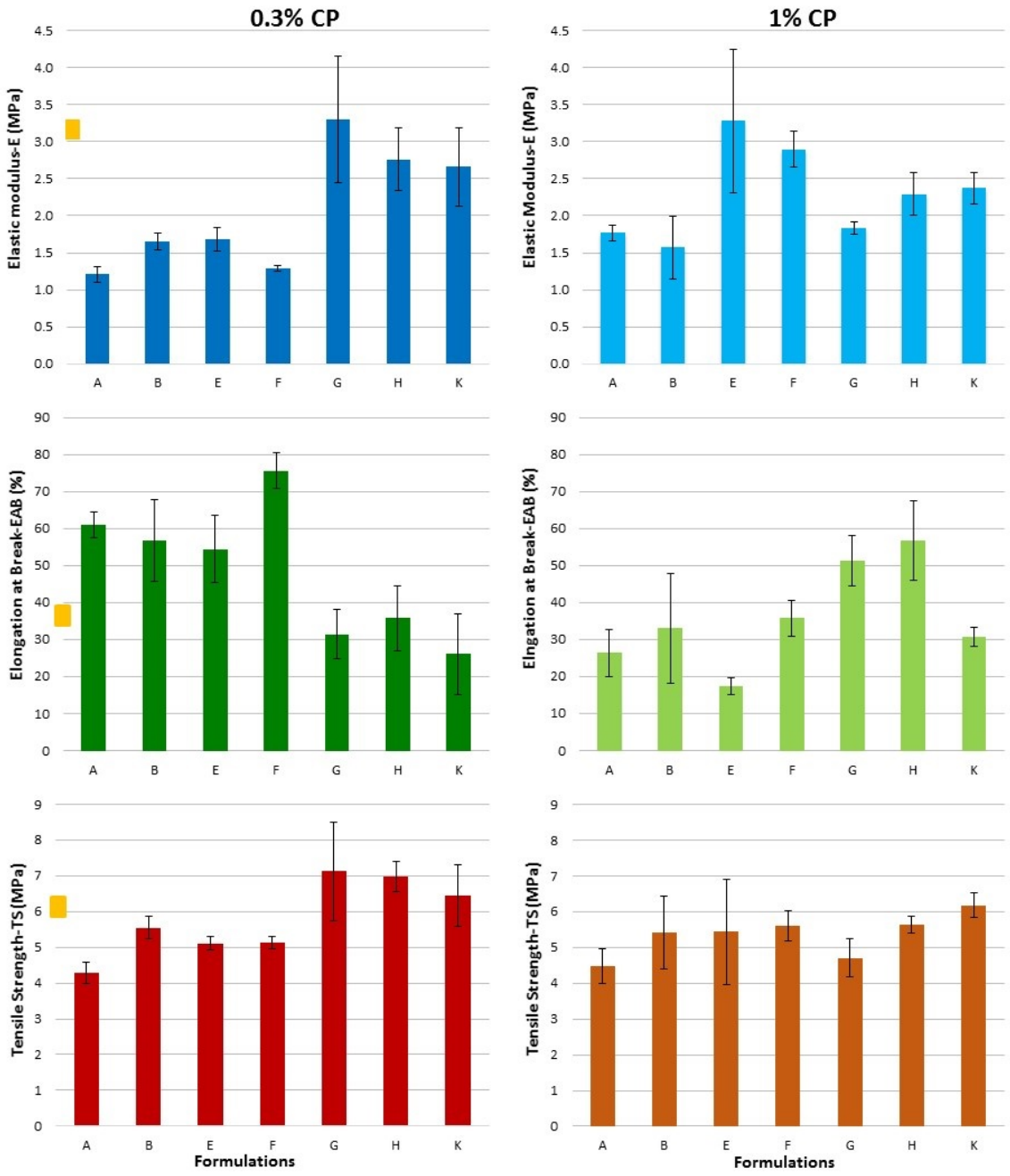


Figure 13: E, EAB, and TS properties of different film formulations with 0.3 and 1% CP at 54-58% RH in 0.03-0.05mm film thickness. Rectangular symbol represents the average values of Control films according to Table 3 (Bonnaillie et al. 2014).

2.2.1.3. A, F, and G formulations with 0.3 and 1.0% (w/w) CP concentrations

Due to the blends of CaCas/Gly film solutions with the addition of CP and the differences in the formulations, the EAB of the A, F, and G films was affected. The A films showed a significant decrease in EAB from 61.01 to 26.38% with 0.3 and 1% CP content, respectively ($p<0.05$) at 55% RH. The EAB of F and G films were not changed significantly ($p>0.05$), unlike the A films. The EAB of F films decreased from 63.97 to 35.78%, and the EAB of G films decreased from 60.25 to 51.38% for with 0.3 and 1% CP content, respectively. Also, the changes in the E and TS values with increasing CP content were insignificant for all three films in the 0.03-0.05mm film thickness at 55% RH ($p<0.05$).

Table 5: The average tensile properties of A, F, and G films with 0.3 and 1% (w/w) CP at ~55% RH.

| **Films name (mixing sequences)** | **CP (%)** | **Average film thickness (mm)** | **E (MPa)** | **EAB (%)** | **TS (MPa)** |
|---|---|---|---|---|---|
| A (Water+CaCas+Gly+CP) | 0.3 | 0.051 | 1.35 ±0.26 | 61.01 ±2.67 | 4.51 ±1.24 |
| | 1.0 | 0.037 | 1.77 ±0.11 | 26.38 ±6.31 | 4.49 ±0.49 |
| F (Water+Gly+CP+CaCas) | 0.3 | 0.038 | 2.06 ±1.07 | 63.97 ±16.19 | 5.36 ±0.97 |
| | 1.0 | 0.040 | 2.90 ±0.24 | 35.78 ±4.87 | 5.60 ±0.42 |
| G (Water+CP+CaCas+Gly) | 0.3 | 0.047 | 2.16 ±1.6 | 60.25 ±40.73 | 5.80 ±1.87 |
| | 1.0 | 0.035 | 1.84 ±0.08 | 51.38 ±6.73 | 4.71 ±0.54 |
| Control A (Water+CaCas+Gly) | 0 | 0.031 | 2.38 ±1.04 | 30.64 ±13.17 | 4.71 ±1.34 |
| Control F (Water+Gly +CaCas) | 0 | 0.033 | 3.35 ±1.51 | 35.08 ±27.72 | 5.99 ±1.43 |

A films with 0.3 and 1% (w/w) CP slightly increased the E and decreased the TS of the films that resulted in the films with less stiff and resistant compared to the Control A films with 2.38 MPa of E, and 4.71 MPa of TS values. However, the EAB of A films with CP increased from 30.64% (w/o CP) to 61.01% (w/ 0.3% CP content), and then decreased to 26.38% (w/ 1% CP content). Due to the addition of CP at the end, pectin had few available sites to bind. Therefore, it caused a loose crosslinks with CaCas and interacted with other molecules (Bonnaillie et al. 2014).

The results showed that F films with CP had more flexible and less resistant properties. F films with 0.3 and 1% CP increased both the E and TS of the films, but they were lower than the E and TS of the Control F films that had 3.35 MPa of E and 5.99 MPa of TS. CP added before the addition of CaCas in the film solution may provide an even dispersion to the pectin molecules in the film solutions, but 0.3% CP may be insufficient to crosslink with all CaCas molecules. Therefore, the crosslinks were loosely formed. The loose crosslinks between CaCas and CP increased the TS and EAB of the F films. However, the films with higher pectin content may increase the chance of the formation of crosslinks that increase E, and reduce EAB and TS of the films (Bonnaillie et al. 2014).

G films with 0.3 and 1% CP decreased both the E and TS of the films compared to the Controls. However, the EAB of G films with CP increased from 30.64% (w/o CP) to 61.01% (at 0.3% CP content), and then slightly decreased to 51.38% (at 1% CP content) unlike the A films with 1% CP. G films with 1% CP indicated the opposite

results to the F films with 1% CP. The reason could be the addition of Gly at the end in the film solutions because it may disturb the crosslinks and hydrogen bonds of the protein chains that reduce the E, and increase the TS of the G films with 1% CP (Bonnaillie et al. 2014).

The results in this section indicated that the mixing sequences of CP in the film solutions altered the mechanical strength of the A, F, and G films because the carboxyl groups of pectin interacted with water molecules before CaCas. When adding CaCas in the film solution after the addition of pectin, CaCas with polar and nonpolar side chains employed between pectin and water molecules. The polar side of CaCas interacted with water molecules, and nonpolar amino acid chains of CaCas bound with pectin molecules (Swaisgood 1992; Chen 2002; BeMiller 2007). Therefore, each compound interacts with each other differently based on the addition and mixing order of the film compounds in the film solutions.

### 2.2.2. Microscopy Images

All microscopy images of the films were captured with a low magnification of 4× to demonstrate the structure of the films in macromolecular level at about 60% RH (Bonnaillie et al. 2014).

2.2.2.1. Controls A and F films Micrographs

Controls A and F films showed a different macroscopic structure even though the compositions and their amounts in the film solutions were the same. The only difference came from the mixing order of Gly (at first or the end) in Table 6 (Bonnaillie et al. 2014)

Table 6: Formulation of Controls A and F (Bonnaillie et al. 2014)

| **Formulation** | **Components 1+2** | **Component 3** | **Component 4** |
|---|---|---|---|
| Control A | Water + CaCas | Gly | - |
| Control F | Water + Gly | CaCas | - |

CaCas hydrated by interacting with water molecules in Control A film solutions. By adding Gly after the mixing of CaCas in the film solutions, the water molecules were not available for Gly, which had a high affinity for water in the solutions (Miner and Dalton 1953). Gly reduced the strength of the protein-protein interactions by interrupting the hydrogen bonds between CaCas and water molecules. Therefore, Control A films showed randomly distributed small particles, smooth, and cloudy areas in microscopic, as shown in Figure 14. The smooth areas may be the reason of hydrophobic interactions of CaCas in water and not interfered by Gly that mostly reduced the strength of weak bonds such as hydrogen bonds (Chick and Ustunol 1998). The cloudy packets circled with red color (shown in Figure 14) may respond to the electrostatic interaction between CaCas and OH groups of Gly attached with the water molecules. In our system, if Gly is mixed with DI water by first, three hydroxyl groups of Gly interact with the water molecules. When CaCas added into the film solution after the mixing of Gly, it interacted with Gly

and unbounded water molecules. Therefore, it provided rough, tight, and randomly distributed small particles of pectin molecules, as pointed with the arrows in Figure 14.

The macroscopic images of Controls A and F films confirmed that molecular changes were correlated to the tensile properties of the films. Control F films were more stretchy, stronger, and stiffer than Control A films, shown in Table 3 even though the tensile properties of both Controls were similar.

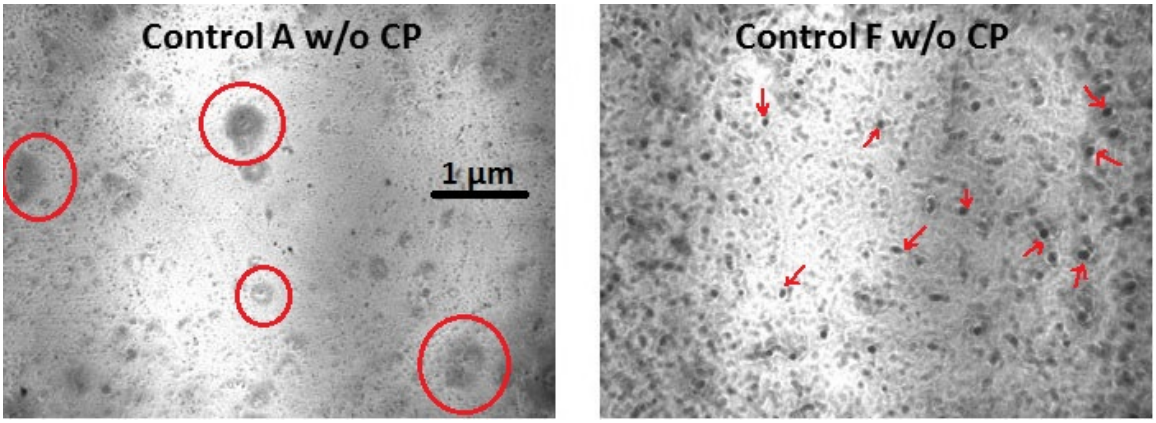


Figure 14: Micrographs of Controls A and F with 4× magnifications.

2.2.2.2. CaCas/Gly/CP Films Micrographs

Figures 15 and 16 show the microscopic images of the films prepared based on the different formulations of the films solutions, which contained 0.3 and 1% (w/w) CP (Bonnaillie et al. 2014).

The formulations of CaCas/Gly films with 0.3% (w/w) CP

The addition of 0.3% (w/w) CP in the film solutions at the different mixing order, all films (the A, B, E, F, G, H, and K films) were observed with a new molecular configuration that composed of either large, heterogeneous, less tight, and aggregated

particles, or homogeneous, more tight, and small particles of the pectin molecules. The A and B films had similar microscopic images because CP was added into the film solution after the mixing of CaCas in DI water that resulted in CaCas interacted with water molecules via both hydrogen bonds and hydrophobic interactions (Maroziene and Kruif 2000). After the addition of a small amount of pectin, it interfered with the interactions to crosslink with $Ca^{+2}$ that caused an uneven distribution of interactions among the molecules. Pedersen and Jorgensen (1991) concluded that casein/pectin solution with $Ca^{+2}$ favored the crosslink between pectin and casein complexes to forms the aggregated structure. Also, the images are in agreement with the tensile properties of A and B films with 0.3% CP, as showed in Figure 13 because the changes in the molecular structure of the films are correlated to the mechanical strength of the edible films (Lacroix et al. 1998; Letendre et al. 2002; Bonnaillie et al. 2014).

E films with 0.3% CP had an open and loose film structure as the F films, but E films had a small, tight and unevenly distributed pectin particles in the microstructure of the films. The F films with 0.3% CP showed a broad dispersed and loose aggregated pectin molecules, as shown in Figure 15. The difference was occurred due to the addition of CP before CaCas in F film solutions, which favored the pectin molecules to disperse well by forming hydrophobic interactions by avoiding water molecules (BeMiller 2007). G films with 0.3% CP demonstrated well dispersed, evenly distributed, small, tight, and homogeneous pectin molecules that were positively correlated with forming a stiffer and stronger film. Therefore, G films with 0.3% CP showed a higher moduli and strength, and

less elastic tensile properties (Bonnaillie et al. 2014). H and K films with a small CP content showed the formation of small and large, heterogeneous, aggregated, and gelled pectin molecules. Also, they had a denser aggregation and large gelled pectin molecules, which probably explained the results of stiffer and stronger H and K films in the section 2.2.1.2 above (Bonnaillie et al. 2014).

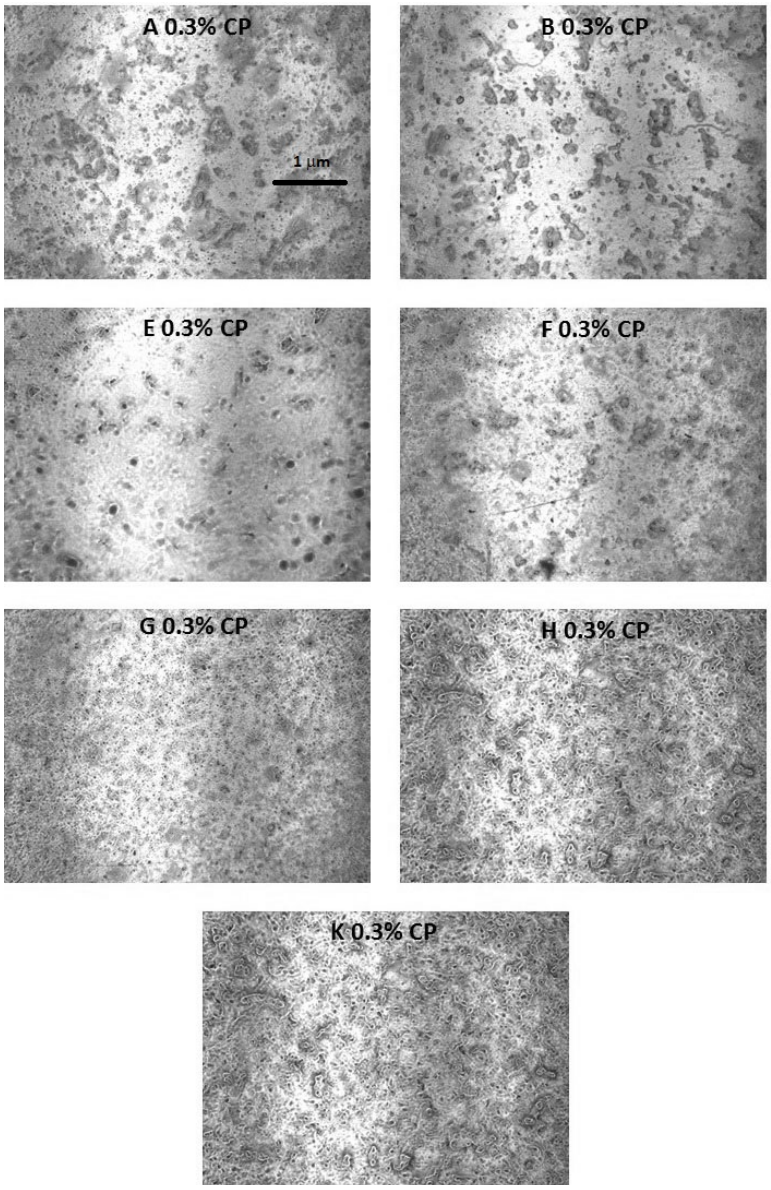


Figure 15: Micrographs of film formulations A to K with 0.3% CP concentration with 4×magnification (Bonnaillie et al. 2014).

The formulations of CaCas/Gly films with 1% (w/w) CP

1% CP addition caused a large and heterogeneous particle formations in A and E films because pectin was added into a viscous solution (15% CaCas solution), and it would not possibly perform a well disperse, which lead pectin molecule to form aggregated particles with $Ca^{+2}$ and other pectin chains (Bonnaillie et al. 2014). H and K films showed that new configuration of molecules occurred. F and G films with 1% CP had a small, tight, and homogeneous particle in Figure 16. In these two films, CP was added in the solution before CaCas; therefore, it dispersed evenly through the solutions that allowed the pectin molecules to contact with more CaCas molecules in the solutions. These microscopic images confirmed the tensile properties of F and G films with 1%, provided in Figure 13 and Table 5. It was obvious that the changes and new configurations in molecular structure affect the tensile properties of films due to the molecular interaction of the film compounds (Bonnaillie et al. 2014).

The various results of tensile properties and different macroscopic images of the films showed that the physical properties of the films were affected by humidity changes, film thicknesses, compositions, preparations, and testing conditions (Bonnaillie et al. 2014).

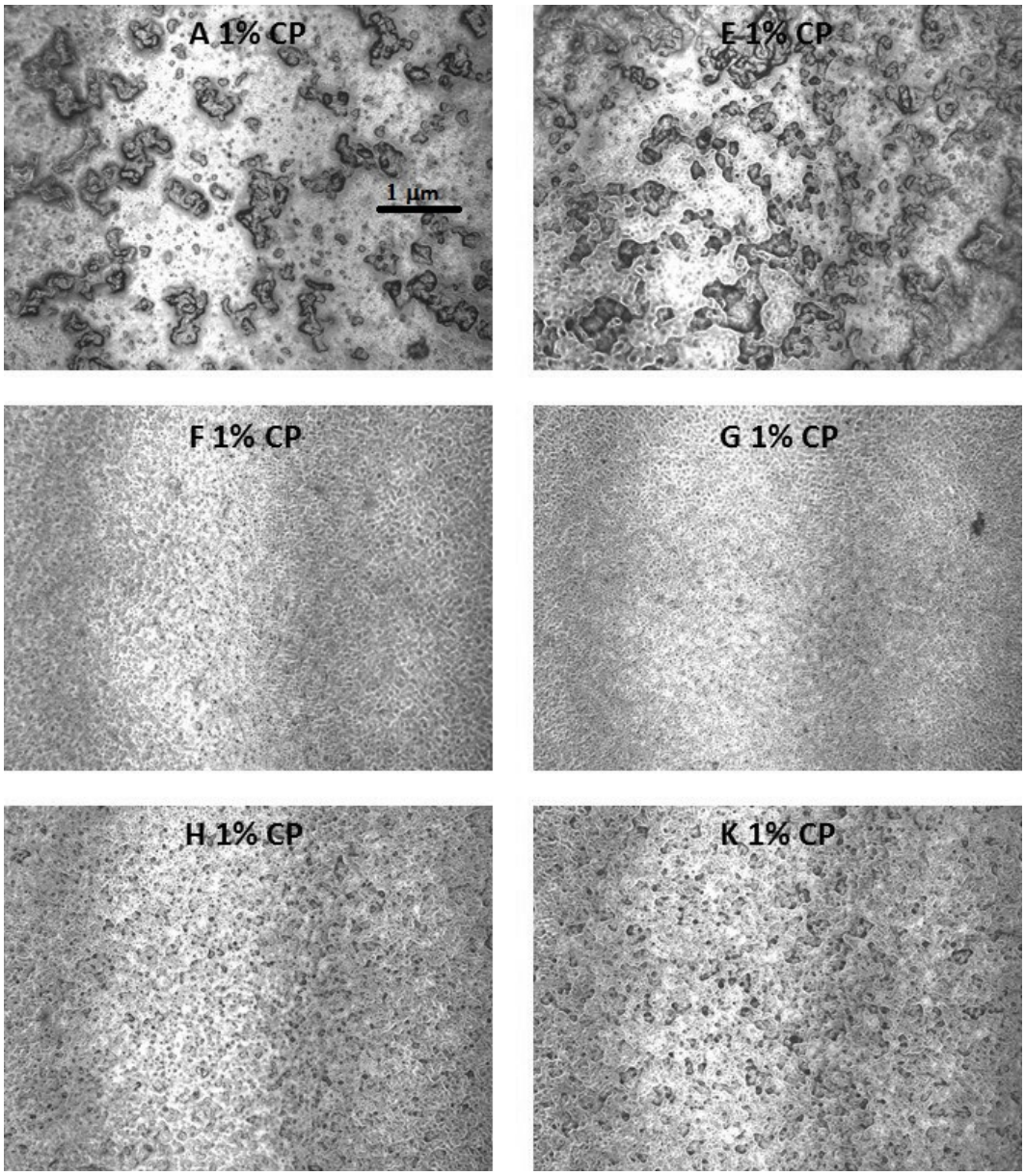


Figure 16: Micrographs of film Formulation A to K with 1.0% CP concentration with 4×magnification (Bonnaillie et al. 2014).

## 2.3. Summary

The values of E, EAB, and TS of all films w/ and w/o CP were obtained from stress-strain curve at different film thicknesses and a narrow range of RH.

The results of Control A films showed that the film thickness had a slight effect on E and TS of the films, which was insignificant ($p>0.05$), while EAB values of the films were significantly affected by the increase of film thickness from 0.01 to 0.144mm at 58-70% RH ($p<0.05$). E of Control A films decreased from 4.3 to 1.4 MPa at 58-65% RH, whereas the E value of the films was 1.9 MPa in 0.02mm film thickness, which was less than the ones with 4.3 MPa in the same film thickness at 58-65% RH, and decreased to 0.7 MPa (in 0.14mm thickness) at 67-70% RH.

The E and TS of Control F films varied and decreased, whereas EAB ranged and greatly affected by increasing film thickness at 59-69% RH ($p<0.05$). The E values of Control F films spread and decreased from 4.81 to 0.78 MPa with increasing the film thickness from 0.025 to 0.134mm at 59-69% RH. The EAB of Control F films ranges from 12.2% to 106.1% with 0.025 to 0.134mm film thicknesses at 59-69% RH. TS of Control F films decreased from 7.4 to 3.8 MPa with over the same range of film thickness and RH. Controls films became less stiff and resistant, and more elastic because network structure became more open and loose with increasing the film thickness at a high RH due to the plasticizing effect of water molecules that reduced the E and TS, and increased

the EAB of the caseinate-based films (Tomasula et al. 1998; Chen 2002; Bonnaillie et al. 2014).

Beyond E and TS values of Control films, EAB of both films boosted in 0.09mm film thickness compared to 0.03mm film thickness at 59-69% RH. EAB values approximately doubled at the 0.03 and 0.09mm of average film thickness. EAB increased from 30.64 MPa in 0.03mm film thickness to 73.64 MPa in 0.09mm film thickness for Control A films. EAB of Control F films increased from 35.08 in 0.03mm thickness to 82.50 MPa in 0.09mm thickness. The thick film favored the edible films to become more elastic and flexible at high RH.

0.3 and 1% (w/w) CP addition and film formulations (A, B, E, F, G, H, and K films), which were differentiated by the mixing sequences of compounds in the film solutions, affected the tensile properties of the CaCas/Gly films. 0.3% CP increased EAB, and reduced E and TS of the A, B, E, and F films while providing stronger, less stiff, and less elastic properties to the G, H, and K films. 1% CP provided an increase in the E, decrease in the EAB, and increase in the TS of A, B, E, and F films. These films became stiffer, less elastic, and stronger, unlike the G, H, and K films. The G, H, and K films solutions with 1% CP resulted in the films with less stiff, more flexible, and more elastic than the films with 0.3% CP concentration (Bonnaillie et al. 2014).

The films made of CaCas/Gly/CP showed a decrease in EAB from 61.01 to 26.38% for A films, 63.97 to 35.78% for F films, and 60.25 to 51.38% for G films with 0.3% and 1% CP content, respectively, in approximately 0.05mm film thickness at 55%

RH. However, the changes in E and TS values with increasing CP content was not significant for all three films in 0.05mm thickness ($p>0.05$) (Bonnaillie et al. 2014).

Microscopy images of films were also captured to observe the molecular structure changes and new configurations of compounds that were used to make the edible films. Control A films had randomly distributed small particles, smooth, and cloudy areas, whereas Control F films had a rough, tight, and mostly homogeneous and randomly distributed small particles of pectin molecules (Bonnaillie et al. 2014).

With adding of 0.3% CP in each film, all films showed a new molecular configuration composed of either large particles, heterogeneous, less tight, aggregated, or homogeneous, tightly aggregated, and small particles of pectin molecules. Both A and B films had similar microscopic images. E films with 0.3% CP showed an open structure as F films, but E films had a small, tight, and heterogeneous pectin particles in the open network structure. F films with 0.3% CP showed a broad spread and loose aggregated pectin molecules. G films with 0.3% CP demonstrated evenly distributed, small, tight, and homogeneous pectin molecules that were positively correlated to the formation of stiffer and stronger film. H and K films with a small CP content showed the formation of small and large, heterogeneous, aggregated, and gelled pectin molecules (Bonnaillie et al. 2014).

Increasing CP content in the film solutions had both positive and negative effects on the molecular structure of the films. 1% CP addition caused a large and heterogeneous particle formations in A and E films while F and G films with 1% CP had a small, tight,

and homogeneous pectin particle distributions throughout the film layer. H and K films showed that new configuration of molecules occurred by forming a tight, small, and large, pectin molecules in the protein network (Bonnaillie et al. 2014).

# 3. EDIBLE COATINGS APPLICATION

## 3.1. Experimental Design

### 3.1.1. Overview

The protein content, milk gain, milk absorption rate, and texture of RTE breakfast cereals coated with CaCas and in blends with Gly, CP, and NFDM solutions were examined. The texture analysis of the flakes with different coatings was performed in both dry state, which is the term used here for the dried flakes coated with the various solutions and wet state of the flakes, which is the term used for the soaked flakes resulting from placing the treated flakes in 1% reduced fat milk at 8 °C for 3 min. Additionally, the surface appearances and morphologies for the different coating treatments on the flakes were observed.

### 3.1.2. Materials and Methods

Refer to the analysis in the section 2.1.2 above, the same calcium caseinate (CaCas), citric pectin (CP), glycerol (Gly), and de-ionized (DI) water were used. Nonfat dry milk powder (NFDM) was purchased from American Casein Co. (Burlington, NJ). Glucose (α-D-Glucose anhydrous, 96%) was purchased from Sigma-Aldrich (St. Louis, MO). Wheaties® (General Mills, Minneapolis, MN) and 1% reduced fat milk (Lehigh Valley Dairy, Pasteurized-Grade A, Lansdale, PA) was purchased from a local grocery store. Lehigh Valley Dairy Farms® provided that 1% reduced fat milk contained 90.75%

water, and 9.25% solids, of which are 8% protein, 25% phosphorous, and some vitamins and minerals. Several 314 mL mist bottles with a non-clogging nozzle (Trudeau Corp. INC, Chicago, IL), were purchased from Walmart (Wyndmoor, PA, US).

Wheaties® (General Mills, Minneapolis, MN) was purchased from a local grocery store (Wyndmoor, PA, US). Wheaties ® contained 81.5% total carbohydrates, which is composed of dietary fiber, sugar, and other carbohydrate, 7.4% (2 g) protein, 1.9% fat, some vitamins and minerals per serving size (27 g), provided by the manufacturer.

### 3.1.3. Compositional Analysis

#### 3.1.3.1. Moisture and Ash Analysis of NFDM Powder

The moisture content and ash content of NFDM were measured in triplicate according to the method of AOAC (1990) by following the same conditions in the sections 2.1.3.1 and 2.1.3.2 above. NFDM contained approximately 6.0% moisture and 6.0% ash.

#### 3.1.3.2. Crude Protein Analysis of CaCas and NFDM Powders

The protein analysis of CaCas and NFDM were carried out according to the method of AOAC 992.23 (AOAC 2000). Nitrogen content of the sample was obtained via EA1112 Nitrogen/Protein Analyzer (Thermo Electron Corp. Waltham, MA, US), known as Combustion Method. Each sample was weighed approx. 20 mg into the tin capsule and squeezed to prevent any sample leakages without damaging to the capsule

surrounded to the samples. Aspartic acid was used for calibration. Each sample analyzed in duplicate, and the average was counted as nitrogen percentage. The percentage of crude protein was calculated based on the protein factor of foods. For example, it is 6.38 for milk and dairy products (Jones 1931; Mosse 1990).

$$Crude\ protein, \% = \%N * protein\ factor$$

%N is the nitrogen percentage of sample

The following test conditions were used: furnace temperature of 950°C for pyrolysis of the samples in pure (99.9%) oxygen; isolation system, which isolates liberated nitrogen gas from other combustion products for subsequent measurement by thermal conductivity detector; and detection system, which interprets detector response as percent of N (w/w). CaCas and NFDM contained 87.7 and 35.9% of protein, respectively.

3.1.3.3. Crude Protein Analysis of Wheaties

The protein content of Wheaties ® was carried out by following the method of AOAC 992.23 (AOAC 2000) for cereal grains and their products by using Nitrogen/Protein Analyzer used the same conditions in the section 3.1.3.2 above. The cereals flakes were ground using a mortar and pestle to have suitable fineness resulting in achieving precision, and the fraction that passed through a 1.4- mm screen was collected and used for the analysis. The sample was weighed approximately 20 mg into the tin capsule and squeezing it to prevent any sample leakages without damaging to the capsule surrounded to the samples. Aspartic acid was used for calibration. Each sample analyzed

in triplicate, and the average was counted as nitrogen percentage. To calculate the percentage of crude protein, protein factor that is 5.83 of whole wheat products (Merrill and Watt, 1973) was used. The result showed that it contained approximately 3-4 g protein per serving size (27 g of Wheaties). However, the manufacturer provided it contained 2 g protein per serving size. The reason of difference might be that the combustion method measures total organic nitrogen, not just protein nitrogen (Jones 1931; Chang 2010).

### 3.1.4. Preparation of Coating Solutions

#### 3.1.4.1. Glucose and CaCas Solutions with Gly and CP

All solutions were prepared with DI water to a constant 15% (w/w) total protein concentration (except glucose solution) by mixing for approximately 2h at room temperature before applying on a cereal product. They were prepared fresh before spraying.

Glucose solution: 15% (w/w) glucose solution was prepared with DI water by stirring at 600 rpm until it dissolved completely.

CaCas/Gly solution: 5 g Gly was mixed with 80 g DI water stirred at 600 rpm for 30 min. Then, 15 g CaCas was added into the solution and continued to mix for 2h to prepare a 100mL coating solution.

CaCas/Gly/CP solutions: At first, 3% (w/w) CP solution was prepared with DI water by stirring at 1200 rpm for 2h (Bonnaillie et al. 2014) because CP is difficult to dissolve in

the solution (Rolin 1993)

After the preparation of CP solution, 5 g Gly was mixed with 80 g DI water stirred at 600 rpm for 30 min. Then, 0.3 g of 3% CP solution (0.009 g of CP powder) was added into the Gly/DI water solution, stirred at 600 rpm for 30 min. 15 g CaCas was added into the solution and continued to mix for 2h.

CaCas/CP solution: 0.3 g of 3% CP solution (0.009 g of CP powder) was taken and added into 85 g DI water in a beaker followed by stirring at 600 rpm for 30 min. Then 14.99 g of CaCas powder was added and continue to mix for 2h at room temperature.

Table 7: The amount of materials for the preparation of coating solutions with constant concentration of protein sources (except glucose solution), 15%.

| Solution name | Amount of dry material (g) | Amount of DI water (g) | % Solution |
|---|---|---|---|
| **Glucose** | 15 | 85 | 15 |
| **CaCas/Gly** | 15/5 | 80 | 20 |
| **CaCas/Gly/CP** | 15/5/0.009 | 80 | 20 |
| **CaCas/CP** | 15/0.009 | 85 | 15 |

3.1.4.2. NFDM, CaCas, and Their Blends with Different Ratios

All solutions were prepared with DI water at constant 15% of total solid concentration by mixing for approximately 2h at room temperature until reaching a complete dissolving before applying on a cereal product. The solutions, CaCas, NDFM, and their blend solutions with different ratios are explained below.

CaCas solution: 15% (w/w) CaCas solution was prepared by dissolving in DI water for 2-3h.

NFDM solution: 15% (w/w) NFDM solution was prepared with DI water by stirring at 600 rpm.

CaCas/NFDM (1:2): 10 g NFDM was mixed with 85 g DI water by stirring at 600 rpm for 30 min. Then 5 g CaCas was added into the solution with continuous stirring at 600-800 rpm.

CaCas/NFDM (2:1): 5 g NFDM was mixed with 85 g DI water by stirring at 600 rpm for 30 min. Then 10 g CaCas was added into the solution with continuous stirring at 600-800 rpm.

CaCas/NFDM (1:1): 7.5 g NFDM was mixed with 85 g DI water by stirring at 600 rpm for 30 min. Then 7.5 g CaCas was added into the solution with continuous stirring at 600-800 rpm.

Table 8: The amount of materials for the preparation of coating solutions with constant concentration of protein sources, 15%.

| **Coating Solutions** | **Amount of dry material (g)** | **Amount of DI water (g)** | **Solution (%) (w/w)** |
|---|---|---|---|
| CaCas | 15 | 85 | 15 |
| NFDM | 15 | 85 | 15 |
| CaCas/NFDM (2:1) | 10/5 | 85 | 15 |
| CaCas/NFDM (1:2) | 5/10 | 85 | 15 |
| CaCas/NFDM (1:1) | 7.5/7.5 | 85 | 15 |

### 3.1.5. Preparation of Treated Breakfast Cereals for Testing

Each solution was poured into the same size plastic mist bottles with adjustable mist nozzle. Each spray dispensed approximately 5 mL of the solutions. 27 g (serving size) of Wheaties® flakes were placed on five 900-$cm^2$ stainless steel mesh trays and sprayed with 5 mL of each solution on one side of each tray at room temperature following with a drying step of 60°C for 1h in a vacuum oven (Vacuum of 0.08 MPa, Jeio Tech Co., Billerica, MA, US). Then, the trays with the flakes were taken out from the oven and twist another side of each tray to apply second spray, again following with drying process. After six spray applications (3 sprays on one side and 3 sprays on another side of each tray) were completed, the trays were placed in a vacuum oven at 60°C for approximately 20h to achieve the complete drying of coatings in Figure 17. Dried coated breakfast cereals were weighed to calculate the amount of changes as a weight resulting in approximately 3 g changes for 27 g (~30 g total) flakes.

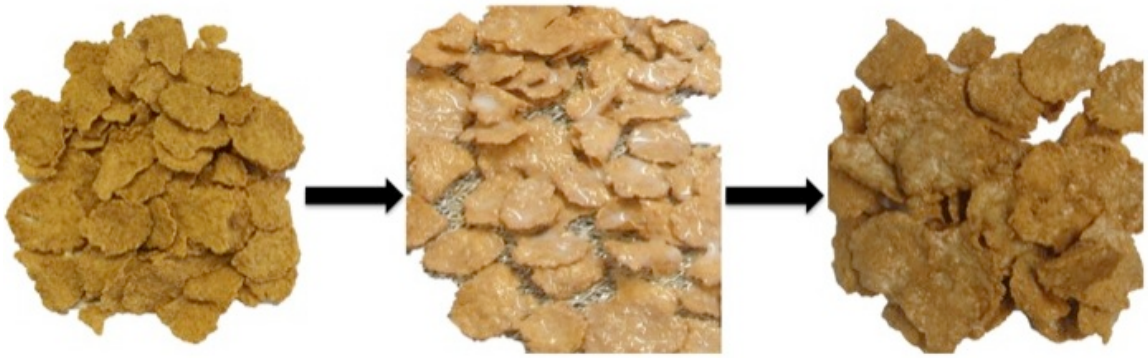

Figure 17: The appearance of sample preparation processing: (1) uncoated Wheaties®, (2) the solution sprayed on flakes, and (3) appearance of flakes after drying at 60°C for 20h.

### 3.1.6. Analysis of Edible Coating on Breakfast Cereals

#### 3.1.6.1. Protein Analysis

The protein content was determined based on measuring the nitrogen percentage via EA1112 Nitrogen/Protein Analyzer according to the method of AOAC 46-30 (American Ass. of Official Analytical Chemists 1990).

The cereals treated with the solutions were ground using a mortar and pestle to have suitable fineness resulting in achieving precision, and the fraction that passed through a 1.4- mm screen was collected and used for the analysis. Each sample was weighed approximately 20 mg into the tin capsule and squeezing it to prevent any sample leakages without damaging to the capsule surrounded to the samples. Aspartic acid was used as a calibration protein. Each sample analyzed in triplicate, and the average was counted as nitrogen percentage. To calculate the percentage of crude protein, the protein contents of treated breakfast cereals were calculated based on 6.2 of protein factor for mix food products (Jones 1931; Mosse, 1990).

$$Crude\ protein, \% = \%N * protein\ factor$$

%N is the nitrogen percentage of sample

#### 3.1.6.2. Milk Absorption Analysis

Milk absorption was measured using a modified method of Luckett and Wang (2012). Approximately 4 g of breakfast cereal (Wheaties ®) was placed in 30 mL of 1% reduced fat milk at 8°C for each minute up to 5 min. The samples were drained on a

2.8-mm stainless steel mesh screen for 10 sec to remove excessive milk on the flakes, and weighed, shown in Figure 18.

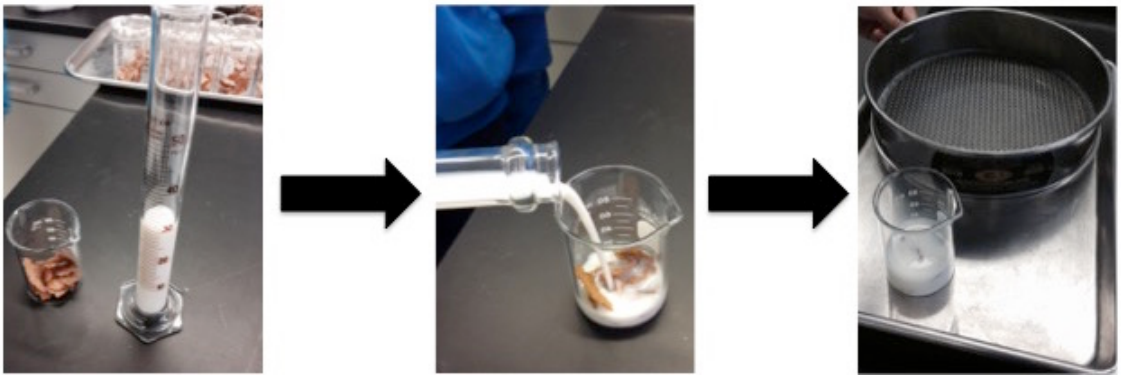

Figure 18: Processing of milk absorption test: (1) 4 g Wheaties ®, (2) pouring 30 mL milk at 8°C for various minutes, and (3) draining the samples on a 2.8-mm stainless steel mesh screen for 10s.

The percent of the milk absorption was calculated by taken percentage of dividing the difference of original flakes and drained flakes with the original flakes weight.

$$\%Milk\ Absorption = \left(\frac{W_1 - W_0}{W_0}\right) * 100$$

Where $W_1$ is the drained weight and $W_0$ is the original flakes weight (serving size 27 g).

3.1.6.3. Surface Morphology

The appearance of treated cereals was photographed using a Canon digital camera (12X Canon Inc., USA) on a light gray cloth background. The surface morphology of flakes with different solution coated was observed using Scanning Electron Microscope, FEI Quanta 200 F (Hillsboro, OR, US). The samples were mounted on stubs and sputter gold coated 80 s x 2. They were observed with Scanning Electron Microscope, with an accelerating voltage of 10 KV in high vacuum mode.

3.1.6.4. Mechanical Properties Measurements

The texture of the coated flakes was measured by following the method of Luckett and Wang (2012) with some modifications. Each cereal treatment was analyzed both the dry state before adding it in milk and wet state after soaking in milk. The force peak (N) (representative of hardness mentioned in the section 1.4.2) and work (N*mm) (representative of crispness mentioned in the section 1.4.2) of the control and coated flakes were obtained.

For dry flakes w/ or w/o coatings, 20 g of each coated flakes were placed flat and spread over the bottom of a Kramer shear cell with 5-blades assembly for a TA.XTPlus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, US) in Figure 19.

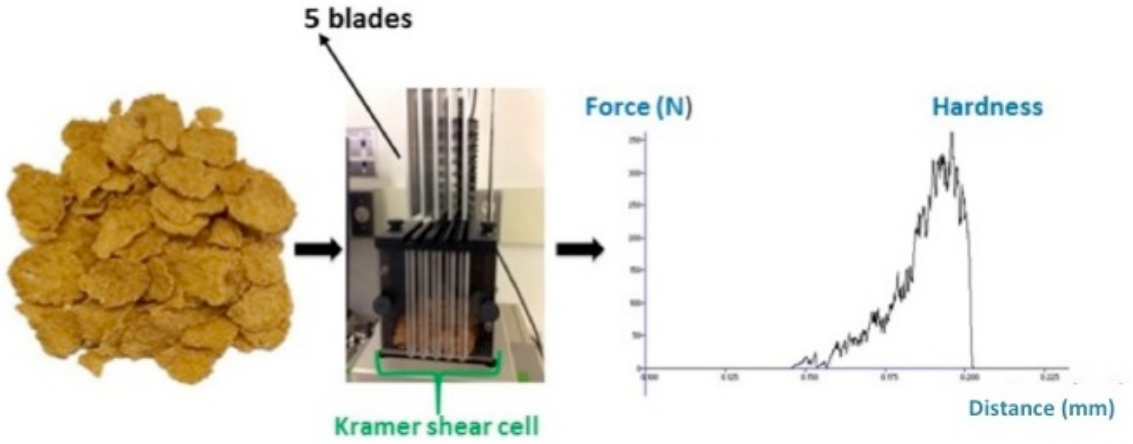


Figure 19: The uncoated sample in a Kramer shear cell with 1 5-blade Texture Analyzer, and obtained Force (N)-Distance (mm) curve.

As the experiment was run, the 5 blades drove at a constant speed through the specimen sample, compressed, and sheared the RTE flakes. Based on the response of blades, the graph was obtained until the blades touched the base of the cell. The 5 blades

provided a data on several positions at the same time.

For the flakes soaked in milk, 20 g of each breakfast flake sample was placed in 150 mL of 1% reduced fat at 8°C for 3 min. The flakes were removed from milk and drained for 5 sec followed by placing them on paper towels to eliminate excessive milk on the flakes with 10 sec in Figure 20. It applied for both side of the flakes by turning the one side to another side of flakes with 5 sec for each side.

Figure 20: Preparation of the flakes placed in milk (at 8°C for 3 min) and drained to measure the mechanical properties.

Afterward, the sample was placed flat and spread over the bottom of the cell. The texture analysis of both the dry flakes and the flakes soaked in milk analysis was carried out by the following conditions: a pre-test speed of 5mm/s, a test-speed of 10mm/s, a post-test speed of 5 mm/s, and a distance of 120mm/s.

3.1.6.5. Statistical Analysis

Differences among protein analysis, milk gain, and Hardness and Crispness of the coated flakes based on the various coatings were evaluated by analysis of variance with means separation (One-way ANOVA), and the means were compared with T-test, performed by using SPSS Version 21 (IBM Corp., London, UK) (provided by The State

University of New Jersey, Rutgers Software System).

## 3.2. Results and Discussion

Preliminary Results:

The spraying method was used to provide a thin and uniform coating layer on the RTE breakfast cereals. Andrade et al. (2012) reported that coating methods were an important parameter for forming a thin and uniform coating layer on the cereals.

In this study, the coating treatments increased the weight of the RTE cereal flakes. The total amount of each coating solution applied was 30 mL (15% total solid content) for 27g of the cereals, and the weight of the coated flakes increased approximately 10% over the pre-coated weight.

### 3.2.1. Protein Content

Figures 21 and 22 represent the increase of protein content (g) of the breakfast flakes provided by the coatings.

The uncoated and glucose coated flakes were considered to contain approximately 2g protein per serving size, which is 27g. CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP coatings provided 3.91, 3.33, 2.59, and 3.19g increase in the protein content for the coated flakes, respectively, compared to the uncoated and glucose coated flakes as shown in Figure 21. Due to the increase of the weight of the breakfast cereals by the coatings, the mass fraction of protein in each coated flakes changed. Therefore,

CaCas/Gly, CaCas/Gly/CP, and CaCas/CP coatings provided a little less protein amount than the CaCas coating even though the 15% of the protein concentration in each solution was kept the same.

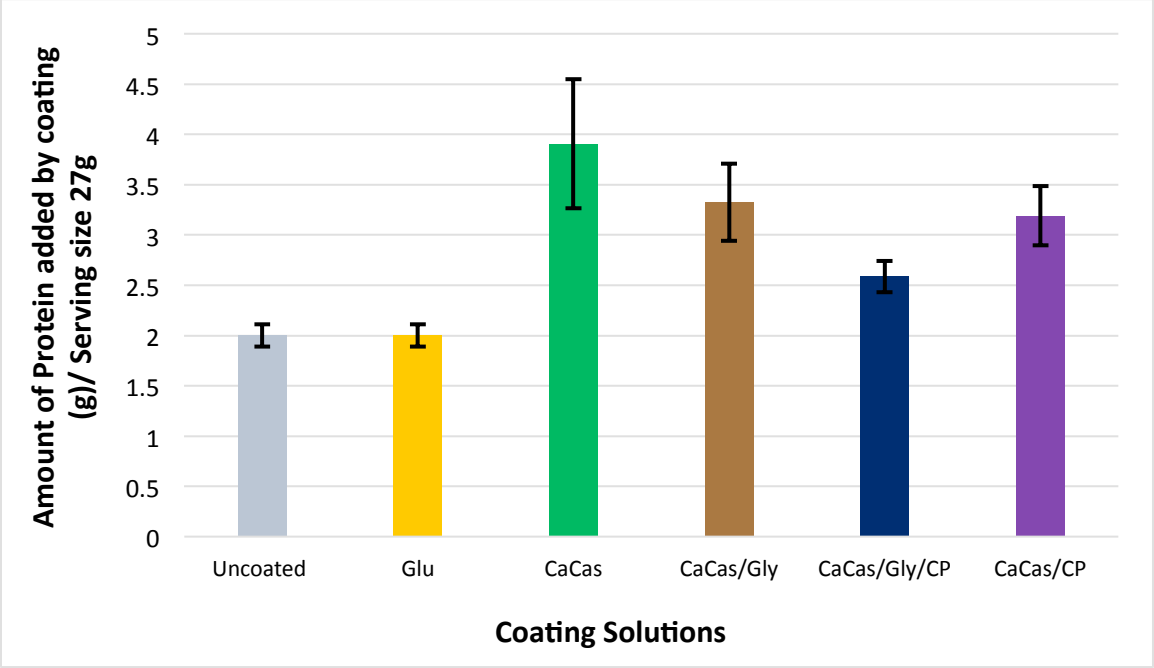


Figure 21: Amount of protein added in cereal flakes with five different solutions and uncoated. All solutions have a 15% (w/w) total protein concentration.

Another application that we examined was the blends of CaCas and NFDM solutions with different ratios (at the same 15% total solid concentration) coated on RTE cereals. The blends did not provide much protein content shown in Figure 22. Among the blends, the higher ratio of CaCas to NFDM in the coating solutions, the coating provided a higher protein due to the high protein content of CaCas. CaCas and NFDM contained approximately 88 and 36% of protein, which was determined in the section 3.1.3.2.

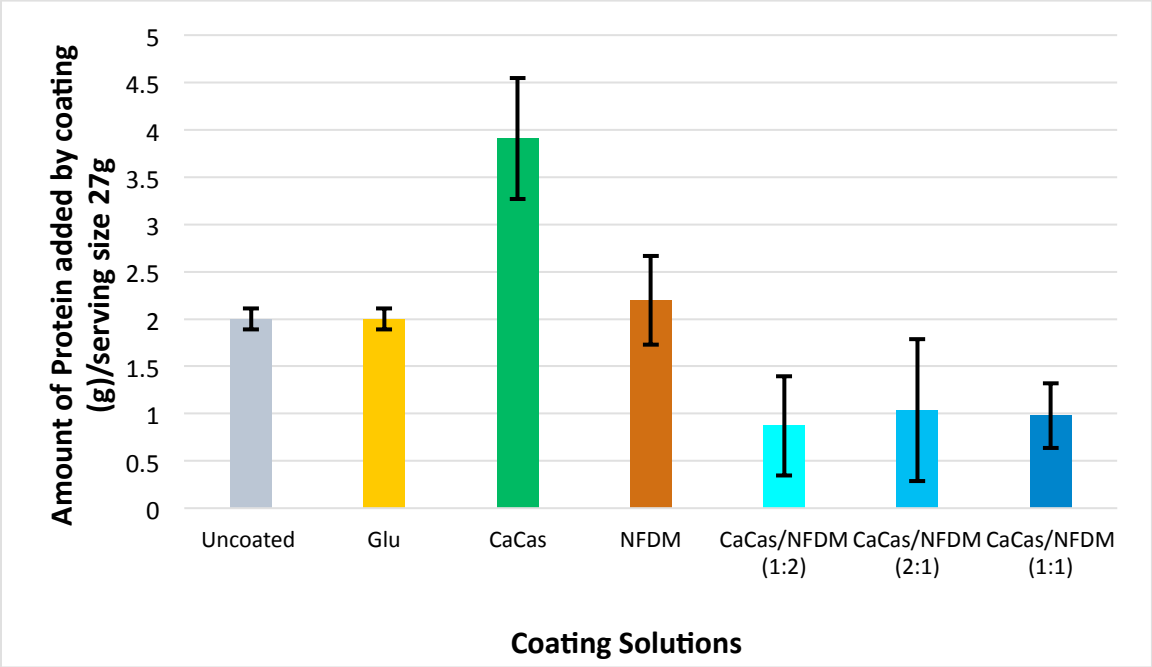


Figure 22: Amount of protein added in cereal flakes coated with six different solutions: glucose, CaCas, NFDM, and blends of CaCas with NFDM with different ratios. All solutions have a 15% (w/w) total solids concentration.

In this study, the result showed that CaCas and in blends with Gly and CP provided protein source for RTE breakfast cereals in this research, shown in Figure 21.

### 3.2.2. Amount of Milk Gain and Rate of Milk Absorption

Figures 23 and 24 represent the amount of milk gain and rate of milk absorption at 8°C at each minute for up to 5 min by the RTE cereal flakes coated with the various solutions. Uncoated and glucose coated flakes were used as the control samples.

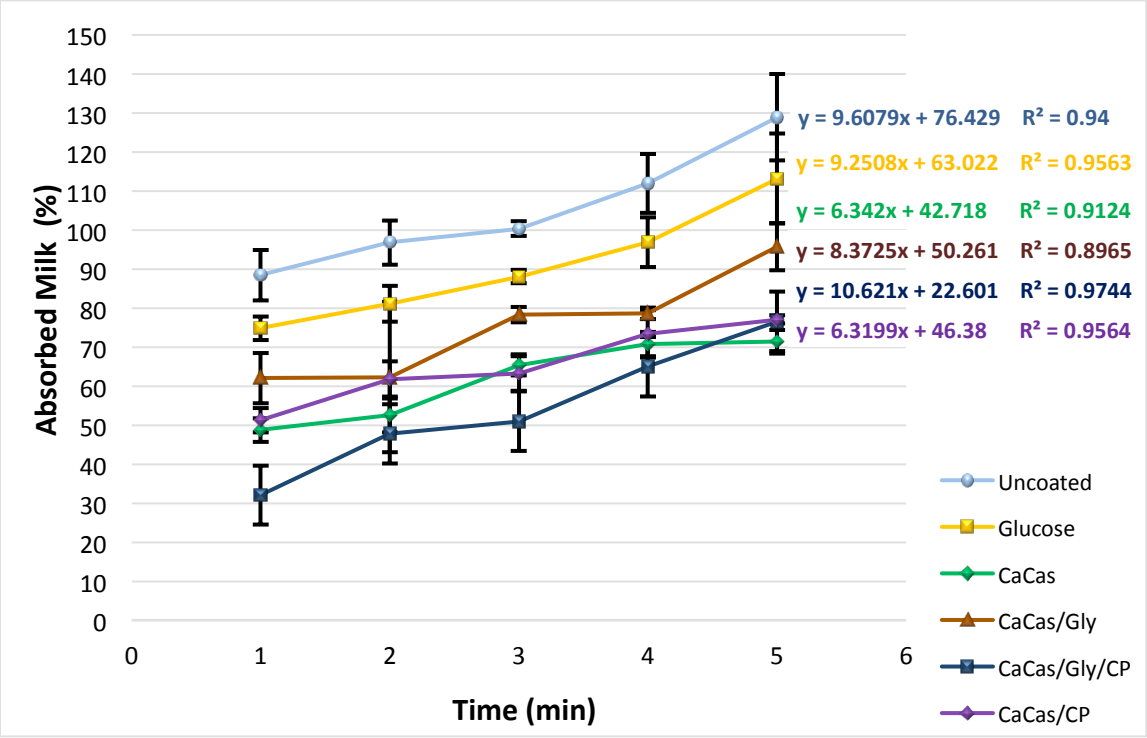


Figure 23: Weight gain of coated cereal during milk absorption of coated samples after soaking in milk for up to 5 min at 8°C ($p<0.05$).

All coating treatments significantly lowered the rate of milk absorption and reduced correlated the milk gain of the coated flakes ($p<0.05$). After 5 min, the uncoated flakes, and glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP coated flakes gained milk showed the by about, 129, 113, 71, 96, 77, and 77%, respectively. The results were equal to the 5.23, 4.58, 2.86, 3.84, 3.04, and 3.11 g of milk gain for 5 min in milk, respectively.

Glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP coatings lowered the total amount of milk absorption compared to the uncoated flakes by 12, 45, 26, 41, and 41% in 5 min, respectively in Figure 23. Referring to the results of section 2.2.1.2 above, a small amount of CP (0.3%) loosely interacts with CaCas, Gly, and water molecules in

the coating solutions induce the open network structure in polymer chains. Therefore, the rate of milk absorption for CaCas/Gly/CP treated flakes was higher than other coatings because liquid milk can easily travel through the coating layer with an open network structure and then reach the dried flakes under the coating layer. Also, the coatings dissolve in milk because CaCas and other coating compounds are water-soluble (Dangaran et al. 2006). CaCas and CaCas/CP coated flakes had similar trends of reducing the amount of milk absorption as well as lowering the rate of milk absorption. Compared to the uncoated and glucose coated samples, CaCas reduced approximately 45 and 38% of milk gain in 5 min, respectively.

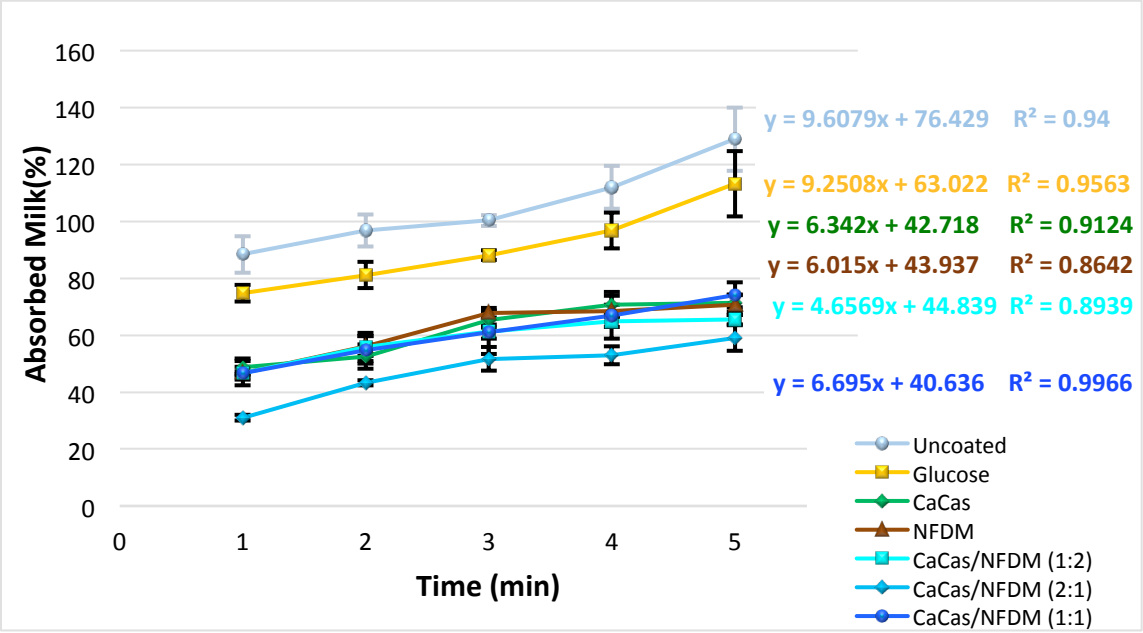


Figure 24: Weight gain during milk absorption of coated samples after soaking in milk for up to 5 min at 8°C ($p>0.05$).

In Figure 24, the uncoated flakes, and glucose, CaCas, NFDM, CaCas/NFDM (1:2), CaCas/NFDM (2:1), and CaCas/NFDM (1:1) coated flakes gained milk by about

129, 112, 71, 70, 65, 59, and 74% respectively, which were equal to 5.23, 4.58, 2.86, 2.81, 2.64, 2.39, and 2.98 g of milk gain in 5 min. The flakes coated with CaCas, NFDM, and the blends of CaCas/NFDM with 1:2, 2:1, and 1:1 ratios significantly reduced the milk absorption of the flakes, which was approximately 50 and 40% reductions of milk gain than uncoated and glucose coated flakes, respectively ($p<0.05$).

The results of the reduction and lowering the milk absorption of the coatings showed that CaCas coating and in blends with Gly and CP had an ability to retain the texture of the flakes in milk that extended the bowl life of RTE breakfast cereals. The less milk gained by the flakes slowed the loss of the flake texture. NFDM and the blends of CaCas and NFDM coatings did not significantly change the hard and crispy texture of the coated cereals in milk ($p>0.05$) and thus CaCas, CaCas/Gly, and CaCas/CP may be beneficial due to the higher protein content of CaCas than NFDM.

Our results showed that the coatings made from milk ingredients would be capable of substitutes for sugar-based coatings on the breakfast cereals to enhance nutritional value and texture properties, and extend the bowl-life of the cereals. Also, the coatings probably protect the coated flakes from gaining moisture from the environment for opened packages.

### 3.2.3. Surface Morphology

#### 3.2.3.1. Surface Appearance

The surface appearance of the flakes is shown in Figures 25 and 26. 15% (w/w) glucose treatment did not form a visible layer on the flakes surface, and instead was absorbed by the flakes. The coated cereals showed a slight change in color. That may be resulted from Maillard reaction between the lysine amino acids, which came from wheat, and glucose, which came from sugar solution, while drying. BeMiller (2007) reported that Maillard reaction occurred during the cooking and drying process based on the amount of reactive amino groups of protein and reduced sugar. A frosted appearance with visible signs of opacity occurred on the surface of flakes treated with 15% (w/w) CaCas solution but it was not evenly adhered to the flake surfaces. The viscosity of coating solution may be the reason of imperfect adherent on the surface because either less viscous fluid can move easily to lower spots of the flakes surfaces until drying or more viscous may not be absorbed well by the flakes. Also the coating solutions may result in a localized accumulation in the creases of rough structure of the flakes due to the rough and fissures structure of RTE cereal flakes.

In Figure 25, CaCas, CaCas/Gly/CP, and CaCas/CP coating solutions led the color of flakes to become darker which resulted from Maillard reaction related to Amadori arragements of lysine (from wheat, calcium caseinate) and galactose from pectin (BeMiller 2007). By manipulating drying temperature-time or drying process, the

desired brown color would be achieved for the RTE flakes. Therefore, the addition of other ingredients may not be required to enhance the color of the flakes in the production of the RTE breakfast cereals (Caldwell and Fast 2000).

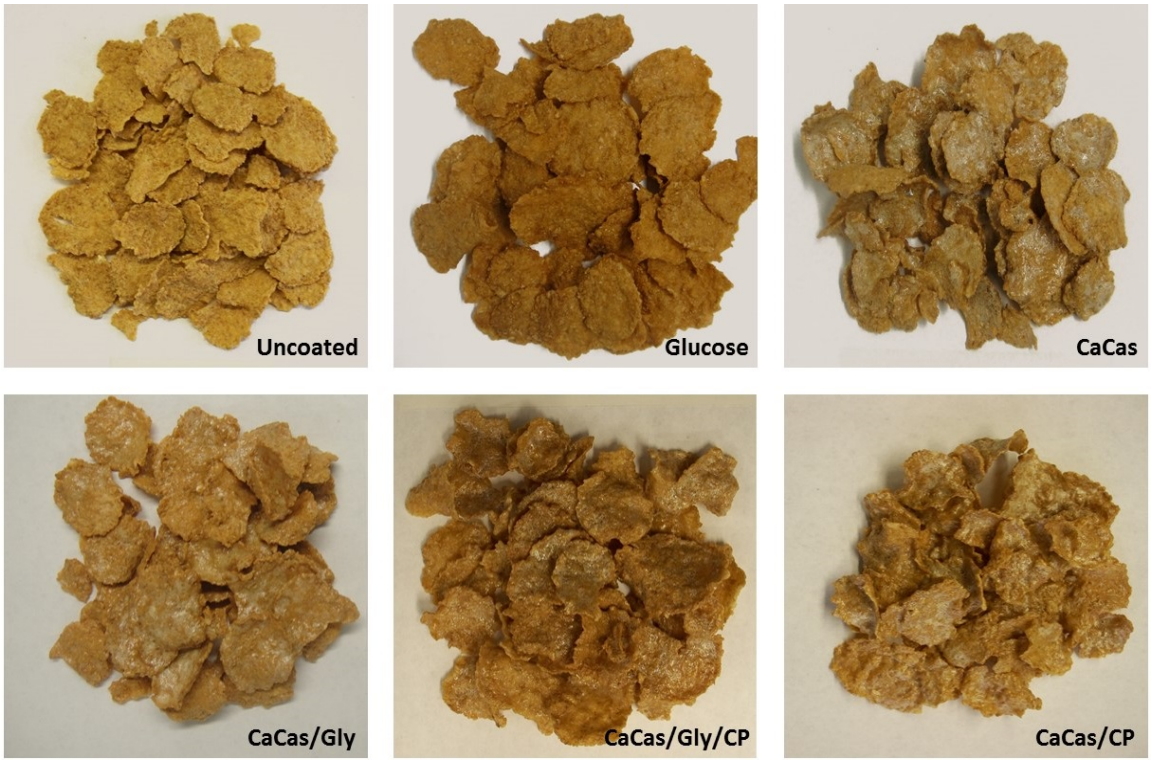


Figure 25: Surface appearance of the uncoated flakes and the dried flakes treated with glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP solutions.

The blends of CaCas and NFDM with different ratios demonstrated a much darker color on the flakes (shown in Figure 26) than the flakes coated with CaCas and along with Gly and CP coatings in Figure 25. Due to the higher lactose content of NFDM, which is approximately 85% lactose in dry weight (Baer et al. 1983), lactosylysine products are probably formed via Maillard reactions because lysine amino acids from wheat flakes and CaCas react with highly reactive lactose (reduce sugar) from NFDM

under heat treatment and this product causes a significant loss of nutritional value (Meltretter et al. 2007), However, NFDM coating slightly changed the color of RTE cereal flakes compared to the blends. NDFM coating provided a little sheen and frosted layer with a little color changes of the flakes.


Figure 26: Surface appearance of uncoated flakes and the dried flakes treated with glucose, CaCas, NFDM, and the blending of CaCas/NFDM solutions with different ratios.

### 3.2.3.2. Scanning Electron Microscopy Observation

Uncoated and Glucose Coated Flakes

In this part, the samples were examined using an accelerating beam at a voltage of 10 KV in high vacuum mode, and magnification of 250× was used.

Uncoated flakes had a crack, fracture, and pore structure in the edge image in Figure 27. With 15% (w/w) glucose coating, RTE cereals attributed to absorb the solution, which filled the fractured and cracked structure, and minimizes the pore sizes inside of the flakes.

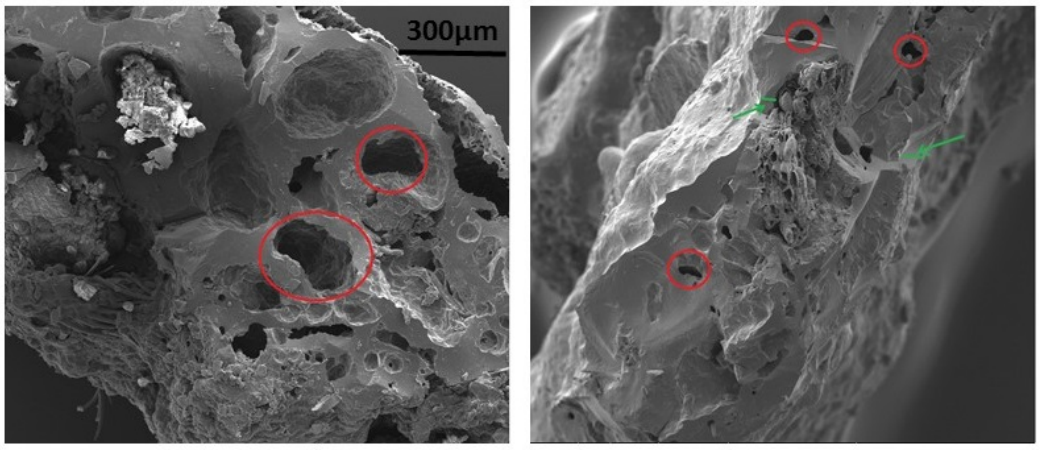


Figure 27: SEM images of the edge sides of uncoated and glucose coated flakes.

The Flakes Coated with Different Solutions

All samples were examined using an accelerating beam at a voltage of 10 KV in high vacuum mode, and magnification of 1,000× was used.

Glucose coatings became the part of cereals flakes, which was absorbed by the flakes, and the coatings filled the fissure, fracture, and crease spots of the flake surfaces. CaCas coatings provided a smoother layer for the surface of flakes, but some pores in layer were formed (pointed out in Figure 28) due to both the evaporation of water molecules as drying and absence of plasticizer (Gly). Thus, plasticizer addition was necessary to improve coating integrity and barrier properties. It facilitated the movement

of polymer chains in the protein network by inserting itself between the protein-protein interactions and water molecules (Chen 2002). Beyond these pores, CaCas provided a smooth and uniform layer to the surface of the cereal flakes, which built a protective layer on the surface of the cereals. The results are in agreement with the results of milk absorption and textural properties because the coating layer reduced the transfer of milk during consumption and inhibited the transfer of moisture during storage after opening the package of RTE breakfast cereals, which maintain a harder and crispier textures of the RTE flakes. Gly and CP additions provided the coating solutions to induce the coating integrity by crosslinking and interacting with $Ca^{+2}$. Since, the amino acids chains of casein in the solution, and all new configurations in the molecular structure had a positive impact on the coating integrity. Therefore, a uniform CaCas with Gly and CP coating of breakfast cereals would provide structural integrity that protect the product from any physical damages during handling, distribution, storage, and marketing.

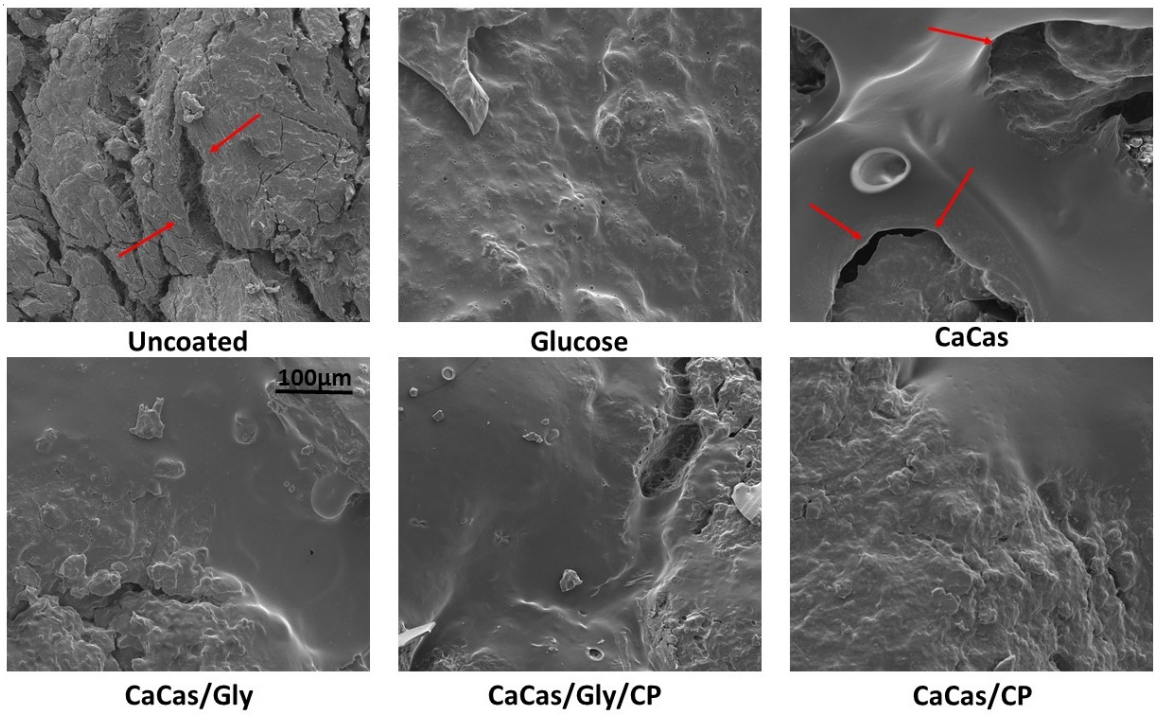


Figure 28: Scanning electron microscopy (SEM) images of uncoated flakes and the flakes coated with glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP solutions.

NFDM treatment showed a uniform coating, but the layer fractured itself during the drying process in Figure 29.

Flakes coated with the blends of CaCas and NFDM solutions followed a different trend compared to the treatments above. Coating integrity and layer appearance of the flakes coated with the blends was neither smooth nor uniform. The reason might be the differences in the molecular structure of CaCas and NFDM. Due to the random coil structure of caseinates, and the casein content of NFDM in a solution, they interfered their stabilities in the solutions by disturbing the molecular interaction of each other. The blends formed some pores, fractured and rough surfaces, which led the treated flakes to

become susceptible to the moisture or milk, and then loose the desired hard and crispy texture of the flakes.

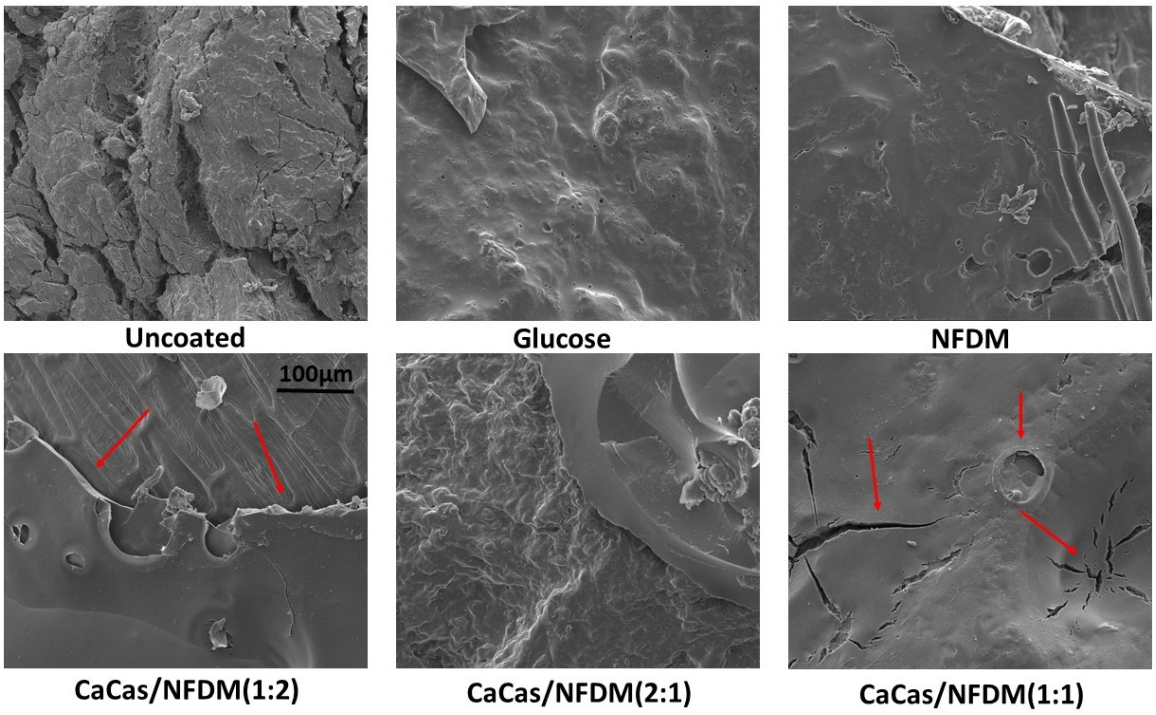


Figure 29: SEM images of uncoated flakes and the flakes coated with glucose, CaCas, NFDM, and the blending of CaCas and NFDM solutions with different ratios.

Besides the coatings, the outer surface of RTE flakes with fissures, fractures, and creases may cause the flaws of the coatings. That would lead the flakes to be exposed by moisture or milk easily, and then it would lose its desired crispy texture in a bowl or an opened package.

### 3.2.4. Mechanical Properties

#### 3.2.4.1. Hardness

Dry state and wet state of the flakes soaking in milk

The results obtained based on the dry state (dried flakes) and wet state of the RTE flakes (the flakes soaking in milk). In the dry state, all cereal coatings in Figure 30 provided harder texture to the RTE cereal flakes compared to the uncoated flakes.

The CaCas and in blends with Gly and CP coatings provided a harder texture to the flakes compared to both the uncoated and glucose coated flakes. The CaCas/CP coating increased the Hardness of the dried flakes by 58% (422.1 N) compared to the Hardness of the uncoated flakes, which was 265.9 N. It was followed by the CaCas/Gly, CaCas, CaCas/Gly/CP, and glucose coatings, which increase the Hardness of the flakes by about, 108.6, 62.8, 46.0, and 24.8 N, respectively. The coatings with CaCas/CP, CaCas/Gly, CaCas, and CaCas/Gly/CP provided an increase in Hardness of the cereal flakes by 131.3, 83.8, 38.0, and 21.1 N, respectively, compared to the Hardness of the flakes coated with glucose solution, which is 290.8 N. The flakes coated with CaCas/CP solution are the hardest flakes (Figure 30) because CaCas and CP can form crosslinks that become closer as water evaporates during drying of the film due to their polar and nonpolar chains (Chen 2002; Bonnaillie et al. 2014).

By soaking in milk for 3 min, both the uncoated and glucose coated flakes lost their Hardness by about, 40 and 20%, respectively. Uncoated flakes lost approximately

half their Hardness in 3 min due to the fissures, fractures, and pore structure of the wheat flakes as mentioned in the section 3.2.3.2 above. The glucose coating maintained the crunchy texture of the flakes than the uncoated ones, but glucose can easily dissolve in water or milk that triggers a loss of RTE cereal texture in a short time.

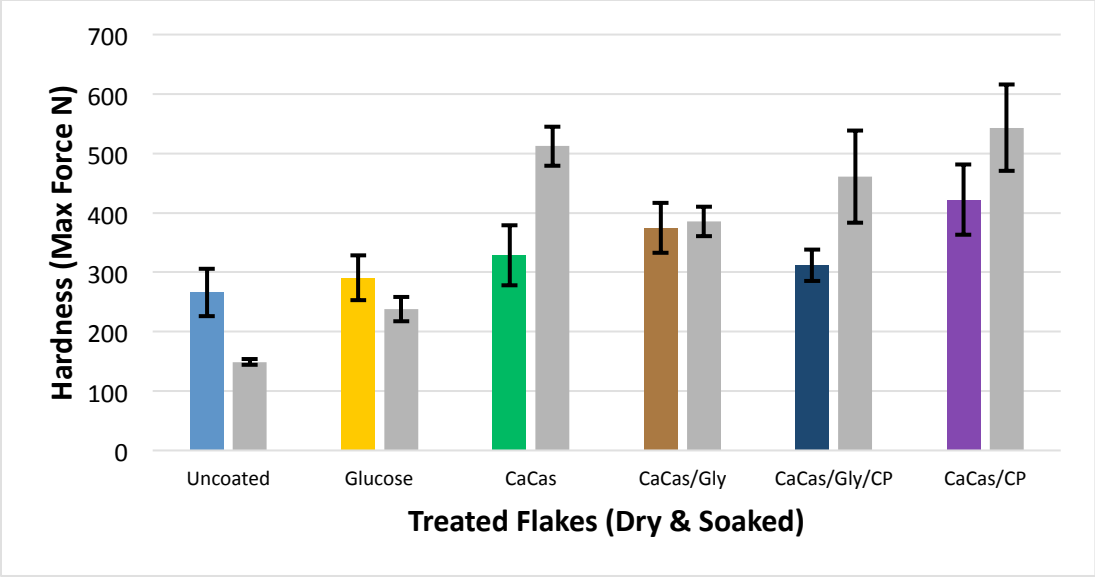


Figure 30: Peak force (hardness) of the flakes treated with the various solutions before (dry state) and after soaking in milk (wet state) at 8°C for 3 min. Colored bars represent the dried flakes; grey bars represent the wet state.

The Hardness of the cereal flakes coated with CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP solutions was negatively correlated with milk absorption after soaking in milk, shown in Figure 30. The outer surface of the protein-coating layer swelled with milk, decreasing the glass transition temperature of the coated RTE flakes and then the layer dissolved in milk that caused a loss in the "crunch sound" associated with the cereal when fractured. Luckett and Wang (2012) reported that absorbing milk affected the texture of RTE cereals that causes the loss of brittleness, texture, and crunchy sounds.

Breakfast cereal products are brittle and generate a loud and high crunchy noise when force applied because they are crispy and crunchy products due to their low density cellular and porous structure (Roudaut et al. 1998).

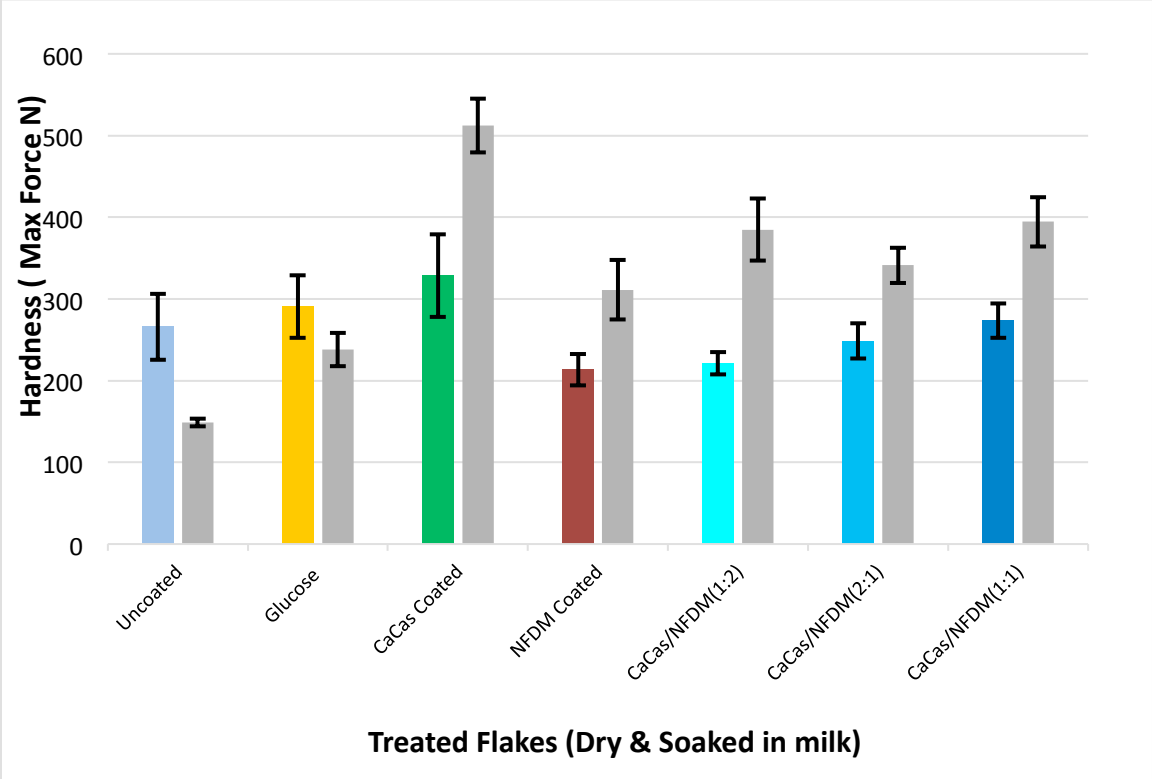


Figure 31: Hardness of flakes treated with different coating solutions before and after soaking in milk at 8°C for 3 min. Colored bars represent the dry state of flakes; grey bars represent the wet state of flakes.

NFDM and the blends of CaCas and NFDM coatings showed a decrease in the peak force in the dried flakes compared to both uncoated and glucose coated cereals in Figure 31. NFDM absorbs moisture from the environment that results in the treated flakes become more bendable and soft because it has a hygroscopic nature, which increases the water content of the dried flakes.

In Figure 31, the texture of the treated cereals retained their Hardness after soaking in milk, which was unexpected. It showed that a delay in the milk absorption occurred in the core of flakes due to the coating layer, which became sticky and rubbery in milk before dissolving. Therefore, more force was required to crush the flakes.

3.2.4.2. Crispness

Dry state and wet state of the flakes soaking in milk

The work on the flakes (represents as crispness) mentioned in section 1.4.2, which is the area under the curve of force and displacement, was analyzed for each coating both for the dry state and wet state of the flakes in 1% reduced fat milk at 8 °C for 3 min.

The flakes coated with CaCas and along with Gly and CP showed a higher Crispness compared to the uncoated and glucose coated cereals. The dried flakes coated with CaCas/Gly/CP provided the highest crispness, which is 2714 N.mm, and it is three times crispier than the uncoated and twice as crispier than the glucose coating, shown in Figure 32. It followed by CaCas/CP, CaCas/Gly, CaCas, and glucose treatments: by about, 2540, 1828, 1657, and 1265 N.mm, respectively, compared to the uncoated flakes, which is 881 N.mm. CaCas/CP, CaCas/Gly, and CaCas increased in Crispness of the flakes, 101, 44, and 30%, respectively compared to the glucose coating. As mentioned previously, the differences in molecular structure of the coating compounds affected the integrity of the coating and structural integrity of the flakes. The coating layers favor the flakes to have a crispier texture.

All coated flakes showed a significant decrease in Crispness of the flakes in milk ($p<0.05$). Uncoated flakes and the flakes coated with glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP lost their crispness in 3 min: approximately 83, 80, 64, 76, 77, and 70%, respectively, compared to their dried forms, which refers to the dried flakes coated with the solutions. However, the flakes coated with CaCas and its blends with Gly and CP retained the texture longer than the uncoated and glucose coated flakes, shown in Figure 32. Based on these results, both the coatings provided a crispier texture in the dried flakes and retained their crispy textures while the flakes were soaking in milk. If the moisture content of these products increases, it results in a soggy, soft texture due to water sorption from the atmosphere or by mass transport from neighboring components (Nicholls et al. 1995).

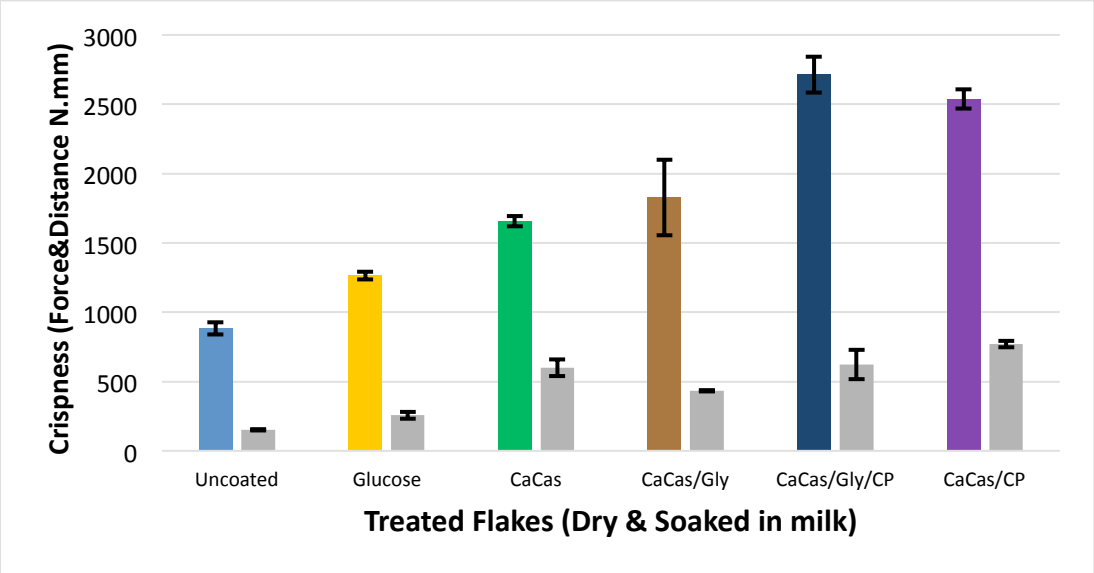


Figure 32: Crispness (N.mm) of flakes treated with different coating solutions before and after soaking in milk at 8°C for 3 min. Colored bars represent the dry state of flakes, grey bars represent the wet state of flakes.

For the dried flakes, the blends of CaCas and NFDM (with 1:2, 2:1, and 1:1 ratios at constant 15% total solid concentration) coatings provided a crispier texture to the flakes, and their contributions were not significantly different from each other in Figure 33 ($p>0.05$). The blends coatings increased the Crispness of the cereal flakes by about, 940, 1079, and 1128 N.mm, respectively compared to the uncoated flakes, which is 881 N.mm in Figure 33. The result showed that the higher the ratio of CaCas to NFDM, the crispier the flakes become. The blends increased the crispness of the flakes, but it was not a significant increase compared to CaCas and NFDM coatings, which has 88% and 64% increase, respectively, compared to the uncoated flakes ($p>0.05$).

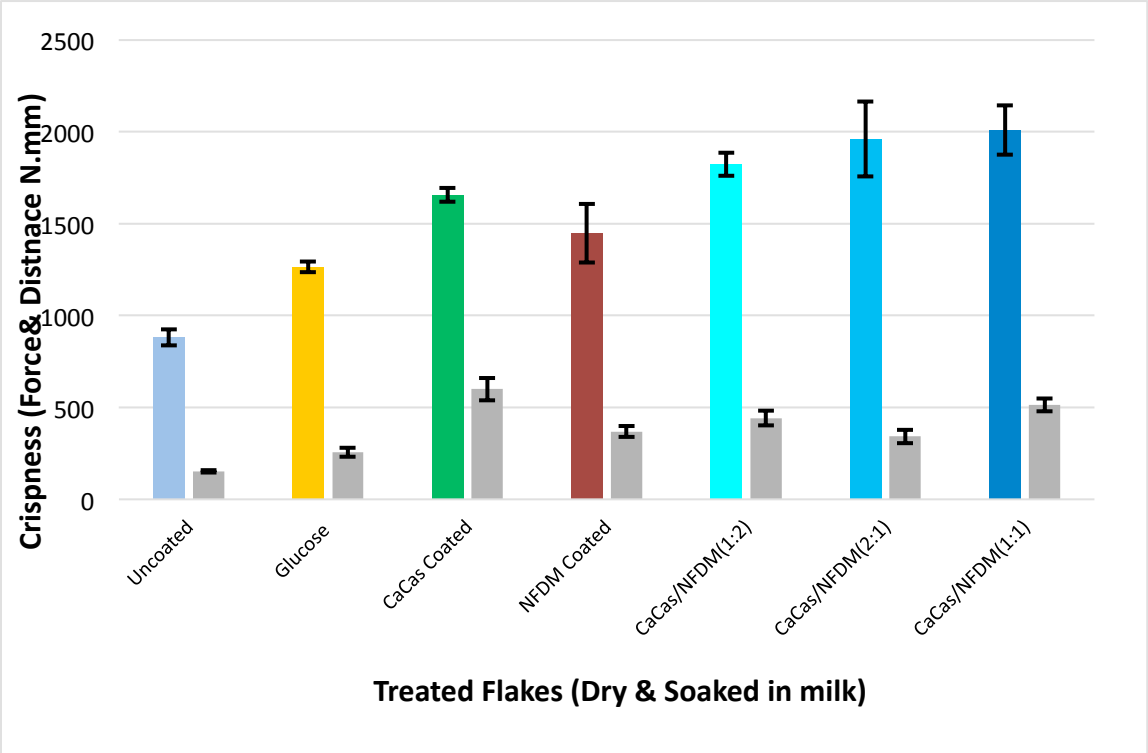


Figure 33: Crispness of flakes treated with different coating solutions before and after soaking in milk at 8°C for 3 min. Colored bars represent the dry state of flakes, grey bars represent the wet state of flakes.

All coated flakes showed a significant decrease in Crispness in milk ($p<0.05$) compared to their dried forms in Figure 33. The uncoated flakes and the ones coated with glucose, CaCas, NFDM, CaCas/NFDM (1:2), CaCas/NFDM (2:1), CaCas/NFDM (1:1) solutions lost most of their crispy texture in milk for 3 min: approximately 83, 80, 64, 75, 76, 83 and 74%, respectively.

Based on all results in the section 3.1, CaCas and in blends with Gly and CP provided a higher protein content, increased bowl-life by reducing the amount of milk absorption, and providing the structural integrity of the flakes that resulted from a harder and crispier texture of coated flakes. However, the blends of CaCas and NFDM did not show constant and correlated values, form a uniform and smooth coating layer, and provide a higher protein content compared to CaCas and in blends with Gly, CP.

### 3.3. Summary

The coating treatments increased the weight of the RTE breakfast cereals. The total amount of each coating solution applied was 30 mL (15% total solid content) for 27 g of Wheaties® breakfast cereals, and the final coated flakes increased approximately 10% over the pre-coated weight.

The CaCas and in blends with Gly and CP coatings provided an increase in protein content of RTE flakes because CaCas contains approximately 88% protein (Chen 2002). CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP provided 3.91, 3.33, 2.59, and 3.19 g increase in protein content of the cereals, respectively, compared to the uncoated

and glucose coated cereal flakes, which contained already 2 g. However, the blends of CaCas and NFDM with different ratios did not provide a much increase in the protein content for the flakes.

All coating treatments lowered the rate of milk absorption and reduced the amount of milk gain. The uncoated flakes and the flakes coated with glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP solutions gained milk by about, 129, 113, 71, 96, 77, and 77%, respectively, after 5 min in 1% reduced fat milk at 8°C. Glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP coatings lowered the amount of milk absorption compared to the uncoated flakes by 12, 45, 26, 41, and 41%, respectively. NFDM and the blends coatings had the similar results of milk absorption, which were 70, 65, 59, and 74%, respectively ($p>0.05$).

The CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP coatings showed a visible coating layer with sheen, frosted, less opaque appearance on the surface of the flakes. Also, the coatings darkened the color of the flakes. The CaCas, CaCas/Gly/CP, CaCas/CP, NFDM coating solutions led the color of flakes to become darker because Maillard reaction occurred between amino acids of CaCas or NFDM and reduced sugar (lactose) with heat. This may help enhance the desired brown color for flakes without adding other color enhancer in the formulation of RTE breakfast cereal production. However, the blends of CaCas and NFDM demonstrated a much darker color flakes. The reason would be the higher lactose content of NFDM, which is approximately 85%

lactose in dry weight (Baer et al. 1983) because lactose favors the Maillard reactions to occur (BeMiller 2007).

Uncoated flakes had a crack, fracture, and pore structure in the edge, and rough, fissure structure on the surface. Glucose coatings became the part of cereals flakes, and the glucose solution was absorbed by the flakes, and then filled the fissure, fracture, and crease spots on the surface of the flakes. CaCas coatings provided a smoother layer to the surface of flakes, but some pores in the layer were formed, whereas CaCas along with Gly and CP solutions provided to induce the coating integrity. NFDM treatment showed a uniform coating, but the layer fractured itself during the drying process resulting from the lack of adhesive property. The blends of CaCas/NFDM coatings formed some pores, fractured and rough surface. The coating integrity and layer appearance of the blends coated flakes were neither smooth, nor uniform.

For the dry state, all cereal treatments (except NFDM and CaCas along with NFDM with 1:2, 2:1, 1:1 ratios at 15% total solid concentration) provided a harder texture to the flakes compared to the uncoated flakes. CaCas/CP coating produced the hardest dry flakes with 58% increase. CaCas/Gly, CaCas, CaCas/Gly/CP, and glucose coatings increased the Hardness of the flakes by about, 41, 24, 17, and 9%, respectively, compared to the uncoated flakes. Compared to the glucose coating, CaCas/CP, CaCas/Gly, CaCas, and CaCas/Gly/CP coatings increased the Hardness of the flakes by 45, 29, 13, and 7%, respectively. NFDM and the blends showed a decrease in the Hardness of the dried flakes compared to both the uncoated and glucose coated cereals.

The uncoated flakes and the flakes coated with the solutions lost their Hardness by soaking in 1% reduced fat milk in 3 min at 8°C. The uncoated flakes lost its Hardness by 40%, and glucose coated flakes lost it by about 20%. The Hardness of the flakes coated with CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP soaking in milk was negatively correlated with the milk absorption because the outer surface of protein-coating layer swelled with milk, creating a sticky/rubbery outer surface, and caused a loss in crunch sounds around the cereals, which required more force to crush the flakes. This negative correlation showed that the milk retention was delayed in the core of flakes due to the coating layer.

All cereal treatments favored the coated flakes to become crispier than both uncoated and glucose coated flakes. Among dried flakes, CaCas/Gly/CP provided the highest crispness, which was three times crispier than uncoated and twice than glucose coating. CaCas/CP, CaCas/Gly, CaCas, and glucose treatments followed the CaCas/Gly/CP with 188, 107, 88, and 43% increase, respectively compared to uncoated flakes. CaCas/CP, CaCas/Gly, and CaCas increased the Crispness of flakes; 101, 44, and 30%, respectively compared to glucose coating. The blends with 1:2, 2:1, and 1:1 ratios of CaCas/NFDM coatings provided a 107, 122, and 128% increase in Crispness, respectively compared to uncoated flakes. All coated flakes showed a decrease in their crispness in milk. Uncoated, glucose, CaCas, CaCas/Gly, CaCas/Gly/CP, and CaCas/CP treatments lost their crispness in 3 min: approximately 83, 80, 64, 76, 77, and 70%, respectively, compared to the dried flakes coated with these solutions. NFDM and the

blends coatings lost most of their crispy texture in milk for 3 min: approximately 83, 80, 64, 75, 76, 83 and 74%, respectively compared to the dried flakes treated with these coatings.

# CONCLUSION

The tensile properties of edible CaCas/Gly films were affected by film thickness, relative humidity (RH), and CP content. CP provided a new molecular configuration through the crosslinks that changed the molecular structure of the film matrix, and affected either positively or adversely the tensile properties of the edible films. Therefore, this study requires more studies to understand and evaluate the efficacy of compositions and environmental changes (RH) of the edible CaCas/Gly films.

Dairy protein-based coatings, based on results, would be a capable substitute of sugar coatings of RTE breakfast cereals to provide an increased protein source, longer bowl life in milk, and crispier textures by forming a uniform, sheen, and protective coating layer on the surface of the flakes. Coating treatments would also enable the protection of the cereals from physical damages (such as crushing) during supply chain, transportation, storage, and handling.

The edible CaCas films and coatings have the potential to increase the utilization of NFDM in both packaging film and coating applications.

# FUTURE WORK

For the first study, since only E, EAB, and TS values of edible CaCas/Gly with a small range of film thickness and CP concentration values were examined at 58-69% RH, different film thicknesses and CP concentration could be tested at a broad range of RH in the future. The edible films could be tested in a controlled RH and temperature to obtain more stable results. In a later stage, different analyses could be performed to determine the molecular interactions of compound in the film solutions besides the tensile properties of the edible films.

For the second study, several coating solutions with 15% concentration were applied on only Wheaties® RTE flakes. The concentration of CaCas coating solution could be increased and applied on the flakes with a different spray technique to achieve additional protein increases and more uniform coatings. CaCas-based coatings could also be tested on other cereals. Further research could include the incorporation of probiotics in the coating solutions to examine the effects of the shelf life of RTE breakfast cereals and activities of probiotics.

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