INTERACTIONS AMONG PROTEINS AND CARBOHYDRATES UNDER THERMAL PROCESSING CONDITIONS AND THEIR EFFECTS ON DAIRY FOULING

by

Yizhe Zhang

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THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Jen-Yi Huang, Chair
Department of Food Science

Dr. Osvaldo H. Campanella
Department of Agricultural & Biological Engineering

Dr. Owen G. Jones
Department of Food Science

Dr. Dharmendra K. Mishra
Department of Food Science

Approved by:
Dr. Arun K. Bhunia

To God for being my rock, my strength, my hope, and my love

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ABSTRACT

In dairy processing, dairy ingredients need to be thermally treated to ensure product quality and safety for an extended shelf life. During thermal processes, milk protein denatures and interacts with other dairy ingredients to form a layer of deposit on heated surfaces, known as fouling which can deteriorate process efficiency and product safety. Milk is a complex mixture of proteins, fats, carbohydrates, minerals and vitamins. The heat-sensitive β-lactoglobulin (β-lg) is known to be a key component in fouling formation (constituting 50% of type A fouling deposits) during milk pasteurization, as β-lg unfolds when heated and exposes the reactive sulfhydryl groups that can interact with other proteins and ingredients to form deposits. Although casein (80% of milk proteins) is known to interact with denatured β-lg, no fouling studies have been performed with particular focus on the effect of casein on whey protein fouling.

Carbohydrates are an ingredient widely added in various dairy products as sweetener, stabilizer, texturizer, and fat replacer. Simple sugars have a protective effect on whey protein denaturation, but their effect on dairy fouling is not known. Polysaccharides can interact with milk proteins through electrostatic and hydrophobic interactions, as well as hydrogen bonding. The addition of polysaccharide (carrageenan) has been reported to cause opposite effects on protein deposition, however, no conclusive mechanism has been proposed to elucidate how protein-polysaccharide interaction at pasteurization temperatures affects the fouling behavior of dairy products.

In this dissertation, different model dairy solutions and real dairy products were used to study the effect of composition, including protein distribution and additions of simple sugars and polysaccharides, on dairy fouling. Fouling deposits were formed and analyzed using a bench-top spinning disc apparatus operating under well-controlled temperatures and shear stresses characterized by computational fluid dynamics simulations. By studying the fouling behavior of camel milk and comparing with bovine milk, milk without β-lg was found to still foul and form deposits containing casein, α-lactalbumin, serum albumin with a reduced thermal resistance due to a more porous structure. Results also showed that the addition of 10 wt% sugar reduced whey protein fouling by more than 30% and affected the structure and adhesion strength of deposits.

Furthermore, the presence of carrageenan in dairy solutions can promote the denaturation of β-lg when heated and form a more compact deposit, resulting in more severe fouling. Overall, this dissertation provides a fundamental understanding of the fouling characteristics of complex dairy products. The knowledge gained is expected to help the dairy industry select suitable ingredients to mitigate or prevent the fouling problem.

CHAPTER 1. BACKGROUND AND INTRODUCTION

1.1 Heat transfer fouling

Fouling can be generally defined as accumulation of unwanted materials on the surfaces of equipment. Heat exchanger is an equipment that transfers heat between hot and cold fluids directly or indirectly. Heat transfer fouling typically takes place on the surfaces of pipes, tubes or plates and has negative impacts on their operation efficiency and sustainability (Ibrahim, 2012). Fouling is a severe problem affecting the economy of processing industries, and was estimated to cost 8−10 billion US dollars which accounts for 0.28−0.35% of US GNP in 1984 (Müller-Steinhagen et al., 1993). The costs associated with fouling include (Bott, 1995; Challa, 2013):

1. Increased capital cost: additional investment in equipment to accommodate the loss of production efficiency because of fouling
2. Additional operation cost: more frequent equipment maintenance as well as extra energy consumption to counterbalance fouling impact
3. Cleaning cost: extensive use of cleaning chemicals and water, and additional treatment for cleaning wastewater as well as increased labor workload
4. Production loss: reduced production capacity because of and more frequent shutdown for cleaning and potential negative effects on product quality

Fouling in heat exchanger is a very complex phenomena, it can be classified into six major categories according to the mechanism of deposit formation on surfaces (Awad, 2011):

1. Particulate fouling: accumulation of particles from heat transfer agents or product flowing in heat exchanger, which is also be called sedimentation fouling.
2. Crystallization/precipitation fouling: crystallized substances (e.g. salts) from saturated solution that then precipitate.
3. Chemical reaction fouling: deposits formed as a result of one or more chemical reactions in the fluid processed without interaction with surface materials.
4. Corrosion fouling: a result of chemical or electrochemical reactions between process surface and fluid to form corrosion products.
5. Biological fouling: attachment and growth of microbial organisms and their products.
6. Solidification fouling (freezing fouling): freezing of liquid onto a cooled surface.

One or more mechanisms can take place at the same time, making fouling a very complex process. Fouling in heat exchangers is a problematic issue in chemical, biological and food processing. Many research efforts have been made in the areas of chemical reaction fouling in petroleum refinery, evaporator fouling in corn processing and sugar refinery, and fouling in biomass boilers (Ibrahim, 2012). This thesis focuses on another critical industrial fouling problem, heat exchanger fouling in dairy processing.

1.2 Fouling in dairy processing

1.2.1 Fouling problem in dairy industry

Heat treatment is an essential operation in dairy processing to eliminate all pathogenic organisms and remove approximately 95% of spoilage-related microbial load for extending products' shelf life (Chandan, 2015). Fouling has been a serious problem in dairy processing ever since the first heat exchanger being introduced in the 1930s (Visser and Jeurnink, 1997). Fouling increases the thermal resistance and pressure drop in heat exchanger, decreasing its processing efficiency (Müller-Steinhagen et al., 1993). Product quality could also be affected by fouling as the temperature required for thermally inactivating the microorganisms cannot be reached. In addition, deposits formed can be a source of biological contamination or a precursor of biofilm formation (Sadeghinezhad et al., 2015). Fouling also leads to the necessity of intensive cleaning. Heat exchangers in milk processing plant need to be cleaned on a daily bases to maintain production efficiency and good hygiene (Bansal and Chen, 2006). Eighty percent of the total operation cost in dairy processing can be attributed to fouling and cleaning (van Asselt et al., 2005). However, the cost associated with fouling is not only in the economic aspect, it also exerts tremendous environmental burden with increased greenhouse gas emission, depletion of water resource, and discharge of cleaning chemicals (Müller-Steinhagen et al., 2009).

1.2.2 Fouling mechanisms

As fouling has various negative impacts on dairy processing, many researchers have been working towards understanding the fouling behavior and underlying mechanisms in order to mitigate or prevent fouling. Milk is a complex mixture. As the average composition shown in Table 1-1, most milk consists of mainly water (87%) and only 13% solids. Lactose, fat, protein and minerals are the major constituents in milk solids. Even though lactose and fat account for portions of milk, protein and minerals are the dominant contributors to milk fouling (Bansal and Chen, 2006).

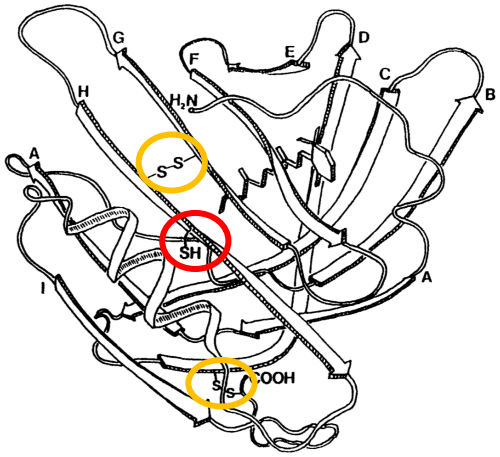
Table 1-1 Average milk composition (Bylund, 2003; Bansal and Chen, 2006)

Constituent	Average concentration (%)
Water	87
Total solids	13
Lactose	4.8
Fat	3.9
Proteins	3.4
Casein	2.6
β-lactoglobulin	0.32
α-lactabumin	0.12
Minerals	0.8

Depending on the final products, milk can be thermally treated with different combinations of time and temperature. The typical thermal processes applied in dairy industry include pasteurization (batch: 63−68 ºC for 30 min; continuous: 72−85 ºC for 15−30 s), ultra-pasteurization (125 ºC for 2−4 s), ultra-high-temperature (UHT) treatment (135−150 ºC for 1−10 s). According to the processing condition, dairy fouling can be generally classified into types A and B (Burton 1968; Bansal and Chen, 2006). Type A fouling also known as protein fouling takes place at temperature between 75 and 110 ºC. The deposit is composed of 50−70% protein, 30−40% minerals and 4−8% fat and appears as a white, soft and spongy milk film. When temperature is above 110 ºC, the deposit formed contains dominantly minerals (70-80%), especially calcium phosphate, and some protein (15−20%) and fat (4−8%). Type B (mineral) fouling forms deposits like hard, compact and granular milk stone.

In type A fouling, whey protein accounts for more than 50% of the deposits, and β-lg is the protein that dominantly controls the fouling process (Bylund, 2003; Gotham et al., 1992). β-lg that accounts for about 65% of bovine whey is a globular protein with each subunit containing 162 amino acids and has a molecular weight of 18 kDa (Visser and Jeurnink, 1997). Two disulfide bonds formed by four of the five cysteines (Figure 1-1a) (Papiz et al., 1986) construct the tertiary structure of β-lg and stabilize its globular quaternary structure (Hammann and Schmid, 2014). The remaining free sulfhydryl (-SH) group (cys-121) is buried in the hydrophobic core in the native state of β-lg. In milk that has a neutral pH, β-lg starts to denature/unfold when temperature is above 65 ºC, and expose the buried sulfhydryl group (Visser and Jeurnink, 1997). The reactive -SH can then interact with similar or other types of proteins such as casein and form aggregates (Jeurnink and Kruif, 1993). Calcium ions in milk can also take part in the fouling process by forming bridges between the proteins attached to heated surfaces and those in liquid (Christian et al., 2002). Bansal and Chen (2006) pointed out a debate that whether fouling deposits are formed by denatured proteins or protein aggregates. Many studies have confirmed that only unfolded β-lg takes part in deposit formation and once protein aggregates, it does not deposit on surface (Blanpain-Avet et al., 2016, 2012; Khaldi et al., 2015). A schematic of fouling process is presented in Figure 1-1b. However, how deposits attach to stainless steel surfaces is still not well understood.

(a)



(b)

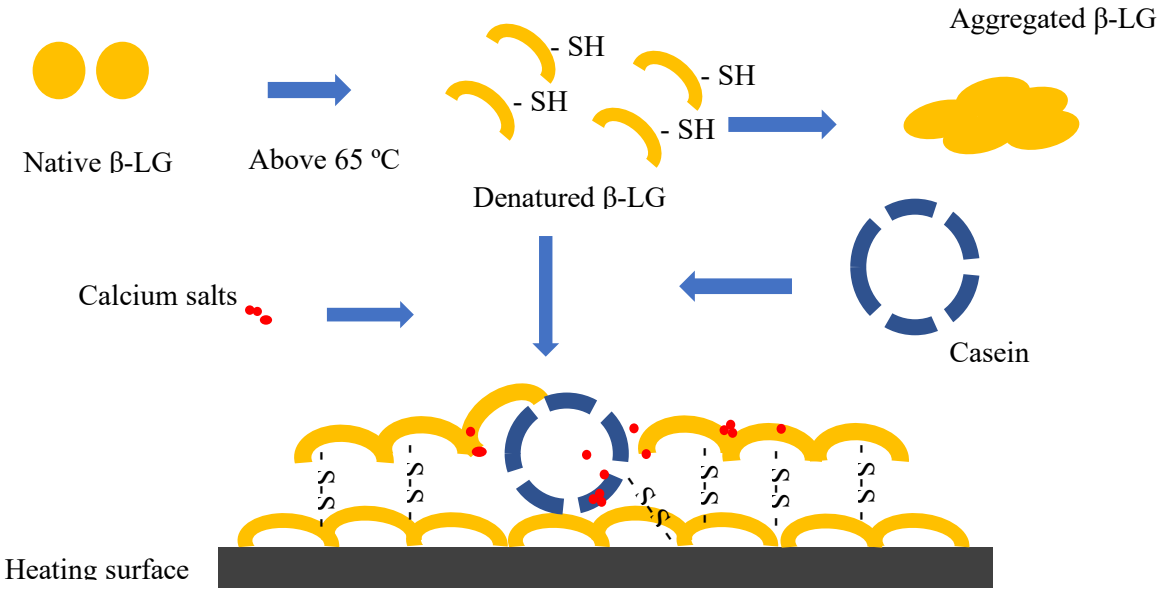


Figure 1-1 (a) Tertiary structure of β-lactoglobulin (Papiz et al., 1986). (b) Mechanism of type A milk fouling (Jimenez et al., 2013; Visser and Jeurnink, 1997).

1.2.3 Fouling factors

Based on the theoretical fouling mechanisms and experimental observations, several factors have been identified to affect the fouling process, which can be generally categorized into four main groups: operating condition, heat exchanger type, and surface characteristics, and dairy composition.

1.2.3.1 Operating condition

Operating conditions of heat treatment of dairy products include temperature, flow, pressure, and presence of air. Temperatures of heat treatment and milk are the most important factors that control fouling formation (Bansal and Chen, 2006). Milk starts to foul only when the surface temperature reaches 70 ºC (Bennett, 2007). Chen and Bala (1998) observed no visible fouling when surface temperature was below 68 ºC even though bulk temperature was up to 84 ºC. As described in the previous section, at heating temperature between 75 and 110 ºC, protein fouling takes place and thermal denaturation is the trigger for deposit formation. Mineral fouling dominates when heating temperature is above 110 ºC. Temperature affects protein denaturation kinetics, higher temperature is generally associated with more fouling in heat exchanger (Sadeghinezhad et al., 2015). Preheating milk was found to reduce fouling as it causes whey protein denaturation and aggregation which could limit fouling formation in subsequent heating processes (de Jong, 1997). Furthermore, temperature changes the nature of fouling, resulting in very different deposit characteristics and composition.

Flow condition is also an important factor in fouling process as it is controlled by both deposit attachment and removal. In most cases, increasing flow rate/turbulence reduces fouling as the enhanced shear stress increases the deposit removal rate (Belmar-Beiny et al., 1993). On the other hand, higher flow velocity could also lead to a better chance of deposit re-entrainment (Rakes et al., 1992).

Processing pressure and air bubbles present in milk also affect fouling behavior. Bennett (2007) found that fouling decreased with an increase of pressure in a tubular heat exchanger which also caused significant differences in the size and shape of deposits formed. When processing pressure

is low, the solubility of air in milk decreases, generating air bubbles on heating surfaces which could significantly increase fouling (Jeurnink, 1995).

1.2.3.2 Heat exchanger type and surface characteristics

Plate heat exchanger is most widely used in dairy processing due to its advantages of better heat transfer performance, higher turbulence and ease of maintenance compared with tubular heat exchanger. However, plate heat exchanger is more prone to fouling (Basal and Chen 2006). The geometry of a heat exchanger affects its heat transfer and flow pattern, and thus fouling formation. The surface of heat exchanger is another important factor, as fouling process is an interaction between liquid and surface, and the attachment of deposits highly depends on the surface characteristics (Basal and Chen 2006). A rougher surface is generally regarded to induce a more severe fouling as they have a larger surface area (Yoon and Lund 1994). Various surface modification techniques targeting on fouling mitigation have been developed. Barish and Goddard (2013), Rungraeng et al. (2012), and Zouaghi et al. (2017) correlated increased surface hydrophobicity and reduced surface energy with fouling reduction. However, opposite findings have also been reported that hydrophilic and amphiphilic surfaces are effective in reducing fouling (Zouaghi et al., 2018a, 2018b).

1.2.3.3 Dairy composition

Besides the crucial role whey protein plays in fouling process, other components in milk or dairy products can interact with whey protein and thus affect fouling. Calcium ion in milk was found to significantly affect whey protein denaturation and aggregation by promoting intermolecular crosslinking of β-lg polymers and strengthening the aggregates (Simmons et al., 2007). Ionic calcium can also create intramolecular electrostatic shielding of the negative charges of β-lg to enhance its aggregation. The tertiary structure of β-lg can be affected by calcium, leading to unfolding and exposing the free thiol group (Joyce et al., 2018). Khaldi et al. (2018) associated the molar ratio of calcium to whey protein with fouling in heat exchangers and found that lower calcium/protein molar ratio yielded a thin deposit with dense structure, and a high ratio led to a thicker deposit with open structure.

Despite the significant role β-lg plays in bovine milk fouling, camel milk that does not contain β-lg was found to form fouling deposits during heating (Felfoul et al., 2016, 2015a, 2015b). Apart from a similar composition of 4.4 % lactose, 3.5% fat, 3.1% protein and 0.79% ash (Al haj and Al Kanhal, 2010) to bovine milk, camel milk has whey protein mainly composed of α-lactalbumin (α-la; 27%), serum albumin (SA; 26%) and immunoglobulins (18%) (Hailu et al., 2016). Felfoul at al. (2015a) observed that α-la and SA were the most affected protein by heat treatment and therefore could be responsible for fouling formation. Furthermore, camel milk deposits were reported to contain less protein (57% in camel, 69% in bovine) but slightly more minerals (35% in camel, 28% in bovine) (Felfoul et al., 2016). Yet, the fouling behavior of camel milk has not been studied under well-controlled temperature and flow, and the milk proteins participating in its fouling process are still not clear.

Fat is generally considered as a minor component in milk deposits (4−8%) (Basal and Chen, 2006). However, some studies observed that deposits formed by whole milk had fat contents of 40–50% (Calvo and de Rafael, 2009; Fang, 1998; Johnson and Roland, 1940). Fang (1998) reported that when the membrane of milk fat globule is damaged by mechanical stress (e.g. shear, turbulence, cavitation), the damaged fat globules tend to coalesce and form larger globules, which could migrate more easily to heated surface and get entrapped in the matrix of fouled protein.

Although casein is the most abundant milk protein (2.6%), it is generally considered heat-stable and does not contribute to milk fouling directly. Casein can attach to denatured whey protein and form β-lg-κ-casein complex (Vasbinder et al., 2003) However, no study has been conducted to investigate the effect of casein on protein deposition at high temperatures.

Besides milk components, other ingredients in dairy products can interact with milk protein and affect its fouling. Sugars are widely used in dairy products as sweetener, flavor and color precursor through Maillard reaction, substrate for fermentation, stabilizer and emulsifier (Voragen, 1998). Sugars have been reported to enhance the thermal stability of whey protein (Timasheff, 1982, Rich and Foegeding, 2000). However, Huang and Goddard, (2015) observed an increased fouling and deposit strength when sugar and cocoa powder were added into whole milk. There is no

comprehensive study focusing on the effect of sugars on protein fouling which thus needs to be further explored.

Polysaccharides are also widely added in dairy products such as flavored milk, ice cream, yogurt and milk shakes to modify their physical, chemical and sensory properties (Burova et al., 2007). Carrageenan is the most commonly used polysaccharide in dairy products which can interact with milk proteins and form complex mainly through electrostatic interactions (Hosseini et al., 2013; Jones et al., 2010; Weinbreck et al., 2004). However, only few studies have investigated the effect of carrageenan on the fouling behaviors of dairy products. Prakash et al. (2010) reported that carrageenan had a stabilizing effect on chocolate milk during UHT processing, with an optimal concentration of 0.03 % at which chocolate milk had the least fouling. Huang and Goddard (2015) found an increase in chocolate milk fouling when 0.1 % carrageenan was added.

1.2.4 Experimental fouling apparatuses

Several apparatuses were applied in past studies to conduct fouling experiments from bench- to pilot-scale. Fouling apparatuses aim to simulate real processing conditions of heat exchangers while making it possible to monitor and collect fouling characteristics online. Table 2-2 summarizes the commonly used fouling apparatuses in literature. Pilot-scale plate heat exchangers have the most similar configuration to simulate the industrial-scale operations and have been used in numerous studies (Blanpain-Avet et al., 2016; Boxler et al., 2014; Sadeghinezhad et al., 2015). The major drawback of this equipment is the need of a large amount of milk or test solutions for one test, leading to a limited number of replicates for each experiment. To reduce sample volume and simplify experimental operations, some lab/bench-scale fouling apparatuses were developed, such as spinning disc apparatus (Huang et al., 2012) and annular tube device (Lv et al., 2015). Apparatuses of smaller scale have more precise controls of processing temperature and flow to improve test reproducibility. They also have the capability of incorporating more sensors for more accurate monitoring of fouling. Apparatuses generally used in other fields have also been applied to study fouling. Yang et al. (2018) used quartz crystal microbalance with dissipation (QCM-D) to reliably measure the fouling rate for a long period of time. The major limitation of QCM-D used is a lower operating temperature (65 ºC) which cannot fully simulate real fouling scenarios.

1.2.5 Fouling measurements

With different fouling apparatuses, parameters associated with fouling can be monitored online to determine fouling behavior, including deposit mass and thickness, heat transfer parameters, pressure drop and acoustic parameters (Award, 2011; Wallhäußer et al., 2012):

1. Direct measures. Direct weighing of deposit mass is the simplest method for describing fouling behavior. Thickness measurement is another way to determine the extent of fouling and also deposit distribution in plate heat exchanger

2. Heat transfer parameters. Thermal resistance of fouling deposits can be determined by the change in heat transfer coefficient during the fouling experiment conducted at a constant heat flux or constant temperature. Fouling behavior determined by this method is based on one assumption that deposits formed do not affect the hydrodynamics of the fluid flow.

3. Pressure drop. Pressures of the inlet and outlet of plate heat exchanger are regularly monitored during processing operation. Fouling formation can decrease the flow area of the flow channel, leading to a pressure drop. This method is not very sensitive to formation of thin deposit layers and cannot identify the location where deposits form. Hence it is often combined with direct measurement of deposit mass and thickness.

4. Acoustic methods. The acoustic parameters of a surface change with fouling formation and can be measured using ultrasound and vibrational methods in transmission and pulse-eco modes. QCM-D described in the previous section is also an acoustic measurement that correlates the change in quartz oscillating frequency with the change in fouling mass.

Table 1-2 Comparison of commonly used apparatuses for dairy fouling studies

Type	Scale	Advantages	Drawbacks
Plate heat exchanger (Boxler et al., 2014; Huang and Goddard, 2015)	Pilot/Bench	Direct representation of industrial continuous thermal processing; with temperature and flow controls; easy operation (bench-scale)	Large amount of experimental materials required; large experimental variations; unable to monitor fouling growth online
Pasteurizer (Zouaghi et al., 2018b)	Pilot	Continuous processing; temperature and flow controls	No temperature difference between bulk and surface; difficulty in online monitoring of fouling
Spinning disc (Rosmaninho and Melo, 2008, 2006)	Bench	Well-controlled temperature and shear, online monitor of fouling; easy operation, small volume of material required	Different flow field from real scenarios; not a continuous processing
Annular tube (Lv et al., 2015)	Bench	Temperature and flow controls, online monitoring of fouling	Not a continuous processing
Couette (Simmons et al., 2007)	Bench	Accurate controls of temperature and shear; easy operation, small volume of material required	Not a continuous processing; unable to monitor fouling online
Quartz crystal microbalance (QCM) (Yang et al., 2018)	Bench	Online monitoring of deposit mass; fouling rate measurement; accurate temperature and flow controls	Low operating temperature than real thermal processing

1.3 Objectives of thesis

The main goal of this dissertation is to gain fundamental understanding of fouling of complex dairy products from the aspect of protein-carbohydrate interaction. Specific objectives include:

1. Investigate the effect of protein distribution on milk fouling by examining the fouling characteristics of camel milk and comparing with bovine milk.

2. Investigate the effect of simple sugars on protein fouling to characterize the fouling behavior of sweetened milk.

3. Investigate the interaction of whey with casein and carrageenan at pasteurization temperature to elucidate the fouling mechanisms of their mixture.

These specific objectives were attained by three separate studies reported in Chapters 2, 3 and 4 of this dissertation. The knowledge gained from this dissertation is expected to serve as the groundwork to help the dairy industry design dairy formulations resulting in reduced fouling.

1.4 References

Al haj, O.A., Al Kanhal, H.A., 2010. Compositional, technological and nutritional aspects of dromedary camel milk. International Dairy Journal 20, 811–821. https://doi.org/10.1016/j.idairyj.2010.04.003

Alizadeh-Pasdar, N., Nakai, S., Li-Chan, E.C.Y., 2002. Principal Component Similarity Analysis of Raman Spectra To Study the Effects of pH, Heating, and κ-Carrageenan on Whey Protein Structure. J. Agric. Food Chem. 50, 6042–6052. https://doi.org/10.1021/jf0116956

Awad, M.M., n.d. Fouling of Heat Transfer Surfaces. Heat Transfer 41.

Bansal, B., Chen, X.D., 2006. A Critical Review of Milk Fouling in Heat Exchangers. Comprehensive Reviews in Food Science and Food Safety 5, 27–33. https://doi.org/10.1111/j.1541-4337.2006.tb00080.x

Belmar-Beiny, M.T., Gotham, S.M., Paterson, W.R., Fryer, P.J., Pritchard, A.M., 1993. The effect of Reynolds number and fluid temperature in whey protein fouling. Journal of Food Engineering 19, 119–139. https://doi.org/10.1016/0260-8774(93)90038-L

Blanpain-Avet, P., André, C., Khaldi, M., Bouvier, L., Petit, J., Six, T., Jeantet, R., Croguennec, T., Delaplace, G., 2016. Predicting the distribution of whey protein fouling in a plate heat exchanger using the kinetic parameters of the thermal denaturation reaction of β-lactoglobulin and the bulk temperature profiles. Journal of Dairy Science 99, 9611–9630. https://doi.org/10.3168/jds.2016-10957

Blanpain-Avet, P., Hédoux, A., Guinet, Y., Paccou, L., Petit, J., Six, T., Delaplace, G., 2012. Analysis by Raman spectroscopy of the conformational structure of whey proteins constituting fouling deposits during the processing in a heat exchanger. Journal of Food Engineering 110, 86–94. https://doi.org/10.1016/j.jfoodeng.2011.12.005

Boxler, C., Augustin, W., Scholl, S., 2014. Influence of surface modification on the composition of a calcium phosphate-rich whey protein deposit in a plate heat exchanger. Dairy Sci. & Technol. 94, 17–31. https://doi.org/10.1007/s13594-013-0142-5

Burova, T.V., Grinberg, N.V., Grinberg, V.Ya., Usov, A.I., Tolstoguzov, V.B., de Kruif, C.G., 2007. Conformational Changes in ι- and κ-Carrageenans Induced by Complex Formation with Bovine β-Casein. Biomacromolecules 8, 368–375. https://doi.org/10.1021/bm060761f

Bylund, Gösta., 2003. Dairy processing handbook. Tetra Pak Processing Systems AB, Lund, Sweden.

Carew, E.B., Stanley, H.E., Seidel, J.C., Gergely, J., 1983. Studies of myosin and its proteolytic fragments by laser Raman spectroscopy. Biophysical Journal 44, 219–224. https://doi.org/10.1016/S0006-3495(83)84294-5

Chandan, R.C., 2015. Dairy Processing and Quality Assurance: An Overview, in: Dairy Processing and Quality Assurance. John Wiley & Sons, Ltd, pp. 1–40. https://doi.org/10.1002/9781118810279.ch01

Changani, S.D., Belmar-Beiny, M.T., Fryer, P.J., 1997. Engineering and chemical factors associated with fouling and cleaning in milk processing. Experimental Thermal and Fluid Science 14, 392–406. https://doi.org/10.1016/S0894-1777(96)00141-0

Cho, Y., Singh, H., Creamer, L.K., 2003. Heat-induced interactions of β-lactoglobulin A and κ-casein B in a model system. Journal of Dairy Research 70, 61–71. https://doi.org/10.1017/S0022029902005642

Christian, G.K., Changani, S.D., Fryer, P.J., 2002. The Effect of Adding Minerals on Fouling from Whey Protein Concentrate: Development of a Model Fouling Fluid for a Plate Heat Exchanger. Food and Bioproducts Processing, Fouling, Cleaning and Disinfection 80, 231–239. https://doi.org/10.1205/096030802321154718

Dalgleish, D.G., Morris, E.R., 1988. Interactions between carrageenans and casein micelles: electrophoretic and hydrodynamic properties of the particles. Food Hydrocolloids 2, 311–320. https://doi.org/10.1016/S0268-005X(88)80028-6

de Jong, P., 1997. Impact and control of fouling in milk processing. Trends in Food Science & Technology 8, 401–405. https://doi.org/10.1016/S0924-2244(97)01089-3

Eftink, M.R., 2000. Intrinsic Fluorescence of Proteins, in: Lakowicz, J.R. (Ed.), Topics in Fluorescence Spectroscopy: Volume 6: Protein Fluorescence, Topics in Fluorescence Spectroscopy. Springer US, Boston, MA, pp. 1–15. https://doi.org/10.1007/0-306-47102-7_1

Elshereef, R., Budman, H., Moresoli, C., Legge, R.L., 2006. Fluorescence spectroscopy as a tool for monitoring solubility and aggregation behavior of β-lactoglobulin after heat treatment. Biotechnology and Bioengineering 95, 863–874. https://doi.org/10.1002/bit.21039

Felfoul, I., Beaucher, E., Cauty, C., Attia, H., Gaucheron, F., Ayadi, M.A., 2016. Deposit Generation During Camel and Cow Milk Heating: Microstructure and Chemical Composition. Food Bioprocess Technol 9, 1268–1275. https://doi.org/10.1007/s11947-016-1714-1

Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., Ayadi, M.A., 2015a. A laboratory investigation of cow and camel whey proteins deposition under different heat treatments. Food and Bioproducts Processing 96, 256–263. https://doi.org/10.1016/j.fbp.2015.09.002

Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., Ayadi, M.A., 2015b. Fouling Behavior of Camel and Cow Milks Under Different Heat Treatments. Food Bioprocess Technol 8, 1771–1778. https://doi.org/10.1007/s11947-015-1529-5

Fitzsimons, S.M., Mulvihill, D.M., Morris, E.R., 2007. Denaturation and aggregation processes in thermal gelation of whey proteins resolved by differential scanning calorimetry. Food Hydrocolloids 21, 638–644. https://doi.org/10.1016/j.foodhyd.2006.07.007

Gotham, S.M., Fryer, P.J., Pritchard, A.M., 1992. β-lactoglobulin denaturation and aggregation reactions and fouling deposit formation: a DSC study. International Journal of Food Science & Technology 27, 313–327. https://doi.org/10.1111/j.1365-2621.1992.tb02033.x

Hailu, Y., Hansen, E.B., Seifu, E., Eshetu, M., Ipsen, R., Kappeler, S., 2016. Functional and technological properties of camel milk proteins: a review. Journal of Dairy Research 83, 422–429. https://doi.org/10.1017/S0022029916000686

Hammann, F., Schmid, M., 2014. Determination and Quantification of Molecular Interactions in Protein Films: A Review. Materials (Basel) 7, 7975–7996. https://doi.org/10.3390/ma7127975

Hosseini, S.M.H., Emam-Djomeh, Z., Razavi, S.H., Moosavi-Movahedi, A.A., Saboury, A.A., Mohammadifar, M.A., Farahnaky, A., Atri, M.S., Van der Meeren, P., 2013. Complex coacervation of β-lactoglobulin – κ-Carrageenan aqueous mixtures as affected by polysaccharide sonication. Food Chemistry 141, 215–222. https://doi.org/10.1016/j.foodchem.2013.02.090

Howell, N., Li-Chan, E., 1996. Elucidation of interactions of lysozyme with whey proteins by Raman spectroscopy. International Journal of Food Science & Technology 31, 439–451. https://doi.org/10.1046/j.1365-2621.1996.00364.x

Huang, J.-Y., John Chew, Y.M., Ian Wilson, D., 2012. A spinning disc study of fouling of cold heat transfer surfaces by gel formation from model food fat solutions. Journal of Food Engineering 109, 49–61. https://doi.org/10.1016/j.jfoodeng.2011.09.034

Huang, K., Goddard, J.M., 2015. Influence of fluid milk product composition on fouling and cleaning of Ni–PTFE modified stainless steel heat exchanger surfaces. Journal of Food Engineering 158, 22–29. https://doi.org/10.1016/j.jfoodeng.2015.02.026

Ibrahim, H.A.-H., 2012. Fouling in Heat Exchangers. MATLAB - A Fundamental Tool for Scientific Computing and Engineering Applications - Volume 3. https://doi.org/10.5772/46462

Ikeda, S., 2003. Heat-induced gelation of whey proteins observed by rheology, atomic force microscopy, and Raman scattering spectroscopy. Food Hydrocolloids, 6th International Hydrocolloids Conference 17, 399–406. https://doi.org/10.1016/S0268-005X(03)00033-X

Ikeda, S., Li-Chan, E.C.Y., 2004. Raman spectroscopy of heat-induced fine-stranded and particulate β-lactoglobulin gels. Food Hydrocolloids 18, 489–498. https://doi.org/10.1016/j.foodhyd.2003.07.003

Jeurnink, T.J.M., Kruif, K.G.D., 1993. Changes in milk on heating: viscosity measurements. Journal of Dairy Research 60, 139–150. https://doi.org/10.1017/S0022029900027461

Jimenez, M., Delaplace, G., Nuns, N., Bellayer, S., Deresmes, D., Ronse, G., Alogaili, G., Collinet-Fressancourt, M., Traisnel, M., 2013. Toward the understanding of the interfacial dairy fouling deposition and growth mechanisms at a stainless steel surface: A multiscale approach. Journal of Colloid and Interface Science 404, 192–200. https://doi.org/10.1016/j.jcis.2013.04.021

Jones, O., Decker, E.A., McClements, D.J., 2010. Thermal analysis of β-lactoglobulin complexes with pectins or carrageenan for production of stable biopolymer particles. Food Hydrocolloids 24, 239–248. https://doi.org/10.1016/j.foodhyd.2009.10.001

Joyce, A.M., Kelly, A.L., O'Mahony, J.A., 2018. Controlling denaturation and aggregation of whey proteins during thermal processing by modifying temperature and calcium concentration. International Journal of Dairy Technology 71, 446–453. https://doi.org/10.1111/1471-0307.12507

Kazi, S.N., 2012. Fouling and Fouling Mitigation on Heat Exchanger Surfaces. Heat Exchangers - Basics Design Applications. https://doi.org/10.5772/32990

Khaldi, M., Croguennec, T., André, C., Ronse, G., Jimenez, M., Bellayer, S., Blanpain-Avet, P., Bouvier, L., Six, T., Bornaz, S., Jeantet, R., Delaplace, G., 2018. Effect of the calcium/protein molar ratio on β-lactoglobulin denaturation kinetics and fouling phenomena. International Dairy Journal 78, 1–10. https://doi.org/10.1016/j.idairyj.2017.10.002

Khaldi, M., Ronse, G., André, C., Blanpain-Avet, P., Bouvier, L., Six, T., Bornaz, S., Croguennec, T., Jeantet, R., Delaplace, G., 2015. Denaturation Kinetics of Whey Protein Isolate Solutions and Fouling Mass Distribution in a Plate Heat Exchanger [WWW Document]. International Journal of Chemical Engineering. https://doi.org/10.1155/2015/139638

Lv, H., Huang, S., Mercadé-Prieto, R., Wu, X.E., Chen, X.D., 2015. The effect of pre-adsorption of OVA or WPC on subsequent OVA or WPC fouling on heated stainless steel surface. Colloids and Surfaces B: Biointerfaces 129, 154–160. https://doi.org/10.1016/j.colsurfb.2015.03.042

Müller-Steinhagen, H., Malayeri, M.R., Watkinson, A.P., 2009. Heat Exchanger Fouling: Environmental Impacts. Heat Transfer Engineering 30, 773–776. https://doi.org/10.1080/01457630902744119

Nigo, R.Y., Chew, Y.M.J., Houghton, N.E., Paterson, W.R., Wilson, D.I., 2009. Experimental Studies of Freezing Fouling of Model Food Fat Solutions Using a Novel Spinning Disc Apparatus. Energy Fuels 23, 6131–6145. https://doi.org/10.1021/ef900668f

Nonaka, Masahiko., Li-Chan, Eunice., Nakai, Shuryo., 1993a. Raman spectroscopic study of thermally induced gelation of whey proteins. J. Agric. Food Chem. 41, 1176–1181. https://doi.org/10.1021/jf00032a002

Nonaka, Masahiko., Li-Chan, Eunice., Nakai, Shuryo., 1993b. Raman spectroscopic study of thermally induced gelation of whey proteins. J. Agric. Food Chem. 41, 1176–1181. https://doi.org/10.1021/jf00032a002

O'Kennedy, B.T., Mounsey, J.S., 2009. The dominating effect of ionic strength on the heat-induced denaturation and aggregation of β-lactoglobulin in simulated milk ultrafiltrate. International Dairy Journal 19, 123–128. https://doi.org/10.1016/j.idairyj.2008.09.004

Palazolo, G., Rodríguez, F., Farruggia, B., Picó, G., Delorenzi, N., 2000. Heat Treatment of β-Lactoglobulin: Structural Changes Studied by Partitioning and Fluorescence. J. Agric. Food Chem. 48, 3817–3822. https://doi.org/10.1021/jf991353o

Papiz, M.Z., Sawyer, L., Eliopoulos, E.E., North, A.C.T., Findlay, J.B.C., Sivaprasadarao, R., Jones, T.A., Newcomer, M.E., Kraulis, P.J., 1986. The structure of β -lactoglobulin and its similarity to plasma retinol-binding protein. Nature 324, 383–385. https://doi.org/10.1038/324383a0

Prakash, S., Huppertz, T., Karvchuk, O., Deeth, H., 2010. Ultra-high-temperature processing of chocolate flavoured milk. Journal of Food Engineering 96, 179–184. https://doi.org/10.1016/j.jfoodeng.2009.07.008

Rahimi Yazdi, S., Corredig, M., 2012. Heating of milk alters the binding of curcumin to casein micelles. A fluorescence spectroscopy study. Food Chemistry 132, 1143–1149. https://doi.org/10.1016/j.foodchem.2011.11.019

Rosmaninho, R., Melo, L.F., 2008. Protein–calcium phosphate interactions in fouling of modified stainless-steel surfaces by simulated milk. International Dairy Journal 18, 72–80. https://doi.org/10.1016/j.idairyj.2007.06.008

Rosmaninho, R., Melo, L.F., 2006. Calcium phosphate deposition from simulated milk ultrafiltrate on different stainless steel-based surfaces. International Dairy Journal 16, 81–87. https://doi.org/10.1016/j.idairyj.2005.01.006

Sadeghinezhad, E., Kazi, S.N., Dahari, M., Safaei, M.R., Sadri, R., Badarudin, A., 2015. A Comprehensive Review of Milk Fouling on Heated Surfaces. Critical Reviews in Food Science and Nutrition 55, 1724–1743. https://doi.org/10.1080/10408398.2012.752343

Schreier, P.J.R., Fryer, P.J., 1995. Heat exchanger fouling: A model study of the scaleup of laboratory data. Chemical Engineering Science 50, 1311–1321. https://doi.org/10.1016/0009-2509(95)98843-4

Seo, J.-A., Hédoux, A., Guinet, Y., Paccou, L., Affouard, F., Lerbret, A., Descamps, M., 2010. Thermal Denaturation of Beta-Lactoglobulin and Stabilization Mechanism by Trehalose Analyzed from Raman Spectroscopy Investigations. J. Phys. Chem. B 114, 6675–6684. https://doi.org/10.1021/jp1006022

Simion (Ciuciu), A.-M., Aprodu, I., Dumitrașcu, L., Bahrim, G.E., Alexe, P., Stănciuc, N., 2015. Exploring the heat-induced structural changes of β-lactoglobulin -linoleic acid complex by fluorescence spectroscopy and molecular modeling techniques. J Food Sci Technol 52, 8095–8103. https://doi.org/10.1007/s13197-015-1949-2

Simmons, M.J.H., Jayaraman, P., Fryer, P.J., 2007. The effect of temperature and shear rate upon the aggregation of whey protein and its implications for milk fouling. Journal of Food Engineering 79, 517–528. https://doi.org/10.1016/j.jfoodeng.2006.02.013

Spagnuolo, P.A., Dalgleish, D.G., Goff, H.D., Morris, E.R., 2005. Kappa-carrageenan interactions in systems containing casein micelles and polysaccharide stabilizers. Food Hydrocolloids, Food Colloids 2004 (Harrogate) 19, 371–377. https://doi.org/10.1016/j.foodhyd.2004.10.003

STEINHAGEN, R., MÜLLER-STEINHAGEN, H., MAANI, K., 1993. Problems and Costs due to Heat Exchanger Fouling in New Zealand Industries. Heat Transfer Engineering 14, 19–30. https://doi.org/10.1080/01457639308939791

Taheri-Kafrani, A., Asgari-Mobarakeh, E., Bordbar, A.-K., Haertlé, T., 2010. Structure-function relationship of beta-lactoglobulin in the presence of dodecyltrimethyl ammonium bromide. Colloids Surf B Biointerfaces 75, 268–274. https://doi.org/10.1016/j.colsurfb.2009.08.045

Vasbinder, A.J., Alting, A.C., de Kruif, K.G., 2003. Quantification of heat-induced casein–whey protein interactions in milk and its relation to gelation kinetics. Colloids and Surfaces B: Biointerfaces, Food Colloids, Biopolymers and Materials Special Issue 31, 115–123. https://doi.org/10.1016/S0927-7765(03)00048-1

Visser, J., Jeurnink, Th.J.M., 1997. Fouling of heat exchangers in the dairy industry. Experimental Thermal and Fluid Science 14, 407–424. https://doi.org/10.1016/S0894-1777(96)00142-2

Wallhäußer, E., Hussein, M.A., Becker, T., 2012. Detection methods of fouling in heat exchangers in the food industry. Food Control 27, 1–10. https://doi.org/10.1016/j.foodcont.2012.02.033

Weinbreck, F., Nieuwenhuijse, H., Robijn, G.W., de Kruif, C.G., 2004. Complexation of Whey Proteins with Carrageenan. J. Agric. Food Chem. 52, 3550–3555. https://doi.org/10.1021/jf034969t

Wong, N.P., 1988. Fundamentals of Dairy Chemistry, 3rd ed. Springer US. https://doi.org/10.1007/978-1-4615-7050-9

Yang, W., Li, D., Chen, X.D., Mercadé-Prieto, R., 2018. Effect of calcium on the fouling of whey protein isolate on stainless steel using QCM-D. Chem Eng Sci 177, 501–508. https://doi.org/10.1016/j.ces.2017.12.004

Zhang, B.Y., Lu, J., Huang, J.-Y., 2019. Effect of sugar on the fouling behavior of whey protein. Food and Bioproducts Processing, Fouling and Cleaning in Food Processing 113, 2–9. https://doi.org/10.1016/j.fbp.2018.10.002

Zouaghi, S., Barry, M.E., Bellayer, S., Lyskawa, J., André, C., Delaplace, G., Grunlan, M.A., Jimenez, M., 2018a. Antifouling amphiphilic silicone coatings for dairy fouling mitigation on stainless steel. Biofouling 34, 769–783.

Zouaghi, S., Six, T., Bellayer, S., Coffinier, Y., Abdallah, M., Chihib, N.-E., André, C., Delaplace, G., Jimenez, M., 2018b. Atmospheric pressure plasma spraying of silane-based coatings targeting whey protein fouling and bacterial adhesion management. Applied Surface Science 455, 392–402. https://doi.org/10.1016/j.apsusc.2018.06.006

CHAPTER 2. FOULING CHARACTERIZATION OF CAMEL MILK WITH COMPARISON TO BOVINE MILK*

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2.1 Abstract

The fouling behavior of raw camel milk was studied under controlled surface temperatures (71−79 ºC) and shear stresses (0.03−3.14 Pa) using a spinning disc apparatus. Camel milk fouling decreased with increasing shear and increased significantly as surface temperature increased. Comparing to bovine milk, camel milk had approximately 76% lower linear fouling rates, and 55% lower final fouling resistances, however, the masses of their dry deposits were not significantly different. Micro-CT scan images revealed a higher porosity of the deposit of camel milk (76%) than bovine milk (55%), which resulted in the lower thermal resistances observed during camel milk fouling. Composition analysis showed that fat (52−62%) and protein (34−43%) were the major constituents in both deposits. SDS-PAGE analysis indicated that casein, α-lactalbumin, serum albumin and peptidoglycan recognition protein were the major proteins contributing to the deposition of camel milk, while β-lactoglobulin and casein were responsible for bovine milk fouling.

Keywords: Milk deposit formation; Thermal resistance; β-lactoglobulin; Casein; α-lactalbumin; Porosity

2.2 Introduction

Camel milk has been an important source of nutrition for nomadic and pastoral cultures in the arid parts of the world for centuries. More recently, there has been a growing interest in camel milk as an alternative to bovine milk and nutraceutical products because of its high nutritional value and therapeutic effects (Khalesi et al., 2017; Zibaee et al., 2015). In addition to prevention of milk allergy in children (Zibaee et al., 2015), the active compounds in camel milk have proved to have

potential treatment effect on diabetes (Agrawal et al., 2011; Malik et al., 2012), cancer (Alhaider et al., 2014; Habib et al., 2013), and autism (AL-Ayadhi and Elamin, 2013). The total camel milk production was estimated to increase by 4.6 times since 1961 to 2.9 million tonnes in 2017 (Singh et al., 2017), and expected to continue growing (Khalesi et al., 2017).

Raw camel milk has a shelf life of 8−9 hours at 37 ºC, and a week at 4−6 ºC (Singh et al., 2017). Although in some countries, camel milk is still consumed unprocessed, there is a growing need for thermal treatment (pasteurization) on milk to ensure product safety and prolong its shelf life (Felfoul et al., 2015b). During thermal processing, camel milk proteins tend to denature and interact with other components, leading to deposit formation on heated surfaces called fouling (Bansal and Chen, 2006; Felfoul et al., 2015a). Fouling is a severe problem in milk processing as the deposit formed can impede heat transfer during heat treatment and also cause pressure drop in piping systems, therefore, intensive cleaning is needed to maintain product quality and safety. In dairy industry, the rapid fouling in heating equipment is responsible for 80% of the production cost (Van Asselt et al., 2005).

There are continuous efforts to understand the behavior and mechanisms of bovine milk fouling in order to develop mitigation strategies. Milk fouling can be generally classified into type A, typical protein fouling taking place at pasteurization temperatures, and type B fouling, which is dominated by mineral fouling formed at temperatures above 110 ºC (Burton, 1968; Changani et al., 1997; Visser and Jeurnink, 1997a). Whey protein, mostly β-lactoglobulin (β-lg), is the governing factor in bovine milk type A fouling (Bansal and Chen, 2006; Petit et al., 2011; Sadeghinezhad et al., 2015). At the temperature above 65 ºC, β-lg starts to denature and unfold, exposing the active amino acid groups that can interact with other milk proteins as well as calcium to form deposit on surface (de Jong et al., 2002; Tuoc, 2015; Visser and Jeurnink, 1997a). Therefore, the rate and distribution of fouling in heat exchanger are closely related to the denaturation and aggregation kinetics of β-lg (de Jong, 1997; Blanpain-Avet et al., 2016; Blanpain-Avet et al., 2012; Petit et al., 2011; Petit et al., 2013; Schreier and Fryer, 1995).

Camel milk has a comparable composition to bovine milk, consisting of 4.4% lactose, 3.5% fat, 3.1% protein and 0.79% ash on average (Al haj and Al Kanhal, 2010). The protein profile of camel

milk, however, is very different from bovine milk. Camel whey proteins do not contain β-lg, which accounts for more than 50% of bovine whey. Instead, α-lactalbumin (α-la; 27%), serum albumin (SA; 26%) and immunoglobulins (18%) are the major proteins in camel milk (Hailu et al., 2016; Konuspayeva et al., 2009). As a result, the knowledge of bovine milk fouling is not applicable to camel milk, and the study on the fouling of this specific dairy product is still very limited. Up to the authors' knowledge, the fouling of camel milk was first and has been only studied by Felfoul and coworkers (2015a, 2015b, 2016). Their research mainly focused on analyzing the chemical changes in milk (*e.g.* free thiol group, composition, protein profile) during heat treatment as well as the surface morphology of fouling deposit. Felfoul et al. (2015a) reported that SA and α-la were the most sensitive proteins in camel milk to heat treatment, implying that they might play an important role in formation of fouling deposit, which, however, was not further examined by their following studies. Furthermore, although Felfoul and coworkers shed light on camel milk fouling, its fouling characteristics were not characterized at well-controlled surface flow and temperature conditions. The effects of shear and temperature on the physicochemical properties of camel milk fouling deposit are still unknown.

In this study, we investigated the fouling behavior of camel milk using a lab-scale spinning disc apparatus, on which fouling deposit can be formed under well-controlled surface temperature and shear stress, simulating the industrial operation. We also analyzed the chemical composition and protein profile of the camel milk fouling deposits for comparison with bovine milk deposits. This study aimed to provide fundamental insights into the effect of protein profile of milk on its fouling during pasteurization, and identify the proteins that participate in the deposition of camel milk. By comparing the fouling characteristics of camel milk with bovine milk, the results of this study are expected to help modify existing mitigation methods for bovine milk fouling for further applications to camel milk processing.

2.3 Materials and Methods

2.3.1 Milk samples

The raw camel milk solution was reconstituted with 95 g of freeze-dried raw camel milk powder (28.45% protein, 23.50% fat and 7.52% ash on dry basis) (Camilk Dairy, DC, USA) and 900 ml

of deionized water. The solution was mixed at room temperature (20−22 ºC) for 2 h then refrigerated (4 ºC) overnight for complete hydration. Fresh raw bovine milk (25.92% protein, 30.50% fat and 4.68% ash on dry basis) was collected from the Dairy Research and Education Center of the Department of Animal Sciences at Purdue University. The raw bovine milk was stored at 4 ºC immediately after collection and tested within 48 h. The pH of all the milk samples were between 6.7 and 6.9 throughout the fouling experiments.

2.3.2 Fouling experiments

Fouling experiments were performed using a spinning disc apparatus (SDA), a detailed description of the SDA is given in Zhang et al. (2018). The temperature of the fouling surface was controlled by circulating ethylene glycol (Semi Grade, BDH Chemicals) at 95−115 ºC. The rotational speed of the spinning disc was adjusted to simulate different levels of fluid flow (hence shear stress) over the fouling surface with the Reynolds numbers (*Re*) from 10350 (50 rpm) to 31250 (150 rpm), which are in the laminar region of a swirl flow (*Re* < 45000) (Kobayashi, 1994). Since the shear stress exerted by the fluid flow on the fouling surface varies with the location, three heat flux sensors coupled with thermocouples were installed at the different locations of the disc, with distances from the disc center of 0.2, 1.7 and 2.8 cm, respectively, in order to better characterize the effect of shear stress on fouling behavior. These sensors measured local temperature and heat flux during the fouling test and recorded the data every 20 s. The overall heat transfer coefficient (U) was determined by the measured heat flux (q) and the temperature difference between heating medium (T_h) and bulk solution (T_b):

$$U = \frac{q}{T_h - T_b} \tag{1}$$

The fouling resistance (R_f) was calculated by the change in the overall heat transfer coefficient over time:

$$R_f = \frac{1}{U_t} - \frac{1}{U_c} \tag{2}$$

where U_t is the overall heat transfer coefficient at time t, U_c is the initial (*i.e.* clean disc surface) overall heat transfer coefficient. As the fouling curve (*i.e.* R_f-t data) featured a linear behavior at the early stage (Figure 1-2), the initial fouling rate was determined by fitting a linear trend line to the fouling curve at the early stage through regression. The end point was determined by inspecting the slope of two consecutive points to find where the slope deviated from the linear trend by more than 0.0001 m^2 K/W min.

2.3.3 Numerical simulation

As the key parameters in milk fouling, the temperature and flow field of the SDA were studied by computational fluid dynamics (CFD) simulation using COMSOL Multiphysics software 5.4 (COMSOL Inc., MA, USA) to calculate the distributions of temperature and shear stress over the fouling surface. The numerical model and boundary conditions used were based on Nigo et al. (2009) and Huang et al. (2012), which had the same geometry (can diameter and vessel volume) as Zhang et al. (2018), but different fluid viscosity (0.0012 Pa s), thermal conductivity (0.6 W/m K), and operating conditions (heating medium temperature: 95−115 ºC, disc rotational speed: 50−150 rpm). A detailed description of the simulation, including governing equations, mesh, and boundary conditions, is given in Supplementary Materials.

2.3.4 Deposit analysis

Proximate composition analysis of the fouling deposits was performed by A & L Greatlake Labotories (Fort Wayne, IN, USA). The content of total crude protein was determined following the AOAC 968.06 method, and calculated based on the standard conversion factor of 6.38. The contents of total fat and total ash were determined using the AACC 32-10 and AOAC 942.05 standards, respectively.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the protein distribution of the fouling deposits. Lyophilized deposit sample was dissolved in Tris-HCl buffer (pH 8.8) to a final concentration of approximately 2 mg/ml. The sample was then heated to 50 ºC and left for 1 h with vortex every 10 min before centrifugation at 15000 × g for 3 min. Twenty microliters of the supernatant were mixed with 2 × Laemmeli sample buffer (1 mg/ml). The insoluble fraction of the deposit was extracted using 2D zwitterionic buffer, consisting of

50 mM Tris-HCl (pH 8.8), 10 mM ethylenediaminetetraacetic acid (EDTA), 5 M urea, 2 M thiourea, 2% w/v 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) and 67 mM dithiothreitol (DTT), with sonication at 30 ºC for 40 min. The mixture was then centrifuged at 15000 × *g* for 3 min and 20 µl of the supernatant were mixed with 2 × Laemmeli sample buffer. The sample was loaded onto a 4−12% Bis-Tris, NuPAGE gradient gel at 200 V for 45 min. Novex Sharp pre-stained protein standard (Thermo Fisher Scientific, Walthan, MA, USA) was used as the protein marker.

Skyscan 1272 X-ray micro-CT (Bruker, Manning Park, MA, USA) was used to scan the 3D structure of the fouling deposits following the protocol of 4.5 µm isotropic pixel size, 50 kV, no filter and 4904 × 4904 × 6276 rec. volume. Image reconstruction was performed using the NRecon software (Micro Photonics Inc., Allentown, PA, USA) and the porosity of the deposit was analyzed using the CTAn software (Bruker, Manning Park, MA, USA).

2.3.5 Statistical analysis

All the fouling experiments were conducted in triplicate by randomized design. One-way analysis of variance (ANOVA) followed by Turkey's pairwise comparison was used to compare the means among different test conditions, with a significance level of 0.05. The statistical analysis was performed using SPSS® Statistics 26 (IBM, Armonk, NY, USA).

2.4 Results and Discussion

2.4.1 Shear stress and temperature distributions over fouling surface

The shear stress and temperature distributions over the disc surface where deposit grew were extracted from CFD simulations (Figure 2-1). The simulation results showed that the shear stress (Figure 2-1a) increased linearly ($R^2 > 0.997$) with the radial position, and reached the maximum values at the disc edge, ranging from 0.6 (50 rpm) to 3.14 Pa (150 rpm). These values were comparable to the shear stresses generated by milk in a typical industry heat exchanger, of 0.48−2.04 Pa for a milk viscosity of 0.0012 Pa s (Simmons et al., 2007), indicating that the SDA is a reliable tool to simulate the actual flow conditions which arise in milk pasteurization. The

surface temperature had a uniform distribution across the disc surface with the temperature difference less than 1 ºC (0.14%) (Figure 2-1b). Therefore, we attributed the variation in fouling behavior with the location mainly to the difference in shear but not temperature. The average surface temperatures corresponding to the three heating medium temperature tested were calculated to be 71.6, 74.8 and 78.3 ºC.

2.4.2 Effect of shear on camel milk fouling

The effect of shear stress on the fouling behavior of camel milk was evaluated by operating the SDA at three rotational speeds, 50, 100 and 150 rpm. Figure 2-2a shows three representative fouling curves obtained under different levels of shear, no induction period was observed in all the cases. The fouling resistance (R_f) exhibited an incipient linear growth period over the first 3−25 min, which lasted longer under lower shear. However, the shear had no significant effect on the linear fouling rate, which was approximately 0.001 m^2 K/W min. The fouling rates then decreased gradually throughout the rest of the 2-h test regardless of the shear stress. Figure 2-2b shows the final R_f at different shear stresses, which is closely related to the total mass of the fouling deposit formed (Huang et al., 2012). The fouling decreased with increasing shear stress, the decrease in the final R_f was more drastic at shear stress below 0.3 Pa, and became gradual as the shear increased. The effect of shear stress on camel milk fouling observed here was comparable to bovine milk fouling (Belmar-Beiny et al., 1993; Simmons et al., 2007). In the laminar flow region, where higher Reynolds number is associated with higher shear. Hence, the increase in the flow rate of liquid can cause decreased fouling because the deposit already formed can be removed at a higher rate by increased shear force (Changani et al., 1997; Kern, 1959).

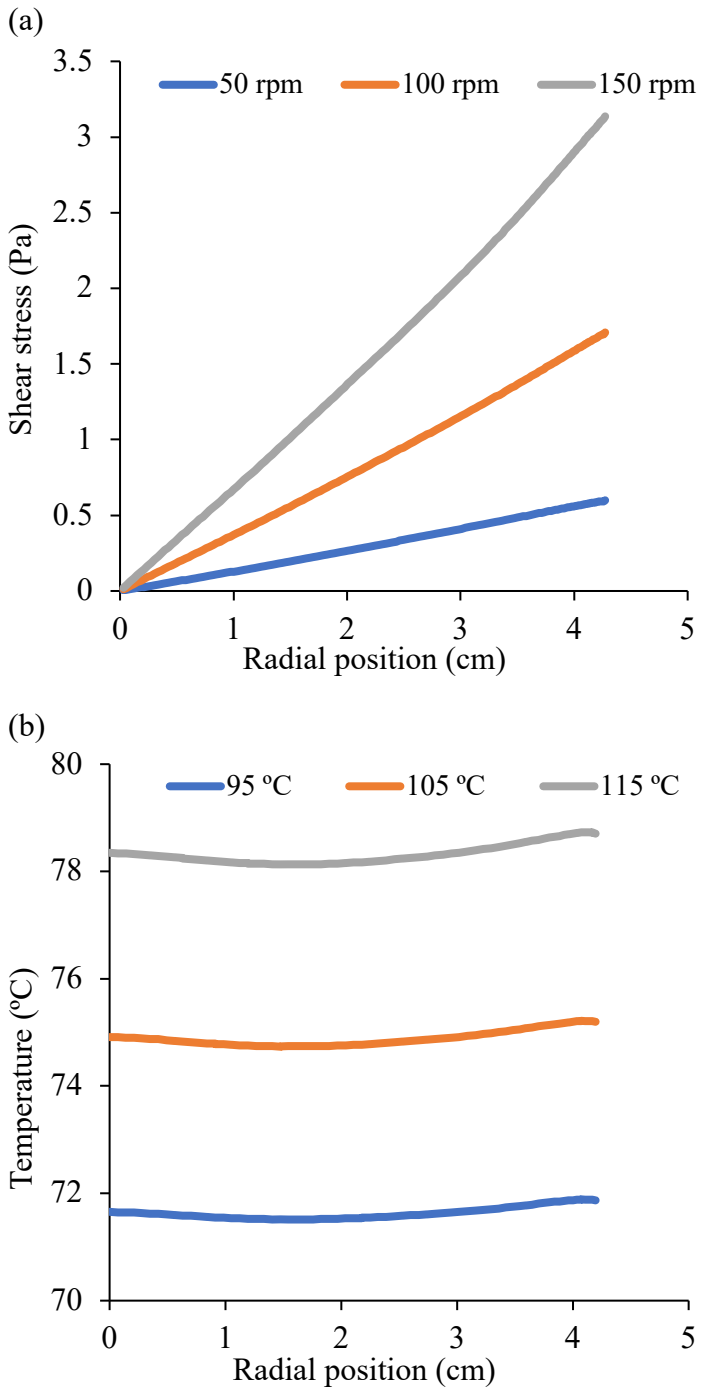

Figure 2-1 CFD simulations of (a) shear stress and (b) temperature distributions over the disc surface.

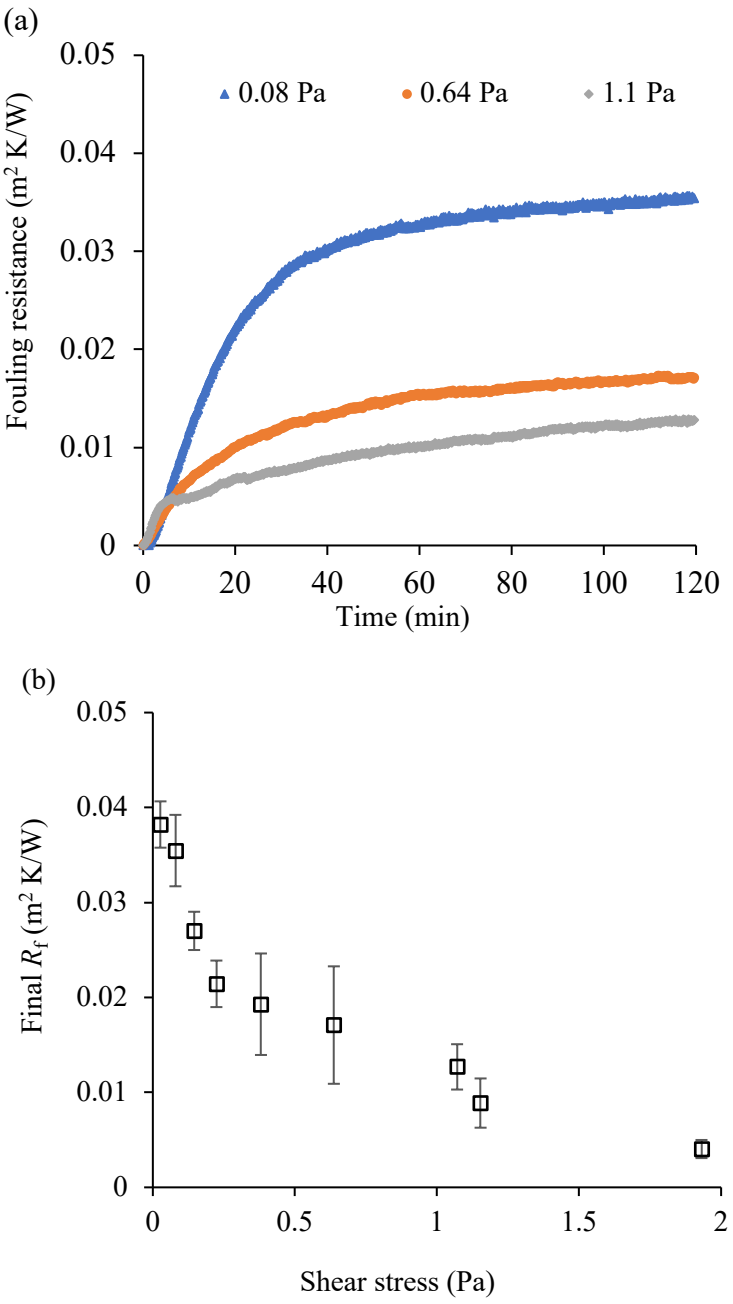

Figure 2-2 (a) Evolution of camel milk fouling under three representative (high, medium and low) shear stresses. (b) Effect of shear stress on final (2 h) fouling resistance (Rf). All the experiments were conducted at the initial (clean) surface temperature of 71.6 ºC. All the data is the average of three replicates, error bar refers to standard deviation.

2.4.3 Effect of temperature on camel milk fouling

2.4.3.1 Fouling characteristics

Milk fouling is known to be strongly affected by the temperature of heated surface (Bansal and Chen, 2006) because temperature is a key factor in the protein denaturation and aggregation kinetics which determine the fouling rate. Previous studies on bovine milk indicated a positive correlation between fouling deposition and whey protein denaturation (Blanpain-Avet et al., 2016; Blanpain-Avet et al., 2012; Petit et al., 2013). The effect of temperature on camel milk fouling behavior was examined in this study by controlling the heating medium temperature at 95, 105 and 115 ºC, which generated the surface temperatures of 71.6, 74.8 and 78.3 ºC, corresponding to the range of industrial pasteurization temperature. A significant increase in fouling was observed with increasing temperature at higher shear stresses because of the higher linear fouling rate (Table 1) and longer linear growth period (Figure 2-3). As shown in Table 2-1, increasing the surface temperature from 71.6 to 74.8 ºC increased the final R_f by 28% at 0.22 Pa and 67% at 0.38 Pa. The further temperature increase to 78.3 ºC increased the final R_f to a smaller extent, of 15% at 0.22 Pa and 19% at 0.38 Pa. In contrast, the final R_f had a larger variation at the shear stress of 0.03 Pa, so although the average value increased by 24% when increasing the surface temperature from 71.6 to 78.3 ºC, no significant difference can be statistically concluded. These fouling characteristics were similar to the findings of bovine milk studies that increase in surface temperature resulted in severe bovine milk fouling (Bansal and Chen, 2006; Rakes et al., 1986), because of accelerated protein denaturation. This is supported by the higher protein content observed in the camel milk fouling deposit formed at higher temperatures (Table 2-2), as described in the following section. However, if the temperature of test milk also increases because of the hotter surface, protein might tend to aggregate in liquid milk instead of depositing on the surface, which would thus result in reduced fouling formation (Petit et al., 2013; Rakes et al., 1986). This could explain the smaller increase in the final R_f observed when the surface temperature further increased from 74.8 to 78.3 ºC. Further investigation of the effect of protein denaturation and aggregation on camel milk fouling at elevated temperature is required. Moreover, it should be noted that the fouling growth decreased more markedly with the shear at 71.6 ºC (Figure 2-3), indicating that the deposits formed at lower temperatures were more susceptible to shear. Simmons

et al. (2007) also reported that deposits formed at higher surface temperatures had higher strength and hence more resistant to shear.

Table 2-1 (a) Fouling characteristics of camel milk at different temperatures and shear stresses, and (b) comparison with bovine milk

(a)

	Final fouling resistance ($\times 10^{-3}$ m^2 K/W)^2			Linear fouling rate ($\times 10^{-3}$ m^2 K/W min)^2		
τ (Pa)	0.03	0.22	0.38	0.03	0.22	0.38
Camel, 71.6 ºC[1]	37 ± 8^{a}	31 ± 4^{a}	22 ± 2^{a}	1.3 ± 5^{a}	1.0 ± 0.4^{a}	0.7 ± 0.5^{a}
Camel, 74.8 ºC[1]	43 ± 7^{a}	40 ± 4^{b}	37 ± 2 ^{b}	1.8 ± 0.4^{a}	1.6 ± 0.3^{ab}	1.4 ± 0.2^{ab}
Camel, 78.3 ºC[1]	46 ± 2^{a}	46 ± 2^{bc}	44 ± 3 ^{c}	2.2 ± 0.3^{a}	2.3 ± 0.6^{b}	1.8 ± 0.3^{b}

(b)

	Final fouling resistance ($\times 10^{-3}$ m^2 K/W)^2			Linear fouling rate ($\times 10^{-3}$ m^2 K/W min)^2		
τ (Pa)	0.03	0.22	0.38	0.03	0.22	0.38
Camel, 71.6 ºC[1]	37 ± 8^{a}	31 ± 4^{a}	22 ± 2^{a}	1.3 ± 5^{a}	1.0 ± 0.4^{a}	0.7 ± 0.5^{a}
Bovine, 71.6 ºC	81 ± 2^{b}	69 ± 2^{b}	48 ± 3^{b}	4.8 ± 0.6^{b}	4.3 ± 0.2^{b}	3.2 ± 0.1^{b}

[1]Initial (clean) surface temperature
[2]All the values represent the mean of triplicate. Values with different letters in the same column were significantly different ($p < 0.05$).

Figure 2-3. Evolution of camel milk fouling at different temperatures and shear stresses: (a) τ = 0.03 Pa; (b) τ = 0.22 Pa; (c) τ = 0.38 Pa.

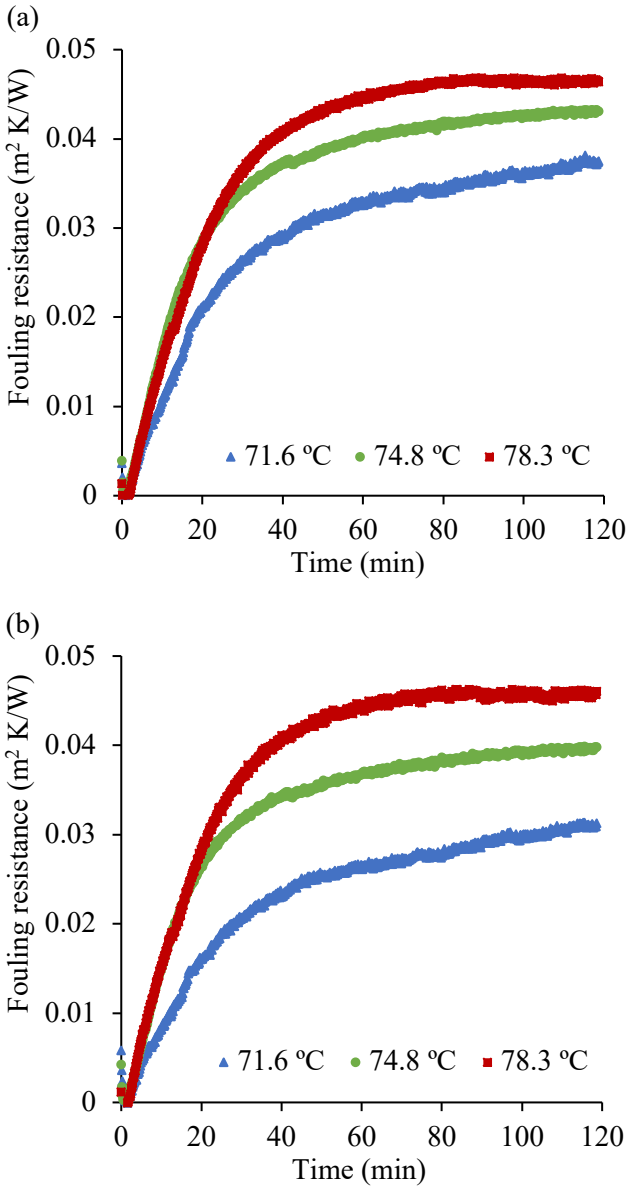

Figure 2-3 continued

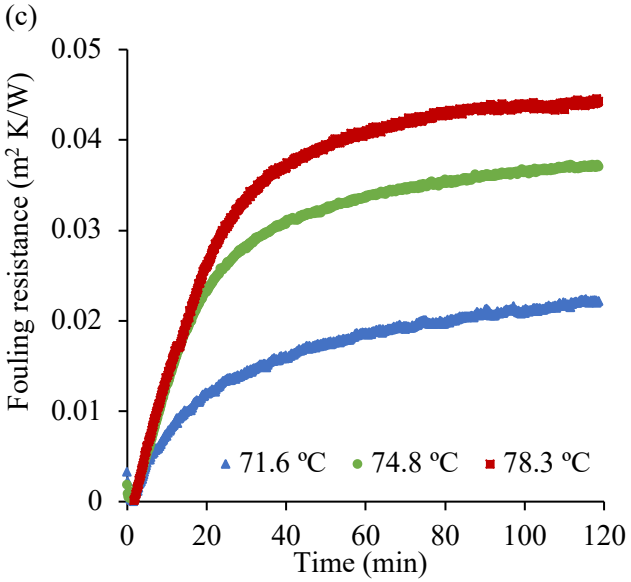

Table 2-2 Mass and composition of camel and bovine milk fouling deposits after 2-h test

	Total mass (dry)[2]	Protein (% DB)[3]	Fat (% DB)[3]	Ash (% DB)
Camel, 71.6 ºC[1]	1.06 ± 0.24[a]	35.10 ± 5.11[a]	61.87 ± 7.25[ac]	3.03 ± 1.29
Camel, 74.8 ºC[1]	1.16 ± 0.17[a]	36.83 ± 1.87[a]	59.54 ± 4.00[ab]	3.64 ± 0.21
Camel, 78.3 ºC[1]	1.24 ± 0.29[a]	43.48 ± 3.54[a]	52.18 ± 7.54[ac]	4.34 ± 0.32
Bovine, 71.6 ºC[1]	0.96 ± 0.14[a]	34.55 ± 3.97	61.96 ± 1.83	3.49 ± 0.30

[1]Initial (clean) surface temperature
[2]Dried by lyophilization, mean of three to five replicates.
[3]Mean of triplicate with standard deviation, except the ash content and bovine milk samples were duplicates due to a limitation of deposits. Values with different letters in the same column were significantly different ($p < 0.05$). Values without standard deviation were the mean of duplicate due to limited amounts of deposits collected for analyses.

2.4.3.2 Physicochemical properties of camel milk fouling deposit

Table 2-2 shows the mass of the camel milk fouling deposit after the 2-h fouling experiment, which increased as surface temperature increased, agreeing with the results of final fouling resistance (Table 2-1). The composition analysis showed that the camel milk fouling deposits were composed mainly of fat (52−62%), protein (35−43%) and ash (3−4%). Slight increases in the protein and ash contents and a decrease in the fat content with increasing surface temperature were observed.

Bennett (2007) reported a similar behavior of bovine milk fouling that deposits formed at higher surface temperature had higher protein content and lower fat content. It is worth noting that although fat has been considered as an insignificant component (< 8%) in milk fouling deposits (Bansal and Chen, 2006; Visser and Jeurnink, 1997b), some studies reported that deposit formed by whole milk had a high fat content, of 40−50% (Calvo and de Rafael, 2009; Fang, 1998; Johnson and Roland, 1940). Fang (1998) reported that when the membrane of milk fat globule is damaged by mechanical stress (*e.g.* shear, turbulence, cavitation), the damaged fat globules tend to coalesce and form larger globules, which could migrate more easily to the heated surface and get entrapped in the matrix of fouled protein.

All the camel milk fouling deposits showed low water solubilities ranging from 10 to 12% based on the BCA essay. The protein distributions of camel milk and its fouling deposits (including both water-soluble and insoluble components) were characterized by SDS-PAGE analysis. Lactoferrin (LF), serum albumin (SA), α-, β- and κ-caseins (CN), α-lactalbumin (α-la) and peptidoglycan recognition protein (PGRP) were the most abundant proteins in camel milk while β-lactoglobulin (β-lg) was not present, as shown in Figure 2-4. This profile aligned well with other studies on camel milk protein (Felfoul et al., 2015b; Genene et al., 2019; Konuspayeva et al., 2009; Omar et al., 2016). Upon heating to 67 ºC, the camel milk protein showed degradation at two unidentified bands of around 50 and 160 kDa. All of the major camel milk proteins (*i.e.* SA, α, β, κ-CN, α-la and PGRP) were also present in the fouling deposit. These results agree with the findings of Felfoul et al., (2015b) that camel showed significantly decreased intensity of α-la, SA and CN bands after heat treatment, indicating their participation in fouling formation. While α-la, PGRP and SA were more concentrated in the water-soluble fraction of the deposit, α-, β-, and κ-CN were the dominant proteins in the water-insoluble fraction, implying that camel casein protein becomes more difficult to dissolve in water after heat-induced aggregation. The CN content of the deposit increased with heating temperature, suggesting that higher temperature can facilitate CN aggregation and deposition.

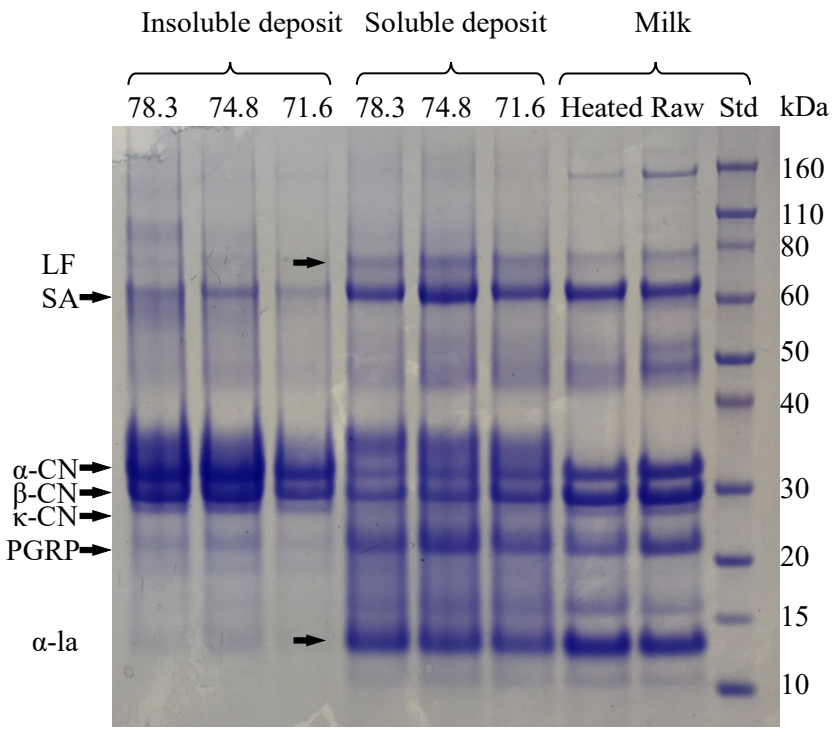

Figure 2-4 SDS-PAGE profiles of camel milk fouling deposits formed at different surface temperatures under 0.22 Pa, and heated and raw camel milk. Std, stand protein marker; LF, lactoferrin; SA, serum albumin; CN, casein; la, lactalbumin.

2.4.4 Comparison between camel and bovine milk fouling

Fouling of reconstituted lyophilized camel milk and raw bovine milk were tested and compared at surface temperature of 71.6 ºC under three levels of shear stress of 0.03, 0.22 and 0.38 Pa. The R_f-t curves of camel and bovine milk showed similar patterns, starting with a linear growth which was followed by a falling rate period before reaching the maximum fouling resistance at the end of the 2-h test (Figure 2-5). Under the same shear, the final fouling resistance and initial fouling rate of the bovine milk were approximately 1.2 and 3 times higher, respectively, than those of camel milk. However, no significant difference was observed between the masses of their dried fouling deposits (Table 2-2). The fouling resistance was estimated based on the change in the heat flux across the wet deposit (*i.e.* containing protein aggregate and entrained liquid milk), which is a function of the thermal conductivity of deposit. However, there was a difficulty in measuring the thermal conductivity of the fouling deposit in its original state (*i.e.* freshly formed on the heated surface). To address the difference in the final fouling resistance, the chemical compositions of the camel and bovine milk, as well as their fouling deposits, were analyzed. Table 2-2 shows that the

camel milk and bovine milk used in this study had a similar protein content of 28 ± 1%, but the camel milk contained more ash (7.52%) and less fat (23.5%) than the bovine milk (4.68% and 30.5%, respectively). However, there was no significant difference between the compositions of their fouling deposits. Therefore, the physical structures of the dry deposits were further analyzed using the micro- CT. The results (Figure 2-6) revealed a higher porosity of the camel milk deposit (76%) compared with the bovine milk deposit (55%). Since liquid milk has a higher thermal conductivity (0.99 W/m K) than its fouling deposit (0.5 W/m K) (Mahdi et al., 2009), deposit with higher porosity can entrap more liquid milk and thus has lower thermal resistance.

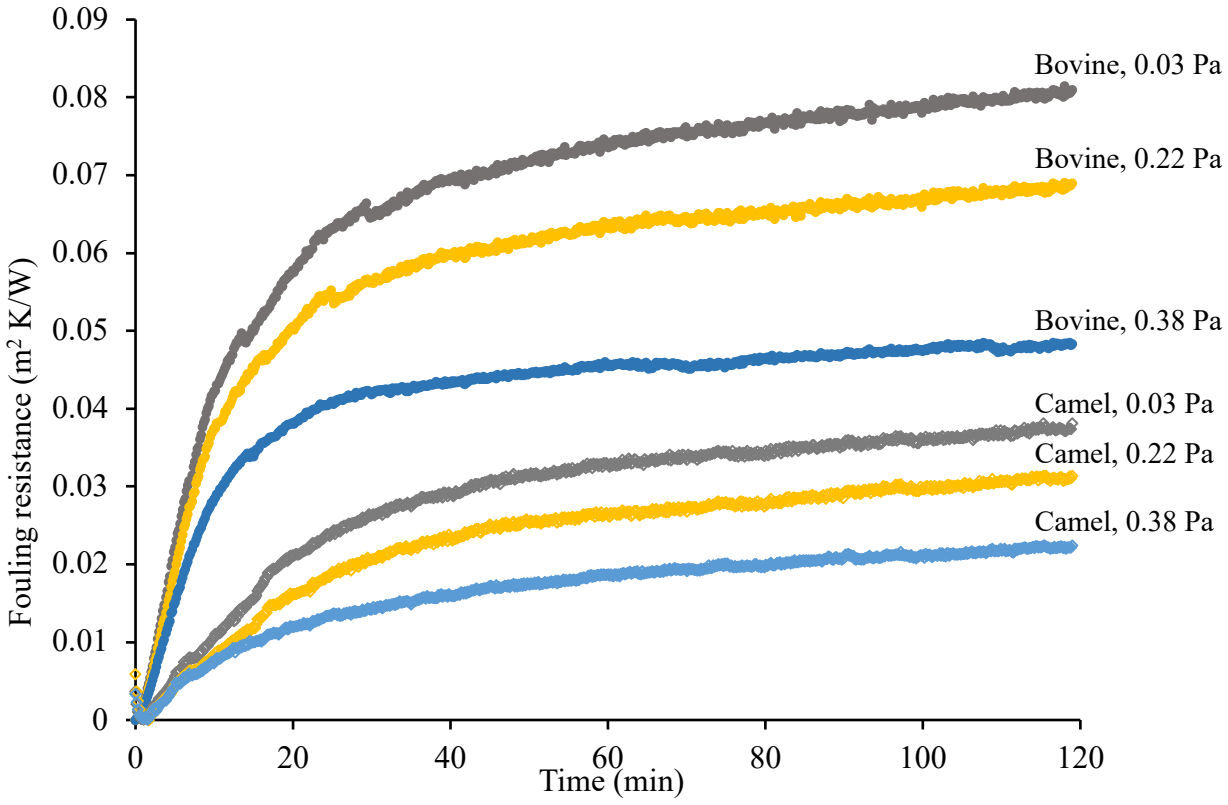

Figure 2-5 Comparison between fouling curves of camel and bovine milk at surface temperature of 71.6 ºC under different shear stresses: 0.03, 0.22 and 0.38 Pa. Each data point is the mean of triplicate.

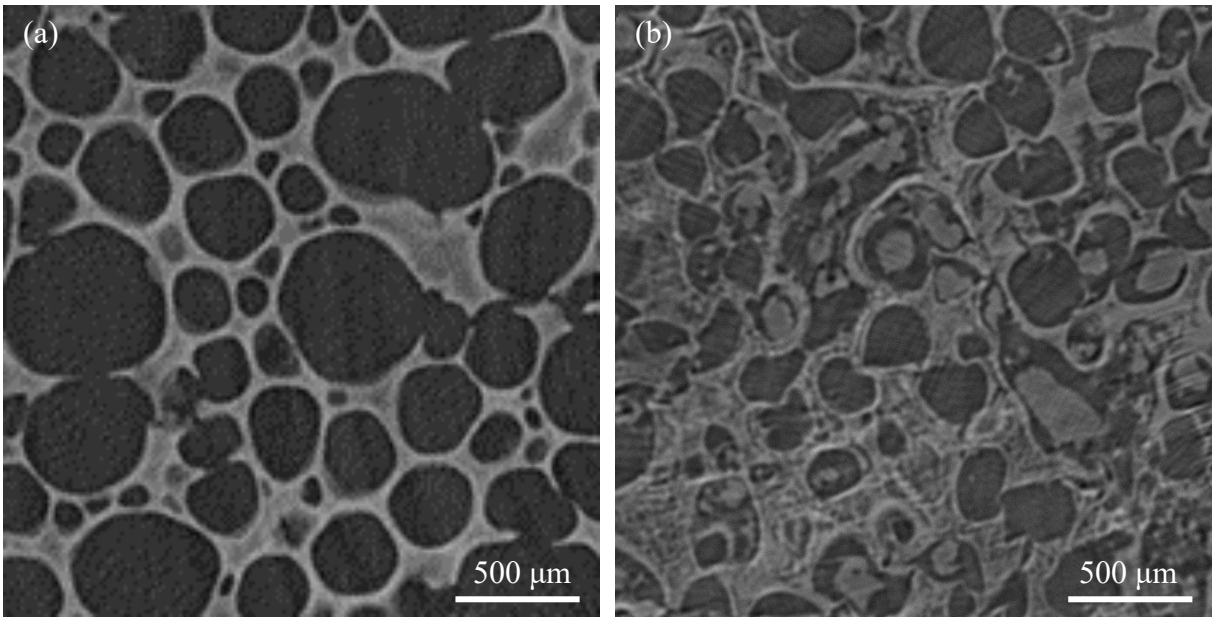

Figure 2-6 X-ray micro-CT images of cross sections of (a) camel and (b) bovine milk fouling deposits formed at 71.6 ºC under 0.22 Pa.

The protein profiles of bovine milk and its fouling deposits were also analyzed for comparison with camel milk. In contrast to camel milk that heat-induced protein degradation was observed at 50 and 160 kDa, heating bovine milk to 67 ºC did not appear to alternate its protein distribution, suggesting that camel milk protein was less thermally stable than bovine milk. This could be supported by the DSC analysis conducted by Felfoul et al. (2015a) that camel milk had an endotherm peak at a lower temperature of 44.8 ºC compared with 81.7 ºC for bovine milk. As shown in Figure 2-7, β-lg and CN were the major proteins in the water-soluble fraction of the bovine milk deposit, and the water-insoluble fraction contained mostly CN. PGRP was not observed in the bovine milk deposit. The SA contents of the camel milk and its deposit were substantially higher than those of both bovine milk samples. Similar compositions of camel milk were also reported by Kappeler (1998) and Omar et al. (2016). Omar et al. (2016) found that bovine milk had a higher κ-CN content than α- and β-CN, however, κ-CN accounted for the smallest amount in the camel milk protein. The camel milk deposit contained no β-lg, its most abundant water-soluble proteins were α-la, PGRP and SA, and its water-insoluble fraction mainly consisted of CN. This agreed with the previous finding that casein micelles can destabilize and attach to the fouling layer (Bennett, 2007; Visser and Jeurnink, 1997b).

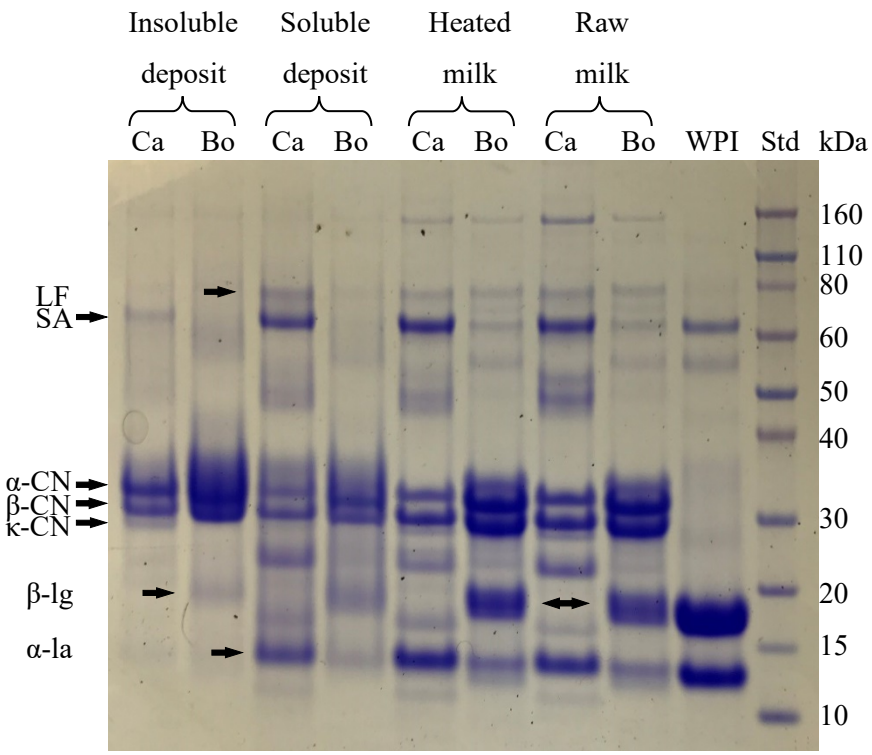

Figure 2-7 SDS-PAGE profiles of camel and bovine milk and their fouling deposits formed at 71.6 ºC under 0.22 Pa. Ca, camel; Bo, bovine; WPI, whey protein isolate; Std, stand protein marker; LF, lactoferrin; SA, serum albumin; α-, β-, κ-CN, α, β, κ-casein; α-la, α-lactalbumin; β-lg, β-lactoglobulin.

In bovine milk fouling, β-lg starts to unfold and denature when being heated, exposing the reactive thiol group (-SH; Cys121) that can interact with β-lg or other milk proteins such as CN and α-la to form aggregates and deposits (Changani et al., 1997; Oldfield et al., 2005; Visser and Jeurnink, 1997a). Like β-lg, SA has one free -SH group (Cys34) (Go et al., 2011) which can provide reactive -SH to induce intramolecular disulfide bond exchange reactions with other protein molecules (Huggins et al., 1951; Nikkel and Foster, 1971) and form large aggregates (Kelly and Zydney, 1994). Compared to bovine milk, camel milk has a significantly higher SA content (Figure 2-7), hence more free thiol groups are available to induce camel milk fouling. α-la is another major camel whey protein. Unlike β-lg and SA, free thiol is not present in native camel whey protein (McGuffey et al., 2005), but camel α-la can form free thiol during heat treatment (Felfoul et al., 2015b; Felfoul, et al., 2017; Lajnaf et al., 2017). The free thiol group in camel α-la can interact with SA, α-la and CN (Havea et al., 2000), leading to the formation of intermolecular disulfide-

bonded aggregates (Genene, et al. 2019; McGuffey et al., 2005). These mechanisms could explain the roles α-la, SA and CN play in camel milk fouling formation.

Since β-lg is generally considered as the dominant protein in milk fouling (Rosmaninho and Melo, 2008; Visser and Jeurnink, 1997a) and CN is a relatively heat-stable protein (Bansal and Chen, 2006), most of previous studies on dairy fouling have focused on whey protein and minerals. However, as shown in Figure 2-7, it is important to note that CN in fact accounted for a significant portion of the proteins in both camel and bovine milk deposits, hence the participation of CN in milk fouling should not be neglected. Therefore, further studies on the effect of CN on milk fouling are needed to help more comprehensively understand milk fouling mechanisms. Moreover, the differences in protein composition observed are associated with the significantly different fouling characteristics of camel milk from bovine milk (*i.e.* fouling resistance and fouling rate) as each protein has distinct physicochemical properties, resulting in the formation of deposits with different microscopic network structures. The interactions among SA, α-la and CN during fouling formation and their effects on deposit structure are still unclear, which need to be further studied in future work.

2.5 Conclusions

In this study, the fouling behavior of raw camel milk under well-controlled surface temperature and flow was investigated, both processing parameters were found to have significant effects. Pasteurization at lower temperature with higher shear stress exerted by flowing milk resulted in reduced fouling, which is thus recommended for industrial operations. This is the first study, to the authors' best knowledge, characterizing the protein profile of camel milk fouling deposit through SDS-PAGE analysis. Although camel milk deposit had a similar chemical composition to bovine milk, their protein profiles were very different. While β-lactoglobulin, the major component of bovine milk protein as well as the key factor in bovine milk fouling, was absent in camel milk, casein, α-lactalbumin, serum albumin and peptidoglycan recognition protein, on the other hand, were found to be responsible for camel milk fouling. Furthermore, camel milk formed more porous deposits compared to bovine milk, resulting in a lower fouling resistance. The knowledge obtained from this work could lead further investigations of denaturation and interaction of different camel

milk proteins, particularly serum albumin, lactalbumin and caseins, at elevated temperatures, in order to better understand the fouling mechanisms of camel milk for mitigation purposes.

2.6 References

Agrawal, R.P., Jain, S., Shah, S., Chopra, A., Agarwal, V., (2011). Effect of camel milk on glycemic control and insulin requirement in patients with type 1 diabetes: 2-years randomized controlled trial. European Journal of Clinical Nutrition 65(9), 1048-1052.

AL-Ayadhi, L.Y., Elamin, N.E., (2013). Camel Milk as a Potential Therapy as an Antioxidant in Autism Spectrum Disorder (ASD). Evidence-Based Complementary and Alternative Medicine 2013, 8.

Al haj, O.A., Al Kanhal, H.A., (2010). Compositional, technological and nutritional aspects of dromedary camel milk. International Dairy Journal 20(12), 811-821.

Alhaider, A.A., Abdel Gader, A.G.M., Almeshaal, N., Saraswati, S., (2014). Camel milk inhibits inflammatory angiogenesis via downregulation of proangiogenic and proinflammatory cytokines in mice. APMIS 122(7), 599-607.

Bansal, B., Chen, X.D., (2006). A Critical Review of Milk Fouling in Heat Exchangers. Comprehensive Reviews in Food Science and Food Safety 5(2), 27-33.

Belmar-Beiny, M., Gotham, S., Paterson, W., Fryer, P., Pritchard, A., (1993). The effect of Reynolds number and fluid temperature in whey protein fouling. Journal of Food Engineering 19(2), 119-139.

Bennett, H.A.E., (2007). Aspects of fouling in dairy processing : a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Engineering at Massey University, Palmerston North, New Zealand. Massey University.

Blanpain-Avet, P., André, C., Khaldi, M., Bouvier, L., Petit, J., Six, T., Jeantet, R., Croguennec, T., Delaplace, G., (2016). Predicting the distribution of whey protein fouling in a plate heat exchanger using the kinetic parameters of the thermal denaturation reaction of β-lactoglobulin and the bulk temperature profiles. Journal of Dairy Science 99(12), 9611-9630.

Blanpain-Avet, P., Hédoux, A., Guinet, Y., Paccou, L., Petit, J., Six, T., Delaplace, G., (2012). Analysis by Raman spectroscopy of the conformational structure of whey proteins constituting fouling deposits during the processing in a heat exchanger. Journal of Food Engineering 110(1), 86-94.

Burton, H., (1968). Section G. Deposits from whole milk in heat treatment plant—a review and discussion. J. Dairy Res. 35(02), 317-330.

Calvo, M.M., de Rafael, D., (2009). Deposit formation in a heat exchanger during pasteurization of CO2-acidified milk. Journal of Dairy Research 62(4), 641-644.

Changani, S.D., Belmar-Beiny, M.T., Fryer, P.J., (1997). Engineering and chemical factors associated with fouling and cleaning in milk processing. Exp. Therm Fluid Sci. 14(4), 392-406.

de Jong, P., te Giffel, M.C., Straatsma, H., Vissers, M.M.M., (2002). Reduction of fouling and contamination by predictive kinetic models. International Dairy Journal 12(2–3), 285-292.

Fang, L., (1998). The effect of milk fat globule membrane damage in the absence of air on fouling in heat exchangers : a thesis presented in partial fulfilment of the requirements for the degree of Master of Technology in Food Technology of Massey University. Massey University.

Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., Ayadi, M.A., (2015a). Fouling Behavior of Camel and Cow Milks Under Different Heat Treatments. Food and Bioprocess Technology 8(8), 1771-1778.
Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., Ayadi, M.A., (2015b). A laboratory investigation of cow and camel whey proteins deposition under different heat treatments. Food and Bioproducts Processing 96, 256-263.
Felfoul, I., Beaucher, E., Cauty, C., Attia, H., Gaucheron, F., & Ayadi, M. A. (2016). Deposit Generation During Camel and Cow Milk Heating: Microstructure and Chemical Composition. Food and Bioprocess Technology, 1-8.
Felfoul, I., Jardin, J., Gaucheron, F., Attia, H., & Ayadi, M. A. (2017). Proteomic profiling of camel and cow milk proteins under heat treatment. Food Chemistry, 216, 161-169.
Genene, A. (2019). Effect of heat treatment on denaturation of whey protein and resultant rennetability of camel milk. Lebensmittel-Wissenschaft + Technologie, 101, (2101) 404-409.
Go, E.P., Zhang, Y., Menon, S., Desaire, H., (2011). Analysis of the Disulfide Bond Arrangement of the HIV-1 Envelope Protein CON-S gp140 ΔCFI Shows Variability in the V1 and V2 Regions. Journal of Proteome Research 10(2), 578-591.
Habib, H.M., Ibrahim, W.H., Schneider-Stock, R., Hassan, H.M., (2013). Camel milk lactoferrin reduces the proliferation of colorectal cancer cells and exerts antioxidant and DNA damage inhibitory activities. Food Chemistry 141(1), 148-152.
Hailu, Y., Hansen, E.B., Seifu, E., Eshetu, M., Ipsen, R., Kappeler, S., (2016). Functional and technological properties of camel milk proteins: a review. Journal of Dairy Research 83(4), 422-429.
Havea, P., Singh, H., Creamer, L.K., (2000). Formation of New Protein Structures in Heated Mixtures of BSA and α-Lactalbumin. Journal of Agricultural and Food Chemistry 48(5), 1548-1556.
Huang, J.-Y., John Chew, Y.M., Ian Wilson, D., (2012). A spinning disc study of fouling of cold heat transfer surfaces by gel formation from model food fat solutions. Journal of Food Engineering 109(1), 49-61.
Huggins, C., Tapley, D.F., Jensen, E.V., (1951). Sulphydryl-disulphide relationships in the induction of gels in proteins by urea. Nature 167(4250), 592-593.
Johnson, J.J., Roland, C.T., (1940). Study of Dairy Cleaning Problems I. Films and Deposits on Hot-Milk Equipment. Journal of Dairy Science 23(5), 457-461.
Kappeler, S., (1998). Compositional and structural analysis of camel milk proteins with emphasis on protective proteins. ETH Zürich.
Kelly, S.T., Zydney, A.L., (1994). Effects of intermolecular thiol–disulfide interchange reactions on bsa fouling during microfiltration. Biotechnology and Bioengineering 44(8), 972-982.
Kern, D.S., RE, (1959). A theoretical analysis of thermal surface fouling. British Chemical Engineering scale 1959 modelling analysis fouling(5), 258--262.
Khalesi, M., Salami, M., Moslehishad, M., Winterburn, J., Moosavi-Movahedi, A.A., (2017). Biomolecular content of camel milk: A traditional superfood towards future healthcare industry. Trends in Food Science & Technology 62, 49-58.
Kobayashi, R., (1994). Review: Laminar-to-Turbulent Transition of Three-Dimensional Boundary Layers on Rotating Bodies. Journal of Fluids Engineering 116(2), 200-211.
Konuspayeva, G., Faye, B., Loiseau, G., (2009). The composition of camel milk: A meta-analysis of the literature data. Journal of Food Composition and Analysis 22(2), 95-101.

Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., Ayadi, M.A., (2017). The effect of pH and heat treatments on the foaming properties of purified α-lactalbumin from camel milk. Colloids and Surfaces B: Biointerfaces 156, 55-61.

Simmons, M.J.H., Jayaraman, P. Fryer, P.J., (2007). The effect of temperature and shear rate upon the aggregation of whey protein and its implications for milk fouling. Journal of Food Engineering 79(2), 517-528.

Mahdi, Y., Mouheb, A., Oufer, L., (2009). A dynamic model for milk fouling in a plate heat exchanger. Applied Mathematical Modelling 33(2), 648-662.

McGuffey, M.K., Epting, K.L., Kelly, R.M., Foegeding, E.A., (2005). Denaturation and Aggregation of Three α-Lactalbumin Preparations at Neutral pH. Journal of Agricultural and Food Chemistry 53(8), 3182-3190.

Nikkel, H.J., Foster, J.F., (1971). Reversible sulfhydryl-catalyzed structural alteration of bovine mercaptalbumin. Biochemistry 10(24), 4479-4486.

Oldfield, D.J., Singh, H., Taylor, M.W., (2005). Kinetics of heat-induced whey protein denaturation and aggregation in skim milks with adjusted whey protein concentration. Journal of Dairy Research 72(3), 369-378.

Omar, A., Harbourne, N., Oruna-Concha, M.J., (2016). Quantification of major camel milk proteins by capillary electrophoresis. International Dairy Journal 58, 31-35.

Petit, J., Herbig, A.L., Moreau, A., Delaplace, G., (2011). Influence of calcium on β-lactoglobulin denaturation kinetics: Implications in unfolding and aggregation mechanisms. Journal of Dairy Science 94(12), 5794-5810.

Petit, J., Six, T., Moreau, A., Ronse, G., Delaplace, G., (2013). β-lactoglobulin denaturation, aggregation, and fouling in a plate heat exchanger: Pilot-scale experiments and dimensional analysis. Chemical Engineering Science 101, 432-450.

Rakes, P.A., Swartzel, K.R., Jones, V.A., (1986). Deposition of Dairy Protein-Containing Fluids on Heat Exchange Surfaces. Biotechnology Progress 2(4), 210-217.

Rosmaninho, R., Melo, L.F., (2008). Protein–calcium phosphate interactions in fouling of modified stainless-steel surfaces by simulated milk. International Dairy Journal 18(1), 72-80.

Sadeghinezhad, E., Kazi, S.N., Dahari, M., Safaei, M.R., Sadri, R., Badarudin, A., (2015). A Comprehensive Review of Milk Fouling on Heated Surfaces. Crit. Rev. Food Sci. Nutr. 55(12), 1724-1743.

Simmons, M.J.H., Jayaraman, P., Fryer, P.J., (2007). The effect of temperature and shear rate upon the aggregation of whey protein and its implications for milk fouling. J. Food Eng. 79(2), 517-528.

Singh, R., Mal, G., Kumar, D., Patil, N.V., Pathak, K.M.L., (2017). Camel Milk: An Important Natural Adjuvant. Agricultural Research 6(4), 327-340.

Tuoc, T.K., (2015). Chapter 20 - Fouling in Dairy Processes, in: Amjad, Z., Demadis, K.D. (Eds.), Mineral Scales and Deposits. Elsevier, Amsterdam, pp. 533-556.

Visser, J., Jeurnink, T.J.M., (1997a). Fouling of heat exchangers in the dairy industry. Exp. Therm Fluid Sci. 14(4), 407-424.

Visser, J., Jeurnink, T.J.M., (1997b). Fouling of heat exchangers in the dairy industry. Experimental Thermal and Fluid Science 14(4), 407-424.

Zhang, B.Y., Lu, J., Huang, J.-Y., (2018). Effect of sugar on the fouling behavior of whey protein. Food and Bioproducts Processing.

Zibaee, S., Hosseini, S.M.A.-R., Yousefi, M., Taghipour, A., Kiani, M.A., Noras, M.R., (2015). Nutritional and Therapeutic Characteristics of Camel Milk in Children: A Systematic Review. Electronic physician 7(7), 1523-1528.

CHAPTER 3. EFFECT OF SUGAR ON THE FOULING BEHAVIOR OF WHEY PROTEIN*

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3.1 Abstract

Fouling is a serious problem in food processing especially in dairy industry. Sugar as a sweetener is very commonly added in various dairy products, however, severe fouling is found in on-site operations when processing sweetened dairy products. While milk fouling has been studied extensively, the compositional effect of sugar in dairy products on their fouling is still elusive. Here, we investigated the effect of different sugars (glucose, fructose and sucrose) on the fouling behavior of whey protein. Fouling formations were conducted using model solutions consisting of whey protein isolate (WPI) and sugars in a spinning disc apparatus under well-controlled temperatures and shear stresses. We found that WPI fouling was reduced for the solutions with 10 wt% of sugar added by more than 30% in terms of fouling resistance and deposit mass, regardless of the type of sugar. We postulate that this reduction was because of the stabilizing effect of sugar on WPI, which was confirmed experimentally by the increased denaturation and aggregation temperatures of whey protein in the sugar-added WPI solutions. The addition of sugar in WPI solution also affected the composition and morphology of the deposits, which had lower protein in content and higher porosity in structure, making them less resistant to shear compared to WPI deposits.

Keywords: Sweetened dairy products; Fouling; Whey Protein; Sugar; Thermal stability; Sloughing

3.2 Introduction

Sweetened dairy products such as ice cream, yogurt and protein drinks play an important role in the market nowadays. Sugars added in dairy products serve as sweetener, flavor and color precursor through browning, substrate for fermentation, stabilizer and emulsifier (Voragen, 1998). Similar to milks, all sweetened dairy products need to be thermally processed to ensure their safety and quality. However, more severe fouling has been observed during their pasteurization in dairy processing plants according to authors' communication with several dairy producers at Indiana Milk Quality Conference on April 12, 2016.

Fouling on heat transfer surfaces affects the efficiency of thermal processes by increasing energy consumption, as well as water and chemical uses for intensified cleaning needs. As a consequence, not only the operation costs but also environmental burdens of processing plants are increased (Muller-Steinhagen et al., 2009). Fouling remains a severe issue in food and bioproduct industries, and was estimated to cost 0.25% ($14.2 billion) of the U.S. gross domestic product (Awad, 2011). In dairy processing particularly, fouling can additionally influence the product quality, and thus thermal processing equipment needs to be cleaned on a daily basis (Bansal and Chen, 2006). Dairy fouling has been studied extensively throughout past decades with a major focus on milk fouling, and many factors have been reported to affect its behavior, such as composition, pH, temperature, and flow conditions (Bansal and Chen, 2006; Jimenez et al., 2013; Visser and Jeurnink, 1997). While whey protein is the major component contributing to milk fouling (Bansal and Chen, 2006), calcium phosphate also plays an important role especially under higher temperature conditions (*i.e.* above 110 ºC) (Rosmaninho and Melo, 2006). Lactose in milk was considered not involved in fouling formation, and fat was regarded to be not a significant factor in milk fouling (Visser and Jeurnink, 1997).

Only few studies, however, have investigated the interaction between protein and sugar under thermal treatment. In the studies on the thermal stabilities of whey protein, sugars showed a stabilization effect on its aggregation (Arakawa and Timasheff, 1982; Rich and Foegeding, 2000; Wijayanti et al., 2014). Goode et al. (2013) reported that whey deposits formed with added sugar exhibited a different adhesive strength on surfaces. Chocolate milk reconstituted by adding sugar and cocoa powder was found to form a thicker fouling layer with higher adhesive strength

compared to whole milk (Huang and Goddard, 2015). However, to the best of our knowledge, there is no systematic study on decoupling the effect of sugar on the fouling behavior of dairy products from their other complex ingredients.

In this study, the fouling behavior of sweetened milk was investigated using model solutions comprising whey protein and glucose, fructose or sucrose, which are the most common monosaccharides and disaccharide found in sweetened dairy products. A lab scale spinning disc apparatus (SDA) was used to conduct fouling experiments under well controlled temperature and shear. The mass, composition and morphology of obtained deposits were analyzed. The effect of sugar on the thermal stability of whey protein was examined by differential scanning calorimetry (DSC) and rheometer.

3.3 Materials and Methods

3.3.1 Model solutions

Whey protein isolate (WPI) containing 90% protein (BiPro, MN, USA) was dissolved in RO water to a concentration of 5 wt% as model milk in this study. The WPI was stirred for 90 min at room temperature then stored overnight at 4 ºC for complete hydration. D-glucose, D-fructose and sucrose (ACS grade, Fisher Chemical, MA, USA) were added, respectively, to WPI solution to a final concentration of 10 wt% as model sweetened milks. The pH of all model solutions remained between 6.75 and 6.95 during all the fouling experiments.

3.3.2 Fouling apparatus

The spinning disc apparatus (SDA) (Huang et al., 2012; Huang et al., 2013; Nigo et al., 2009) was used with some modifications to perform fouling tests. A schematic of the SDA configuration is shown in Figure 3-1*a*. The principal component is a motor-driven cylinder that is located centrally within a 1 L jacketed vessel, the base of cylinder is immersed in the sample solution. A circulating water/glycol bath is used to control the temperature of the disc. The temperature of the test solution in the vessel is maintained by the water jacket fed by a second circulator. Three thin-foil heat flux

sensors containing thermocouple (FRM-090-T, Wuntronic, Germany) are imbedded inside the 316 stainless steel (SS) disc (Figure 3-1*b*) to measure the local heat flux and temperature.

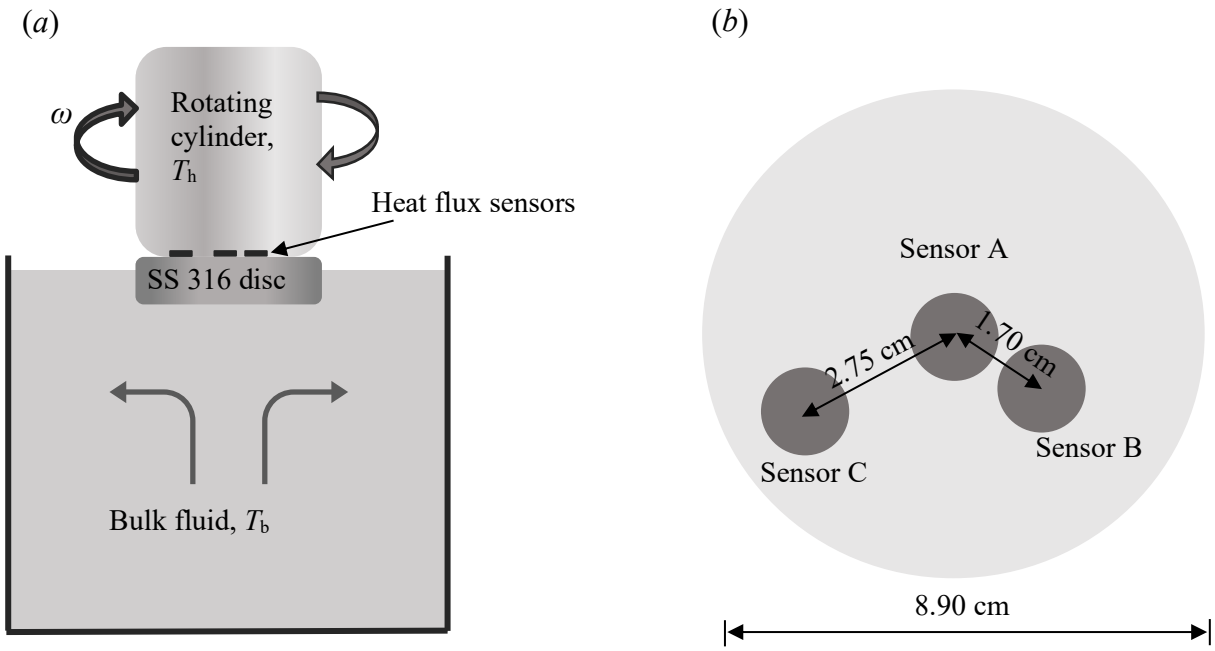


Figure 3-1 Schematics of the (*a*) SDA operation, and (*b*) position of heat flux sensors (A, B and C) in the fouling disc (not to scale).

3.3.3 Fouling experiments

Before each fouling test, the model solution was preheated to 58±1 ºC at which no denaturation and aggregation of whey protein occurred (Erabit et al., 2014; Nicolai et al., 2011). The fouling disc was heated by the circulating heating medium (*i.e.* water/glycol) with temperature (T_h) set at 95 ºC. After fouling experiment started, the temperature of the bulk solution (T_b) soon reached an equilibrium at 68±1 ºC and remained constant throughout the test. The flow of the bulk solution was controlled by the disc rotational speed (ω), which was set at 5.2 rad/s, providing a laminar flow condition ($Re \approx 5000$). All the fouling tests were conducted for a period of 2 h. Immediately after the 2 h test, the can temperature was quickly decreased to below 40 ºC. The deposits on the disc surface were rinsed with RO water to remove any bulk liquid attached, and then carefully removed using a plastic spatula and freeze dried for mass measurement and further analyses. A randomized complete block design was employed to evaluate the effect of adding three different sugars. All the experiments described were carried out in triplicate.

In this study, the deposit growth was quantified by its thermal resistance, R_f. The R_f was determined by the change in overall heat transfer coefficient (U) over time:

$$R_f = \frac{1}{U_t} - \frac{1}{U_c} \quad (1)$$

where U_t is the overall heat transfer coefficient at time t, U_c is the initial (*i.e.* clean disc surface) overall heat transfer coefficient. The U values were determined by the measured heat flux (q) and temperature difference between heating medium and bulk solution:

$$U = \frac{q}{T_h - T_b} \quad (2)$$

3.3.4 Numerical simulation

To calculate the shear stress (τ) values across the disc surface, computational fluid dynamics (CFD) simulation of the flow pattern of the SDA were performed using COMSOL Multiphysics software 5.3 (COMSOL, Inc, MA, USA). This work adapted the numerical model proposed by Huang et al. (2012) for the study on food fat fouling, having the same boundary conditions but different geometry (can diameter and vessel volume), fluid properties including viscosity (0.001 Pa s for all model solutions) and density (WPI = 1002.5 kg/m^3, glucose-WPI = 1042.5 kg/m^3, fructose-WPI = 1040 kg/m^3, sucrose-WPI = 1037.5 kg/m^3), and operating parameters (rotational speed and temperatures).

3.3.5 Deposit analysis

Protein content of the deposits was analyzed by the A & L Great Lakes Laboratories, Inc. (Fort Wayne, IN, USA) using the total protein method (AOAC 968.06).

Scanning electron microscope (SEM) analysis of deposits was conducted using FEI Quanta 3D FEG (FEI Company, OR, USA) under high vacuum at an accelerating voltage of 5 kV. Sample was prepared by cutting a small piece of deposits then air dried for 24 h before mounted on carbon tape and sputter coated with platinum.

3.3.6 Thermal stability characterization

To characterize the thermal stability of model solutions, the Discovery DSC (TA instruments, DE, USA) was used to determine the thermal transition temperature of protein. A sample of 20−25 mg was placed in a Tzero pan with hermetic lid (TA Instrument, DE, USA), and an empty pan was used as reference. The sample was tempered at 30 ºC for approximately 5 min for equilibrium before heated to 90 ºC at a heating rate of 5 K/min. The pH of the sample was maintained at 6.89±0.01.

The Rheometer ARES-G2 (TA instruments, DE, USA) with concentric cylinder geometry was used to measure the apparent viscosity of model solutions under heating conditions. A sample of approximately 25 mL was loaded and then subject to steady shear at a fixed shear rate of 60 1/s, and also a linear temperature ramp from 30 to 90 ºC at 3 K/min.

Preliminary tests were conducted using samples with different WPI concentrations, and the results showed that the thermal transition temperature was independent of WPI concentration. Therefore, in order to obtain better signal resolution and more reproducible results, the WPI concentrations of all model solutions were increased to 10 wt% for all DSC and rheological tests.

3.4 Results and discussion

3.4.1 Effect of sugar on whey protein fouling characteristics at low shear stress

During the 2 h fouling experiments, the heat flux and the temperature difference between heating media and bulk solution were recorded for calculating fouling resistance, R_f (Section 3.3.3). The evolution of fouling resistance was presented as fouling curves in Figures 3-2 and 3-4. Although all the fouling tests were conducted in triplicate, only the most representative data set is shown here for simplicity purposes. The maximum value observed in the fouling curve (R_{fM}) and the value at the end of experiment (R_{fF}) were extracted to characterize the fouling behavior of model solution (Table 3-1). The variation of each mean shown in Table 3-1 demonstrates the reproducibility of the fouling tests

Figure 3-2 shows the fouling curves of different model solutions at the shear stress of 0.02 Pa. The WPI deposits built up immediately after the experiment started, which was indicated by the linear increase in the R_f over the first 10 min. The fouling rate then started to decrease after the R_f reached the transition point of approximately 0.07 m^2 K/W at about 20 min, and the following increase in R_f was more gradual, indicating that the deposition rate and the removal rate were approaching equilibrium (Kern, 1959; Muller-Steinhagen, 2011). This can be attributed to that when approaching the transition point, the strength of the deposits became weaker because the deposits were formed at decreased surface temperature (Matthias, 1987), making them more susceptible to the applied shear which was constant throughout the experiments. This asymptotic fouling behavior (Awad, 2011) of WPI solution was similar to the fouling curves reported by Belmar-Beiny et al. (1993) and Boxler et al. (2013). In the cases of sugar-WPI fouling, the R_f at which deposition and removal reaching an equilibrium were much lower than that of WPI fouling, suggesting that weaker deposits were formed with the presence of sugars.

The fouling curves of different model sweetened milks also showed an asymptotic pattern. However, following a similar initial fouling stage to the WPI solution, the transition point was around 0.04 m^2 K/W, much lower in the case of sugar-WPI solutions. The subsequant plateau (sucrose) and decrease (glucose and fructose) of R_f after reaching the transition point were observed, resulting in the smaller R_{fF} compared to the WPI solution.

Table 3-1 summarized the key characteristics of the fouling curves of different test solutions obtained at different shear stresses. While the WPI solution had the highest R_{fM} and R_{fF}, the addition of sugar significantly decreased both values regardless of the type of sugar. Adding glucose and fructose decreased the R_{fM} and R_{fF} by more than 50%, and adding sucrose decreased the R_{fM} and R_{fF} by 30%. All these findings indicated that sugars acted as an inhibitor of whey protein fouling.

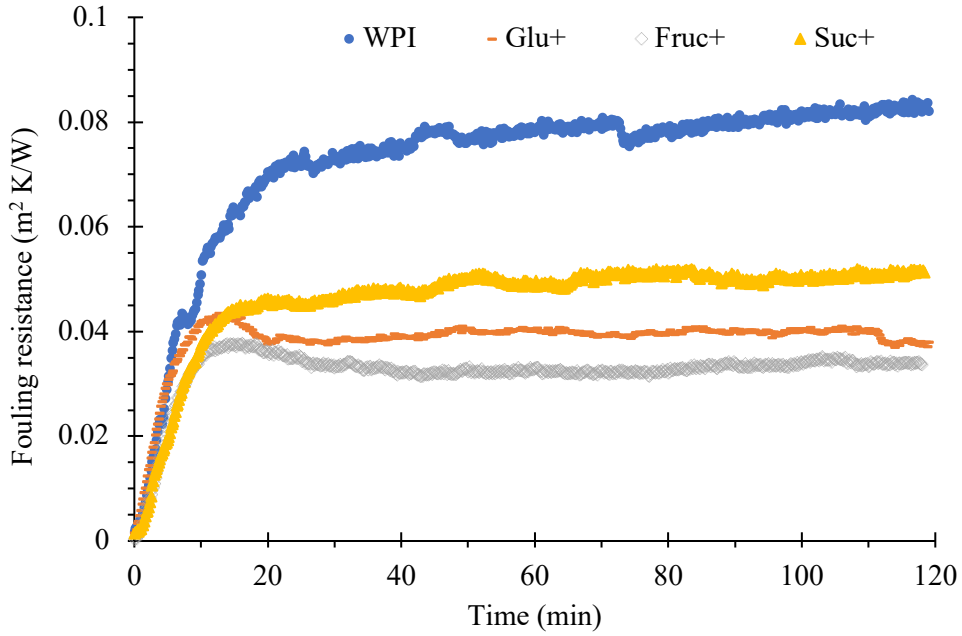


Figure 3-2 Fouling profiles of different model solutions at shear stress of 0.02 Pa. WPI: 5% WPI solution, Glu+: 10 wt% glucose and 5 wt% WPI; Fruc+: 10 wt% fructose and 5 wt% WPI, Suc+: 10 wt% sucrose and 5 wt% WPI. T_h = 95 ºC, T_b = 60 ºC.

Table 3-1 Fouling characteristics of different model solutions under different shear stresses

	R_{fM} (m^2 K/W)*			R_{fF}(m^2 K/W)*		
τ (Pa)	0.02	0.12	0.2	0.02	0.12	0.2
WPI[1]	0.077^{a}±0.011	0.055^{a}±0.011	0.026^{a}±0.003	0.074^{a}±0.014	0.051^{a}±0.014	0.024^{a}±0.003
Glu+[2]	0.038^{bc}±0.005	0.024^{b}±0.006	0.007^{b}±0.006	0.034^{b}±0.004	0.018^{b}±0.002	0.005^{b}±0.004
Fruc+[2]	0.035^{c}±0.006	0.024^{b}±0.003	0.009^{b}±0.001	0.03^{b}±0.004	0.018^{b}±0.003	0.007^{b}±0.001
Suc+[2]	0.054^{b}±0.004	0.037^{ab}±0.008	0.011^{b}±0.008	0.05^{b}±0.009	0.031^{ab}±0.01	0.009^{b}±0.007

*All the values represent the mean of three replicates. Values with different letters in the same column were significantly different (p<0.05)
[1]WPI: 5 wt% WPI solution
[2]Sugar+: 5 wt% WPI solution with 10 wt% sugar added

Whey protein denaturation and aggregation were found to be the key controlling steps in fouling (Bansal and Chen, 2006; Blanpain-Avet et al., 2016; Blanpain-Avet et al., 2012; de Jong, 1997). Therefore, DSC and rheometer were used to study the influence of sugar on the thermal stability of whey protein in order to better understand the inhibition effect of sugar on WPI fouling. In the

DSC tests, the model solutions showed endothermic transition at the temperatures between 65 and 85 ºC with the midpoint transition temperature (T_m), a parameter indicating protein denaturation, between 74.1 and 78.9 ºC (Figure 3-3*a*). The T_m of WPI solution was the lowest, of 74.1 ºC, which was similar to the previous study (Fitzsimons et al., 2007) (Table 3-2). Adding 10 wt% of different sugars all increased the T_m of whey protein significantly, indicating that sugar had the stabilizing effect on whey denaturation (Boye and Alli, 2000). Furthermore, the glucose-WPI mixture exhibited the highest T_m compared to fructose and sucrose.

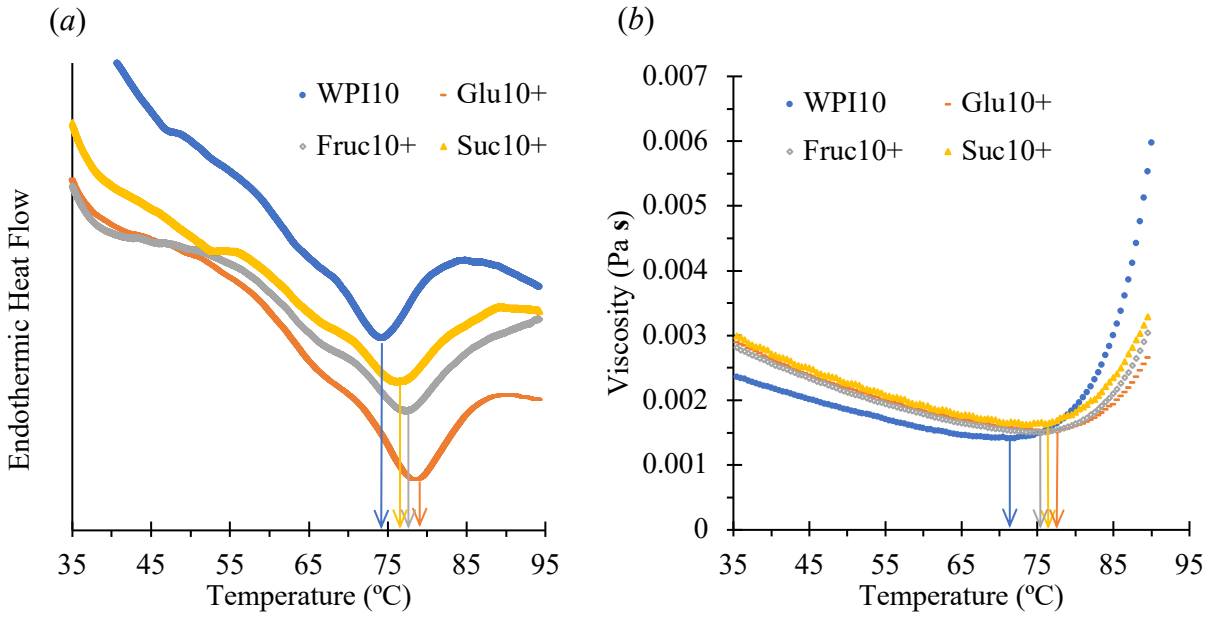


Figure 3-3 (*a*) Normalized heat flow curve (heating rate = 5 K/min), and (*b*) apparent viscosity (heating rate = 3 K/min) of different model solutions. WPI10: 10 wt% WPI solution, Sugar10+: 10 wt% WPI solution with 10 wt% sugar added. The arrows show the respective T_m and T_{ag}.

Table 3-2 Denaturation and aggregation temperatures of different model solutions

Sample	T_m (ºC)*	T_{ag} (ºC)*
WPI10[1]	$74.1^{b}\pm0.1$	$71.3^{c}\pm0.4$
Glu+[2]	$78.9^{a}\pm0.5$	$76.5^{a}\pm0.1$
Fruc+[2]	$76.4^{b}\pm1.2$	$75.1^{b}\pm0.6$
Suc+[2]	$76.4^{bc}\pm0.4$	$74.5^{b}\pm0.0$

*All the values represent the mean of three replicates. Values with different letters in the same column were significantly different ($p<0.05$)
[1]WPI10: 10 wt% WPI solution
[2]Sugar+: 10 wt% WPI solution with 10 wt% sugar added

The results of rheological tests (Figure 3-3*b*) show that the apparent viscosity of the model solutions decreased as temperature increased until the critical point, where the viscosity started to increase drastically. This change in viscosity can be attributed to the association of partially denatured protein molecules which formed aggregates (Singh and Havea, 2003). Therefore, the temperature of the critical point can be regarded as the aggregation temperature (T_{ag}) of the model solution. While the WPI solution had the lowest T_{ag}, of 71.3 ºC, the addition of sugars increased the T_{ag} of whey protein by 3.2−5.2 ºC, among which glucose showed the most significant increase (Table 3-2). Furthermore, at the temperature higher than T_{ag}, slower increases in the viscosity of sugar-WPI mixtures were observed, which may indicate that sugars can decrease the extent of whey aggregation. This finding was consistent with the DSC results and also agreed with previous studies, showing that sugar can increase the thermal stability of whey protein (Garrett et al., 1988; Kulmyrzaev et al., 2000; Rich and Foegeding, 2000; Wijayanti et al., 2014). Table 3-2 summarizes the T_m and T_{ag} values of different model solutions.

This stabilizing effect of sugar on whey protein could be attributed to the decrease in thermodynamic affinity of protein surface in the presence of sugar, which inhibits denatured protein molecules from aggregation (Kulmyrzaev et al., 2000). Garrett et al. (1988) also suggested that sugar increases the hydrophobic interaction between nonpolar amino acids, thus stabilizing the protein and hindering its unfolding and aggregation. As a result, protein denaturation and aggregation processes which are the key controlling steps in the fouling mechanism of whey protein (Bansal and Chen, 2006; Jimenez et al., 2013) are retarded by sugar, and fouling can thus be reduced. This agrees with our findings that fouling curves of sugar-WPI solutions had lower R_{fM} and R_{fF} than WPI solution, suggesting that the interaction between sugar and whey protein molecules inhibited further protein denaturation and aggregation.

From a heat transfer point of view, fouling is controlled by the thermal driving force (Huang et al., 2012), which is the difference between disc or deposit surface temperature and protein aggregation temperature. In this study, the increased aggregation temperature of whey protein due to the addition of sugar reduced the thermal driving force of fouling formation, leading to lower R_f values.

3.4.2 Effect of sugar on whey protein fouling characteristics at higher shear stress

To investigate the effect of shear on the fouling behavior of sweetened dairy products, same fouling tests were conducted at increased shear stresses. Figure 3-4 shows the fouling curves of different model solutions at the shear stresses $\tau = 0.12$ (Figure 3-4*a*) and 0.2 Pa (Figure 3-4*b*). Similar to the fouling profiles obtained at 0.02 Pa (Figure 3-2), deposits grew immediately after the test started at 0.12 Pa and the R_f increased rapidly over the first 10 to 15 mins, regardless of solution. The R_f of sugar-WPI solutions stopped growing at lower transition points, of 0.024−0.037 m^2 K/W, while the WPI solution reached an average R_{fM} of 0.055 m^2 K/W (Table 3-1). This reduction in R_f caused by sugar addition was also significant at 0.2 Pa, where the R_{fM} was decreased by more than 50%. There were clear decreases in both R_{fM} and R_{fF} (Table 3-1) with increasing shear stress, which agreed with previous studies that increasing shear rate or Reynolds number of the fluids decreased whey protein fouling (Belmar-Beiny et al., 1993; Khaldi et al., 2015). This decrease can be mainly attributed to the increased removal rate, which increases with shear stress (Huang et al., 2013).

After the transition point, the WPI solution featured a saw-tooth behaviour at higher shear stresses, representing the competing deposition and removal processes. A similar fluctuation in fouling resistance was observed by Boxler et al. (2014) in their study on WPI fouling on C/H/Si-coated surfaces. The sudden drop in R_f was due to deposit sloughing where larger chunks of deposits were removed by the shear (Awad, 2011; Challa et al., 2015; Challa et al., 2017). At higher shear, the removal force is stronger. When the deposits become thicker (*i.e.* higher R_f), the new deposits form at a decreased temperature of deposit-liquid interface, the lower thermal driving force can make the attachment strength of newly formed deposits weaker (Epstein, 1983; Matthias, 1987). Therefore, sloughing is more likely to take place. In contrast, the R_f values of all the sugar-WPI solutions remained relatively unchanged after the transition point. This suggests that sugar-WPI deposits have a different property from WPI deposits. A further discussion about deposit analysis will be mentioned in Section 3.3.3.

Images of the deposits on disc surface taken at the end of fouling experiments were shown in Figure 3-5. All the treatments with sugar addition had a smaller fouling area compared to WPI. It can be seen that on the surface further from the center, where shear stress was higher, fewer or no deposits were formed. These images correspond with the fouling characteristics of sugar-WPI

solutions obtained at the highest shear stress (0.2 Pa), and support the findings that the sugar-WPI deposits were more susceptible to shear.

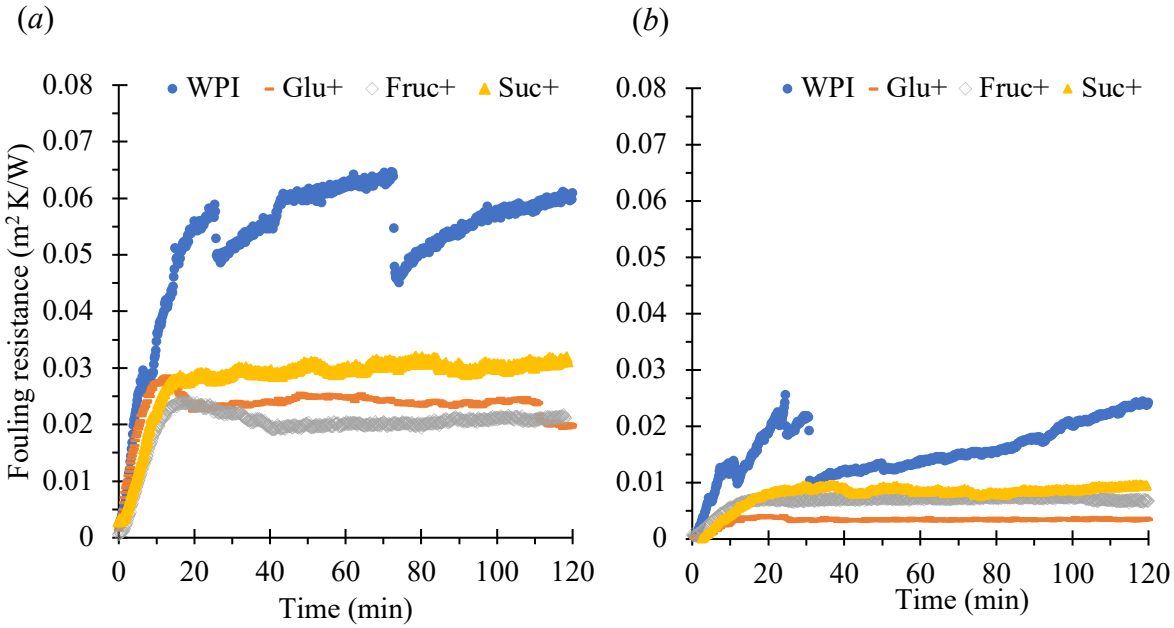


Figure 3-4 Fouling profiles of different model solutions at shear stresses of (*a*) 0.12 Pa, and (*b*) 0.2 Pa. WPI: 5% WPI solution, Glu+: 10 wt% glucose and 5 wt% WPI; Fruc+: 10 wt% fructose and 5 wt% WPI, Suc+: 10 wt% sucrose and 5 wt% WPI. T_h = 95 ºC, T_b = 60 ºC.

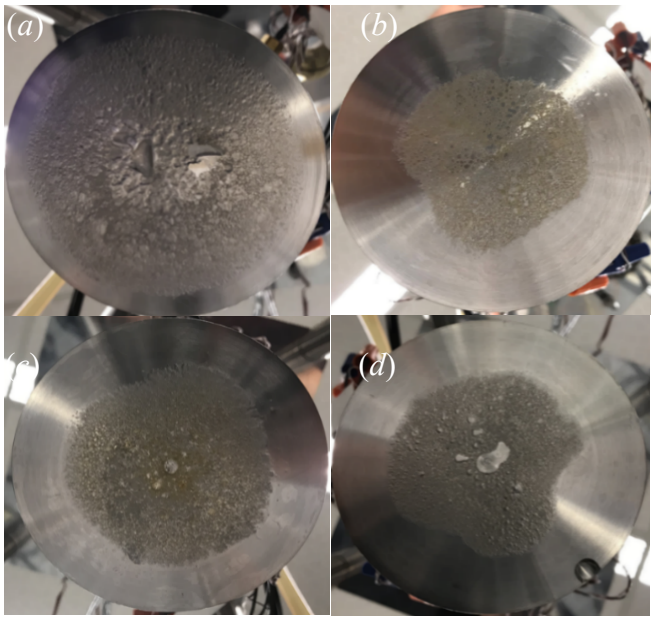


Figure 3-5 Visualization of deposits on disc surface after 2-h fouling tests: (a) 5 wt% WPI, (b) 10 wt% glucose and 5 wt% WPI, (c) 10 wt% fructose and 5 wt% WPI, (d) 10 wt% sucrose and 5 wt% WPI. Th = 95 ºC, Tb = 60 ºC.

3.4.3 Effect of sugar on deposit properties

Deposit mass, composition and morphology were characterized to study the effect of sugar on the properties of whey protein deposits. Table 3-3 shows the dry deposit mass per disc area formed by different model solutions, in which the WPI deposits had the largest value, of 28.41 g/m^2. Adding sugars all significantly reduced the deposit mass by 40%−65%, and the glucose-WPI solution produced the least deposits while the fructose-WPI one produced the most.

Protein content of the deposits also decreased by 6−8.8% with added sugars (Table 3-3). This suggests that sugar was involved in the deposits formed, which is supported by the brown color shown in the glucose-WPI and fructose-WPI deposits (Figure 3-5, Glu+ and Fruc+), resulting from the Millard reaction since no brown color was observed in the case of non-reducing sugar sucrose. However, in this study, Millard reaction, the chemical reaction between reducing sugars and amino acids of whey protein, did not cause a significant difference in the fouling characteristics (*i.e.* R_{fM}, R_{fF}, deposit mass) among the three sugar-WPI solutions in most cases.

Figure 3-6 shows the SEM images of deposits obtained from different model solutions, in which a clear change in the deposit structure caused by added sugars can be seen. The WPI deposits had a more smooth and compact structure, and fewer pores were observed. In contrast, with sugar addition, the deposits presented a more porous structure. The fructose-WPI deposits had fewer pores and was more compact compared to glucose and sucrose. This observation agreed with the larger amounts of final deposits obtained with the cases of WPI and fructose-SPI solutions, as shown in Table 3-3.

As discussed in Section 3.3.2, fouling characteristics at different shear stresses suggested that WPI deposits had a higher attachment strength compared to sugar-WPI deposits. This difference in deposit strength can be supported by the deposit morphology observed in Figure 3-6. The compact structure of WPI deposit demonstrated a stronger cross-linking of denatured whey protein. On the contrary, the added sugars retarded the protein cross-linking, resulting in a porous structure with lower strength. The pores formed in sugar-WPI deposits can be due to the sugar-rich liquid entrained in the solid matrix of deposits, which causes no fouling (Challa et al., 2015). The deposit morphology may also help explain the sloughing behavior observed during WPI fouling but not in the cases of sugar-added model solutions. This could be because with a more compact structure,

the deposits are more likely removed in larger pieces due to the stronger connection within the deposits. However, although all the evidence in this study suggested that sugar-WPI solutions formed weaker deposits, there was difficulty in direct quantitative measurements of deposit strength. This was because the rheological properties of the deposits formed by the SDA could not be analyzed *in situ*. When the deposits are removed from the fouling surface, they are not in their original state and the results obtained with post-analysis might hence be inaccurate. Therefore, a gauging sensor, such as fluid dynamic gauge (Chew et al., 2004), could be incorporated into the SDA system and would be able to give an indication of the deposit strength.

Table 3-3 Deposit dry mass per fouling surface area and protein content of WPI fouling deposits with the presence and absence of different sugars

	Dry mass/disc area (g/m^2)*	Protein content (%)
WPI[1]	$28.41^{a}\pm2.89$	92.50
Glu+[2]	$9.97^{c}\pm1.21$	84.34
Fruc+[2]	$17.04^{b}\pm2.38$	84.82
Suc+[2]	$11.75^{bc}\pm1.24$	86.95

*All the values represent the mean of three replicates. Values with different letters in the same column were significantly different ($p<0.05$)

[1]WPI: 5 wt% WPI solution

[2]Sugar+: 5 wt% WPI solution with 10 wt% sugar added

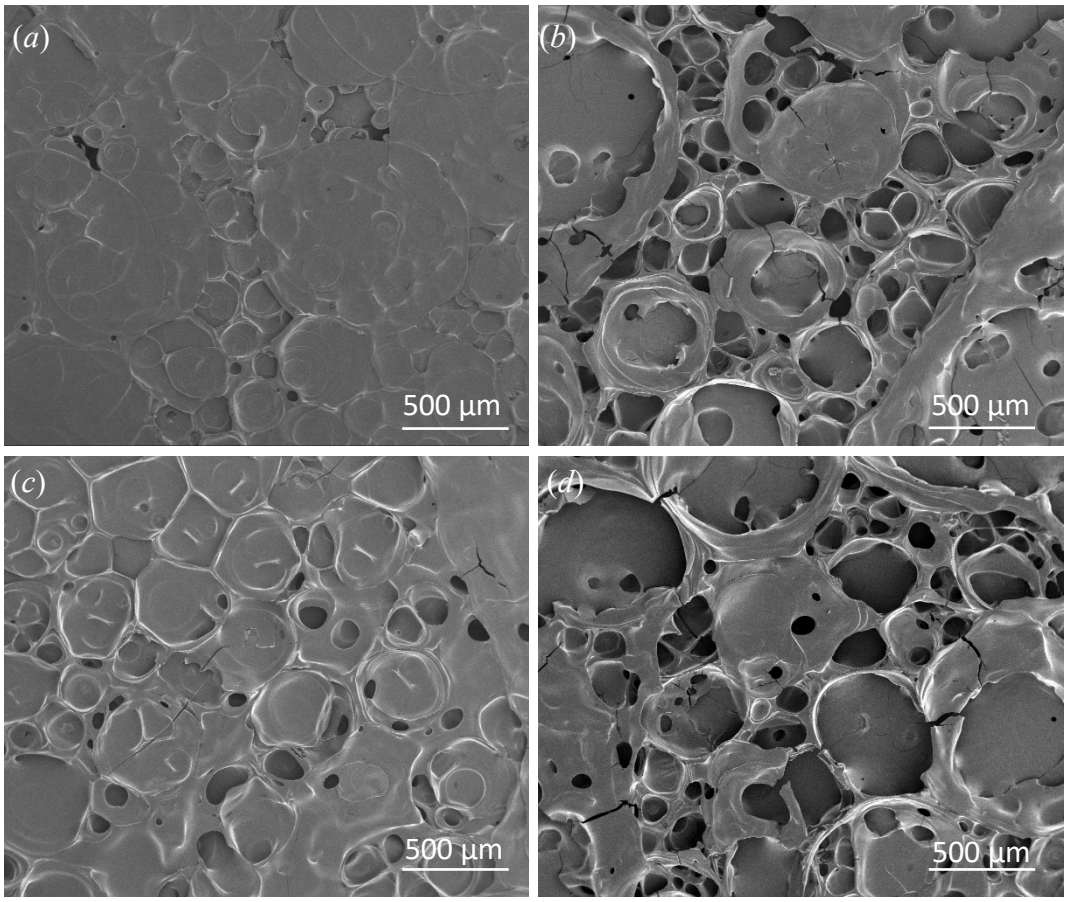


Figure 3-6 SEM images of deposits generated by different model solutions: (*a*) 5 wt% WPI, (*b*) 10 wt% glucose and 5 wt% WPI, (*c*) 10 wt% fructose and 5 wt% WPI, (*d*) 10 wt% sucrose and 5 wt% WPI.

3.5 Conclusions

This study investigated the fouling behavior of sweetened dairy products using model solutions containing whey protein and different sugars. All the fouling tests were conducted by a spinning disc apparatus under various shear stresses. We found that all sugars added to whey protein solution retarded fouling with reduced fouling resistance and deposit mass. This agreed with the increased thermal stability of whey protein by sugar addition, confirmed by DSC and rheological tests. The sugar-added solutions showed a very different fouling profile from the whey protein solution, especially under increased shear. Sugar was found to affect the deposit composition and structure, leading to the deposits more susceptible to shear compared to the whey protein deposits. The knowledge obtained from ternary system (*i.e.* water, whey protein and sugar) in this work can

be extended to investigate the fouling behavior of more complex model solutions *e.g.* addition of starch or gum, the other major components for thickening purposes. Such a quaternary mixture would closely resemble the composition of sweetened dairy products.

3.6 References

Arakawa, T., Timasheff, S.N., 1982. Stabilization of protein structure by sugars. Biochem. 21, 6536-6544.

Awad, M.M., 2011. Fouling of Heat Transfer Surfaces, Heat Transfer - Theoretical Analysis, Experimental Investigations and Industrial Systems. InTech.

Bansal, B., Chen, X.D., 2006. A critical review of milk fouling in heat exchangers. Compr. Rev. Food Sci. Food Saf. 5, 27-33.

Belmar-Beiny, M., Gotham, S., Paterson, W., Fryer, P., Pritchard, A., 1993. The effect of Reynolds number and fluid temperature in whey protein fouling. J. of Food Eng. 19, 119-139.

Blanpain-Avet, P., André, C., Khaldi, M., Bouvier, L., Petit, J., Six, T., Jeantet, R., Croguennec, T., Delaplace, G., 2016. Predicting the distribution of whey protein fouling in a plate heat exchanger using the kinetic parameters of the thermal denaturation reaction of β-lactoglobulin and the bulk temperature profiles. J. of Dairy Sci. 99, 9611-9630.

Blanpain-Avet, P., Hédoux, A., Guinet, Y., Paccou, L., Petit, J., Six, T., Delaplace, G., 2012. Analysis by Raman spectroscopy of the conformational structure of whey proteins constituting fouling deposits during the processing in a heat exchanger. J. of Food Eng. 110, 86-94.

Boxler, C., Augustin, W., Scholl, S., 2013. Fouling of milk components on DLC coated surfaces at pasteurization and UHT temperatures. Food and Bioprod. Process. 91, 336-347.

Boye, J.I., Alli, I., 2000. Thermal denaturation of mixtures of α-lactalbumin and β-lactoglobulin: a differential scanning calorimetric study. Food Res. Int. 33, 673-682.

Challa, R., Johnston, D., Singh, V., Tumbleson, M., Rausch, K., 2015. Fouling characteristics of model carbohydrate mixtures and their interaction effects. Food Bioprod. Process. 93, 197-204.

Challa, R., Zhang, Y.B., Johnston, D., Singh, V., Engeseth, N., Tumbleson, M.E., Rausch, K.D., 2017. Evaporator fouling tendencies of thin stillage and concentrates from the dry grind process. Heat Transfer Eng. 38.

Chew, J.Y.M., Paterson, W.R., Wilson, D.I., 2004. Fluid dynamic gauging for measuring the strength of soft deposits. J. of Food Eng. 65, 175-187.

de Jong, P., 1997. Impact and control of fouling in milk processing. Trends Food Sci. Technol. 8, 401-405.

Epstein, N., 1983. Thinking about heat transfer fouling: A 5 × 5 Matrix. Heat Transfer Eng. 4, 43-56.

Erabit, N., Flick, D., Alvarez, G., 2014. Formation of β-lactoglobulin aggregates during thermomechanical treatments under controlled shear and temperature conditions. J. of Food Eng. 120, 57-68.

Garrett, J.M., Stairs, R.A., Annett, R.G., 1988. Thermal Denaturation and Coagulation of Whey Proteins: Effect of Sugars1. J. of Dairy Sci. 71, 10-16.

Goode, K.R., Bowen, J., Akhtar, N., Robbins, P.T., Fryer, P.J., 2013. The effect of temperature on adhesion forces between surfaces and model foods containing whey protein and sugar. J. of Food Eng. 118, 371-379.
Huang, J.-Y., John Chew, Y.M., Ian Wilson, D., 2012. A spinning disc study of fouling of cold heat transfer surfaces by gel formation from model food fat solutions. J. of Food Eng. 109, 49-61.
Huang, J.Y., 2013. Fundamental Features of Food Fat Freezing Fouling. University of Cambridge.
Huang, J.Y., Chew, Y.M.J., Wilson, D.I., 2013. Investigating the effect of starting mode on food fat gel layer formation on cold surfaces. J. of Food Eng. 119, 454-463.
Huang, K., Goddard, J.M., 2015. Influence of fluid milk product composition on fouling and cleaning of Ni-PTFE modified stainless steel heat exchanger surfaces. J. of Food Eng. 158, 22-29.
Jimenez, M., Delaplace, G., Nuns, N., Bellayer, S., Deresmes, D., Ronse, G., Alogaili, G., Collinet-Fressancourt, M., Traisnel, M., 2013. Toward the understanding of the interfacial dairy fouling deposition and growth mechanisms at a stainless steel surface: A multiscale approach. J. of Colloid and Interface Sci. 404, 192-200.
Kern, D.S., RE, 1959. A theoretical analysis of thermal surface fouling. Br. Chem. Eng. 4, 258-262.
Khaldi, M., Blanpain-Avet, P., Guérin, R., Ronse, G., Bouvier, L., André, C., Bornaz, S., Croguennec, T., Jeantet, R., Delaplace, G., 2015. Effect of calcium content and flow regime on whey protein fouling and cleaning in a plate heat exchanger. J. of Food Eng. 147, 68-78.
Kulmyrzaev, A., Bryant, C., McClements, D.J., 2000. Influence of Sucrose on the Thermal Denaturation, Gelation, and Emulsion Stabilization of Whey Proteins. J. of Agric. and Food Chem. 48, 1593-1597.
Matthias, B., 1987. Fouling of heat transfer surfaces. Chem. Eng. Technol. 10, 113-125.
Muller-Steinhagen, H., 2011. Heat transfer fouling: 50 years after the Kern and Seaton model. Heat Transfer Eng. 32, 1-13.
Muller-Steinhagen, H., Malayeri, M.R., Watkinson, A.P., 2009. Heat exchanger fouling: environmental impacts. Heat Transfer Eng. 30, 773-776.
Nicolai, T., Britten, M., Schmitt, C., 2011. β-Lactoglobulin and WPI aggregates: Formation, structure and applications. Food Hydrocolloids 25, 1945-1962.
Nigo, R.Y., Chew, Y.M.J., Houghton, N.E., Paterson, W.R., Wilson, D.I., 2009. Experimental Studies of Freezing Fouling of Model Food Fat Solutions Using a Novel Spinning Disc Apparatus. Energy Fuels 23, 6131-6145.
Rich, L.M., Foegeding, E.A., 2000. Effects of Sugars on Whey Protein Isolate Gelation. J. of Agri. Food Chem. 48, 5046-5052.
Rosmaninho, R., Melo, L.F., 2006. Calcium phosphate deposition from simulated milk ultrafiltrate on different stainless steel-based surfaces. Int. Dairy J. 16, 81-87.
Singh, H., Havea, P., 2003. Thermal Denaturation, Aggregation and Gelation of Whey Proteins, in: Fox, P.F., McSweeney, P.L.H. (Eds.), Advanced Dairy Chemistry—1 Proteins: Part A / Part B. Springer US, Boston, MA, pp. 1261-1287.
Visser, J., Jeurnink, T.J.M., 1997. Fouling of heat exchangers in the dairy industry. Exp. Therm. Fluid Sci. 14, 407-424.
Voragen, A.G.J., 1998. Technological aspects of functional food-related carbohydrates. Trends Food Sci. Technol. 9, 328-335.

Wang, W., Zhong, Q., 2014. Improved thermal stability of whey protein–maltodextrin conjugates at pH 5.0 by d-Glucose, sucrose, d-cellobiose, and lactose. Food Hydrocolloids 41, 257-264.
Wijayanti, H.B., Bansal, N., Deeth, H.C., 2014. Stability of Whey Proteins during Thermal Processing: A Review. Compr. Rev. Food. Sci. Food Saf. 13, 1235-1251.

CHAPTER 4. INTERACTIONS OF WHEY WITH CASEIN AND CARRAGEENAN DURING PASTEURIZATION AND THEIR EFFECTS ON PROTEIN DEPOSITION

4.1 Abstract

Carrageenan is extensively used as texturizer, stabilizer and fat replacer in dairy products such as flavored milk, ice cream, yogurt and milk shake. These dairy products have to be thermally processed to ensure their safety and quality, which, however, causes more severe fouling problem than milk processing. Although there have been many studies on dairy fouling, the majority focused on milk ingredients, especially whey protein, and only little, if anything, is known about the effects of other milk proteins like casein and non-milk stabilizers such as carrageenan gum. Therefore, the fouling mechanisms of complex dairy products are still elusive. In this study, we prepared different model dairy solutions with whey protein isolate (WPI; 2 wt%), casein (0.5 wt%) and carrageenan (0.03 wt%) and characterized their fouling behaviors under well-controlled temperature and flow. The addition of casein into WPI solution significantly increased its fouling resistance but formed deposits with apparent ruptures because casein reduced the thermal stability of whey. Adding carrageenan increased the fouling of WPI solution with formation of more compact deposits. As evidenced by Fluorescence and Raman spectroscopy analyses on the changes in protein structures and increased exposure of tryptophan residues, we confirmed that the presence of casein and carrageenan can facilitate the denaturation of β-lactoglobulin, the critical step in fouling formation, which explains the increased fouling observed. This work sheds light on the interaction of milk proteins with polysaccharides under thermal processing conditions and is expected to provide the dairy industry with knowledge of designing dairy formulation with reduced fouling tendency.

Keywords: Dairy fouling; β-lactoglobulin; Denaturation; Tryptophan; Protein structure

4.2 Introduction

Thermal treatment is an essential operation in dairy processing to inactivate microorganisms, improve product quality, and safety and extend shelf life (Ramesh and Arun. 2011). Heat sensitive milk proteins tend to denature and interact with other dairy components, forming undesirable deposits on heated surfaces known as fouling (Visser and Jeurnink, 1997). Fouling in dairy industry has been a serious issue as it decreases heat transfer efficiency and increases pressure drop (de Jong, 1997). Furthermore, product quality and safety can be deteriorated by fouling deposits, hence intensified cleaning is needed in dairy processing (Bansal and Chen, 2006; Sadeghinezhad et al., 2015)

Many studies have been carried out to address the challenges of dairy fouling. Milk is a complex matrix, in which whey protein, especially β-lactoglobulin (β-lg) is the component leading to fouling formation at pasteurization temperatures and is predominant in milk deposits (Bansal and Chen, 2006). Upon heating, β-lg unfolds to expose the hydrophobic core containing reactive thiol groups that can interact with other milk components then attach to processing surfaces to form deposits (Bansal and Chen, 2006; Jimenez et al., 2013; Khaldi et al., 2018; Visser and Jeurnink, 1997). Calcium also plays an important role in fouling formation by facilitating the unfolding and linking of β-lg. Moreover, calcium can form bridges between reactive species and deposit layer (Bansal and Chen, 2006; Changani et al., 1997; Christian et al., 2002; O'Kennedy and Mounsey, 2009).

While casein is the most abundant protein in milk (82 %) (Wong, 1988), it is generally considered heat stable and its effect on protein deposition was not specifically studied in in the past. However, in our previous study (Zhang et al., 2020), a significant amount of casein was observed in the fouling deposits of raw milk, indicating the contribution of casein to fouling formation. Thus, it is necessary to characterize the interaction between casein and whey at high temperatures and investigate its effect on milk fouling.

In addition to milk, other dairy products such as flavored milk, ice cream, yogurt, milk shake are important foods on the market nowadays. However, their pasteurization result in more severe fouling (Huang and Goddard, 2015; Prakash et al., 2010; Zhang et al., 2019). Kappa carrageenan

(κ-car) is a linear, anionic polysaccharide and extensively used as texturizer, stabilizer and fat replacer in these dairy products (Burova et al., 2007). The interactions of carrageenan with milk whey and casein proteins have been studied. Carrageenan can form complexes with whey protein and β-lg mainly through electrostatic interactions (Weinbreck et al., 2004; Hosseini et al., 2013; Jones et al., 2010). Furthermore, κ-car can bind to the surface of casein micelles and stabilize them from macroscopic phase separation (Dalgleish and Morris, 1988; Spagnuolo et al., 2005). κ-car can also bind to β-casein by electrostatic interactions (Burova et al., 2007). Only few studies, however, have investigated the effect of carrageenan on the fouling behavior of dairy products. Prakash et al., (2010) studied the fouling of chocolate milk with different contents of carrageenan at ultra-high temperature condition, and found that κ-car helped stabilize chocolate milk and reduce its fouling with an optimal concentration of 0.03 %. Huang and Goddard, (2015) reported an increase in fouling formation when adding 0.1 % carrageenan into chocolate milk. Despite the apparent dependency of cholate milk fouling on carrageenan concentration, how carrageenan interacts with other milk components under thermal processing conditions and thus the fouling mechanisms of such a complex colloidal system are still unknown.

In this study, model solutions containing whey protein and calcium, the key components in milk deposits, were used to study the effects of casein and carrageenan on dairy fouling. In addition, the interactions among carrageenan, whey and casein were characterized at molecular level to gain fundamental insights into the fouling mechanisms of dairy products with the addition of carrageenan.

4.3 Materials and Methods

4.3.1 Model solutions

Whey protein isolate (WPI; 88% protein; biPro, Eden Prairie, MN, USA), micellar casein (87% protein; BulkSupplements, Henderson, NV, USA), and κ-car (Alfa Aesar, Tewksbury, MA, USA) were used to prepare the test solutions. For all the fouling experiments, we mixed WPI powder (2 wt%) and free calcium (80 ppm) from calcium chloride dihydrate (99%; Thermo Fisher Scientific, Waltham, MA, USA) in deionized water at ambient temperature (20−22 ºC) for 2 h as the base

model solution (control) to represent dairy products. The model solutions with casein and/or carrageenan were prepared by mixing the base solution, with casein/carrageenan powder to a final concentration of 2 wt% WPI, 0.5 wt% casein and/or 0.03 wt% carrageenan at ambient temperature for 4 h. The pH of the model solutions were in the range of 6.9−7.0 throughout the fouling experiments. All the model solutions were stored at 4 ºC for 16−18h until each fouling test for complete hydration.

4.3.2 Fouling experiments

Fouling experiments were performed using a spinning disc apparatus (SDA) to simulate controlled temperature and shear conditions for fouling formation on a stainless steel surface. A detailed description of the SDA is given in Zhang et al. (2019). The disc surface temperature was controlled by circulating ethylene glycol at 100 ºC (T_h), and the temperature of the test solution (T_b) contained in a 1-L jacketed vessel was regulated by a second circulator operating with water at 60 ºC. The SDA has three heat flux sensors coupled with thermocouples at different locations of the disc with distances of 0.2, 1.7 and 2.8 cm from the disc center, respectively. Heat flux and temperatures were recorded by a data logger every 20 s during fouling experiments. The overall heat transfer coefficiency (U) was determined by the measured heat flux (q) and the temperature difference between heating medium (T_h) and test solution (T_b):

$$U = \frac{q}{T_h - T_b} \tag{1}$$

The fouling resistance (R_f) was then calculated by the change in the overall heat transfer coefficient over time:

$$R_f = \frac{1}{U_t} - \frac{1}{U_c} \tag{2}$$

where U_t is the overall heat transfer coefficient at time t, U_c is the initial (*i.e.* clean disc surface) overall heat transfer coefficient. Fouling curve (R_f-t) was used to describe the fouling behavior.

For all fouling experiments, model solutions were preheated to 60 ºC, and the rotational speed of the disc was set at 5.2 rad/s, creating a laminar flow condition (Re ≈ 2500−5000). The test solution temperature reached an equilibrium of 68 ºC very quickly after the fouling experiment started and remained constant over the 2-h test. After each test, the disc was quickly cooled to below 35 ºC before removing the fouled disc. The deposits were rinsed three times with deionized water to remove the residue of test solution and carefully removed using a plastic spatula, then freeze-dried for further analyses.

4.3.3 Deposit structure

Deposit microstructure was characterized by scanning electron microscope (SEM) imaging. Freeze-dried deposits were cut into small pieces and coated with platinum before analyzed using a FEI Quanta 3D FEG SEM (FEI Company, Hillsboro, OR, USA) under high vacuum at an accelerating voltage of 5 kV.

4.3.4 Thermal analysis

The thermal transition temperature and enthalpy of milk proteins were analyzed using a differential scanning calorimeter (DSC 2000, TA Instrument, New Castle, DE, USA). To study the effects of casein and carrageenan on the thermal stability of WPI, four samples: (*i*) 10 wt% WPI, (*ii*) 10 wt% WPI + 2.5 wt% casein, (*iii*) 10 wt% WPI + 0.3% carrageenan, and (*iv*) 10 wt% WPI + 2.5 wt% casein + 0.3% carrageenan, were prepared following the protocol described in Section 4.3.1 for analysis. Here we increased the concentrations of the components by five times without altering their ratio due to the limitation of DSC sensitivity. Test solution (~20 mg) was sealed in a DSC Tzero hermetic aluminum pan and heated from 20 to 95 ºC at a rate of 5 ºC/min. DSC curve was analyzed using TRIOS software (TA Instrument, New Castle, DE, USA). The value reported is the mean of five measurements for each sample.

4.3.5 Fluorescence spectroscopy

Intrinsic fluorescence measurements were performed using a Cary Eclipse fluorescence spectrophotometer (Agilent, Santa Clara, CA, USA) with a quartz cell of 10 mm path length. Fluorescence spectra were taken at excitation wavelengths of 292 and 274 nm at a scan rate of 120

nm/min. The emission wavelength was recorded between 200 and 300 nm (Simion et al., 2015). Ten milliliters of model solution was placed in a 15-ml falcon tube and heated in a water bath of 80 ºC for 10 mins then cooled down at 4 ºC for 1 h. All heated and unheated samples were then centrifuged (10,000 × *g*) at 16 ºC for 10 min to remove undesired large aggregates. The supernatant of each sample was diluted (1:4) with deionized water and equilibrated at 25 ºC prior to fluorescence measurement.

4.3.6 Raman spectroscopy

The Raman spectra of model solutions and their fouling deposits were measured using a Bruker MultiRAM system (Bremen, Germany) equipped with a 1064 nm laser and a liquid nitrogen-cooled Ge detector. The spectra were detected over the range of 400−1900 cm^{-1} under a fixed measurement condition (laser power: 300 mW, resolution: 4 cm^{-1}, aperture: 4 mm, 100 scans). Spectra accusation and analysis were performed using OPUS software (Bruker Optics, Billerica, MA, USA). Spectra were baseline-corrected and normalized to the intensity of the phenylalanine peak at 1006 cm^{-1}. All samples were freeze-dried prior to Raman measurement.

4.3.7 Statistical analysis

Fouling experiments were conducted using a randomized design with triplicates. One-way analysis of variance (ANOVA) followed by Turkey's pairwise comparison was used to compare the means among different samples, with a significance level of 0.05. The statistical analysis was performed using SPSS® Statistics 26 (IBM, Armonk, NY, USA).

4.4 Results and Discussion

4.4.1 Fouling behavior of model solutions

4.4.1.1 Fouling characteristics

The fouling profiles (*i.e.* R_f-*t* curves) of four model solutions at different shear stresses are presented in Figure 4-1. For all the cases tested, fouling curves had no induction period, deposits began to build up immediately after the experiments started at different linear rates, which lasted

for different lengths of time (linear increase period). The fouling rate then slowed down due to the decrease in the deposit surface temperature when deposit became thicker, which decreased the driving force of fouling formation (*i.e.* temperature difference between deposit surface and test solution). After reaching the maximum value, the R_f either remained stable showing an asymptotic behavior, or decreased at different levels under elevated shear depending on the solution, indicating sloughing (shear-off) of deposits with weaker strength. Table 4-1 summarizes the initial fouling rate and maximum R_f of different solutions under different shear stresses.

As the control of this study, 2% wt WPI formed the least fouling among all the model solutions regardless of the shear stress applied. This was because it had the lowest initial fouling rate and shortest linear increase period. While the curves obtained with 0.03 and 0.22 Pa showed an asymptotic pattern, deposit sloughing was observed when the shear increased to 0.38 Pa. Adding casein (CN) to WPI solution significantly increased its initial fouling rate by 83% (0.38 Pa)−163% (0.03 Pa), resulting in approximately two-fold maximum R_f (Table 4-1). However, marked deposit sloughing was observed for all the cases, suggesting that the CN-WPI deposits were more susceptible to shear removal, which agreed with the smaller increase in fouling rate observed at higher shear.

Although the initial fouling rate only moderately increased by 7−18% following the addition of carrageenan (Car) into WPI solution, the maximum R_f showed 52−61% increases. This was because the fouling curve of Car-WPI solution had a longer linear increase period hence the fouling rate started to slow down later than the WPI solution, allowing its R_f to reach a higher value. Furthermore, slight sloughing was observed for Car-WPI fouling. In contrast, adding Car largely decreased the initial rate of CN-WPI fouling by 29% (0.03 Pa) −35% (0.38 Pa). However, Car-CN-WPI deposits were more resistant to shear than CN-WPI deposits, resulting in the highest R_f among the model solutions tested. It is important to note that the fouling of Car-CN-WPI and Car-WPI had very similar patterns. The presence of CN increased the initial fouling rate and also prolonged the initial increase period, therefore, Car-CN-WPI solution formed more fouling, especially at lower shear stresses.

Shear showed a significant effect on the fouling behavior of the model solutions that the R_f decreased with increasing shear stress (Figure 4-1), as expected. Moreover, the time for the R_f to reach its maximum value increased as the shear increased. As fouling is a dynamic process consisting of deposit formation and removal on surfaces (Kazi, 2012), increasing shear/Reynolds number can increase the removal rate hence reducing the net fouling rate. Similar findings were widely reported in previous studies on dairy fouling (Belmar-Beiny et al. 1993; Simmons et al., 2007; Zhang et al., 2020; Zhang et al., 2019).

Table 4-1 Fouling characteristics of model solutions at different shear stresses

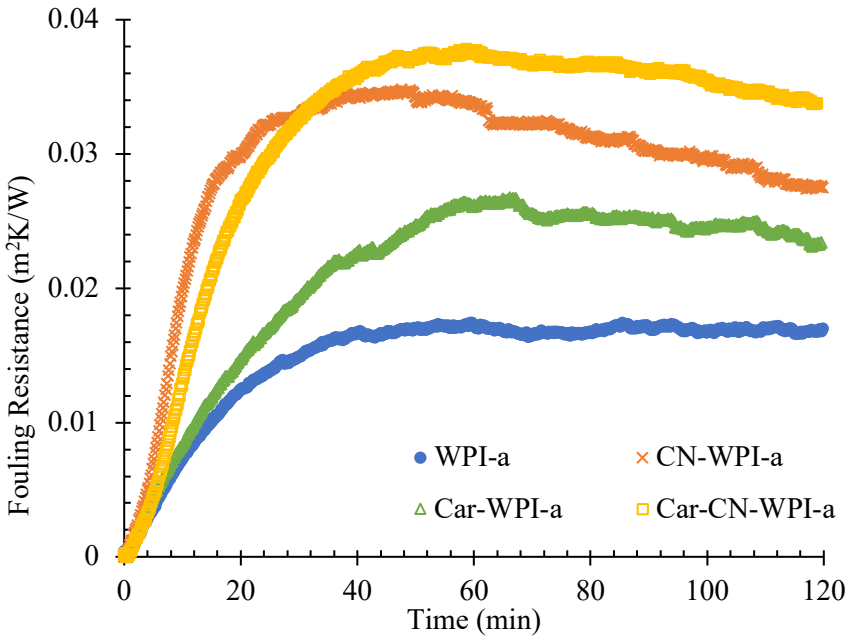
	Maximum fouling resistance ($\times 10^{-3}$ m^2 K/W)[2]			Initial fouling rate ($\times 10^{-3}$ m^2 K/W min)[2]			Deposit dry mass (per disc area; g/m^2)[2]
τ (Pa)	0.03	0.22	0.38	0.03	0.22	0.38	
WPI[1]	17.8 ± 3.1^{a}	18.0 ± 3.4^{a}	16.9 ± 1.7^{a}	0.80 ± 0.0^{a}	0.87 ± 0.06^{a}	0.93 ± 0.06^{a}	62.61± 5.73^{b}
CN-WPI[1]	35.4 ± 1.1^{bc}	36.0 ± 0.9^{bc}	31.7 ± 1.8^{b}	2.1 ± 0.06^{b}	1.9 ± 0.15^{b}	1.7 ± 0.06^{b}	43.37 ± 3.91^{a}
Car-WPI[1]	27.1 ± 2.8^{b}	28.4 ± 3.7^{b}	27.3 ± 5.3^{b}	0.90 ± 0.1^{a}	0.93 ± 0.06^{a}	1.1 ± 0.15^{a}	75.98 ± 7.20^{c}
Car-CN-WPI[1]	38.5 ± 4.8^{c}	40.0 ± 6^{c}	34.2 ± 4.2^{b}	1.5 ± 0.45^{b}	1.3 ± 0.5^{ab}	1.1 ± 0.36^{a}	71.71 ± 5.98^{bc}

[1]WPI: 2% WPI; CN-WPI: 2% WPI +0.5% Casein; Car-WPI: 2% WPI +0.03% Carrageenan; Car-CN-WPI: 2% WPI +0.5% Casein +0.03% Carrageenan

[2]All the values represent the mean of triplicate. Values with different letters in the same column were significantly different ($p < 0.05$).

Figure 4-1 Evolution of fouling of model solutions at different shear stresses: (a) τ = 0.03 Pa; (b) τ = 0.22 Pa; (c) τ = 0.38 Pa. Each data point is the mean of triplicate.

(a)



(b)

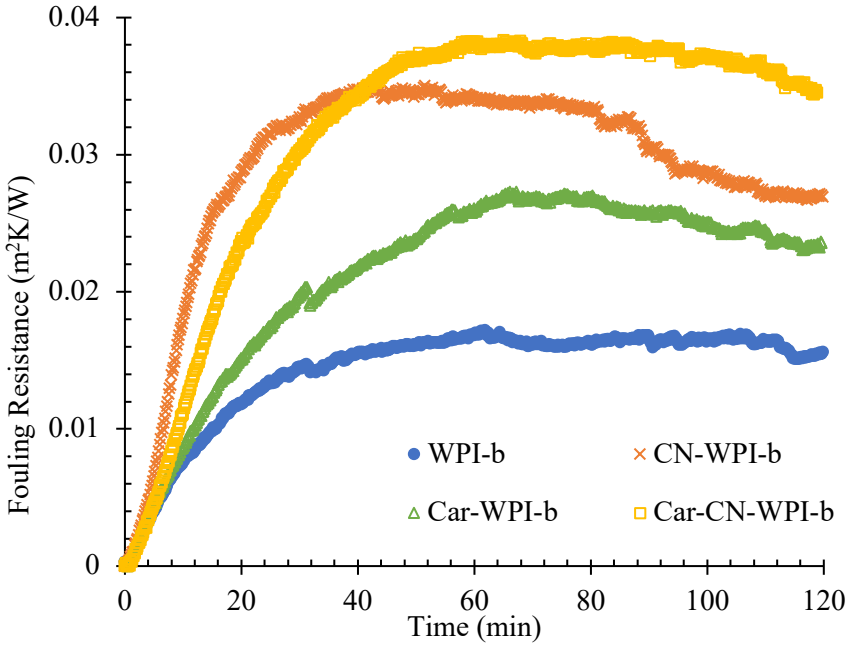
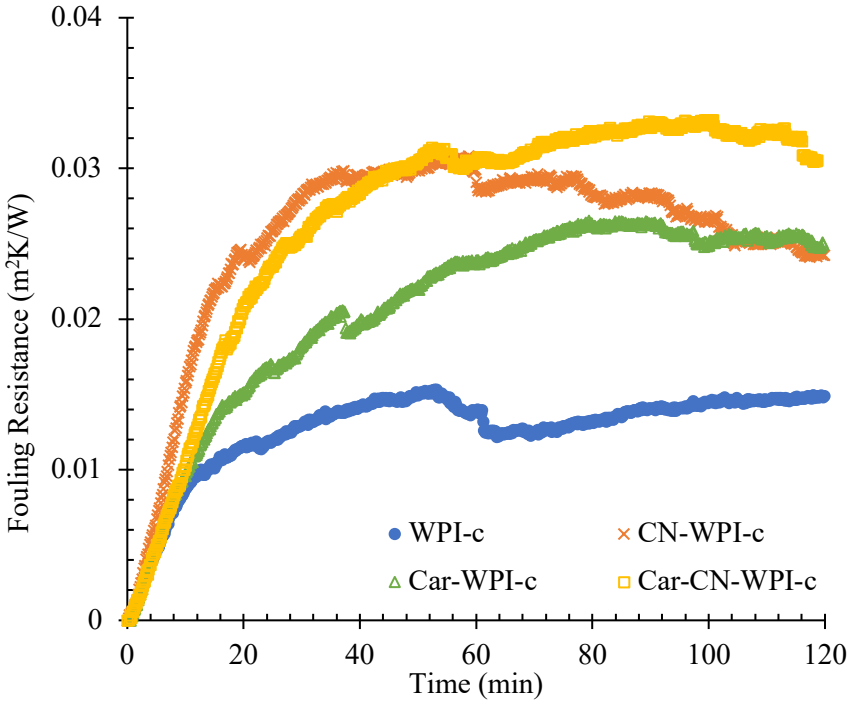


Figure 4-1 continued

(c)



4.4.1.2. Deposit analysis

Since the mass of the freshly formed (wet) deposits cannot be measured very accurately with our experimental set-up due to the large variation in the amount of liquid droplets remaining on the deposit surface which cannot be well controlled. Therefore, the dry mass of the deposit collected after the 2-h fouling test was presented in this study, as shown in Table 4-1. Although CN addition to WPI solution resulted in a higher R_f at the end of the 2-h test (Figure 4-1), it decreased the deposit mass by 32%. Similarly, Car-CN-WPI solution formed slightly less deposit than Car-WPI solution. In contrast, following the trend observed in the R_f results, adding Car to both the WPI and CN-WPI solutions generated more deposits, although the increase of deposit mass (15−21%) was not proportional to that of R_f. It has to point out that the R_f was obtained via in situ measurement (at where the heat flux sensors are located) for wet deposits, which is a function of wet deposit mass, and deposit density and thermal conductivity, and thus does not directly correlate with the dry deposit mass.

Figure 4-2 shows the SEM images of the deposit microstructure. Although the four deposits have distinct appearances, it is clear that they all showed porous structures, indicating that the deposits consisted of a continuous solid matrix with liquid entrained in pores. Compared to the WPI deposits that only a few small fractures were observed (Figure 4-2a), adding CN to WPI solution formed a largely fractured matrix (Figure 4-2b), which well supports the lowest deposit mass obtained as well as the marked sloughing observed in the fouling curves (Figure 4-1). On the other hand, with Car added, both Car-WPI (Figure 4-2c) and Car-CN-WPI (Figure 4-2d) deposits were more compact and no fracture was observed. This explains their higher mass and also results in higher cohesive strength to mitigate deposit removal by shear during fouling, compared to WPI and CN-WPI deposits, respectively (Figure 4-1). Moreover, Car-CN-WPI deposits contained larger pores than Car-WPI deposits, hence had a lower mass.

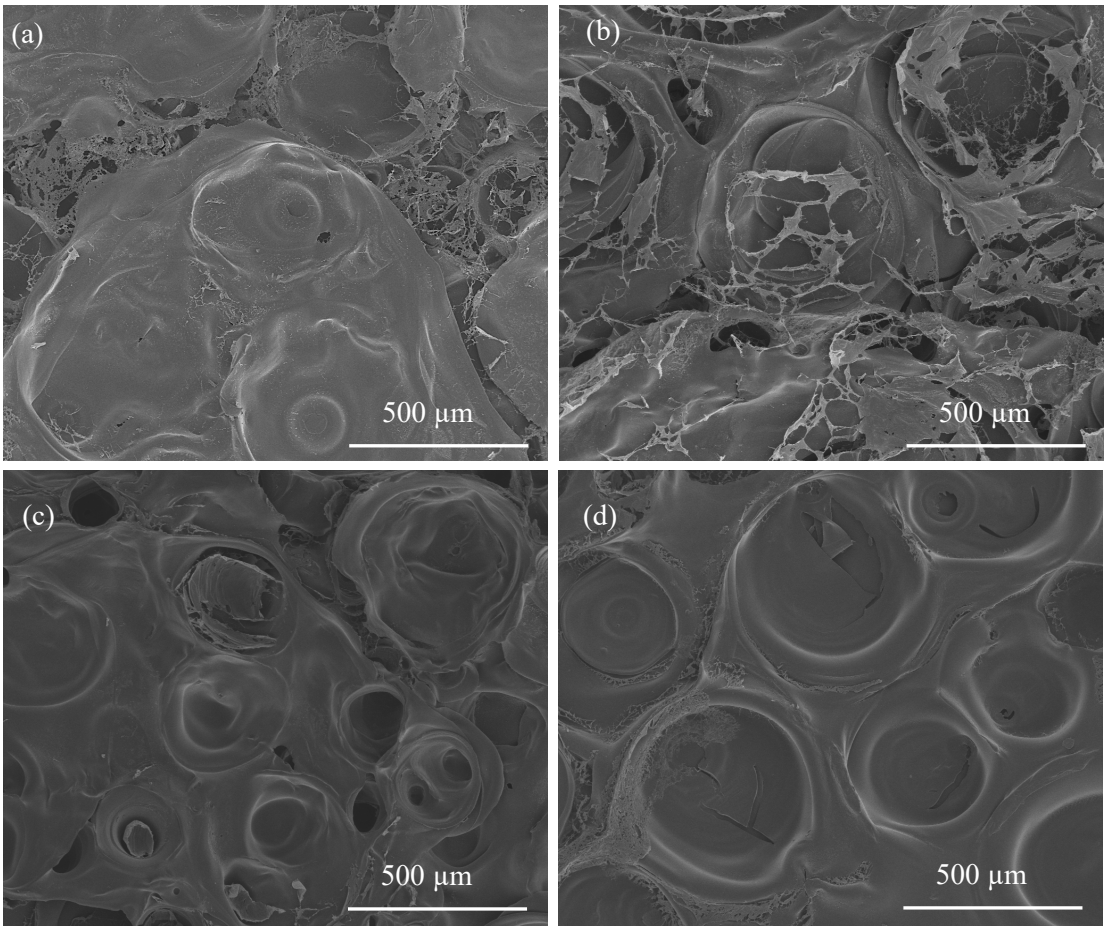


Figure 4-2 SEM images of deposits of model solutions: (a) 2% WPI; (b) 2% WPI +0.5% Casein; (c) 2% WPI +0.03% Carrageenan; (d) 2% WPI +0.5% Casein +0.03% Carrageenan

4.4.2 Effect of casein on whey stability

To explain the changes in the fouling characteristics induced by CN addition, the effect of CN on the thermal stability of WPI was examined. As whey protein denaturation is generally recognized as the controlling mechanism in milk fouling (Bansal and Chen, 2006; Blanpain-Avet et al., 2012; de Jong, 1997; Jimenez et al., 2013), the denaturation temperature (T_m) and enthalpy change (ΔH) of mixtures with the same ratio of WPI, CN and Car to the model solutions were determined by DSC analysis, as shown in Table 4-2. Single endothermic peaks centered between 72 and 75 ºC were observed for the mixtures tested that corresponded to β-lg denaturation (Fitzsimons et al., 2007). However, denaturation of CN was not detected over the temperature range analyzed (25−95 ºC). Adding CN to WPI solution significantly decreased the T_m from 73.29 to 72.05 ºC, and also the ΔH by 41%. These decreases resulting from the presence of CN suggest that CN may promote β-lg denaturation by making β-lg less thermally stable. This result agreed with Cho et al. (2003) that β-lg loses its native-like structure faster when CN is present. There was also a significant decrease in T_m when CN was added to Car-WPI mixture.

Representative fluorescence spectra of heated and unheated WPI and CN-WPI solutions at excitation wavelength of 272 nm are presented in Figure 4-3a. When heated, the peak fluorescence intensity of WPI and CN solutions increased by 20% and 38 %, respectively, the former also had a red-shift of the peak wavelength by 4 nm. Although adding CN to WPI solution did not change its spectra before heating, thermal treatment caused a larger increase in peak intensity to CN-WPI (28%) than WPI solution. Tryptophan residue is an intrinsic fluorophore and can be used as a sensitive indicator of structural changes of protein by showing different peak florescence wavelength and intensity (Rahimi Yazdi and Corredig, 2012). In β-lg, two tryptophan residues contribute to its fluorescence intensity, Trp-19 buried in the hydrophobic interior, and Trp-61 exposed to surrounding environment (Eftink, 2000; Elshereef et al., 2006). The increase in fluorescence intensity and red-shift of the spectra peak observed indicate more tryptophan exposed to the environment caused by β-lg denaturation after heating (Palazolo et al., 2000). CN also has a number of tryptophan residues. β-casein and κ-casein contain one Trp (143 and 76), α_{s1}- and α_{s2}-casein have two Trp at positions 164/199 and 109/193, respectively. The increased fluorescence intensity of CN solution observed following thermal treatment could be attributed to

a tertiary structural change of CN which represents its denaturation. CN denaturation, however, was not detected in the DSC analysis, which could be because of the limitation of its sensitivity. The decreased T_m and ΔH as well as the increased fluorescence intensity due to the presence of CN suggest that CN-WPI solution is more susceptible to heat-induced denaturation. These findings also prove the participation of CN in fouling and can explain the higher initial fouling rate of CN-WPI solution than that of WPI solution (Figure 4-1). However, there is no direct evidence of interaction between WPI and CN during fouling formation.

Table 4-2 Denaturation temperature (T_m) and enthalpy change (ΔH) of milk protein and carrageenan mixtures

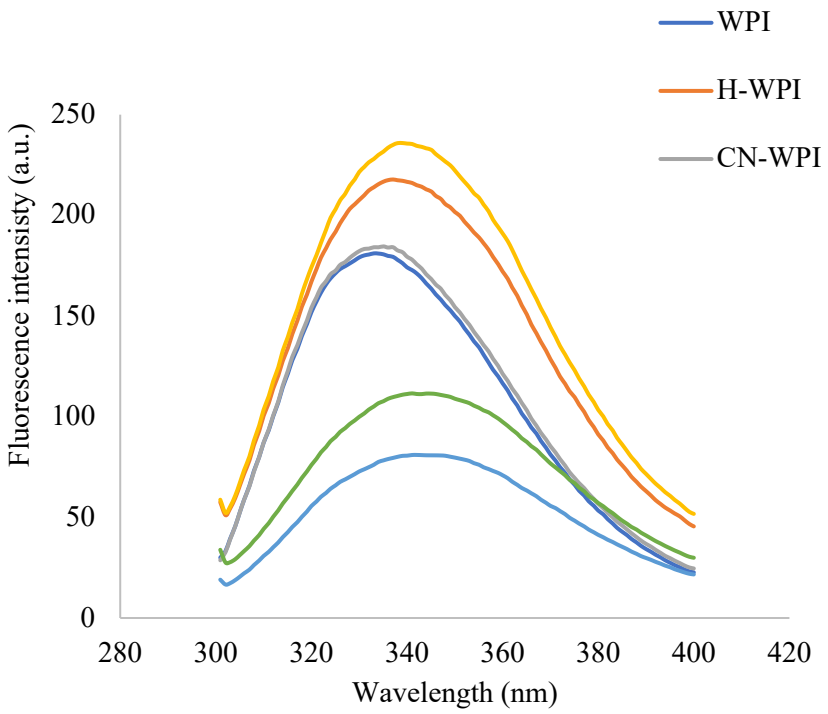
	T_m (°C)	ΔH (J/g)
WPI[1]	73.29 ± 0.14[a]	0.76 ± 0.10[a]
CN-WPI[1]	72.05 ± 0.21[b]	0.45 ± 0.06[b]
Car-WPI[1]	73.52 ± 0.16[a]	0.63 ± 0.09[a]
Car-CN-WPI[1]	72.83 ± 0.40[c]	0.76 ± 0.13[a]

[1]WPI: 10% WPI; CN-WPI: 10% WPI +2.5% Casein; Car-WPI: 10% WPI +0.3% Carrageenan; Car-CN-WPI: 10% WPI +2.5% Casein +0.3% Carrageenan.
[2]All the values represent the mean of five replicates. Values with different letters in the same column were significantly different ($p < 0.05$).

4.4.3 Effect of carrageenan on protein stability

Car did not show a significant effect on the T_m of WPI solution (Table 4-2), which was because of the strong electrostatic repulsion between β-lg and Car at neutral pH which limits formation of complex (Jones et al. 2010). On the other hand, the T_m of CN-WPI solution increased significantly with addition of Car, suggesting an improved thermal stability. This can be related to the decreased initial fouling rate of Car-CN-WPI solution compared to CN-WPI solution, as shown in Table 4-1.

(a)



(b)

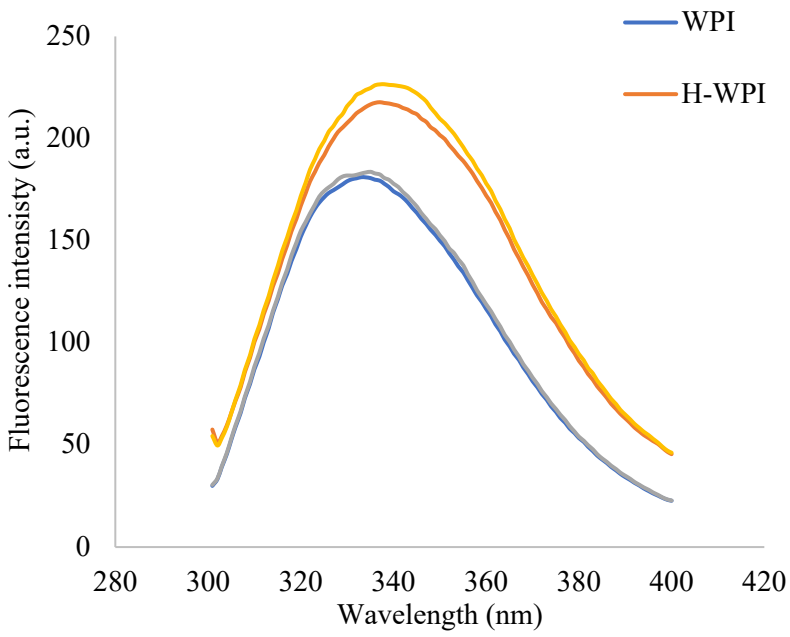


Figure 4-3 Effects of (a) CN, and (b) Car on the emission spectra of WPI solution at λ_{ex} 292 nm.

While the peak fluorescence intensity of WPI solution increased from 181 to 218 (a.u.) after heating, Car-WPI solution showed a larger increase from 183 to 227 (a.u.). When heated, Trp-61

that is normally buried in the hydrophobic core of β-lg can be exposed because of β-lg unfolding (Simion et al., 2015). This enlarged increase in fluorescence intensity by adding Car suggests Car could promote β-lg denaturation and expose more Trp. This finding agrees with the results of fouling experiments that Car-WPI solution showed higher R_f (Figure 4-1) and formed more deposits (Table 4-1) than WPI solution. Furthermore, a small red-shift of the peak fluorescence wavelength of heated WPI solution was observed after Car was added, which indicates the transfer of Trp from a hydrophobic to a more hydrophilic environment (Taheri-Kafrani et al., 2010). On the contrary, for the CN-Car solution, adding Car did not change the spectra of both unheated and heated samples (data not shown).

4.4.4 Raman spectroscopy analysis

The Raman spectra of the heated solution as well as fouling deposits of four model solutions are presented in Figure 4-4. Figure 4-4a showed the tentative assignment of Raman bands in the 500−1800 cm^{-1}region based on previous studies (Alizadeh-Pasdar et al., 2002; Blanpain-Avet et al., 2012; Howell and Li-Chan, 1996; Nonaka et al., 1993a). These bands provide qualitative information about the secondary structure and microenvironment of aromatic or hydrophobic side chains.

The major peak shown around 1660 cm^{-1} represents amid I band which relates to mostly in-plane peptide C=O stretching and partly in-plane N-H bending vibrations (Ikeda and Li-Chan, 2004). The frequency and intensity of the amide I band correspond with conformational changes in the secondary structure of proteins (Alizadeh-Pasdar et al., 2002; Seo et al., 2010). The peak at 1660 cm^{-1} relates to α-helix structure and the bands around 1670 cm^{-1} are associated with β-sheet and other disorder structures (Carew et al., 1983; Ikeda and Li-Chan, 2004). Compare to WPI solution that features a peak at 1660 cm^{-1} relating to α-helix structure, the amide I band of WPI deposits showed an obvious shift to 1670 cm^{-1}, suggesting a structural transformation of WPI to β-sheet and other disorder structures after heating and deposition (Figure 4-4a). Moreover, the increasing intensity of the 1670 cm^{-1} band during fouling formation indicated an increase in the content of β-sheet protein in the samples. Figure 4-4d compares the Raman spectra (740-960 cm^{-1}) of the

deposits from different model solutions WPI and Car-WPI deposits contained more β-sheet protein than deposits formed by samples with CN added.

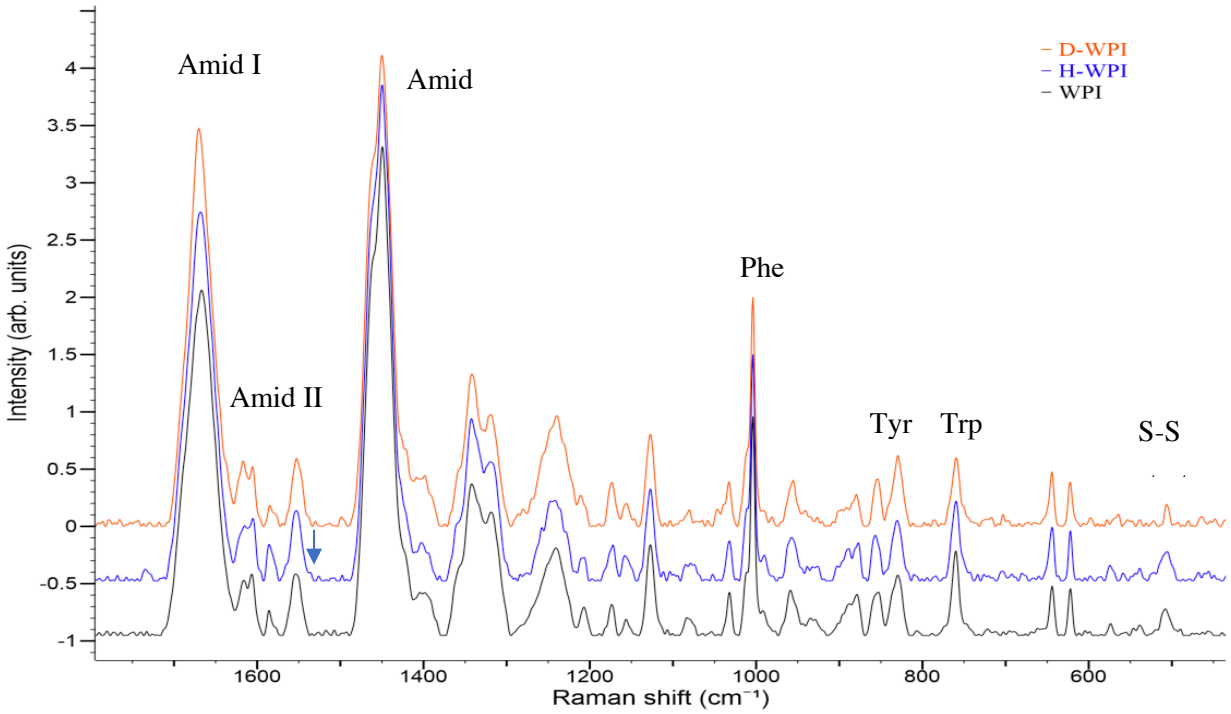
Comparing with unheated solution, their deposits showed a lower band intensity around 940 cm^{-1} (Figure 4-4c), among which Car-CN-WPI had the lowest value followed by Car-WPI and CN-WPI, while WPI showed the highest intensity. The band around 940 cm^{-1} represents the skeletal C-C stretching and its intensity is proportional to the content of α-helix protein (Howell and Li-Chan, 1996; Ikeda, 2003). As α-helix is the most heat-labile secondary structure of β-lg (Ikeda and Li-Chan, 2004), this result suggests a decrease in the heat stability of β-lg when Car and CN are present, which supports the finding that adding Car and CN increased the fouling of WPI solution, as shown in Figure 4-1.

The normalized Raman band at 760 cm^{-1} is associated with indole-ring vibrations of Trp residues and known to be sensitive to the environmental polarity of Trp (Ikeda and Li-Chan, 2004; Nonaka et al., 1993b). A lower intensity means Trp is more exposed to a polar environment rather than buried. Native WPI had the highest intensity of the 760 cm^{-1} band, indicating more buried Trp, and the intensity decreased with heating (Figure 4-4b) and deposition (Figure 4-4c). Heated WPI had the highest intensity followed by Car-WPI, CN-WPI and Car-CN-WPI, suggesting that adding Car and CN can enhance Trp exposure to the surrounding environment. As Trp exposure is regarded as an indicator of β-lg denaturation, This finding proves that the presence of Car and CN can promote β-lg denaturation and thus fouling formation, which is supported by the same trend of the 760 cm^{-1} band intensity shown by the protein structures of the deposits from different model solutions (Figure 4-4c).

Another noticeable change was the intensity ratio of 855/830 cm^{-1} (I_{855}/I_{830}) (Figure 4-4c) that reflects the nature of hydrogen bonding and ionized state of the phenolic hydroxyl group in the tyrosine (Tyr) side chain (Howell and Li-Chan, 1996; Nonaka et al., 1993b). Car-CN-WPI deposit had the highest I_{855}/I_{830} and WPI deposit had the lowest ratio among the deposit samples, suggesting that Tyr residues could be strongly hydrogen-bonded in the deposits formed with Car and CN (Ikeda and Li-Chan, 2004).

Figure 4-4 (a) Raman spectra (with tentative band assignment between 400 and 1800 cm^{-1}) of native WPI solution, heated WPI solution and WPI deposit. Truncated Raman bands of heated model solution between 740 and 860 cm^{-1} region. Truncated Raman band of deposit from different model solutions between (c) 740 and 960 cm^{-1}, and (d) 1200 and 1700 cm^{-1}.

(a)



(b)

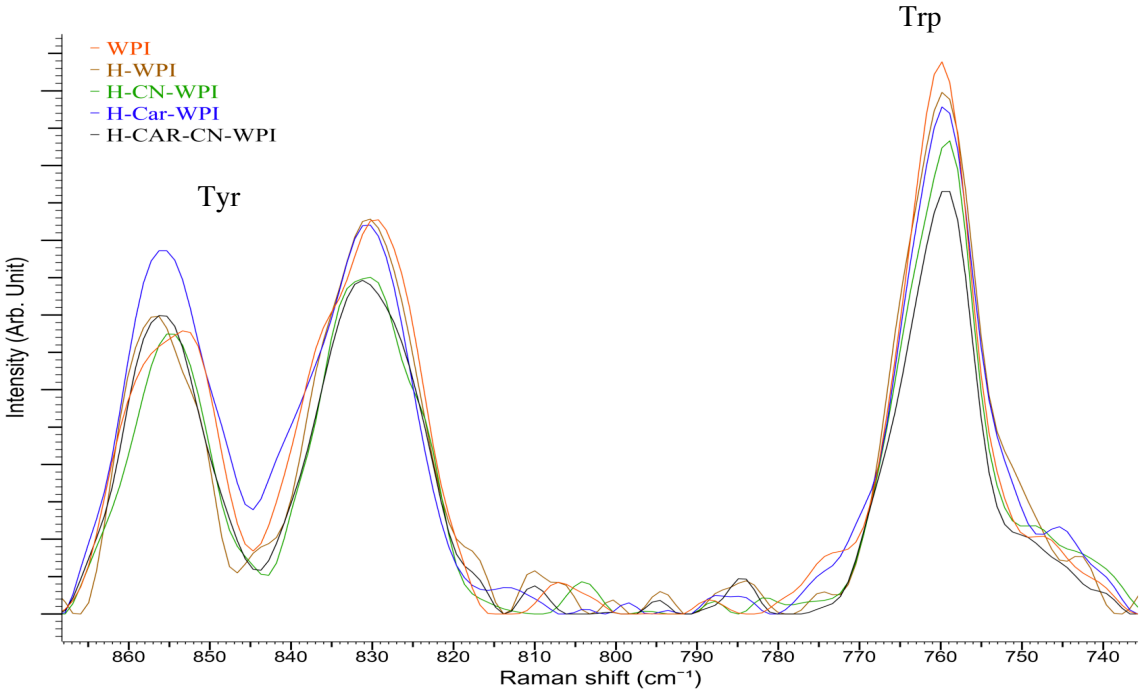
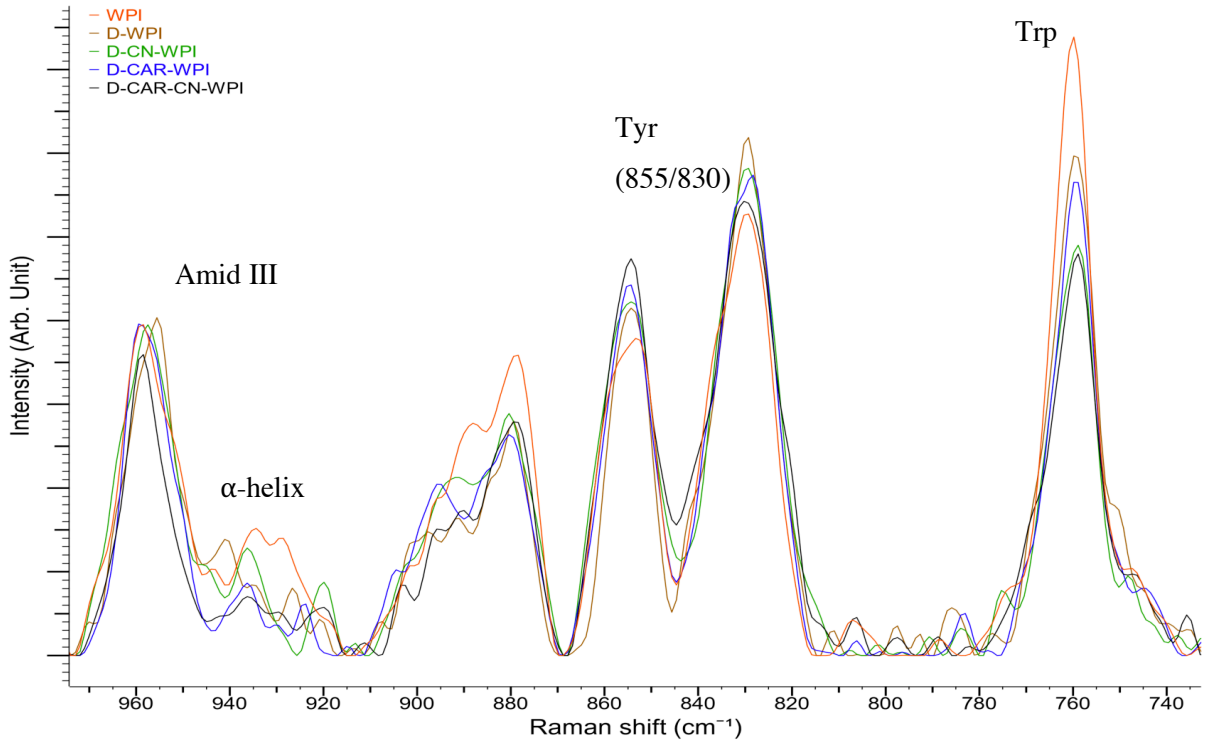
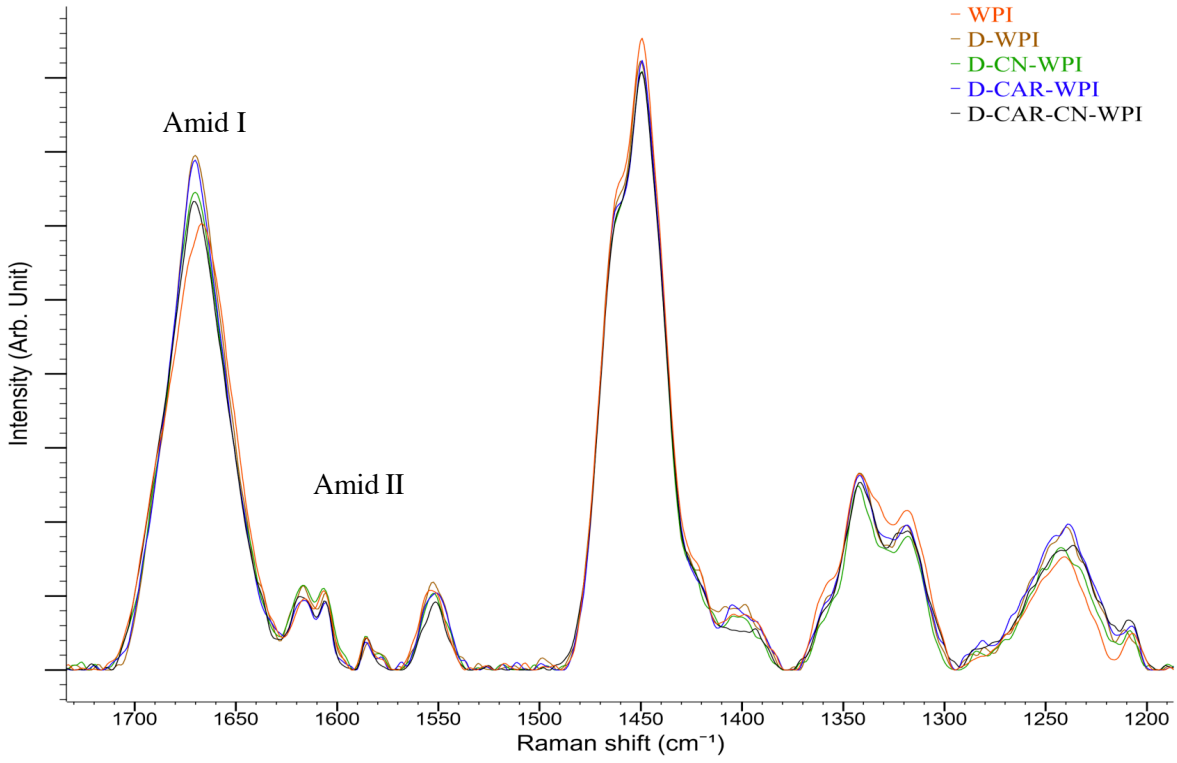


Figure 4-4 continued

(c)



(d)



4.5 Conclusion

In this study, the fouling behaviors of model dairy solutions composed of WPI (2 wt%), CN (0.5 wt%) and Car (0.03 wt%) was characterized under well-controlled temperature and flow. Adding CN to WPI solution increased the fouling resistance because CN reduced the denaturation temperature of WPI. However, CN-WPI deposit had a lower dry mass compared to WPI deposit due to its fractured structure. The addition of Car increased both the fouling resistance and deposit mass owing to the formation of more compact deposits. Fluorescence and Raman spectroscopy analyses on protein structure and Trp residues in β-lg proved that Car and CN can make β-lg less heat-stable and promote its denaturation when heated, leading to increased fouling. This study provides fundamental understanding of the interactions of CN and Car with whey under thermal processing conditions and how they affect protein fouling. This knowledge can serve as the groundwork for studying the fouling of real dairy products, and ultimately helping the food industry select proper ingredients to mitigate or prevent dairy fouling.

4.6 References

Al haj, O.A., Al Kanhal, H.A., 2010. Compositional, technological and nutritional aspects of dromedary camel milk. International Dairy Journal 20, 811–821. https://doi.org/10.1016/j.idairyj.2010.04.003

Alizadeh-Pasdar, N., Nakai, S., Li-Chan, E.C.Y., 2002. Principal Component Similarity Analysis of Raman Spectra To Study the Effects of pH, Heating, and κ-Carrageenan on Whey Protein Structure. J. Agric. Food Chem. 50, 6042–6052. https://doi.org/10.1021/jf0116956

Awad, M.M., n.d. Fouling of Heat Transfer Surfaces. Heat Transfer 41.

Bansal, B., Chen, X.D., 2006. A Critical Review of Milk Fouling in Heat Exchangers. Comprehensive Reviews in Food Science and Food Safety 5, 27–33. https://doi.org/10.1111/j.1541-4337.2006.tb00080.x

Belmar-Beiny, M.T., Gotham, S.M., Paterson, W.R., Fryer, P.J., Pritchard, A.M., 1993. The effect of Reynolds number and fluid temperature in whey protein fouling. Journal of Food Engineering 19, 119–139. https://doi.org/10.1016/0260-8774(93)90038-L

Blanpain-Avet, P., André, C., Khaldi, M., Bouvier, L., Petit, J., Six, T., Jeantet, R., Croguennec, T., Delaplace, G., 2016. Predicting the distribution of whey protein fouling in a plate heat exchanger using the kinetic parameters of the thermal denaturation reaction of β-lactoglobulin and the bulk temperature profiles. Journal of Dairy Science 99, 9611–9630. https://doi.org/10.3168/jds.2016-10957

Blanpain-Avet, P., Hédoux, A., Guinet, Y., Paccou, L., Petit, J., Six, T., Delaplace, G., 2012. Analysis by Raman spectroscopy of the conformational structure of whey proteins constituting fouling deposits during the processing in a heat exchanger. Journal of Food Engineering 110, 86–94. https://doi.org/10.1016/j.jfoodeng.2011.12.005

Boxler, C., Augustin, W., Scholl, S., 2014. Influence of surface modification on the composition of a calcium phosphate-rich whey protein deposit in a plate heat exchanger. Dairy Sci. & Technol. 94, 17–31. https://doi.org/10.1007/s13594-013-0142-5

Burova, T.V., Grinberg, N.V., Grinberg, V.Ya., Usov, A.I., Tolstoguzov, V.B., de Kruif, C.G., 2007. Conformational Changes in ι- and κ-Carrageenans Induced by Complex Formation with Bovine β-Casein. Biomacromolecules 8, 368–375. https://doi.org/10.1021/bm060761f

Bylund, Gösta., 2003. Dairy processing handbook. Tetra Pak Processing Systems AB, Lund, Sweden.

Carew, E.B., Stanley, H.E., Seidel, J.C., Gergely, J., 1983. Studies of myosin and its proteolytic fragments by laser Raman spectroscopy. Biophysical Journal 44, 219–224. https://doi.org/10.1016/S0006-3495(83)84294-5

Chandan, R.C., 2015. Dairy Processing and Quality Assurance: An Overview, in: Dairy Processing and Quality Assurance. John Wiley & Sons, Ltd, pp. 1–40. https://doi.org/10.1002/9781118810279.ch01

Changani, S.D., Belmar-Beiny, M.T., Fryer, P.J., 1997. Engineering and chemical factors associated with fouling and cleaning in milk processing. Experimental Thermal and Fluid Science 14, 392–406. https://doi.org/10.1016/S0894-1777(96)00141-0

Cho, Y., Singh, H., Creamer, L.K., 2003. Heat-induced interactions of β-lactoglobulin A and κ-casein B in a model system. Journal of Dairy Research 70, 61–71. https://doi.org/10.1017/S0022029902005642

Christian, G.K., Changani, S.D., Fryer, P.J., 2002. The Effect of Adding Minerals on Fouling from Whey Protein Concentrate: Development of a Model Fouling Fluid for a Plate Heat Exchanger. Food and Bioproducts Processing, Fouling, Cleaning and Disinfection 80, 231–239. https://doi.org/10.1205/096030802321154718

Dalgleish, D.G., Morris, E.R., 1988. Interactions between carrageenans and casein micelles: electrophoretic and hydrodynamic properties of the particles. Food Hydrocolloids 2, 311–320. https://doi.org/10.1016/S0268-005X(88)80028-6

de Jong, P., 1997. Impact and control of fouling in milk processing. Trends in Food Science & Technology 8, 401–405. https://doi.org/10.1016/S0924-2244(97)01089-3

Eftink, M.R., 2000. Intrinsic Fluorescence of Proteins, in: Lakowicz, J.R. (Ed.), Topics in Fluorescence Spectroscopy: Volume 6: Protein Fluorescence, Topics in Fluorescence Spectroscopy. Springer US, Boston, MA, pp. 1–15. https://doi.org/10.1007/0-306-47102-7_1

Elshereef, R., Budman, H., Moresoli, C., Legge, R.L., 2006. Fluorescence spectroscopy as a tool for monitoring solubility and aggregation behavior of β-lactoglobulin after heat treatment. Biotechnology and Bioengineering 95, 863–874. https://doi.org/10.1002/bit.21039

Felfoul, I., Beaucher, E., Cauty, C., Attia, H., Gaucheron, F., Ayadi, M.A., 2016. Deposit Generation During Camel and Cow Milk Heating: Microstructure and Chemical Composition. Food Bioprocess Technol 9, 1268–1275. https://doi.org/10.1007/s11947-016-1714-1

Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., Ayadi, M.A., 2015a. A laboratory investigation of cow and camel whey proteins deposition under different heat treatments. Food and Bioproducts Processing 96, 256–263. https://doi.org/10.1016/j.fbp.2015.09.002

Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., Ayadi, M.A., 2015b. Fouling Behavior of Camel and Cow Milks Under Different Heat Treatments. Food Bioprocess Technol 8, 1771–1778. https://doi.org/10.1007/s11947-015-1529-5

Fitzsimons, S.M., Mulvihill, D.M., Morris, E.R., 2007. Denaturation and aggregation processes in thermal gelation of whey proteins resolved by differential scanning calorimetry. Food Hydrocolloids 21, 638–644. https://doi.org/10.1016/j.foodhyd.2006.07.007
Gotham, S.M., Fryer, P.J., Pritchard, A.M., 1992. β-lactoglobulin denaturation and aggregation reactions and fouling deposit formation: a DSC study. International Journal of Food Science & Technology 27, 313–327. https://doi.org/10.1111/j.1365-2621.1992.tb02033.x
Hailu, Y., Hansen, E.B., Seifu, E., Eshetu, M., Ipsen, R., Kappeler, S., 2016. Functional and technological properties of camel milk proteins: a review. Journal of Dairy Research 83, 422–429. https://doi.org/10.1017/S0022029916000686
Hammann, F., Schmid, M., 2014. Determination and Quantification of Molecular Interactions in Protein Films: A Review. Materials (Basel) 7, 7975–7996. https://doi.org/10.3390/ma7127975
Hosseini, S.M.H., Emam-Djomeh, Z., Razavi, S.H., Moosavi-Movahedi, A.A., Saboury, A.A., Mohammadifar, M.A., Farahnaky, A., Atri, M.S., Van der Meeren, P., 2013. Complex coacervation of β-lactoglobulin – κ-Carrageenan aqueous mixtures as affected by polysaccharide sonication. Food Chemistry 141, 215–222. https://doi.org/10.1016/j.foodchem.2013.02.090
Howell, N., Li-Chan, E., 1996. Elucidation of interactions of lysozyme with whey proteins by Raman spectroscopy. International Journal of Food Science & Technology 31, 439–451. https://doi.org/10.1046/j.1365-2621.1996.00364.x
Huang, J.-Y., John Chew, Y.M., Ian Wilson, D., 2012. A spinning disc study of fouling of cold heat transfer surfaces by gel formation from model food fat solutions. Journal of Food Engineering 109, 49–61. https://doi.org/10.1016/j.jfoodeng.2011.09.034
Huang, K., Goddard, J.M., 2015. Influence of fluid milk product composition on fouling and cleaning of Ni–PTFE modified stainless steel heat exchanger surfaces. Journal of Food Engineering 158, 22–29. https://doi.org/10.1016/j.jfoodeng.2015.02.026
Ibrahim, H.A.-H., 2012. Fouling in Heat Exchangers. MATLAB - A Fundamental Tool for Scientific Computing and Engineering Applications - Volume 3. https://doi.org/10.5772/46462
Ikeda, S., 2003. Heat-induced gelation of whey proteins observed by rheology, atomic force microscopy, and Raman scattering spectroscopy. Food Hydrocolloids, 6th International Hydrocolloids Conference 17, 399–406. https://doi.org/10.1016/S0268-005X(03)00033-X
Ikeda, S., Li-Chan, E.C.Y., 2004. Raman spectroscopy of heat-induced fine-stranded and particulate β-lactoglobulin gels. Food Hydrocolloids 18, 489–498. https://doi.org/10.1016/j.foodhyd.2003.07.003
Jeurnink, T.J.M., Kruif, K.G.D., 1993. Changes in milk on heating: viscosity measurements. Journal of Dairy Research 60, 139–150. https://doi.org/10.1017/S0022029900027461
Jimenez, M., Delaplace, G., Nuns, N., Bellayer, S., Deresmes, D., Ronse, G., Alogaili, G., Collinet-Fressancourt, M., Traisnel, M., 2013. Toward the understanding of the interfacial dairy fouling deposition and growth mechanisms at a stainless steel surface: A multiscale approach. Journal of Colloid and Interface Science 404, 192–200. https://doi.org/10.1016/j.jcis.2013.04.021
Jones, O., Decker, E.A., McClements, D.J., 2010. Thermal analysis of β-lactoglobulin complexes with pectins or carrageenan for production of stable biopolymer particles. Food Hydrocolloids 24, 239–248. https://doi.org/10.1016/j.foodhyd.2009.10.001

Joyce, A.M., Kelly, A.L., O'Mahony, J.A., 2018. Controlling denaturation and aggregation of whey proteins during thermal processing by modifying temperature and calcium concentration. International Journal of Dairy Technology 71, 446–453. https://doi.org/10.1111/1471-0307.12507

Kazi, S.N., 2012. Fouling and Fouling Mitigation on Heat Exchanger Surfaces. Heat Exchangers - Basics Design Applications. https://doi.org/10.5772/32990

Khaldi, M., Croguennec, T., André, C., Ronse, G., Jimenez, M., Bellayer, S., Blanpain-Avet, P., Bouvier, L., Six, T., Bornaz, S., Jeantet, R., Delaplace, G., 2018. Effect of the calcium/protein molar ratio on β-lactoglobulin denaturation kinetics and fouling phenomena. International Dairy Journal 78, 1–10. https://doi.org/10.1016/j.idairyj.2017.10.002

Khaldi, M., Ronse, G., André, C., Blanpain-Avet, P., Bouvier, L., Six, T., Bornaz, S., Croguennec, T., Jeantet, R., Delaplace, G., 2015. Denaturation Kinetics of Whey Protein Isolate Solutions and Fouling Mass Distribution in a Plate Heat Exchanger [WWW Document]. International Journal of Chemical Engineering. https://doi.org/10.1155/2015/139638

Lv, H., Huang, S., Mercadé-Prieto, R., Wu, X.E., Chen, X.D., 2015. The effect of pre-adsorption of OVA or WPC on subsequent OVA or WPC fouling on heated stainless steel surface. Colloids and Surfaces B: Biointerfaces 129, 154–160. https://doi.org/10.1016/j.colsurfb.2015.03.042

Müller-Steinhagen, H., Malayeri, M.R., Watkinson, A.P., 2009. Heat Exchanger Fouling: Environmental Impacts. Heat Transfer Engineering 30, 773–776. https://doi.org/10.1080/01457630902744119

Nigo, R.Y., Chew, Y.M.J., Houghton, N.E., Paterson, W.R., Wilson, D.I., 2009. Experimental Studies of Freezing Fouling of Model Food Fat Solutions Using a Novel Spinning Disc Apparatus. Energy Fuels 23, 6131–6145. https://doi.org/10.1021/ef900668f

Nonaka, Masahiko., Li-Chan, Eunice., Nakai, Shuryo., 1993a. Raman spectroscopic study of thermally induced gelation of whey proteins. J. Agric. Food Chem. 41, 1176–1181. https://doi.org/10.1021/jf00032a002

Nonaka, Masahiko., Li-Chan, Eunice., Nakai, Shuryo., 1993b. Raman spectroscopic study of thermally induced gelation of whey proteins. J. Agric. Food Chem. 41, 1176–1181. https://doi.org/10.1021/jf00032a002

O'Kennedy, B.T., Mounsey, J.S., 2009. The dominating effect of ionic strength on the heat-induced denaturation and aggregation of β-lactoglobulin in simulated milk ultrafiltrate. International Dairy Journal 19, 123–128. https://doi.org/10.1016/j.idairyj.2008.09.004

Palazolo, G., Rodríguez, F., Farruggia, B., Picó, G., Delorenzi, N., 2000. Heat Treatment of β-Lactoglobulin: Structural Changes Studied by Partitioning and Fluorescence. J. Agric. Food Chem. 48, 3817–3822. https://doi.org/10.1021/jf991353o

Papiz, M.Z., Sawyer, L., Eliopoulos, E.E., North, A.C.T., Findlay, J.B.C., Sivaprasadarao, R., Jones, T.A., Newcomer, M.E., Kraulis, P.J., 1986. The structure of β -lactoglobulin and its similarity to plasma retinol-binding protein. Nature 324, 383–385. https://doi.org/10.1038/324383a0

Prakash, S., Huppertz, T., Karvchuk, O., Deeth, H., 2010. Ultra-high-temperature processing of chocolate flavoured milk. Journal of Food Engineering 96, 179–184. https://doi.org/10.1016/j.jfoodeng.2009.07.008

Rahimi Yazdi, S., Corredig, M., 2012. Heating of milk alters the binding of curcumin to casein micelles. A fluorescence spectroscopy study. Food Chemistry 132, 1143–1149. https://doi.org/10.1016/j.foodchem.2011.11.019
Rosmaninho, R., Melo, L.F., 2008. Protein–calcium phosphate interactions in fouling of modified stainless-steel surfaces by simulated milk. International Dairy Journal 18, 72–80. https://doi.org/10.1016/j.idairyj.2007.06.008
Rosmaninho, R., Melo, L.F., 2006. Calcium phosphate deposition from simulated milk ultrafiltrate on different stainless steel-based surfaces. International Dairy Journal 16, 81–87. https://doi.org/10.1016/j.idairyj.2005.01.006
Sadeghinezhad, E., Kazi, S.N., Dahari, M., Safaei, M.R., Sadri, R., Badarudin, A., 2015. A Comprehensive Review of Milk Fouling on Heated Surfaces. Critical Reviews in Food Science and Nutrition 55, 1724–1743. https://doi.org/10.1080/10408398.2012.752343
Schreier, P.J.R., Fryer, P.J., 1995. Heat exchanger fouling: A model study of the scaleup of laboratory data. Chemical Engineering Science 50, 1311–1321. https://doi.org/10.1016/0009-2509(95)98843-4
Seo, J.-A., Hédoux, A., Guinet, Y., Paccou, L., Affouard, F., Lerbret, A., Descamps, M., 2010. Thermal Denaturation of Beta-Lactoglobulin and Stabilization Mechanism by Trehalose Analyzed from Raman Spectroscopy Investigations. J. Phys. Chem. B 114, 6675–6684. https://doi.org/10.1021/jp1006022
Simion (Ciuciu), A.-M., Aprodu, I., Dumitraşcu, L., Bahrim, G.E., Alexe, P., Stănciuc, N., 2015. Exploring the heat-induced structural changes of β-lactoglobulin -linoleic acid complex by fluorescence spectroscopy and molecular modeling techniques. J Food Sci Technol 52, 8095–8103. https://doi.org/10.1007/s13197-015-1949-2
Simmons, M.J.H., Jayaraman, P., Fryer, P.J., 2007. The effect of temperature and shear rate upon the aggregation of whey protein and its implications for milk fouling. Journal of Food Engineering 79, 517–528. https://doi.org/10.1016/j.jfoodeng.2006.02.013
Spagnuolo, P.A., Dalgleish, D.G., Goff, H.D., Morris, E.R., 2005. Kappa-carrageenan interactions in systems containing casein micelles and polysaccharide stabilizers. Food Hydrocolloids, Food Colloids 2004 (Harrogate) 19, 371–377. https://doi.org/10.1016/j.foodhyd.2004.10.003
STEINHAGEN, R., MÜLLER-STEINHAGEN, H., MAANI, K., 1993. Problems and Costs due to Heat Exchanger Fouling in New Zealand Industries. Heat Transfer Engineering 14, 19–30. https://doi.org/10.1080/01457639308939791
Taheri-Kafrani, A., Asgari-Mobarakeh, E., Bordbar, A.-K., Haertlé, T., 2010. Structure-function relationship of beta-lactoglobulin in the presence of dodecyltrimethyl ammonium bromide. Colloids Surf B Biointerfaces 75, 268–274. https://doi.org/10.1016/j.colsurfb.2009.08.045
Vasbinder, A.J., Alting, A.C., de Kruif, K.G., 2003. Quantification of heat-induced casein–whey protein interactions in milk and its relation to gelation kinetics. Colloids and Surfaces B: Biointerfaces, Food Colloids, Biopolymers and Materials Special Issue 31, 115–123. https://doi.org/10.1016/S0927-7765(03)00048-1
Visser, J., Jeurnink, Th.J.M., 1997. Fouling of heat exchangers in the dairy industry. Experimental Thermal and Fluid Science 14, 407–424. https://doi.org/10.1016/S0894-1777(96)00142-2
Wallhäußer, E., Hussein, M.A., Becker, T., 2012. Detection methods of fouling in heat exchangers in the food industry. Food Control 27, 1–10. https://doi.org/10.1016/j.foodcont.2012.02.033

Weinbreck, F., Nieuwenhuijse, H., Robijn, G.W., de Kruif, C.G., 2004. Complexation of Whey Proteins with Carrageenan. J. Agric. Food Chem. 52, 3550–3555. https://doi.org/10.1021/jf034969t

Wong, N.P., 1988. Fundamentals of Dairy Chemistry, 3rd ed. Springer US. https://doi.org/10.1007/978-1-4615-7050-9

Yang, W., Li, D., Chen, X.D., Mercadé-Prieto, R., 2018. Effect of calcium on the fouling of whey protein isolate on stainless steel using QCM-D. Chem Eng Sci 177, 501–508. https://doi.org/10.1016/j.ces.2017.12.004

Zhang, B.Y., Lu, J., Huang, J.-Y., 2019. Effect of sugar on the fouling behavior of whey protein. Food and Bioproducts Processing, Fouling and Cleaning in Food Processing 113, 2–9. https://doi.org/10.1016/j.fbp.2018.10.002

Zouaghi, S., Barry, M.E., Bellayer, S., Lyskawa, J., André, C., Delaplace, G., Grunlan, M.A., Jimenez, M., 2018a. Antifouling amphiphilic silicone coatings for dairy fouling mitigation on stainless steel. Biofouling 34, 769–783.

Zouaghi, S., Six, T., Bellayer, S., Coffinier, Y., Abdallah, M., Chihib, N.-E., André, C., Delaplace, G., Jimenez, M., 2018b. Atmospheric pressure plasma spraying of silane-based coatings targeting whey protein fouling and bacterial adhesion management. Applied Surface Science 455, 392–402. https://doi.org/10.1016/j.apsusc.2018.06.006

CHAPTER 5. CONCLUSIONS AND RECOMMENDED FUTURE WORK

5.1 Conclusions

This dissertation has reported an investigation of milk protein and carbohydrate interactions and their effects on fouling process. The studies were carried out with whey protein-based model solutions and real milks using a spinning disc apparatus where fouling resistance was monitored under controlled temperature and flow. Although the key role that β-lactoglobulin denaturation of plays in the fouling of complex model systems is confirmed, β-lactoglobulin-free camel milk also formed significant fouling because of the activation of free thiol in serum albumin and α-lactalbumin upon heating. Furthermore, stabilized β-lactoglobulin was observed with presence of simple sugars that resulted in reduced fouling. In contrast, additions of casein and carrageenan make β-lactoglobulin less heat-stable and promote its denaturation, leading to increased fouling. This dissertation has expanded the scope of β-lg- and calcium-focused fouling studies to investigate more complex dairy systems (i.e. different protein distributions, addition of different carbohydrates). The results obtained are expected to guide future research efforts to systematically increase the complexity of model dairy solutions and ultimately elucidate the fouling mechanisms of different milks and dairy products. This fundamental understanding is important to help the dairy industry develop and implement proper preventive and remediative strategies against fouling problem.

5.2 Recommended future work

Based on the findings of this study, the recommendations for future work include:

1. Investigate the effect of individual milk protein (e.g. α-la, SA) on milk fouling to further refine the proposed fouling mechanism.

2. Investigate the interaction between carbohydrates and milk proteins at different concentration ratios and their concentration effect on fouling.

3. Systematically study the effect of concentration ratio of casein to whey protein on milk fouling with the presence of calcium in the system.

4. Based on the developed model dairy solutions, adding other types of polysaccharides such as starch and pectin to study their interactions with milk protein and effects on dairy fouling.

5. Conduct fouling experiments under UHT processing conditions to study the effect of carbohydrates on type B fouling.

6. Extend the knowledge gained from this study to conduct fouling experiments using a pilot-scale plate heat exchanger to better simulate industrial processing scenarios.