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Assessing Impact of Food Structure on Oral Tribology and In-Vitro Digestion of Dairy Products

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ASSESSING IMPACT OF FOOD STRUCTURE ON ORAL TRIBOLOGY AND IN-VITRO DIGESTION OF DAIRY PROTEINS

by

Lamis Ali

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ABSTRACT

Assessing Impact of Food Structure on Oral Tribology and In-vitro Digestion of Dairy Proteins

by

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Utah State University, 2024

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Texture, mouthfeel, and digestibility of dairy products contribute to consumer satisfaction and nutrient efficiency. In this study, we have divided our research into two main objectives. First, we assessed the tribological properties of eight different types of commercial dairy products. For the second objective, we investigated the disintegration and protein release in selected three dairy products. Eight different dairy products were assessed; solid (cheddar, cheese curd, Parmesan), semi-solid (cottage cheese, ricotta cheese, yogurt), and liquid (milk, whey protein beverage). Samples were tested for tribological behavior, particle size analysis, SDS PAGE. Tribology analysis was conducted on an MCR-302 rheometer to evaluate mouth feel and breakdown properties for the eight samples. Particle size distribution was measured using (Anton Paar 1190 model, Graz, Austria) to investigate the particle size effect on mouthfeel. Data showed distinct differences between samples based on the Stribeck curve at several sliding speeds. Interestingly, liquid dairy products exhibited significantly higher friction ($p<0.05$) compared to semi solid and solid dairy products. This observation could be attributed to variations in the food structures, protein, and moisture content of the products.

Subsequentially, three dairy products (Cheddar cheese, ricotta cheese and milk) with distinct structures were selected for in-vitro digestion using the INFOGEST protocol to evaluate the disintegration and protein release at different time intervals in gastric and intestinal phase. Results displayed a clear distinction between liquid semi solid, and solid samples during in-vitro digestion at different time points (G0, G 15, G 30, G 60, I 5, I 30. I 60, I 120, I 180). For instance, liquid dairy products, such as milk, demonstrated a significantly faster release of soluble protein compounds in the gastric phase compared to solid dairy products like cheddar cheese. We attribute these differences in protein breakdown rates during in-vitro digestion to variations in protein content and food structure. By evaluating both tribological properties and in-vitro digestibility, this research provides valuable insights for the food industry to better understand texture, mouthfeel, and protein release mechanisms in dairy products. Such knowledge will aid in the development of new dairy products, improve nutritional quality, and enhance consumer satisfaction.

(123 Pages)

PUBLIC ABSTRACT

Assessing Impact of Food Structure on Oral Tribology and In-Vitro Digestion of Dairy Proteins

Lamis Ali

In this research, we focused on understanding the critical elements impacting consumer experience and the nutritional value of dairy products, specifically their texture, mouthfeel, and protein breakdown in the gastrointestinal tract. Our study aimed to accomplish two main goals. First, we performed analysis of the tribological attributes of various commercially dairy products. The second objective was to investigate the process of disintegration and protein release in selected dairy products.

The study included an assessment of eight dairy products of varied consistencies: solid like cheddar, cheese curd, and parmesan; semi-solid such as cottage cheese, ricotta cheese, and yogurt; and liquid represented by milk and a whey protein beverage. To achieve our objectives, we used variety of techniques like tribology, particle size examination, and SDS PAGE. We measured the samples' mouthfeel via tribology. Furthermore, we examined the particle size distribution, a significant factor affecting mouthfeel.

The findings indicate differences among the samples based on the Stribeck curve at various sliding speeds. Liquid dairy products displayed considerably higher friction ($p<0.05$) compared to semi-solid and solid dairy items, likely due to their unique food structures, protein levels, and moisture content.

The second phase involved the in-vitro digestion of three structurally distinct dairy products using the INFOGEST protocol. The purpose was to understand the process of disintegration and protein

release during the gastric and intestinal phases at specific time intervals. The outcomes highlighted the differences among liquid, semi-solid, and solid samples at different stages of the in-vitro digestion process (G0, G15, G30, G60, I5, I30, I60, I120, I180). For instance, liquid dairy items, such as milk, displayed a significantly quicker release of soluble protein compounds during the gastric phase when compared to solid dairy products like cheddar cheese. These varied protein degradation rates were primarily due to the differences in protein content and food structure.

By examining the tribological properties and in-vitro digestibility of dairy products, this study offers valuable insights into the mechanics of texture, mouthfeel, and protein release. The findings of this research can help food industry professionals develop innovative dairy products, augment their nutritional worth, and boost consumer satisfaction.

This thesis is dedicated to my daughter, Shaam AbuZwayyed, my mom Badria Abdo, and my dad Adnan Manassra

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Lamis Ali.

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LIST OF ABBREVIATIONS

AAs	= Amino Acids
N	= normal force
PSA	= Particle size analysis
D (4,3)	= volume weighted mean particle size.
SSF	= Simulated Salivary Fluid
SGF	= Simulated Gastric Fluid
SIF	= Simulated Intestinal Fluid
HCl	= hydrochloric acid
$CaCl_2(H2O)_2$	= Calcium chloride dihydrate
GP	= Gastric phase
IP	=Intestinal phase
CN	= Casein

CHAPTER 1

INTRODUCTION

Food digestibility and protein release in the human gastrointestinal system is governed by both food texture and food composition including protein and fat content. For example, soft cheeses should disintegrate faster than hard and elastic cheeses in the oral and gastrointestinal phase due to their composition and texture. In addition to texture, structural attributes of the food materials can play an important role in protein digestion and nutrient delivery. Apart from this, protein types such soluble, colloidal, acid coagulated, and rennet coagulated can have a strong influence on protein breakdown in the gastrointestinal tract. For example, liquid milk proteins are digested more quickly than rennet gels with the same composition (Lorieau et al., 2018). Therefore, significant impact of food structure on rate of protein breakdown and nutrient delivery is anticipated.

Many physico-chemical changes take place during the processing of milk, which have a big impact on the structure and composition of the final dairy products. Important variables like pH and temperature also have an impact on structure and functionality of dairy products. Casein and whey protein, the two main proteins found in milk, account for a sizeable amount of their composition. Casein makes up 80% of all milk proteins while whey protein makes up the remaining 20%. The properties and functionality of diverse dairy products are greatly influenced by proportion of these proteins and processing conditions. Dairy products, such as cheese, yogurt, and milk, provide significant amounts of essential nutrients to the consumers, in the form of

protein, calcium, vitamins, and minerals (Fulgoni et al., 2011). Furthermore, the appeal of these dairy products isn't just limited to their nutritional value, they also offer a rich taste and texture that many consumers find enjoyable. According to several studies, food purchase and intake is influenced by the appearance and texture of food (Rioux, Turgeon, et al., 2012), such as creaminess, thickness, brittleness, sliminess, stickiness, etc. (Wijk, Priz, et al., 2006). Unique food matrix structures can also affect digestion and absorption of these nutrients (Sharma et al., 2020). Therefore, it is important to consider the impact of food structure on digestion and nutrient release.

Food digestion is a complex process that starts with physical breakdown of the food in the oral phase followed by enzymatic breakdown in the gastric tract and ends up with maximum breakdown of proteins, fat, and carbohydrates in the intestinal phase where most of the food absorption takes place (Fang et al., 2016). While eating, food is exposed to complex steps in the oral phase, such as mastication, chewing, mixing with saliva, and finally formation of a bolus ready for swallowing (Brodkorb et al., 2019; Prakash, Tan, et al., 2013). As the food breakdown starts in the mouth, texture, and mouthfeel during food consumption is one of the vital elements that contribute significantly towards consumer satisfaction. However, this can also impact the overall food breakdown in the gastro-intestinal tract. Another factor that affects sensorial perception is the interacting surfaces between oral surfaces and food particles, including tongue, palate, teeth, and cheeks (Nguyen et al., 2017). Those interactions can be understood by studying their frictional behavior by simulating interacting surfaces inside the mouth during oral processing through tribological measurements (Sharma et al., 2022). Tribology is the study of friction, wear, and lubrication that helps explain the phenomena involving interacting surfaces that are in relative motion. Hence, it is helpful to investigate tribological properties of different food materials with varying compositions (Chen and Stokes, 2012). Tribology can be followed by the performing of

in vitro digestion (INFOGEST) which is a standardized method used to mimic in vivo food digestion. The purpose of this method is to help understand the impact of food composition and structure on human health, by studying the relationship between food composition, structure, and their nutrient release, through simulating the physiological status of the upper gastrointestinal tract, including oral, gastric, and small intestine stages (Brodkorb et al., 2019). Though there are some studies on studying digestion of dairy products, however, it is still unclear if consuming dietary proteins in various structural configurations impacts the delivery of nutrients, the bioavailability of dietary components and overall human health. Assessing the impact of food structure on in-vitro digestion is a current research focus.

Therefore, this research aims at understanding how differences in food structure in different dairy products would affect the breakdown and rate of release of the proteins under simulated gastrointestinal conditions

Research Hypothesis

Overall hypothesis: The diverse range of dairy products, encompassing liquid, semi-solid, and solid forms, exhibits varying structural properties due to the type (casein or whey) and state of protein (native, renneted, or coagulated) present in these products. We anticipate that these structural differences will contribute to distinct rates of protein digestion and nutrient release within the gastrointestinal tract, following the mechanical breakdown during the oral phase. Thus, it is hypothesized that changes in food structure may directly impact the efficiency and degree of in-vitro digestion in dairy products.Cheddar, as a solid cheese with a compact protein network, is expected to display a slower rate of protein digestion since the gastrointestinal enzymes require additional time to break down the protein matrix, due to its solid physical state and the dense protein network. Conversely, milk, being a liquid with a relatively homogeneous dispersion of both casein micelles and whey proteins, is likely to exhibit a more rapid rate of protein digestion and nutrient compared to cheddar. This is due to the ease with which gastrointestinal enzymes can access and act upon the dispersed proteins in milk.

Objectives

1. Study the mechanical properties, such as rheology, texture, and tribological properties of the selected dairy products
2. Study the impact of food proteins on in-vitro digestibility of proteins and their rate of release.

CHAPTER 2

LITERATURE REVIEW

Importance of dairy products

Milk and dairy products are one of the most essential foods for all ages, such as infants, young adults, and elderly. They provide our body with essential amino acids, essential fatty acids, enough calcium phosphate, and a good energy source due to their sugar and fat content (Kubicová, Predanocyová, Kádeková et al., 2019). According to a study published by Wolfe et al. (2017), Dairy proteins, in particular whey and casein are high-quality proteins that provide essential amino acids for human nutrition and promoting muscle protein synthesis.

The basic principles of food production involve a series of steps such as determining food composition, processing foods, developing food formulations, and maintaining optimum storage conditions (Chen et al., 2015). Food composition has a significant impact on nutrient quality evaluation. However, it is unknown whether food items with similar compositions, including proteins, carbohydrates, lipids, vitamins, and minerals, but with different delivery structure will have the same nutritional values or not (Lamothe et al., 2017). Health improvement and energy requirements rely on good nutrition especially for elderly people, as it is recommended to increase the intake of good quality protein as we get older (Norton et al., 2021). Additionally, the maintenance and growth of muscle mass are essential for overall health and fitness and can be obtained from dairy products containing proteins.

The nutritive value of dairy products depends upon the interaction between nutrients in the dairy matrix structure (Thorning et al., 2017). Accordingly, the food matrix plays a significant role in altering the nutritional properties of food. Their unique matrix structures can affect digestion and absorption in addition to their nutrient content.

Milk is composed of water, fat, protein, and carbohydrates with the fat and protein molecules dispersed in a water-based solution. This structure facilitates the digestion and absorption of nutrients in milk. On the other hand, the act of curdling and pressing the milk results in a distinctive matrix structure for cheese. Digestion and absorption of cheese can be impacted by the concentrated protein and more solid or liquid form of fat present in cheese. Compared to soft cheeses, hard cheeses like cheddar, Parmesan have a more compact structure that might make them more difficult to digest (Watson et al. 2017). Yogurt is another dairy product with soft gel structures. The fermentation method is used to manufacture yogurt causes the breakdown of lactose and the production of lactic acid, which can make it easier to digest for those who are lactose intolerant. Yogurt also has a special acid coagulated protein matrix structure. Besides, it is produced by the addition of probiotics, which are beneficial microorganisms known for their health advantages (Watson et al., 2017).

Casein and Whey proteins

Casein and whey protein are two principal dairy protein components that can be isolated from milk. The separation of milk into its two main components, curds, and whey, is the initial step in the production of casein and whey proteins. Because of their distinctive protein profile and functionality, these ingredients are gaining importance among food and beverage manufacturers (Carter et al., 2021).

The amino acid (AA) content of proteins, as well as their digestibility and bioavailability, is the

most important aspect in evaluating the quality of a protein (Lorieau et al., 2018). With an 80% casein content in total milk protein, they are recognized as an important source of supplying essential AAs. Casein protein production begins with the separation of milk through acid coagulation or rennet coagulation to obtain curd, a colloidal suspension when mixed with an acidic substance. On the other hand, whey proteins, which represents 20% of the total milk protein, are globular proteins that exhibits solubility across a wide range of pH levels and are sensitive to heat (Lorieau et al., 2018).

In terms of protein metabolism in our body, protein digestion significantly activates protein synthesis in the muscles (Burd et al., 2019). Previous studies suggest greater postprandial protein retention was obtained for whey compared to casein, which is related to the faster digestion and nutrient release from whey. Both proteins (casein and whey) have different molecular make up, therefore, may exhibit different rates of digestion and release of nutrients even after having same total protein content. However, caseins and whey proteins contain all the amino acids required for the synthesis of muscle protein (Penning et al. 2011).

Accordingly, a faster digestion and absorption were obtained for whey compared to caseins, due to the higher anabolic properties of whey which tend to increase the release of plasma amino acid availability, moreover, activate faster muscle protein synthesis (Biorie et al., 1997; Dangin et al., 2001).

Disintegration and ingestion of dairy foods

Foods undergo significant size reduction during the human digestive process to assist the release of embedded nutrients for eventual intestinal absorption. The mouth and stomach are the primary components where foods are mechanically broken down into small pieces, whereas the small intestines are the primary site of nutrient absorption (Kong et al., 2008). The size and

physical properties of the food particles, as well as the structure of the food, might influence the rate and complexity of digestion. For example, while smaller food particles may be easier to digest, larger food particles may take longer to digest. Additionally, before they can be further broken down by enzymes, some food structures may need to undergo more mechanical processing in the mouth.

During ingestion, mastication, and swallowing, a complex set of physicochemical, physiological, and biochemical reactions begin in the human mouth and throat, which dramatically change the composition, structure, and properties of food. Food interacts with the surfaces of the tongue, mouth, and throat, changes its pH, and temperature, which affects activity of digestive enzymes, encounters a complicated flow profile, and may be physically broken down into smaller pieces by chewing (McClements et al., 2008).

Food processing can also alter how products break down since some processed foods may have additional substances that can inhibit or slow down digestion. For example, cheese texture and composition can affect cheese in-vitro digestion. Soft and ripened cheeses that possess a high-fat content and hydrolyzed proteins such as aged cheddar have a higher disintegration rate compared to the elastic cheeses such as mozzarella and Swiss cheese (Kong and Singh et al., 2008). Depending upon the food structure, the ingested food is converted into smaller food particles with a variation in particle size (0.82–3.04 μm) (Jalabert-Malbos, Mishellany-Dutour, Woda et al., 2007). On the other hand, semi solid foods already resemble the bolus created for swallowing, hence minimum mastication is required in the mouth cavity (De Wijk et al., 2004). Overall, digestion is a complicated process that goes through different phases and depends on the response of the digestive system as well as the structure and functionality of the food.

Oral processing

Oral processing involves the physical and chemical modifications that take place in food as it is chewed, mixed with saliva, and prepared for swallowing. This process is also known as "mastication." It is the first stage of digestion and impacts how food tastes, feels, and whether you like it or not (De Wijk et al., 2004). Food and the mouth cavity interact mechanically, chemically, and biologically during the complicated process of oral food processing. These interactions result in destruction of food structure, and formation of bolus (Chen et al., 2015). In the oral phase, the understanding of the characteristics of the food bolus included two meanings: (1) final evaluation of food preferences based upon gustatory and textural perception, (2) mechanical characteristics, such as particle size and cohesiveness that have been understood by sensory receptors (Jalabert-Malbos et al., 2007). Hence, consumer appreciation for food products is determined by quality attributes of the oral texture (Prakash, Tan et al., 2013). Structural breakdown of food in the oral phase is a substantial segment of the digestion process. Changes in the textural properties of food while breaking down in the oral phase is suggested by Chen (2015) to be the most important part of overall food disintegration. Food is structurally broken down during the dynamic process of oral processing to be easily swallowed and imparted for further digestion (Chen et al., 2015).

Tribology

A new rheological technique known as tribology has been applied to simulate food texture and mouthfeel perception within the oral stage (Chen et al., 2012). Tribology is the science of friction between two rubbing and interacting surfaces in a relative motion in the presence or absence of lubricant. It is applied to assess the frictional behavior of lubricants (Godoi et al., 2017).

The lubrication behavior of food can be represented by a Stribeck curve (Fig. 2.1). The curve includes the coefficient of friction on the y-axis against different parameters such as speed, viscosity, and load on the x-axis (Prakash et al., 2013).

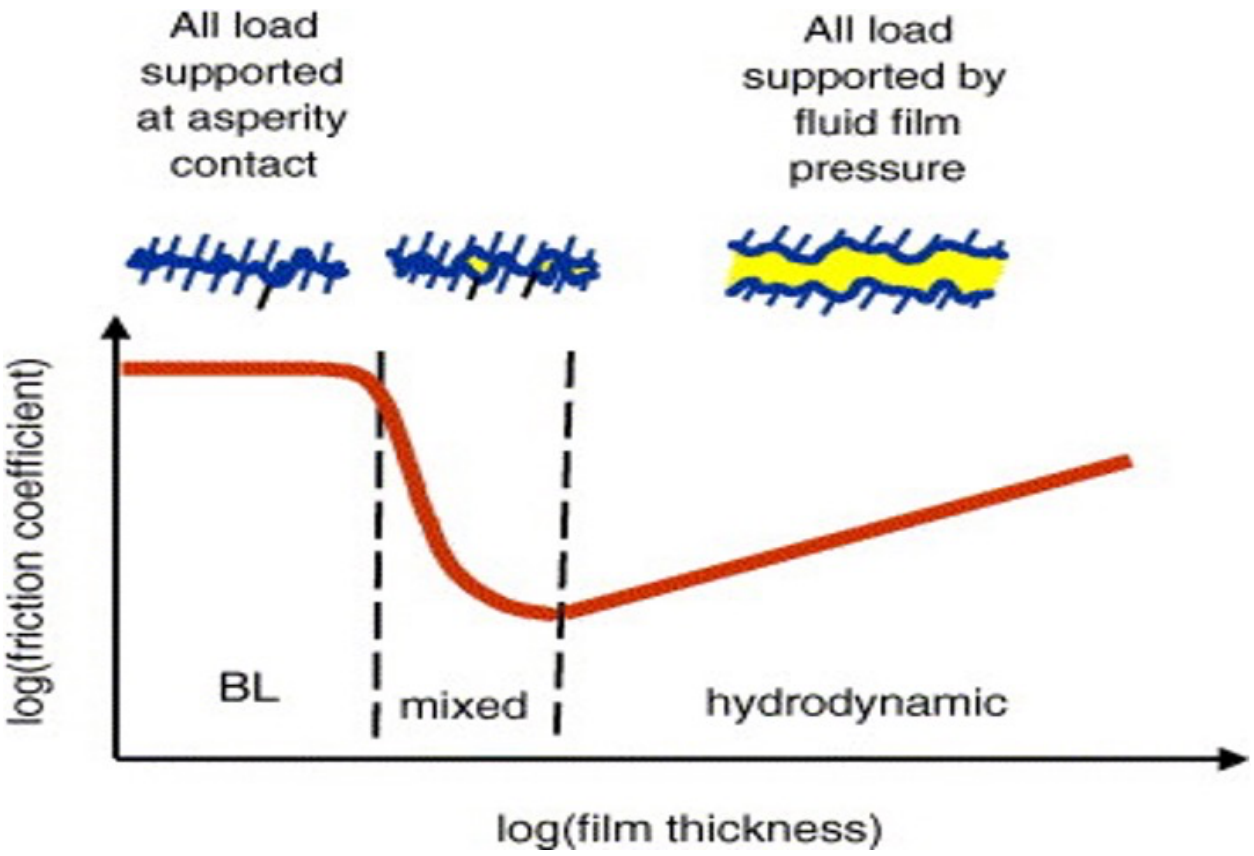

Figure 2.1: Typical Stribeck curve illustrating three distinct regimes: (1) Boundary layer with direct sample contact, (2) Mixed regime leading to partial surface separation, and (3) Hydrodynamic regime characterized by full surface separation due to a thin fluid layer. Adapted from (De Vicente et al., 2006).

A Stribeck curve can be divided into three friction regimes (Fig. 2.2): (1) boundary, (2) mixed, and (3) hydrodynamic. The boundary regime is spotted at which friction is created between two interacting surfaces at very low speeds (Godoi et al., 2017). After the boundary regime comes the mixed regime at which there is an increase in the sliding speed that tends to induce partial separation of food. This can happen at the two interacting surfaces, the tongue, and palate. This is followed by a complete separation of the interacting surfaces by a thin fluid layer, this is known as the hydrodynamic regime (Sudhakar et al., 2020).

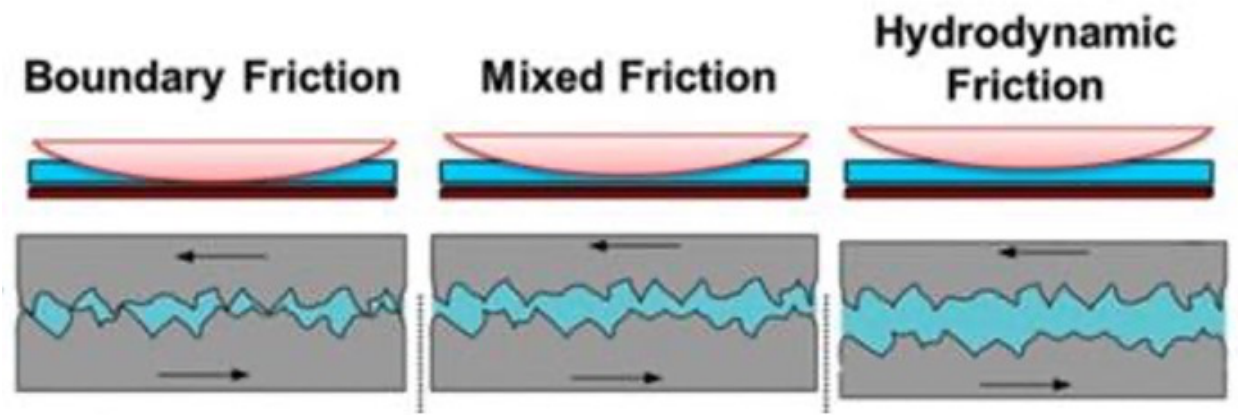

Figure 2.2: The three different regimes in the stribeck curve (boundary, mixed, and hydrodynamic, were adapted from Anton Paar). In the Boundary friction, the sample is in direct contact. The Mixed friction often results in partial separation of the interfaces. Meanwhile, in the Hydrodynamic friction, there's a complete separation between interacting surfaces due to a thin fluid layer

In-vitro digestion

Simulating digestion in the upper gastrointestinal system has become more important in

the attempt to acquire a mechanistic understanding of how food structure and composition impact health (Brodkorb et al., 2019). In-vitro digestion is a simple method used to mimic in vivo food digestion. This is achieved by mimicking in vivo factors and conditions prevailing during digestion, such as the existence of enzymes, pH, salt concentration, and other factors (Figure 2.3). This method is applied to help understand the impact of food composition and structure on nutrient release, by simulating the physiological status of the upper gastrointestinal tract, including oral, gastric, and small intestine stages (Brodkorb et al., 2019).

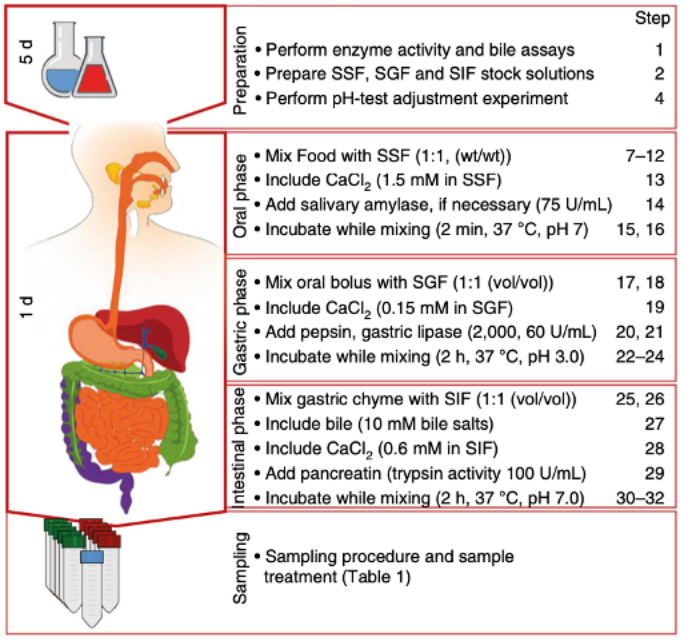

Figure 2.3: Flow diagram of the digestion method (INFOGEST) including preparation, oral phase, gastric phase, and the intestinal phase, adapted from Brodkorb et al. (2019).

Available in-vitro digestion systems can be divided into two models, static models which represent a fixed ratio of food samples to enzymes during digestion. On the other hand, the dynamic model can be used to obtain more accurate simulation, for example, there is a variation in enzymatic secretion between infants and elderly people, which requires accurate simulation, since static models can't capture the continuous changes and interactions that occur over time, dynamic model are becoming more popular (Minekus et al., 2014). Minekus (2014) considered that most of the measurements and the studies used in-vitro digestion methods, are due to their

applicability of being fast, less work intense, and less cost, with no ethical restrictions compared to in-vivo models.

Gastric emptying

The breakdown of bulk food into smaller particles within a range of 1-2mm right before emptying stomach is achieved by mechanical and chemical action in the stomach, which eventually affects the absorption of nutrients in the intestine (Kong, Singh et al., 2008).

Furthermore, the degradation and emptying time of food in the stomach is affected by different factors, such as the amount of meal, calories, viscosity, and the physical properties of the meal including the structure and texture (Kong, Singh et al., 2008).

The breakdown properties of ingested dairy products are affected by their physical state. As it was shown in the past that longer gastric emptying time was observed for rennet gel cheese (352 min) compared with acid gels (159 min), and milk took the least emptying time (114 min) (Fang et al., 2016).

Gastric Acidity Profile

Upon consumption, dairy proteins embark on their digestive journey, starting in the acidic environment in the stomach. The stomach typically maintains a pH of 1.5 to 3.5. When the pH is around 2.0, the abundance of H+ ions can result in the protonation of most proteins. The extent of this protonation largely depends on a protein's isoelectric point (pI). For instance, casein's pI is 4.6, as reported by Day et al. (2014). When the pH dips below 4.6, most whey proteins acquire a net positive charge, as seen in acid whey. This allows them to bind with cationic polymers in ion

exchange processes. Conversely, in sweet whey with a pH of about 5.5, most whey proteins display a net negative charge (Goulding et al., 2020).

In this acidic environment, dairy proteins experience structural transformations, exposing their internal sequences. This may render them more accessible to digestive enzymes. Pepsin, the primary proteolytic enzyme in the stomach, thrives in this environment. It activates from its precursor, pepsinogen, under such acidity (Sah et al., 2016) and demonstrates peak activity between pH 1.5 and 2. As dairy proteins unfold, they become prime targets for pepsin, facilitating their fragmentation into smaller peptides. This protein breakdown rate, led by pepsin, can be influenced by various elements like structural differences in dairy proteins, as noted by Yang et al. (2023). Other influential factors include the type of protease, the substrate, the duration of hydrolysis, and the immediate conditions.

Interestingly, the net charge of proteins plays a pivotal role in their enzymatic degradation. Those with a positive charge may enhance their enzymatic interactions, increasing hydrolysis rates (O'Dowdyer et al. 2022). While a protein's charge can reshape its structure and solubility, the sequence of its amino acids predominantly dictates enzyme specificity. However, a positive charge might guide enzymes to their action sites or even modify these binding locales. Increased solubility due to a positive charge in the stomach's acidic environment can increase the interactions with enzymes such as pepsin, possibly optimizing protein degradation.

On the other hand, inherent buffering capacity of dairy proteins can temper the pH drop during digestion, possibly extending the digestion duration (Wang et al., 2018). Yet, as digestion advances, pH disparities among samples diminish (Wang et al., 2018). Notably, as pH nears the isoelectric point of 4.6, dairy proteins, especially caseins, experience isoelectric precipitation. This change in charge curbs electrostatic repulsion, in turn affecting protein stability (Cabero et al.,

2017). Intriguingly, while significant portions of casein endure even after an hour in gastric digestion, they succumb completely to intestinal proteases. This phenomenon may result from the acidic pH in the stomach approaching isoelectric point of casein, prompting casein aggregation, and thus reducing its susceptibility to pepsinolysis (Nguyen et al., 2016).

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CHAPTER 3

INVESTIGATE THE TRIBOLOGICAL PROPERTIES, PARTICLE SIZE, AND PROTEIN TYPE IN THE DIFFERENT DAIRY PRODUCTS.

ABSTRACT

Dairy products consist of fat, proteins, and carbohydrates, each of these can impact their textural, sensorial, and nutritional attributes and affect nutrient release and absorption. Additionally, they are produced in solid and liquid forms, thus making it challenging to assess their properties, such as composition, texture, and nutrient release. Tribology is the study of friction, wear, and lubrication and can explain the phenomena involving numerous interacting surfaces under relative motion. Hence, it is helpful to investigate the tribological properties of dairy products with varying compositions. This study investigated the tribological properties of 8 dairy products: solids (cheddar, cheese curd, parmesan), semi-solid (cottage cheese, ricotta cheese, yogurt) and liquid (milk, whey protein beverage). One gram of grated sample was extracted and equilibrated to 22°C for 30 min, and subsequently heated up to 37°C in a water bath with 5 min of holding time. Afterward, 1 mL of DI water was added to the samples and mixed for 2 min before loading them onto the MCR-302 Anton Paar rheometer for tribological measurements. The tests were performed using polydimethylsiloxane (PDMS) pins within one minute of the set time.

Friction between the sample surface and glass ball was provided for calculating the friction factor and sliding velocity friction factor (μ) for different products showing a clear distinction between samples on the Stribeck curve at several sliding speeds. The friction factor also varied with different normal forces. At the same time, μ for liquid dairy products i.e., milk was 1.5 at 0.01–0.1 mm/s sliding velocity, which was significantly higher ($P < 0.05$) than cottage cheese and solid cheeses. We observed that semi-solids had the second highest friction factor 0.54 at 0.1 mm/s of sliding velocity ($P < 0.05$). Similarly, the parmesan cheese belonging to the solid foods had a friction factor of 0.32 at 0.1–1 mm/s sliding velocity. This could be due to differences in the food structures, fat content, and moisture content of products. This study would further the understanding of the tribological behavior of food products with different compositions, assisting the dairy industry in the development of premium products with higher consumer satisfaction.

Introduction

Texture and mouthfeel during consuming food products is one of the vital elements that tend to contribute significantly towards consumer satisfaction. The composition of food also plays a crucial role in determining the texture and mouthfeel of various products, which further influences consumer preferences (Sharma Khanal et al., 2019, 2020). Dairy products display an extensive variety of textural and oral-sensory characteristics, which can be attributed to their distinct compositions and processing methods. These products contain primary components such as proteins, fats, and carbohydrates, which contribute to their unique structures. Dairy products can range from a soluble form, such as milk and whey, to a semi-solid acid-coagulated form like (Ricotta, Cottage cheese, yogurt), and even to a rennet-coagulated form found in cheese (Cheddar, Curd, and parmesan). Although they share similar compositions, these dairy products possess diverse structures that result in distinct mouthfeel experiences.

The food trapped between the tongue and palate undergoes microstructural breakdown through the combined action of salivary enzymes, shear forces, and sliding speeds in the mouth as the food goes through oral processing (Godoi et al., 2017). Oral processing majorly encompasses various stages such as biting, mastication, particle size reduction, mixing, lubrication, bolus formation, and swallowing. Throughout the mastication process, both solid and soft food matrices undergo size reduction, contingent upon their physical attributes and the individual's chewing habits, including factors like masticatory force, salivary secretion, and duration until swallowing (Aguilera, 2019). Those interactions can be understood by studying their frictional behavior by simulating interacting surfaces inside the mouth during oral processing through tribological measurements (Lamichhane et al., 2022; Sharma, 2022).

Tribology is the study of friction, wear, and lubrication that helps explain the phenomena involving interacting surfaces that are in relative motion. Hence, it is ideal for investigating the tribological properties of different food materials with varying compositions (J. Chen & Stokes, 2012). It also offers the ability to evaluate sophisticated properties of food during oral processing, which cannot be assessed through rheology alone (Sudhakar et al., n.d.).

The lubricant of food in the oral phase is affected by the food type, physical state, protein, fat, and moisture content. Tribometer is widely used to measure the mouthfeel of different food products including lubricant properties of food, such as slipperiness, smoothness, and creaminess. Stribeck curve was developed to evaluate the tribological behavioral response under different sliding speeds, and Newtonian lubricants (J. Chen & Stokes, 2012). This curve calculates the friction coefficient based on the rate of friction force to the applied load determining the lubricant properties of food (Bornhorst & Paul Singh, 2014). Friction is the resistant motion of two surfaces sliding over each other. the coefficient of friction (μ) is calculated as the ratio of the friction force (F, N) to the normal force (N, N), $\mu = F/N$ (Zad Bagher Seighalani et al., 2021; Zad Bagher Seighalani & Joyner, 2019).

The curve includes the coefficient of friction on the y-axis against different parameters such as speed, viscosity, and load on the x-axis (Godoi et al., 2017). The Stribeck curve can be divided into three friction regimes: (1) boundary, (2) mixed, and (3) hydrodynamic. The boundary regime is spotted at which friction is created between two interacting surfaces at very low speeds (J. Chen, 2015; Morales-Celaya et al., 2012; Pondicherry et al., 2018; Sharma Khanal et al., 2020) by the mixed regime at which there is an increase in the sliding speed causing partial separation of food, on the two interacting surfaces, the tongue, and palate. Followed by a

complete separation of the interacting surfaces by a thin fluid layer, this is known as the hydrodynamic regime.

However, understanding and addressing textural and oral-sensory aspects of food products create major challenges for developers and manufacturers. These issues originate from a lack of understanding of the physiological processes involved in the sense of texture and mouthfeel, as well as a lack of data on customer preferences for tactile and kinesthetic characteristics (Aguilera, 2019; Baier et al., 2009; Bryant et al., 1995; J. Chen, 2015; Guinard & Mazzucchelli, 1996). Tribology is an innovative method that can be employed to better understand the textural properties of food associated with mouthfeel and sensory aspects.

In this study, we investigated the combined effect of the protein type, physical state, and normal force on the frictional behavior of eight different products (Cheddar, Curd, Parmesan, Ricotta, Yogurt, Cottage cheese, Milk, and Whey). The samples were subjected to tribological measurements at three different normal forces and three runs to mimic the human oral processing. As part of this study, we have also evaluated the physicochemical properties, particle size and extent of proteolysis in the samples. Overall, this study would assist in understanding the behavior of different dairy foods with variable structural attributes during the oral processing and thus improving the understanding of material properties that impact the mouthfeel of dairy products, thereby greatly benefiting the food and beverage industries.

Materials and methods

Dairy products

Eight dairy products were selected to provide a diverse range of food structures and protein compositions. These dairy products were commercially available and sourced from different locations, including Cheddar, curd, and ricotta purchased from Gossner (Logan, UT), parmesan, cottage cheese, and yogurt obtained from Walmart (Logan, UT), and whey collected from Aggies Creamery (Logan, UT). The selected dairy products were classified into three groups based on their physical properties, namely solid, semi-solid, and liquid.

The solid category included curd, Cheddar, and parmesan, which provided a comprehensive representation of the textural spectrum within solid dairy products, ranging from relatively soft curd to hard, aged cheeses like parmesan. The semi-solid group comprised yogurt, ricotta, and cottage cheese, offering a diverse sampling of cultured dairy products with distinct viscoelastic properties and protein structures. Finally, the liquid category consisted of milk and whey, allowing for the investigation of dairy products in their most fluid state, as well as the analysis of protein-rich liquid by-products of cheese production. The rationale behind the selection of these eight dairy foods was to ensure a comprehensive evaluation of dairy products with varying physical properties, protein content, and processing methods. This diversity facilitates a more robust understanding of the relationships between food structure, protein composition, and the functional properties of dairy products.

Proximate analysis

The moisture content of the eight dairy products was analyzed using CEM Turbo Technology Rapid Moisture Analyzer (Matthews, NC). Two square pads were placed on the scale and tared. A sample weighing around 2.0 ± 0.5 g was then measured on the gravimetric scale and compressed with a hand presser to enlarge the surface area. Following this, the sample was processed in the machine for approximately 4 min at 110 °C. The moisture content for each sample was displayed on the screen. This process was repeated in triplicate for all samples, and the values were recorded.

The Babcock method was employed to quantify the fat content in eight diverse dairy samples. Cheese samples (9 g), semi-solid, and liquid samples (18 g) were each introduced into a 50% paley bottle, followed by the addition of 10 grams of distilled water at a temperature of 60 °C. Subsequently, 17.5 mL of concentrated sulfuric acid was incorporated in three separate aliquots. The resulting solution was mixed until a homogeneous dark brown color was observed. The bottles were then centrifuged for 5 min, after which water heated to 60 °C was added to adjust the contents to the base of the neck level of the bottle. Upon centrifuging for an additional 2 min, water at 60 °C was introduced to facilitate fat flotation in the neck of the bottle. The sample underwent final centrifugation for 1 min. With the inclusion of 4-5 drops of glymol, the fat column's length was determined by measuring the demarcation between fat and glymol to the lower meniscus.

The Protein quantification was conducted using CEM Sprint Rapid Protein Analyzer (CEM, Matthews, NC). The iTag reagent adheres to protein through an acid group. Rapid identification of the aromatic region of the iTag molecule is achieved via colorimetry, as it absorbs light. The dye within the iTag solution associates with proteins in the sample, resulting in a

reduction in absorbance. The protein content of the sample is determined by comparing the absorbance values. A sample cup was placed on the weighing balance connected to the protein analyzer and was tared. Subsequently, dairy samples based on the type of the products (0.200-0.600 g) were introduced into the cup, and the system was equilibrated. Upon achieving equilibrium, the analysis was executed for a duration of 4–5 min. The protein content of the sample was displayed on the screen and measured in triplicate.

Particle size analysis

The particle size distribution of the eight dairy products was measured by the principle of static light scattering using a Particle size analyzer, Anton Paar 1190 model (Anton Paar, Graz, Austria), operating with Kalliope software (Anton Paar, Graz, Austria). The PSA could measure particles within the range of 0.4-2500 μm. Particle size analysis helped to understand the rate of particle disintegration. One g/mL of the sample had been grated and equilibrated to room temperature; afterward, the sample had been held in a water bath for 5 min at 37 °C. Then, it was diluted with 10 mL of DI water and mixed for 2 min. Background measurements had been checked before loading the samples. The obscuration rate was maintained within 5–30% while loading the sample. Various particle size parameters, such as D [4,3], D 10, D 50, and D 90, were obtained in triplicates for each sample.

SDS gel electrophoresis

The SDS page was performed using the Bio-rad Mini-Protean Tetra system, to evaluate the extent of proteolysis in the eight dairy food samples. The individual protein fractions were separated based on their molecular weight. The SDS PAGE was performed using a 4-20 % precast Mini-Protean, 15 well, polyacrylamide gel that was purchased from Bio-Rad (Bio-Rad laboratories, Hercules, CA). The running buffer was prepared by diluting 100 mL of the concentrated buffer solution with 900 mL of ultrapure water, achieving a 1:10 dilution. For the 4x sample buffer, it was prepared by mixing 250 mM Tris-HCl (pH 6.8), 4% Lithium dodecyl sulfate (LDS), 40% w/v glycerol, 0.02% bromophenol blue, and 15% beta-mercaptoethanol, which was freshly added during the preparation. Subsequently, the protein sample was prepared for electrophoresis by combining three parts of the sample with one part of the 4X sample buffer, effectively diluting the sample for optimal resolution during the SDS-PAGE run. Afterward, pre-run was performed for 30 min at 100 V. Samples were added to the sample buffer to obtain the final concentration of one mg protein in one mL of sample buffer. Afterward it was heated at 95 ℃ for five min in a water bath to ensure adequate denaturation of the proteins present in the samples, afterward cooled down to equilibrate room temperate and vortex before loading. 10 µL sample buffer containing 1 µg of sample per µL was loaded into the wells to ensure the loading of equal amount of protein in each of the wells present in the gel. Bio Rad® Precision Plus protein dual color standards was used as marker and contained a mixture of 12 recombinant proteins (2–250 kD) with nine blue-stained bands, and three pink reference bands (2, 25, 75 kD). The SDS PAGE was conducted in duplicates at 100 V for all the eight samples.

Tribological properties measurement

The tribological measurements were performed using an Anton Paar MCR 302 rheometer (Anton Paar, Graz, Austria), using PDMS pins (T-PID) attached to it a glass ball geometry using a ball-on-three -pin test configuration (Pondicherry et al., 2018). The sample was run at 37 ℃ to mimic body temperature. Three different normal forces, 1 N, 1.5 N, and 2 N were applied with three replicates. One gram of the sample was grated and equilibrated to 22 ℃ for 30 min. Solid and semi-solid samples were grated using pestle mortar equipment. subsequently, the sample was heated up to 37 ℃ in a water bath with 5 min of holding time. Afterward, one mL of DI water was added to the samples and mixed for 2 min before loading the sample on the rheometer. The relationship between the friction between the sample surface and glass ball and sliding velocity was expressed by an extended Stribeck curve fig. 3.1.

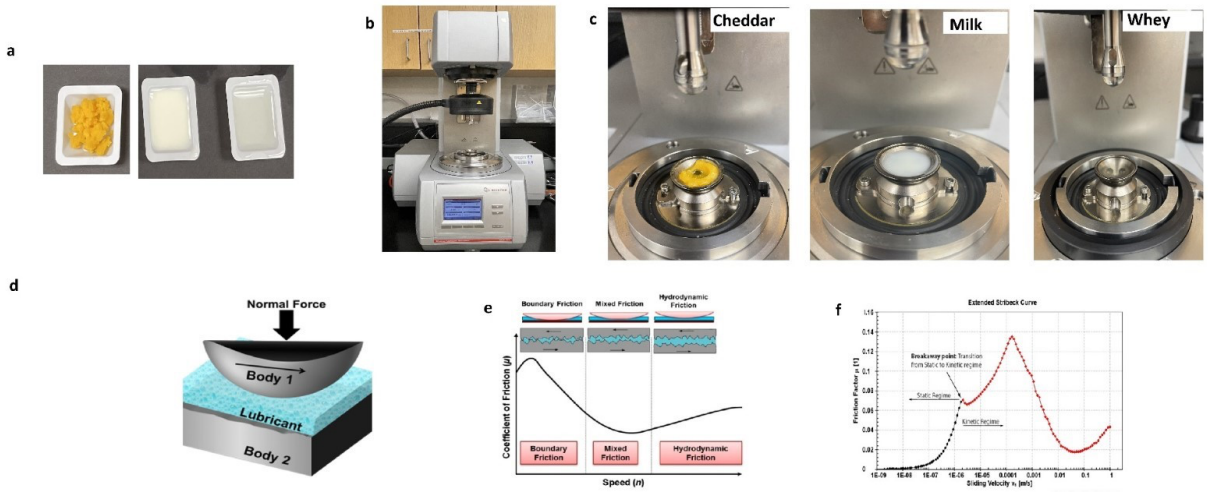

Figure 3.1: Tribological properties testing of dairy products (a) in the rheometer using the PDMS pin setup and schematic representation of simplified (d) and extended (e) Stribeck curves. Figure 4 (d-f) were adapted from https://wiki.anton-paar.com/en/basics-of-tribology/.

Statistical Analysis

The data was analyzed for significant differences ($P < 0.05$) at $\alpha=0.05$ in the origin lab 2023b. The mean values for the runs and normal force applied were compared among the samples and letter superscripts were presented to indicate the significant differences among the means.

Results and discussion

Proximate analysis

Eight dairy foods range from solid types to semi-solids like ricotta, down to liquids are presented in Fig. 3.1. Among the solid, semi solid, and liquid samples analyzed in this study, Cheddar cheeses had the highest amount of protein 24 %, followed by curd and Parmesan cheeses at 23.3 and 21.6 %, respectively. Protein plays very important role in formation of right body and texture in the cheese. It also influences elastic and brittle characteristics in the cheese. The protein content in semisolid samples used in this study, was found in the range of–4.7-12.5%, which was significantly lower ($p<0.05$) than that of the solid products. Lower quantity of protein would imply weaker body and soft texture. At the same time, the liquid dairy products (whey and milk) had the lowest measured protein content that ranged from 1.02 to 3.21 %. Abundance of aqueous phase in the liquid samples is expected to modulate interaction of digestive enzymes with soluble proteins.

The protein, moisture and fat content of the samples can affect their rheological and tribological properties. The differences in the protein contents and their types in the samples can be attributed to the way they are manufactured. For example, cheeses such as Cheddar, are primarily manufactured from milk by rennet action, and consist of caseins that form the 3-D protein network in the cheese (Lucey et al., 2003). The cheese manufacturing process involves removal of whey in some cases such as Cheddar (90% removal) while cottage cheese contains more amount of whey. During cheese making almost all the whey proteins get transferred to the whey but into a large liquid volume, therefore protein content in whey is much lower (~1%) than milk and cheese. Similarly, the protein content in milk is lower than the cheeses because during cheese making milk solids gets concentrated. Yogurt, had a lower protein content than cottage and other cheeses,

because base material for yogurt manufacture, mostly is milk. In terms of moisture content in the samples, we observed the reverse trend where the moisture content in the liquid products were measured to be highest at 93% in whey. At the same time, the moisture content in the semi solid dairy products ranged between 73–85% and that of the solids ranged between 32–37% moisture. The solid samples contained 30-33 % fat, the highest amongst the eight samples studied in this work, whereas whey samples had the least fat content. Because of these compositional differences, particularly in relation to protein content and type, differences in tribological behavior, particle size and digestion behavior were also expected.

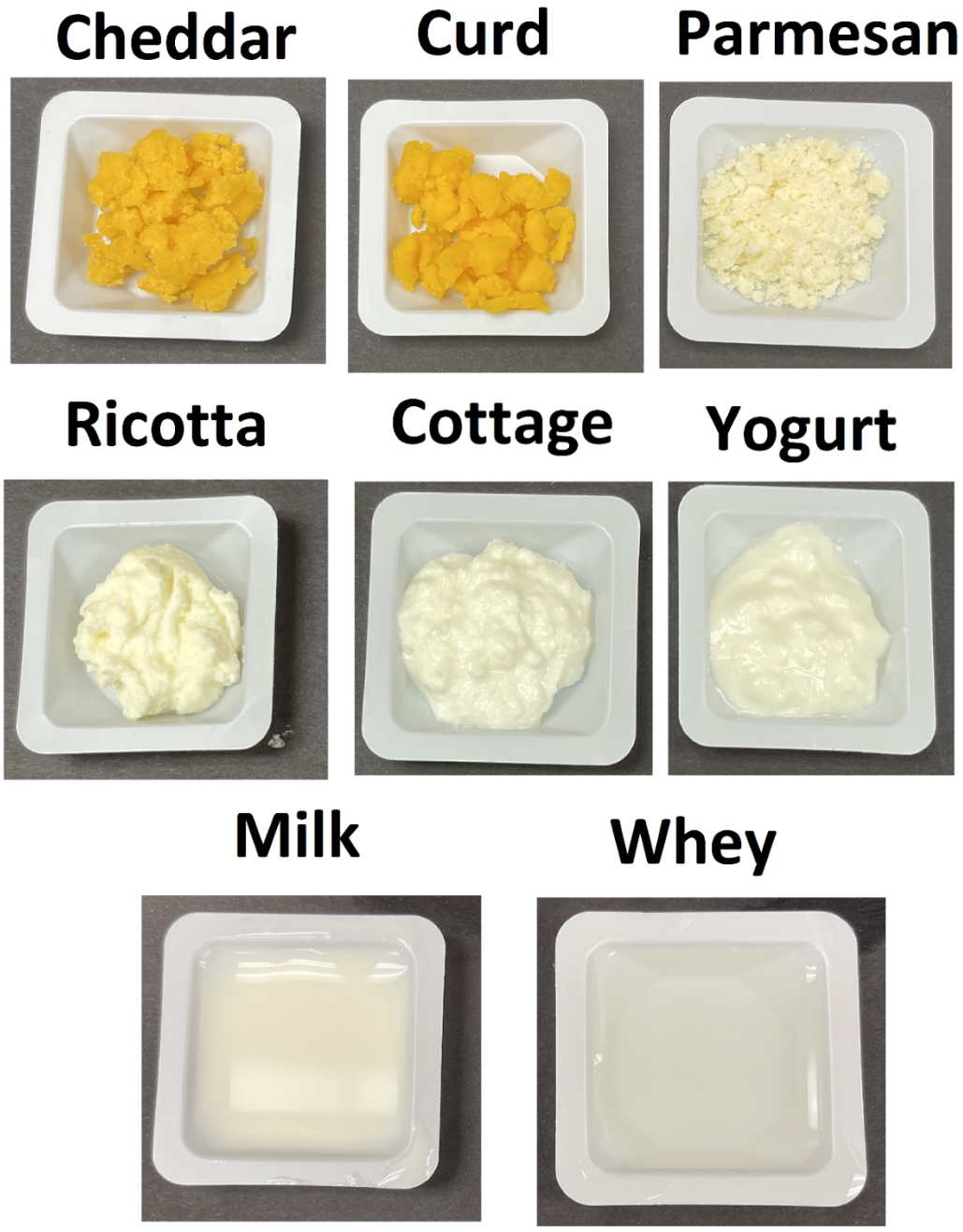

Figure 3.2: Sample specimen containing solid, semi-solid and liquid dairy products used for tribological measurements.

Particle size distribution

Impact of shearing on particle size reduction of eight different dairy foods was studied. The average particle size for the solid products ranged between 48–58 μm, whereas the semi-solid products had a larger particle size with yogurt having an average particle size of 143.5 μm (Table 3.1) (Figure 3.3). At the same time, we did not observe a significant difference ($p<0.05$) between the particle sizes of the solid cheese samples. In the case of liquids, we observed the widest range of particle sizes amongst all the samples.

Milk exhibited a monomodal particle size distribution for its fat, protein, and mineral components, highlighted by a minor peak at 1.35 μm. This peak represents a small population of particles ranging from 0.2 to 8 μm, which can be attributed to the presence of fat globules, as indicated by Godoi et al., 2017. According to (Godoi et al., 2021), casein proteins are essentially spherical particles with diameters ranging from 50 to 600 nm, with an average diameter of approximately 200 nm. This narrow particle size distribution is associated with the soluble form of proteins and fats, which are present with a high moisture content, as detailed in (Table 3.1).

On the other hand, whey beverages present a multimodal particle size distribution, with sizes ranging from 0.8 μm to about 600 μm. The proportion of larger particles is due to protein aggregation (casein fines), forming larger structures. In particular, the most substantial particles can be traced back to casein aggregation during cheese manufacturing (Guralnick, Panthi, Bot, et al., 2021; Guralnick, Panthi, Cenini, et al., 2021).

Particle size in cheese can be affected by the size of the protein and fat particles dispersed within the cheese matrix (Bornhorst & Paul Singh, 2014; Guralnick, Panthi, Bot, et al., 2021).

Several factors contribute to particle size, including the protein content, milk composition, processing techniques, and cheese variety. Protein plays a crucial role in the structure and texture of cheese as the casein network tends to trap fat globules and water, giving cheese its characteristic texture. Type of protein present in the food matrix can affect the size and distribution of protein particles, influencing the overall particle size. Cheeses with higher protein content may exhibit brittleness which can cause formation of smaller size particles upon shearing depending upon amount and duration of shear applies during sample preparation. This is related to the complex protein matrix with a relatively low moisture content that led to a brittle texture. When cheese is grated during sample preparation, these brittle cheeses contribute to a breakage of particles into smaller, hence more uniform pieces.

In our case, solid samples with a higher protein content had a lower particle size amongst the cheese samples (Table 3.1). This is because a higher concentration of proteins promotes a more compact brittle protein network due to a lack of fat particles, resulting in a finer texture and smaller particle size (Chen et al., 1996). Cheeses with a higher protein content tend to have a smoother and more homogeneous texture.

However, other factors can also impact particle size. For example, the composition of cheese milk can be one such factor that can affect the cheese texture and therefore their tribological behavior. The fat content and casein-to-whey protein ratio can affect the protein network formation and particle size. In addition, processing techniques, including curd cut size and extent of stirring, can also have a significant influence over the size and distribution of particles present in the cheese. Furthermore, different cheese varieties have distinct characteristics, including particle size. For example, some cheeses like Cheddar or Swiss have a characteristic grainy texture with larger protein particles, while others like cream cheese or cottage cheese have smaller particle sizes,

resulting in a smoother texture (C. M. Chen et al., 1996; J. Chen & Stokes, 2012). While the protein content is a significant factor influencing particle size in cheese, it is vital to consider other factors such as milk composition, processing techniques, and cheese variety to fully understand and control particle size in different cheese types.

Table 3. 1: Physicochemical properties and particle sizes of solid, semi-solid and liquid dairy samples.

Samples	Protein (%)	Fat (%)	Moisture (%)	D 10 (µm)	D 50 (µm)	D 90 (µm)	Mean size volume D [4,3] (µm)
Cheddar	24.24±0.21 [a]	33.00±0.00 [a]	37.02±0.17 [f]	10.98±0.73 [bc]	47.29±1.32 [c]	114.46±3.31 [c]	58.4±1.64 [c]
Cheddar Curd	23.25±0.17 [b]	30.83±0.16 [b]	37.74±0.70 [f]	8.70±1.53 [bcd]	48.61±3.12 [c]	100.47±7.13 [c]	54.4±2.32 [c]
Parmesan	21.64±0.04 [c]	32.33±0.16 [a]	32.24±0.91 [g]	11.48±0.46 [b]	43.691±1.19 [c]	84.38±2.73 [c]	48.3±1.35 [c]
Ricotta	11.07±0.15 [e]	14.00±0.28 [c]	73.06±0.55 [e]	5.17±0.61 [de]	41.50±3.12 [c]	97.10±10.69 [c]	49.1±4.65 [c]
Cottage	12.52±0.08 [d]	3.43±0.06 [e]	76.67±0.28 [d]	11.77±0.97 [b]	56.2±10.14 [bc]	196.5±19.11 [b]	87.0±11.7 [b]
Yogurt	4.47±0.19 [f]	3.66±0.16 [e]	85.50±0.16 [c]	37.95±1.13 [a]	130.1±3.43 [a]	241.5±3.27 [a]	143.5±3.02 [a]
Milk	3.21±0.01 [g]	3.16±0.16 [d]	88.04±0.06 [b]	0.74±0.006 [e]	1.33±0.00 [d]	2.31±0.01 [d]	1.53±0.001 [d]
Cheddar cheese whey	1.02±0.008 [h]	0.50±0.00 [f]	93.03±0.008 [a]	6.122± 1.81[cd]	86.21±16.59 [b]	185.0±5.93 [b]	95.86±8.27 [b]

Note: The values are presented as Mean ± SD. Different lower-case and upper-case superscripts show significant differences ($p<0.05$) between values within rows and columns, respectively.

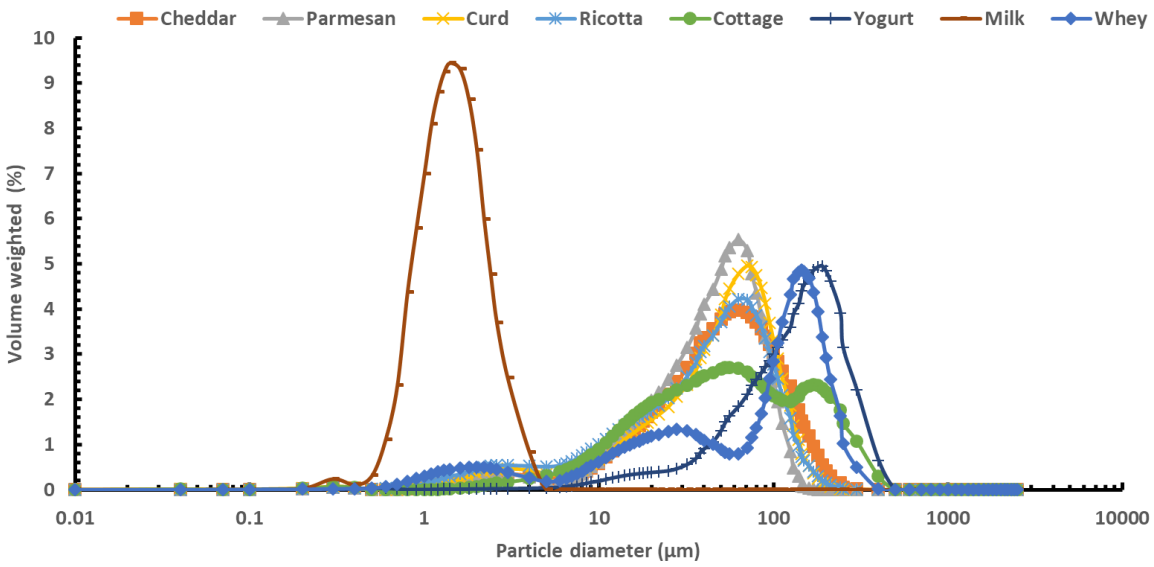

Figure 3.3: Particle size distribution of solid, semi-solid and liquid dairy products subjected to tribological testing.

Tribological measurements

Tribology refers to the study of friction, lubrication, and wear between interacting surfaces. The normal force is the force exerted perpendicular to the contact surface when an external load or weight is applied to the cheese. The tribological measurements were performed on the eight dairy samples and their properties were analyzed under the effect of the normal and shear stress applied. In addition, the testing involved the application of runs for accurately predicting their tribological behavior at the stationary and mobile phases or oral phase of digestion.

Effect of normal force

Normal force has a significant effect on predicting the tribological properties of the dairy products as it can cause variation in breakdown of the samples during the chewing or mastication process. Ease of mastication and swallowing is linked with the friction behavior which in turn is affected by particle size and surface characteristics. In this study, we observed a higher friction factor (μ) at lower normal forces for all the samples Figure 3.5. This could be attributed to the higher breakdown of the samples during the application of a larger normal force that helps in reducing the particle size of the samples and thus facilitating a better mixing with the saliva that can result in a lower friction factor (μ) (Figure 3.5) (Table 3.2–3.4).

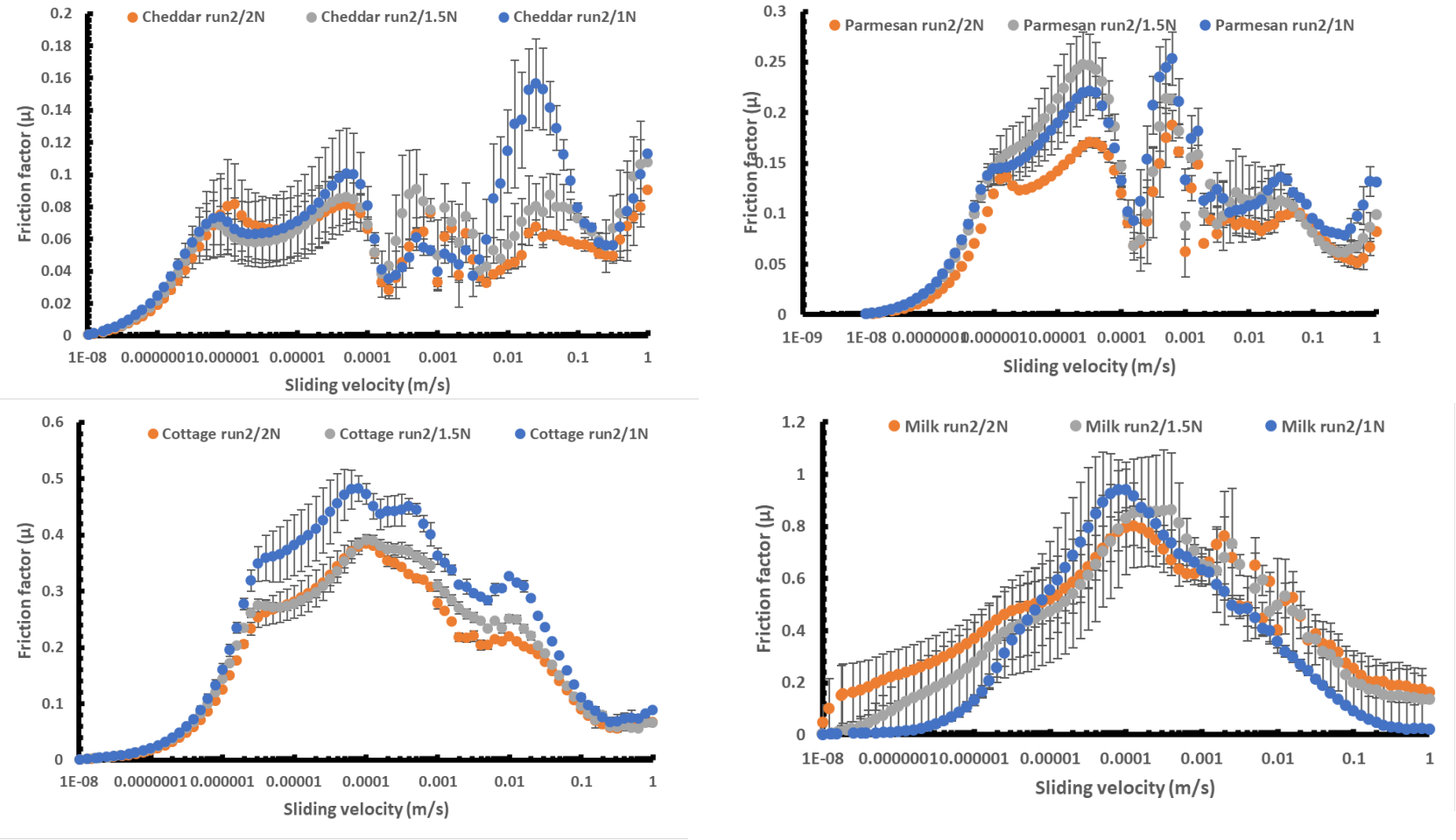

Figure 3.4: Effect of normal forces on solid, semi solid, and liquid dairy products during the second run of the tribological measurements performed at run 2.

Table 3. 2: Effect of normal force applied and runs conducted on the peak friction factor (μ) of the three solid dairy products.

	Peak friction factor (μ)								
	Cheddar			Curd			Parmesan		
Normal force (N)	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
1	0.234 ± 0.17^{aA}	0.174 ± 0.02^{abA}	0.173 ± 0.05^{bA}	$0.192\pm.011^{aA}$	0.350 ± 0.03^{aA}	0.394 ± 0.01^{aB}	0.225 ± 0.02^{aA}	0.220 ± 0.03^{aA}	0.264 ± 0.03^{aA}
1.5	$0.175\pm.018^{aAB}$	0.123 ± 0.01^{aAB}	0.119 ± 0.01^{aAB}	0.414 ± 0.22^{bA}	0.491 ± 0.21^{abA}	0.866 ± 0.04^{aA}	$0.144\pm.02^{bAB}$	0.247 ± 0.03^{aA}	0.177 ± 0.01^{abB}
2	$0.155\pm.017^{aB}$	0.10 ± 0.02^{aB}	0.097 ± 0.01^{aB}	0.171 ± 0.01^{aA}	0.243 ± 0.01^{aA}	0.238 ± 0.01^{aB}	0.121 ± 0.01^{aB}	0.192 ± 0.02^{aA}	0.157 ± 0.01^{aB}

Note: The values are presented as Mean ± SD. Different lower-case and upper-case superscripts show significant differences ($p<0.05$) between values within rows and columns, respectively.

Table 3.3: Effect of normal force applied and runs conducted on the peak friction factor (μ) of the semi-solid dairy products.

Normal force (N)	Peak friction factor (μ)								
	Ricotta cheese			Cottage cheese			Yogurt		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
1	0.205±.01^{aA}	0.272±.02^{aA}	0.235±0.01^{aA}	0.468±.02^{aA}	0.503±.04^{aA}	0.455±0.15^{aA}	0.315±.003^{bA}	0.518±.014^{aA}	0.473±0.027^{aA}
1.5	0.180±.02^{aA}	0.260±.06^{aA}	0.214±0.02^{aA}	0.349±.01^{aB}	0.390±.01^{aB}	0.376±0.002^{aAB}	0.240±.008^{bB}	0.454±.01^{aB}	0.404±0.01^{aB}
2	0.188±.006^{aA}	0.256±.05^{aA}	0.192±0.02^{aA}	0.322±.02^{aB}	0.388±.01^{aB}	0.358±0.01^{aB}	0.181±.001^{bB}	0.295±.025^{aC}	0.310±0.018^{aC}

Note: The values are presented as Mean ± SD. Different lower-case and upper-case superscripts show significant differences ($p<0.05$) between values within rows and columns, respectively.

Table 3.4: Effect of normal force applied and runs conducted on the peak friction factor (μ) of the liquid dairy products.

	Peak friction factor (μ)					
	Milk			Whey		
Normal force (N)	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
1	1.05 ± 0.14^{aA}	1.01 ± 0.16^{aA}	0.81 ± 0.009^{aA}	$0.53\pm.023^{bA}$	1.01 ± 0.04^{aA}	1.17 ± 0.01^{aA}
1.5	0.67 ± 0.20^{aA}	0.85 ± 0.21^{aA}	0.80 ± 0.16^{aA}	0.54 ± 0.06^{bA}	1.05 ± 0.06^{aA}	1.21 ± 0.10^{aA}
2	0.67 ± 0.07^{aA}	0.81 ± 0.06^{aA}	0.88 ± 0.07^{aA}	0.40 ± 0.04^{bA}	0.69 ± 0.66^{aB}	0.84 ± 0.05^{aB}

Note: The values are presented as Mean ± SD. Different lower-case and upper-case superscripts show significant differences ($p<0.05$) between values within rows and columns, respectively.

Due to the application of the normal force on solid and semi solid samples, they can undergo wear at the same time due to the consequential action of the shearing and compression. Specifically for cheeses, when a normal force is applied, it compresses the material, thereby affecting the texture and structure of the cheese, leading to changes in its mechanical properties. Higher normal forces would result in greater compression, which can make the cheese denser and alter its tribological properties (Sharma Khanal et al., 2020; Sharma, 2022, 2022). Furthermore, the normal force applied to the samples can be influenced by the contact area between the cheese and the interacting surface (J. Chen & Stokes, 2012; Sudhakar et al., n.d.). A higher normal force leads to a larger contact area, increasing the number of intermolecular interactions and adhesion between the cheese and the surface it is in contact with. This can affect the frictional behavior between the cheese and other materials. At the same time, the normal force plays a role in the frictional forces experienced by the cheese. A higher normal force can increase the frictional force between the cheese and its surroundings, thereby affecting the ease with which the sample layers move. However, in our case we observed an opposite trend where the samples showed a lower friction factor (μ) at higher normal force applied (Fig. 3.5) (Table 3.2–3.4). This could be due to their physicochemical properties and the release of fat from the samples structure which may have resulted in forming a lubrication layer and reducing the friction at higher normal forces. Formation of this type of layer can also impact in-vitro digestion behavior of milk proteins present in various dairy products.

In the oral processing, during mastication, the presence of saliva acts as a lubrication medium. A higher normal force can increase the wear on the cheese, particularly when it interacts with surfaces that are abrasive or have a rough texture, affecting the overall mouthfeel.

Along with the normal force, the particle size of food products can influence the frictional behavior between surfaces. Smaller particles tend to have a higher specific surface area, leading to increased interactions and higher frictional forces between particles and surfaces. This can result in increased resistance to sliding or movement. Furthermore, larger particles in food products can act as abrasive agents, causing more significant wear on contacting surfaces. This can be particularly important in processing equipment, where larger particles can cause increased erosion and damage to the machinery. In addition, the particle size can affect the lubrication properties of food products. Smaller particles have a higher tendency to form colloidal suspensions or emulsions, which can act as lubricants between interacting surfaces and retard the digestion process. These colloidal systems can reduce friction and wear by providing a protective film or boundary layer. In terms of the mouthfeel and sensory perception during the oral phase of food digestion, particle size plays a crucial role in the perception of texture and mouthfeel of food products. Larger particles can contribute to a gritty or coarse texture, while smaller particles can create a smoother and more homogeneous mouthfeel. The particle size can also impact the processing characteristics, stability and digestibility of food products. Smaller particles can enhance the dispersibility and solubility of ingredients, making them more suitable for formulations or manufacturing processes. Additionally, controlling the particle size distribution is essential to achieve desired rheological properties, such as viscosity and flow behavior, which can affect the stability and nutritive performance of food products.

Effect of the runs

In this study, we performed the tests with the samples at three consecutive runs and three normal forces, among which the 1 N normal force was adopted to compare the effectiveness of the runs on the samples, run 1, 2, and (Figure 3.5) (Table 3.2–3.4). In the case of solid samples such

as Cheddar and parmesan cheeses, the friction factor (μ) for the runs 2 and 3 were similar in the static phase, around (0.07), whereas the run 1 showed a lower friction factor of (0.01). Beyond the static phase, there were dissimilarities for the way the friction factors for different runs. In the case of Cheddar cheese sample, we observed that the run 3 had a higher friction factor (μ) than the run 2 whereas in case of parmesan, run 2 was higher in terms of friction factor (μ) than the run 3. This could be attributed to their differences in the protein content and fat present in the samples which could have contributed to the dissimilarities in the frictional behavior of the samples during different runs. In the case of cottage cheese, we observed a similar behavior as to that of parmesan where the run 2 had a higher friction factor (μ) (0.50) than the run 3, (0.45) just beyond the static region. This could be attributed to the higher presence of lubrication during run 3 on the geometry surface compared to run 2. In case of milk, on the other hand, we observed run 1 had the highest friction factor (μ) beyond the static region (Table 3.2–3.4).

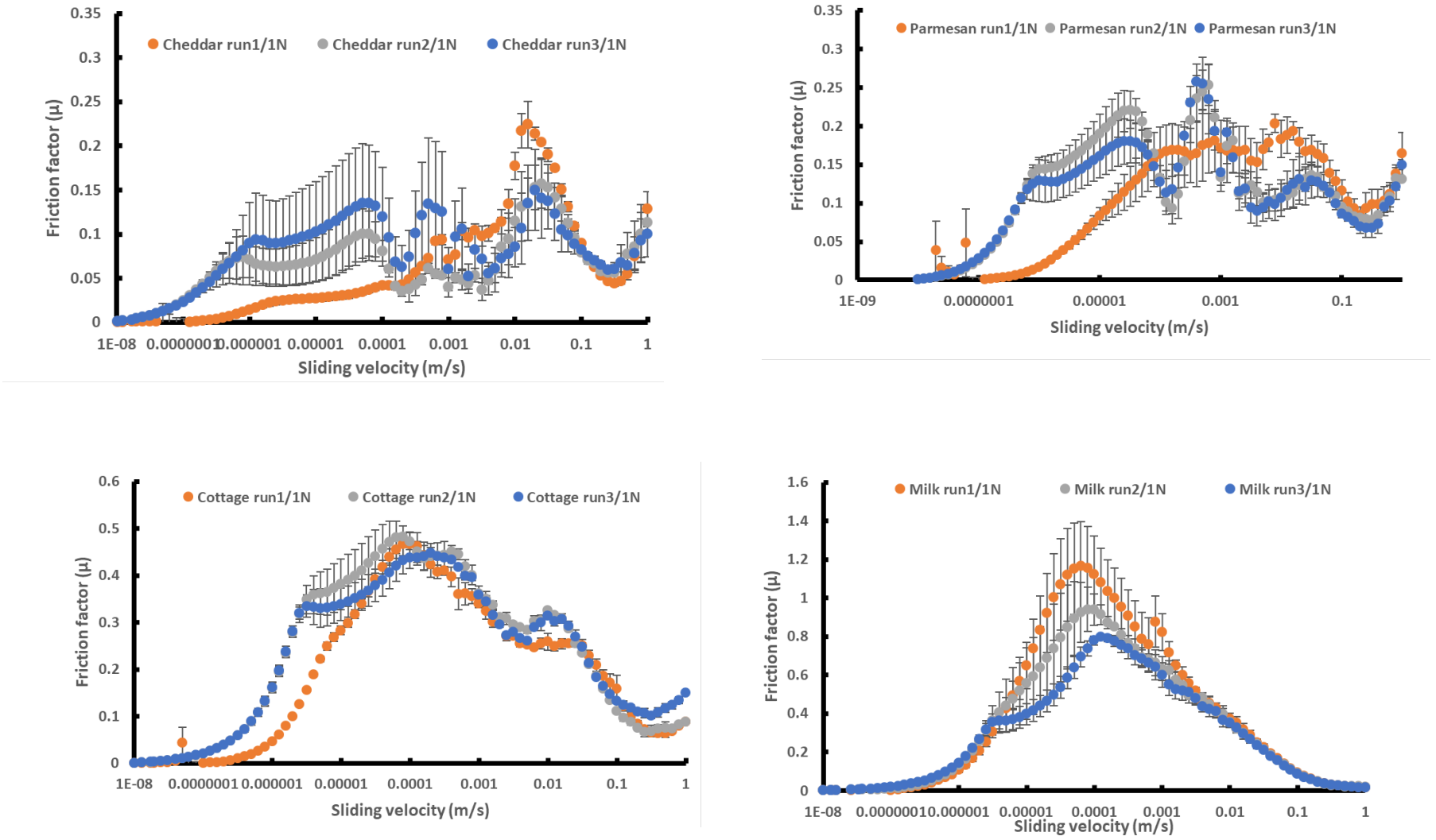

Figure 3.5: Effect of the three runs at 1 N normal force on solid, semi solid, and liquid dairy products during tribological measurements.

During the running in action of the tribological measurements using a rheometer involving PDMS pins, the run 1 is when the contact between the ball and the sample occurs in the static, and with the subsequent runs (repetitive breakdown), the contact changes between sample's interfacial layers during the high shear action of the ball (Lamichhane et al., 2022; Pondicherry et al., 2018). During the first run, the surface of the PDMS pins is subjected to a running in action which assists in even out the surface asperities due to the wear action of the pins (Pondicherry et al., 2018; Sharma Khanal et al., 2019, 2020).

Beyond the static region in the extended stribeck curve, within the kinetic region where the shearing action of the ball on the PDMS pins applies both the shear and the normal forces to the sample, Within the boundary regime in the kinetic region where the applied rotation is slow, the samples show a higher friction followed by the mixed friction regime where the presence of the small amount of lubricant reduces the friction factor. During the region, the sample breaks down into smaller chunks and the particle size is lower, thus mimicking the chewing process in the oral phase of the digestion (Lamichhane et al., 2022). The hydrodynamic regime, which follows immediately after the mixed friction regie, has a higher amount of sample providing lubrication during the testing. As a result, the friction factor (μ) is low during the hydrodynamic regime of the testing for all the three runs. Previously, researchers have observed that the data for the run 1 can be variable, this was attributed towards the differences in the physical and rheological behaviors of the samples (Pondicherry et al., 2018).

For both the samples, the dissimilarities between the runs were minimal at the higher sliding velocities such as 0.001 (m/s). This could be attributed to the phenomenon of stick slip that tends to occur at the higher sliding velocities where the sample particles tend to move past each other due to the shearing and normal force application during the testing.

Effect of composition and physical state

The tribological properties of all the samples at run 2 and 1 N normal force applied was shown in Fig. 6. The liquid samples such as whey and milk showed a higher friction factor (μ) compared to the solid samples such as Cheddar and parmesan cheeses. The friction factor (μ) for milk was 0.92 whereas for whey it was 0.96, which was significantly higher than Cheddar cheese at 0.1. water inherently has more friction factor than milk (Baier et al., 2009). Furthermore, solid, semi-sloid and liquid samples showed a similar profile in the static regime where we did not observe any difference based on their physicochemical properties and physical states. Once the glass ball began to move under the application of a normal stress (in mixed and hydrodynamic regime), the samples showed a variable profile with the increasing sliding velocity (Fig.3.7).

The friction factor (μ) for fat tends to be lower than water (Baier et al., 2009). This could be one of the reasons why we observed a higher friction factor (μ) for milk and whey compared to Cheddar cheese samples studied in this work. These friction factors can be investigated to find their relationship with digestibility of proteins. Which could be related to accessibility of substrate for the digestive enzymes. The presence of a fat layer between the glass balls used for shearing the samples on the PDMS pins may have provided lubrication during the testing which may have caused the friction factor (μ) to be lower for samples with a higher fat content. Since in this study, we have experimented the tribological properties at 37 ℃ which could have caused the fat to move to the sample surface during the testing, resulting in a lower friction factor. Comparing tribological data for all samples, it was evident that Cheddar cheese, yogurt, ricotta cheese and milk had most distinctive tribological behavior suggesting that particle size, protein type and moisture content had greater influence on the frictional behavior.

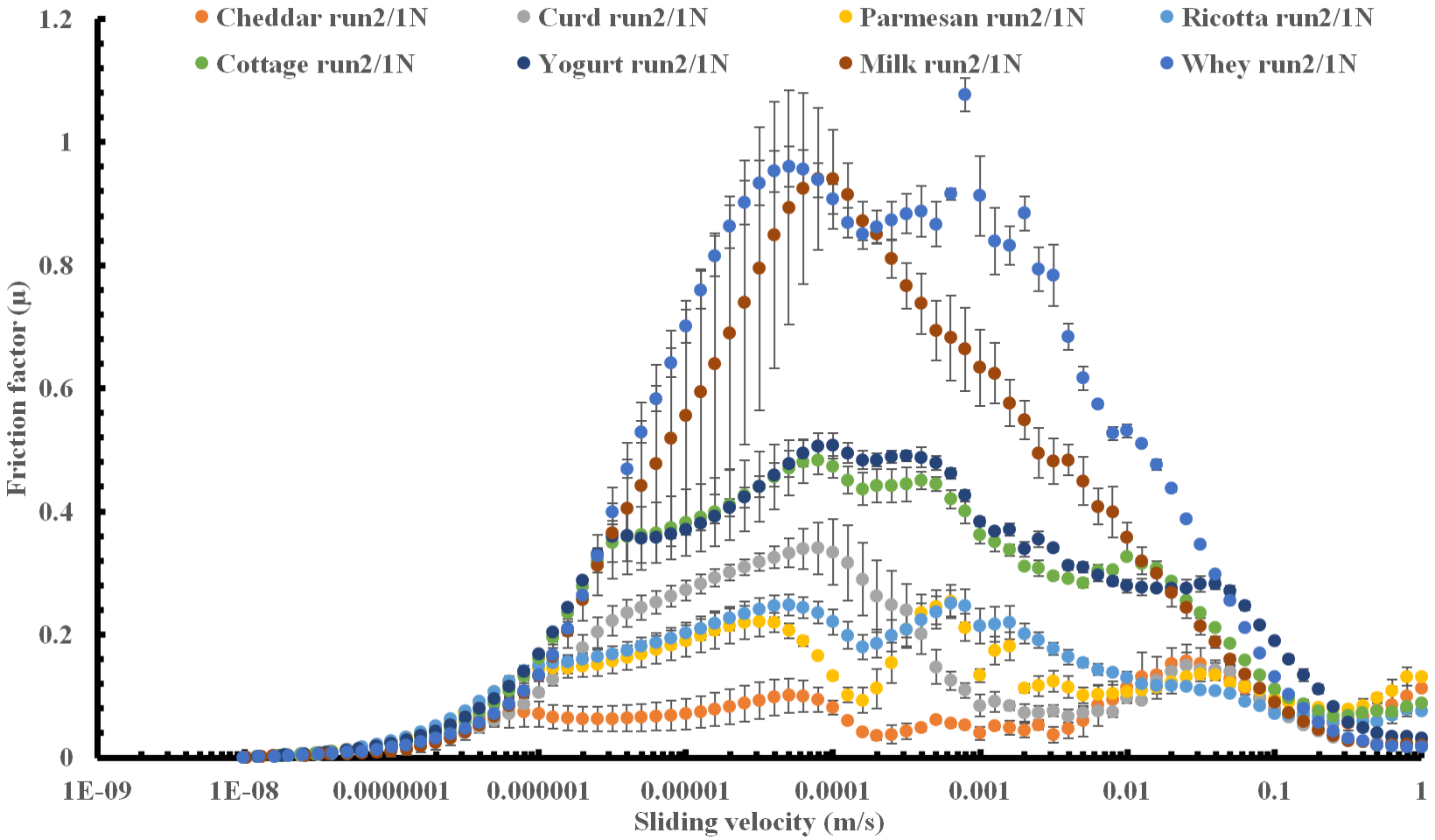

Figure 3.6: Effect of composition and physical state on the tribological behavior of dairy products at 1 N normal force.

SDS PAGE

SDS Page patterns of the eight dairy samples is presented in Fig. 3.7. The samples of solid dairy products, Cheddar, Curd, and Parmesan cheeses exhibited dominant casein (CN) bands, demonstrating the presence of coagulated CN fraction due to rennet action in the cheese-making process. Extra bands corresponding to β-lg were also observed due to residual serum proteins in the cheese samples. Notably, Parmesan showed a greater number of bands with higher intensity, indicative of greater proteolysis that occurs during the longer aging process for this type of cheese.

Ricotta and cottage cheeses, along with yogurt acid and heat coagulated products, make up the semi-solid category. Ricotta demonstrated dominance in both CN and whey proteins, as its manufacturing process mainly involves heat-coagulated whey protein. Therefore, this product is anticipated to have unique digestion behavior compared to other products. Conversely, the CN and whey protein bands in Cottage cheese were like solid cheeses (Cheddar and parmesan), indicating presence of rennet coagulated caseins. Yogurt, which contains all milk proteins, was characterized by the presence of both CN and whey proteins but in degraded form (Wroblewska et al., 2012).

In the liquid category, represented by pasteurized milk and whey, we observed different characteristics. Milk compromised of most of intact protein bands with absence of breakdown products. In whey, a complete disappearance of CN was observed, with distinct β-lg bands clearly evident.

Based on these observations, three samples Cheddar, ricotta cheese and milk were selected from different categories for the study's second objective due to their distinct structural attributes.

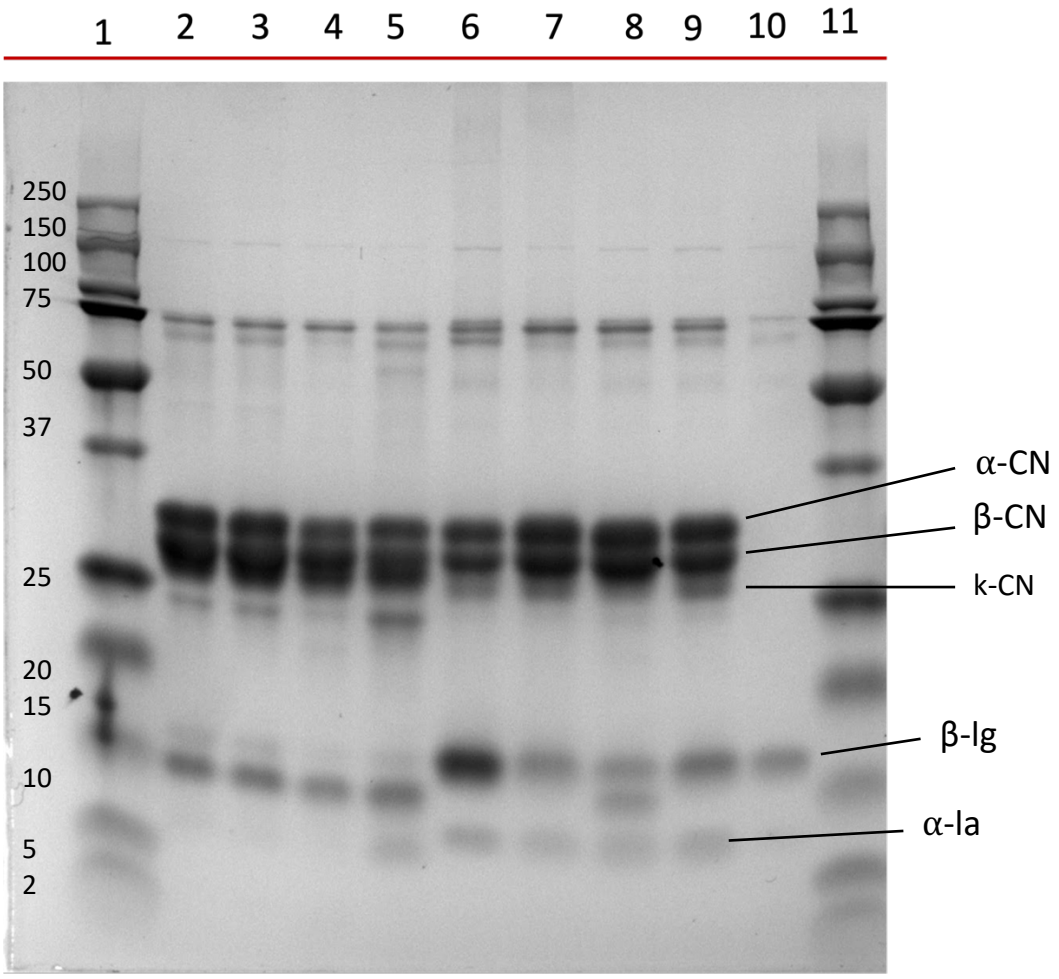

Figure 3.7: Reducing SDS PAGE analysis of solid, semi solid, and liquid samples in the study, 2-10 represent 2. Cheddar cheese, 3. Cheddar cheese, 4. curd, 5. Parmesan, 6. Ricotta, 7. yogurt, 8. cottage, 9. milk, and 10. whey samples, respectively. 1 and 11 contained Bio Rad® Precision Plus protein dual color standards (mixture of 10 recombinant proteins, 10-250 kDa) that were used as markers. The bands were identified based on their molecular weights.

Conclusions

A significant difference in tribological properties was found among various dairy products, with a clear distinction visible in the Stribeck curves. Solid, semi-solid, and liquid forms (such as cheddar, cottage cheese, and milk) differed significantly in their friction factors and sliding velocity friction factor (μ) under various normal forces and sliding speeds. Liquid dairy products, like milk, showed a significantly higher friction factor than cottage cheese and solid cheeses, suggesting a fundamental difference due to their unique compositions and structures. Given these findings, it is evident that tribology can have a substantial impact on their texture and sensory appeal. Interestingly, solid cheeses like Cheddar cheese demonstrated lower friction factors, potentially due to their different protein, fat, and moisture contents, further influencing their tribological properties. In addition to this, semi-solid products presented a unique tribological behavior, indicating that the physical state of the product significantly impacts its tribology. These differences hint towards the use of tribological measures in improving the sensory properties of dairy products, particularly those related to texture and mouthfeel. SDS PAGE analysis clearly indicated fundamental differences in the molecular pattern of the milk proteins in various dairy products.

Therefore, our findings suggest the potential for tailoring the composition and physical state of dairy products to achieve optimal tribological properties. This can assist in improving the sensory appeal of these products, ultimately leading to higher consumer satisfaction in the dairy industry

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CHAPTER 4

IN-VITRO DIGESTION OF MILK PROTEINS PRESENTS IN LIQUID, SEMI SOLID, AND SOLID DAIRY PRODUCTS

ABSTRACT

In this study, three dairy foods, i.e., Cheddar cheese, Ricotta and milk were subjected to In-vitro digestion using INFOGEST protocol to understand their disintegration and the protein release at different time intervals in each of the digestive stage (Gastric phase and Intestinal phase). Structural breakdown of food materials in oral phase was simulated by mixing artificial saliva to the sample using pestle mortar. The mixed bolus was then transferred to the stomach and intestinal phase and subjected to mechanical and enzymatic reactions at 37 ºC for further digestion and protein release. Pepsin enzyme (2000 IU/mL) was used to perform in the gastric phase responsible for protein disintegration at pH 2-3. Whereas trypsin (100 IU/ml) was used for the disintegration of protein into polypeptide fragments in the intestinal phase at pH 7. Samples collected at different time points were centrifuged at 10,000 rcf for 20 mins. Both supernatant and pellet run on the SDS-PAGE and UREA-PAGE for studying molecular breakdown of proteins.

The digestion and protein release for different types of dairy products shows a clear distinction between liquid, semi solid and solid samples during invitro digestion at different time points. The disintegration and release of soluble protein compounds for liquid dairy products such

as milk was significantly faster (60% soluble protein release in 1 h) in gastric phase as compared with solid dairy products such as cheddar cheese (40% soluble protein release in 1 h), and semi solid such as ricotta (25% soluble protein release in 1 h). Electrophoresis images indicate that the rennetted caseins present in cheese were digested faster in gastric phase as compared with the whey proteins present in the liquid milk. This can be attributed to the fact renneted caseins had more open structure as compared to the whey proteins giving easier access to the digestive enzymes. The rate of protein breakdown in the intestinal phase in cheese and milk product were similar. Differences in the rate of protein breakdown during in-vitro digestion could be attributed to the protein content and food structure. These results may assist the food industry to improve the nutritional quality and design functional dairy products by optimizing processing methods.

Introduction

Food digestibility and the breakdown process during digestion are multi-stage processes, involving a variety of physiological interactions. These different stages result in the release of nutritional constituents, effectively supplying our bodies with necessary nourishment through the liberation and absorption of proteins (Hiolle et al., 2020). The efficiency and extent of this process are fundamentally governed by factors such as food texture, and composition (Khanal et al., 2020). The sequential degradation of diverse food structures involves several distinct yet interconnected stages, initiated by the macrostructural breakdown of food during the oral phase. This initial phase incorporates physical actions such as biting and mastication, as well as the enzymatic activity of saliva. Subsequently, the partly digested food proceeds to the stomach and small intestine, wherein it undergoes further enzymatic, biochemical, and mechanical transformations. Most of the nutrient absorption occurs within the gastrointestinal tract, underlining its pivotal role in overall digestion and nutrient release. Among the several food groups, dairy products play an important role in human nutrition at all stages of life. Dairy products are an important part of our diets considering it's high in protein, calcium, and fat. Milk and its products, such as cheddar and ricotta cheeses, are particularly high in proteins, such as casein and whey (Kubicová et al., 2019). Casein is the most abundant protein in milk, accounting for around 80% of total milk protein, with whey protein accounting for the remainder (Barbe et al., 2014). The processing methods employed in milk production greatly influence the composition and structure of the resulting dairy products. Critical parameters such as pH and temperature employed during processing significantly impact their nutritional content by changing their structure. Furthermore, the type of protein used directly affects the quality and nutritional profile of the final dairy product. This study provides an in-depth exploration of in-vitro digestion, specifically focusing on protein release in different dairy

structures, including milk, cheddar, and ricotta. By understanding the impact of dairy product structure on protein digestibility, this study can provide considerable insights for food formulation with optimized nutritional value. It is anticipated that these findings will contribute to the existing body of knowledge on these fundamental processes and their implications for human nutrition. Both food texture and food composition, including protein and fat content, govern food digestibility and protein release in the human gastrointestinal tract. Food disintegration takes place initially in the mouth (Kong, Singh, et al., 2008), followed by further digestion in the stomach and small intestine, leading to nutrient absorption predominantly in the gastrointestinal tract. By examining the process of food digestion from the initial oral phase through to nutrient absorption in the gastrointestinal tract, this study aims to provide a comprehensive understanding of dairy food digestion and its influences on the protein release affected by their distinct structures.

Materials and methods

Sample preparation

Three distinct dairy products namely, milk, ricotta, and Cheddar cheese were obtained from the Aggies Creamery at Utah State University (Logan, Utah). These dairy products were chosen based on various factors such as their physical states solid, semi-solid, and liquid, the type of coagulant used, and the protein composition. To facilitate further analysis, the solid and semi-solid samples were grinded using a pestle and mortar. Subsequently, each product underwent a systematic process that simulated the oral, gastric, and intestinal phases. This procedure was instrumental in assessing the extent of disintegration and the rate of protein release.

Proximate analysis

The proximate composition of the three selected samples, namely Cheddar, Ricotta, and Milk, was analyzed for protein, fat, moisture content, and pH. Protein content was analyzed using a CEM Sprint Rapid Protein Analyzer (Matthews, NC). Approximately 0.400-0.500 grams of each sample were added to the sample cup, the test was performed to determine the percentage of protein in each sample. Moisture content was measured using a CEM Turbo Technology Rapid Moisture Analyzer (Matthews, NC). Approximately 3.0 ± 0.5 g sample of sample was placed between two sample pads, the machine was then used to measure the moisture content.

The Babcock method was utilized to determine the percentage of fat in the three samples. Around 9 g of Cheddar sample, and 18 g of ricotta, and milk was added to a 50% Paley bottle, combined with 17.5 ml of sulphuric acid, and centrifuged to separate the fat layer. The obtained fat layer was measured as a percentage of fat in the cheese sample. For pH measurement, a calibrated pH meter

was used. The electrode was submerged into the samples, ensuring the electrode was not touching the container's sides or bottom. After the reading was stabilized, the pH value was recorded. All measurements were performed in triplicates for each sample. *Invitro* digestibility of dairy samples

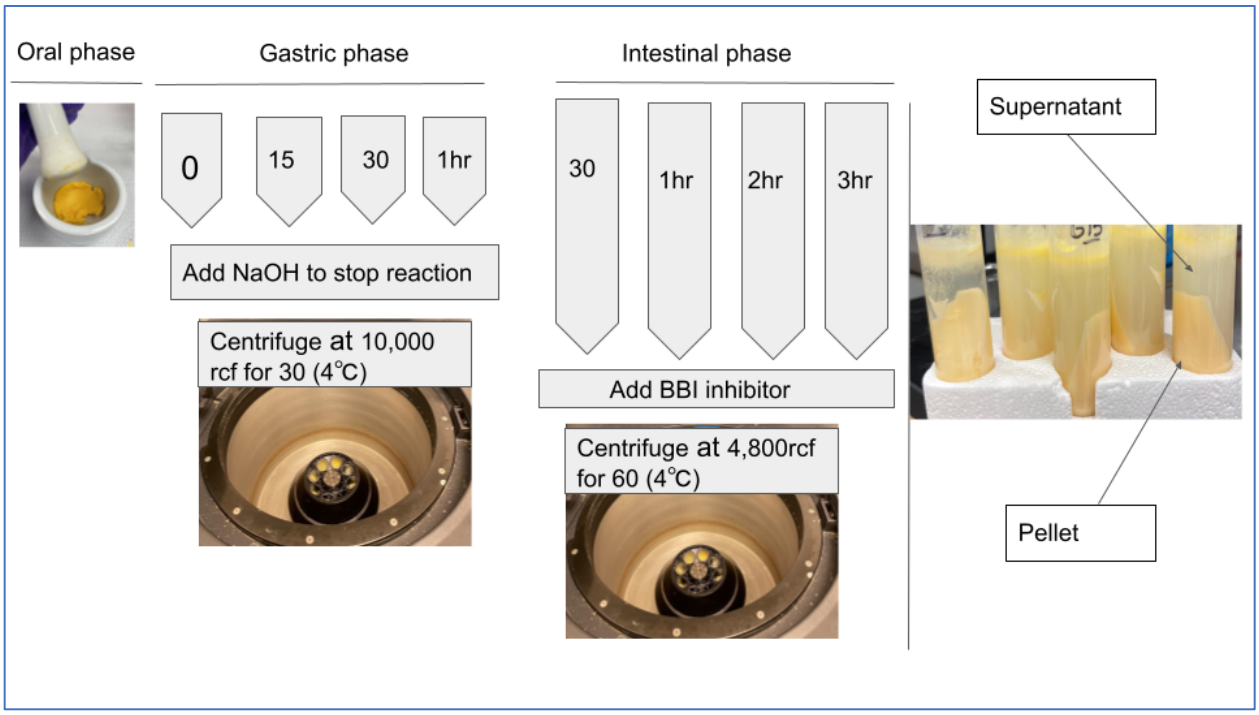

Figure 4.1: Schematic design of invitro digestion during gastric and intestinal phases at different time intervals.

Simulated in-vitro digestion method was used to mimic the human digestion process (Brodkorb et al., 2019), thereby simulating the physiological conditions including pH, temperature, and digestive fluids, to study the release of nutrients in different dairy products figure 4.1.

Three different dairy structures were selected for in-vitro digestion. To simulate the oral phase of digestion, dairy samples were first diluted with a prewarmed simulated salivary fluid (SSF) at a

ratio of 1:1 (wt/wt) to obtain a swallowable bolus with a paste-like consistency. Afterward, the sample was incubated at 37 ℃ for 2 min. After obtaining the digested sample from the oral phase and measuring the volume, preheated simulated gastric fluid (SGF) at 37 °C was added to the oral bolus with 1:1 ratio (%V/V).

The pH was adjusted to 2.00 by adding 6M of hydrochloric acid (HCl) mimicking gastric pH. Calcium chloride dihydrate $CaCl_2(H_2O)_2$ was added to achieve a final concentration of 0.15 mM in SGF. During the conditioning phase, 2,000 U/mL of prepared porcine pepsin was made up in 37 °C water solution to obtain 30 mg in a 15 mL tube. Then 4 mL of prewarmed DI water was used (Table 4.1).

After the conditioning phase was completed, 2 mL of porcine pepsin solution of 2,000 U/mL was added. Water was added to obtain 1x concentration of the SGF. pH was checked and maintained at 2.00. Afterward, the sample was incubated at 37 ℃, then mixed sufficiently in the Titrando for one hour from the point at which pepsin was added. The samples were collected at different time intervals; 0, 15, 30 and 60 min. Afterward, the enzyme reaction was stopped by adding 6M NaOH. For further digestion in the intestinal phase Bile salt solution was prepared by dissolving 350 mg bile salt in 6 mL prewarmed SIF (37 ℃) in a 15 mL tube by using a vortex. To achieve better digestion, bile salt was dissolved 20 -30 min prior to starting the intestinal phase, so that the bile salts are completely dissociated. Then SIF electrolyte was added to the gastric chyme to get the final ratio of 1:1 (%V/V). After the pH was adjusted to 7.0 with the addition of 6M NaOH, bile salt solution was added to the SIF/gastric chyme to obtain the final concentration of 10mM. The solution was kept stirred in the Titrando at 37 ℃ for 30 min for complete bile solubilization. Then Calcium chloride dihydrate solution was added to get a concentration of 0.6 mM in SIF. Based upon the pancreatin activity, 8000 U/mL of pancreatin

solution was prepared by mixing it in SIF electrolyte stock solution, with a final concentration of 100 U/mL in the final mixture (Table 4.1). During the conditioning phase of the intestinal protocol, 0.4 g pancreatin was dissolved in 10 mL SIF solution. The 10 mL pancreatin enzyme solution was added to the digesta. After pancreatic enzyme was added, the sample was incubated at 37 ºC and mixed using a rotating wheel/ shaking incubator for 3 hours, samples were collected at different time intervals (I5, I30, I60, I 120, I 180). A 100 µL of Bowman-Birk Inhibitor (BBI) solution (0.05g/L) in water per mL of intestinal digesta was added to inhibit pancreatin enzymes. Once the targeted inhibition occurs, the digests were centrifuged at 10,000 rcf for 20-40 mins to get supernatant and pellet components. The supernatant was collected. At the same time both the supernatant and pellet were frozen at -80 ℃ and freeze-dried for further test experiments.

Table 4.1: Infogest invitro digestion protocol with 10 g of food throughout the three phases (oral, gastric, intestine).

Digestion phase	Oral	Gastric	Intestinal	
Food or digests	10 g of food samples	20 mL from oral phase	40 mL from gastric phase	
1.25X electrolyte stock solution	8 mL	16 mL	16mL(total volume of SIF, including 10mL pancreatin and 6mL bile salts, dissolved in SIF)	
$CaCl_2(H_2O)^2$ (0.3 M)	50 µL	10 µL	80 µL	
Enzyme activity (U/mL)		2000 U/mL	250 U/mL	
Enzymes		Pepsin	Trypsin in Pancreatin	Bile salts
Concentration of enzyme/bile solution		16 mg/ mL	266 mg/mL	530 mg/mL
Volume of enzyme/bile to added		2 mL	10 mL	6mL
Final volume	20 mL	40 mL	80 mL	

Particle size distribution

The average particle size and particle size distribution of the three digested dairy products were examined using a laser-light diffraction Anton Paar 1190 model (Anton Paar, Graz, Austria) operating with Kalliope software (Anton Paar, Graz, Austria). Digested samples were immediately collected during gastric phase (G0, G15, G30, G60), and after 5, 30, 60, 120, 180 min in the intestinal phase. A certain volume of the digested sample was introduced into the dispersion unit until a laser obscuration range between 5-30% was achieved.

The particle size of the selected dairy products, namely milk, ricotta, and Cheddar cheese, were characterized using the volume-weighted average (%). volume weighted mean diameter D [4,3] was obtained in triplicates for each sample.

UREA-PAGE

The disintegration of proteins during gastro-intestinal digestion at various time points was examined by analyzing the profile of protein release in the supernatant and the protein content retained in the pellet post-centrifugation, using a urea-PAGE method. This analysis was conducted using urea Polyacrylamide Gel Electrophoresis (PAGE), performed on a precast 15% Bio-Rad TBE polyacrylamide gel (Bio-Rad, Hercules, CA), following the protocols of Ryne et al., 2004 and Sharma Khanal et al., 2019. The sample buffer was prepared by combining 0.75 g of Tris (hydroxymethyl)- methylamine, 49 g of urea, 0.7 mL of β-mercaptoethanol, and 0.10 g of bromophenol blue in ultrapure water, resulting in a final volume of 100 ml. Digested dairy samples were incorporated into this sample buffer to achieve a final concentration of one mg of protein per

one ml of the buffer. These solutions were subsequently heated at 55 °C for 15 min, then allowed to cool to room temperature before being loaded onto the gel. Mini-Protean TBE-Urea Precast Gels, procured from Bio-Rad (Bio-Rad Corp., California, USA), were pre-run at 180 V for 30 min. Following this, 10 mg of proteins (equivalent to 10 μL of samples) were loaded into the wells. The urea PAGE was then conducted at 120 V for 90 min, and the voltage was increased to 180 V once the bands reached the bottom of the gel. Post-run, the gels were rinsed with ultrapure water, for 5 min. They were then stained for an hour using Coomassie Brilliant Blue G 250 (Sigma Aldrich, St. Louis, MO). After the designated staining period, the gels were de-stained with ultrapure water and stored overnight at refrigerated temperatures. Images of the gels were obtained using an Analytikjena Image Scanner (Analytikjena, Jena, Germany). Sodium caseinate was used as a control for intact casein.

Soluble Nitrogen and protein release

The calculation of protein release was achieved by transforming nitrogen into a percentage of protein. This was done by multiplying the nitrogen value by a conversion factor of 6.38 (Moubois et al., 2016). The soluble nitrogen present in the samples post in-vitro digestion was quantified using the Vario Max Cube model, a multifunctional elemental analyzer. This assessment yields insights into the degree of protein digestion and release, given that an elevation in soluble nitrogen content is associated with increased protein hydrolysis levels.

The formula to calculate the protein release percentage (PR%) is given by (Fang et al., 2016):

PR % = CS (WS)/p) $P_0$100. In this formula, 'Cs' symbolizes the protein concentration present in the supernatant after time 't'. 'Ws' signifies the weight of the supernatant (in grams) after the same

period, 't'. 'P_0' is the initial protein weight in the cheese sample (in grams), and ' p ' is the supernatant's density, assumed to be 1 g/L.

Disintegration rate

During the GP and IP stages, the digested samples were collected at different time points. Sodium hydroxide was added at the GP stage to stop the reaction, and Bowman Birk Inhibitor BBI was added during the IP stage. Immediately after being digested, these samples were transferred to an iced bath until they were centrifuged. The resultant digesta was composed of both liquid and solid phases. Centrifugation was done for the gastric phase samples at 10,000 rcf for 30 minutes at 4 C. The intestinal phase samples were centrifuged at 4800 rcf for 60 minutes at 4^{o}C. In the meantime, the pellets and supernatants were then separated. Samples disintegration contributed to the separation of dairy components into the liquid phase throughout digestion (Khanal et al., 2020). This was calculated based on this equation:CD (%) = (W_0- W_p)/W_0 * 100, whereas, W_0 = weight (g) of initial dairy sample prepared for the digestion. W_p= weight (g) of dry pellet after digestion at each phase.

Statistical analysis

Triplicate experiments were conducted to ensure the accuracy and reproducibility of the results. To assess the statistical significance of differences between various samples, a one-way

analysis of variance (ANOVA) was employed, with a post-hoc Tukey's test, predetermined significance level of 5% ($p < 0.05$) to compare different samples.

Results and discussion

Proximate analysis

The proximate composition of the three experimental samples is presented in Table 4.1. The average protein content for the Cheddar samples was found to be 24.24 %, the fat content averaged 33.00%, and the moisture content was 37.02 %. Cheddar exhibited the highest protein and fat percentages among the three samples, while having the least moisture content. In contrast, the ricotta samples demonstrated significantly lower protein and fat content, with mean values of 11.07% and 14.00%, respectively. This is attributed to higher moisture content of ricotta cheese 73.06% w/w. Moisture and other solids (fat, and protein) usually have inverse relationship. Ricotta cheese is prepared by heat and acid coagulation at slightly higher pH 5.9 (compared to other cheese varieties) (Farkye et al., 2017). These conditions lead to retention of more moisture in the coagulum. In fact, lower amount of proteins and fat in the ricotta was a result due to higher retention of moisture (Farkye et al., 2004). The Milk samples contained the lowest percentage of both proteins (3.21%) and fat (~3.5%) as compared to Cheddar and ricotta cheese, owing to its higher moisture content (88.04%). Milk contains both caseins and whey proteins in soluble forms, whereas proteins in ricotta cheese are acid and heat coagulated with

less solubility. On the hand, proteins present in the aged Cheddar cheese are rennet coagulated caseins which are further broken down into smaller protein and peptide fractions during ripening. Compositional differences within these products can also form a basis for the differences in the final structure and protein breakdown pattern during in-vitro digestion.

Table 4.2: Proximate composition of Cheddar, Ricotta, and Milk samples

Samples	Protein (%)	Fat (%)	Moisture (%)	pH
Cheddar	22.2±0.46 [a]	33.00±0.5 [a]	31.66±0.61[a]	5.1±0.00 [a]
Ricotta	10.69±0.20 [b]	10.60±0.16 [b]	73.61±0.87 [b]	5.9±0.01[b]
Milk	3.54±0.03 [c]	3.66±0.16 [c]	87.42±0.53 [c]	6.6±0.00 [c]

Values with different superscript letters indicate significant difference within the column at 5% level of significance. Values are presented as average ± SE.

Disintegration behaviour

The changes in particle size distribution of digested whole milk samples during gastric and intestinal tract is shown in Figure 4.2 A, B. The multimodal distribution in both gastric and intestinal phase was observed indicating presence of multiple components in the whole milk

digesta. A peak around 73 μm suggest large population of acid coagulated casein particles due to HCl addition in the gastric phase. These coagulated particles may have some entrapped fat within the network (Ye et al., 2011). Small peaks around 30 and 50 μm may indicate presence of disintegrated casein particles due to enzymatic and mixing action in the gastric and intestinal phase. A slight leftward shift of the peak was observed in the particle size distribution during gastric digestion indicating a change in the macroscopic breakdown of the casein particles over time. This slight shift suggests that pepsin enzyme and the mechanical force in the stomach are effective in breaking down larger coagulated milk particles into smaller ones. However, this trend was more pronounced in the intestinal phase suggesting that proteolytic enzymes present in commercial pancreatin were helping degrading the microstructure to a greater extent.

The volume weighted mean particles size $d_{4,3}$ also showed decreasing trend with digestion time Figure 4.2, C. The decrease in the $d_{4,3}$ was more pronounced in the gastric phase than intestinal phase owing to both acid and mechanical agitation. There was a slight increase in $d_{4,3}$ while switching from gastric to intestinal phase which could be attributed to changes in the voluminosity of caseins due to changes in the pH from 2.0 to 7.0 (Tunick et al., 2016) or coalescence of fat particles. A subsequent decrease in the $d_{4,3}$ of these aggregates was observed in the rest of intestinal phase. According to Liu et al., 2019 aggregates formed during the gastric phase tend to disintegrate in the intestinal phase due to enzymatic action of proteases and lipases. Figure 4.2, D. demonstrates the process of disintegration of milk protein particles during digestion. The initial disintegration rate for the milk matrix at a temperature of 37 ^{o}C begins at approximately 60%, attributed to the soluble nature of proteins present in the milk. Over the course of 4 hrs. of digestion time, the disintegration increased from 60% to 81%. There was a minor decrease in disintegration observed after a 30-minute digestion period in the gastric phase,

which may be associated with the coagulation effect of milk due to the stomach's acidic environment a pH of 2. As the digested milk transitions into the intestinal phase, the disintegration percentage begins to increase again, ranging between 65-81%. This contributes to the idea that most of the milk structure disintegration occurs within the intestinal phase.

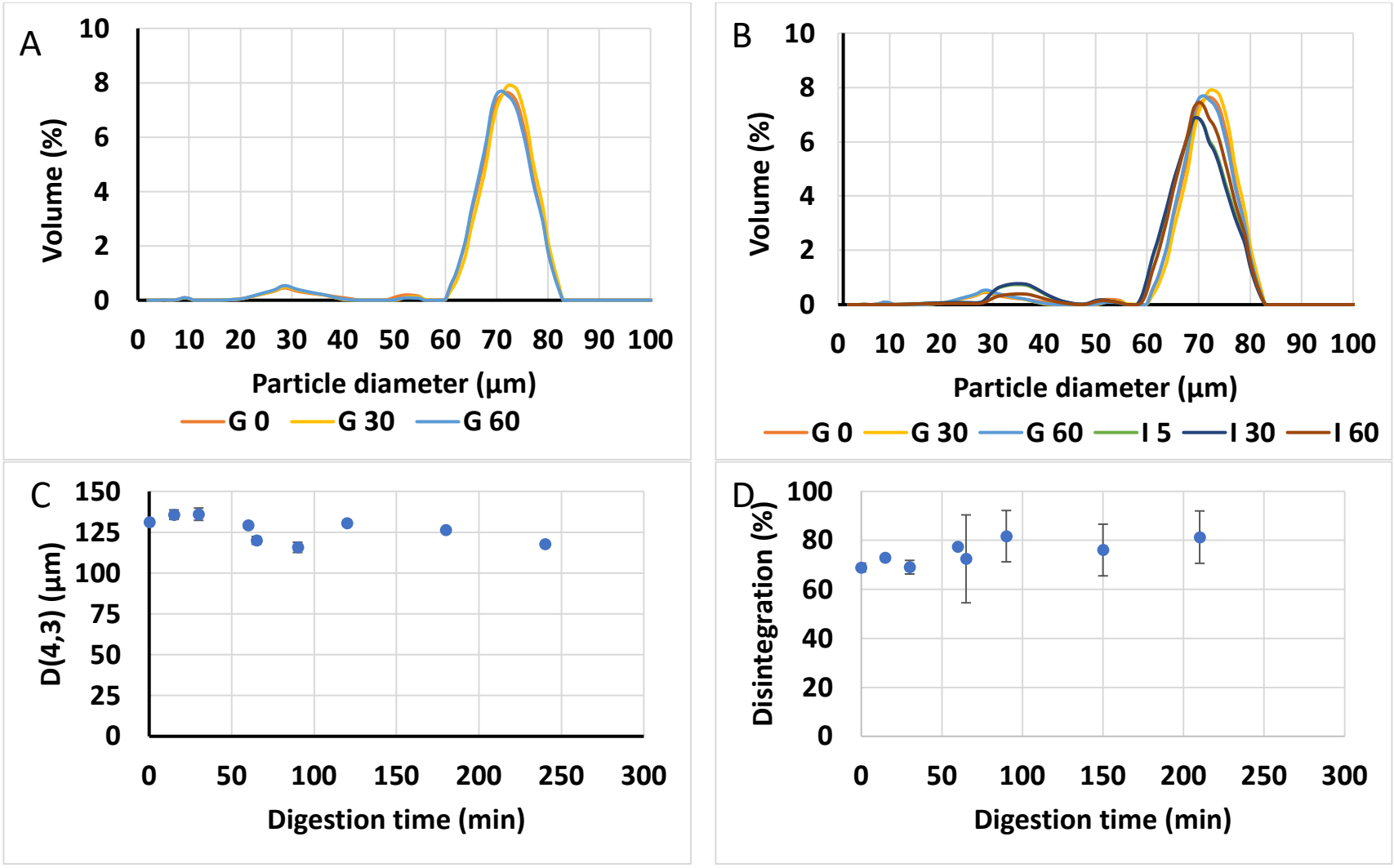

Figure 4.2: Changes in the particle size distribution of whole milk digesta during gastric (A) and intestinal (B) in-vitro digestion (G0, G30, G60; I5, I30 and I60). Volume-weighted mean diameters (d_{43}) for whole milk (C) during in-vitro gastrointestinal digestion (G0,

G15, G30, G60, I 5, I 30, I60, I120, I180). Disintegration percentage (D) of whole milk matrix during in-vitro gastrointestinal digestion. Data represents mean ± standard error of mean (n=3).*

The disintegration behavior of ricotta during digestion is shown in Figure 4.3. Initially, at the onset of the gastric phase (0 min), the particle size population exhibited an almost monomodal distribution, ranging from 2.0 to 104 μm with presence of peak around 70 μm. By the time reaching to 30-minute interval (G30), a decline in particle size was observed with left ward shift of population from 70 μm to 65 μm. A new particle population within a smaller size range (<50 μm) started appearing with another peak around 30 μm. By the end of the gastric phase (60 min), the peak had shifted leftward (towards smaller particle size), revealing a bimodal particle size distribution pattern, clearly indicating formation of smaller aggregates from the large aggregates. This shift implies a disintegration of particle sizes throughout the digestion process, potentially due to the influence of enzymes on food particles or mechanical force at the gastric stage. In the intestinal phase, the disintegration of particles continued rapidly, peak shifting further to the left and causing an expansion shoulder in the second peak's range. These demonstrate continued breakdown of particles underlining the action of pancreatin and bile salts on the structural breakdown of the heat coagulated soft texture of ricotta. Similar pattern of rapid digestion during the intestinal phase was observed for soft gels (Guo et al., 2016). The volume-weighted mean particle sizes $d_{4,3}$ for ricotta cheese decreased steadily throughout the digestions process as shown in Figure 4.3C. The data indicates a steady decrease in the $d_{4,3}$ volume-weighted mean particle size (from 115 to 35μm) during the initial hour of gastric phase digestion. This trend suggests ongoing structural degradation at this stage, which could be affected by gastric enzymatic activity, mechanical factors, or a mixture of both. As the digestion progresses into the intestinal phase, an initial increase in the particle size is observed within the first 30 min I5-I30, from 55 to 65μm. A similar increase in particle size was also observed in the case of whole milk digestion. This increase might be related to the aggregation of protein and fat particles due to pH change from 2.0 to 7.0 or

due to the enzymatic action of proteases and lipases causing precipitation proteins or flocculation of fat. Subsequently, a decrease in the d 4,3 values is observed until the final observation point in the intestinal phase, where it reaches 51.6 μm. Figure 4.3, D provides information on the disintegration process of the ricotta matrix, measured as a percentage of matrix dispersion, during digestion at 37 °C. The initial disintegration percentage was 39% at G0 (lower than whole milk), which progressively increased up to 53% at the final point of the gastric digestions (G60). This gradual increase of the disintegration of the structure in the gastric phase can be attributed to the soft texture of the ricotta cheese and high whey protein content.

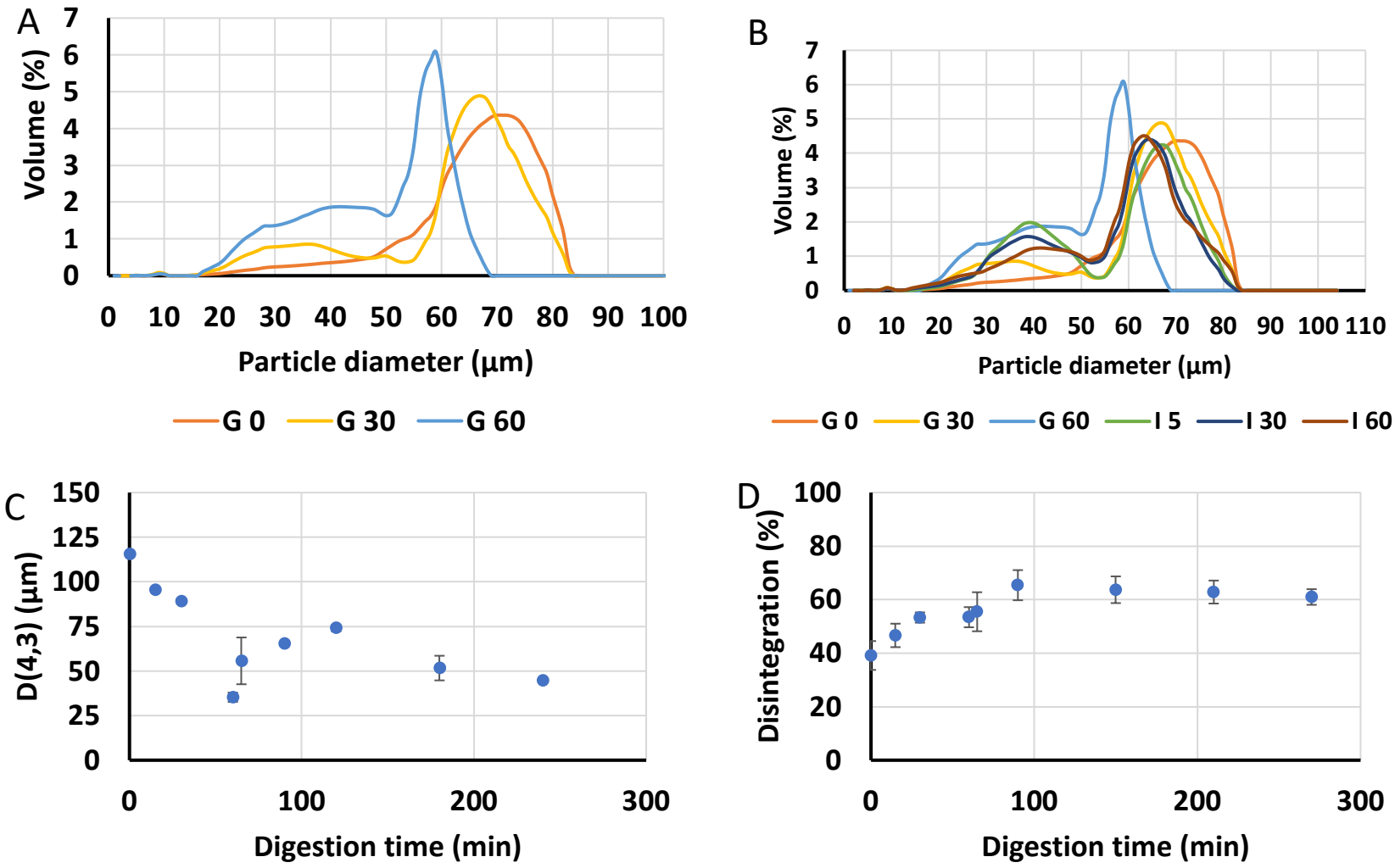

Figure 4.3: Changes in the particle size distribution of ricotta cheese digesta during gastric (A) and intestinal (B) in-vitro digestion (G0, G30, G60). Volume-weighted mean diameters (d_{43}) for ricotta cheese (C) during in-vitro gastrointestinal digestion (G0, G15,

G30, G60, I 5, I 30, I60, I120, I180). Disintegration percentage (D) of ricotta cheese matrix during in-vitro gastrointestinal digestion. Data represents mean ± standard error of mean (n=3).

The particle size distribution for digested cheddar during the gastric phase ranges between 20-82 μm as shown in Figure 4.4 A. A leftward shift in the particle size distribution peak was observed within the first hour of gastric digestion, indicating presence of smaller size particle. Moreover, a monomodal distribution was the pattern for the initial hour of digestion.

As the digestion progressed into the intestinal phase, the particle distribution assumed a bimodal pattern, as presented in Figure 4.4, B, indicating further breakdown of the cheese matrix. Figure (4.4, C) displays the volume-weighted mean particle size $d_{4,3}$ for Cheddar cheese digesta. Initially, the $d_{4,3}$ for cheddar increased from 101 μm at the onset of digestion to 130 μm in the gastric phase. This increase could potentially be due to the aggregation of protein and fat particles present in the cheddar cheese. However, a subsequent continuous decline in $d_{4,3}$ was observed, reaching 77.8 μm by the end of the gastric phase. This decrease suggests that the aggregated particles broke down due to enzymatic action and agitation. Following this, an increase in particle size was observed within the first 5 min in the intestinal phase (like whole milk and ricotta cheese), This fluctuation continued until a plateau was reached towards the end of digestion. The initial increase might be due to larger particles of added enzymes and bile salts, while the subsequent decrease could result from the action of pancreatin. The disintegration rate for cheddar cheese is illustrated in Figure 4.3D. When compared to ricotta and milk (Figures 4.2 and 4.3), Cheddar cheese showed a lower extent of disintegration i.e., 10.6% at G0, which might be linked to its compact structure. Nevertheless, disintegration consistently increased during the entire digestive process, including both gastric and intestinal phases, due to enzymatic and mechanical action.

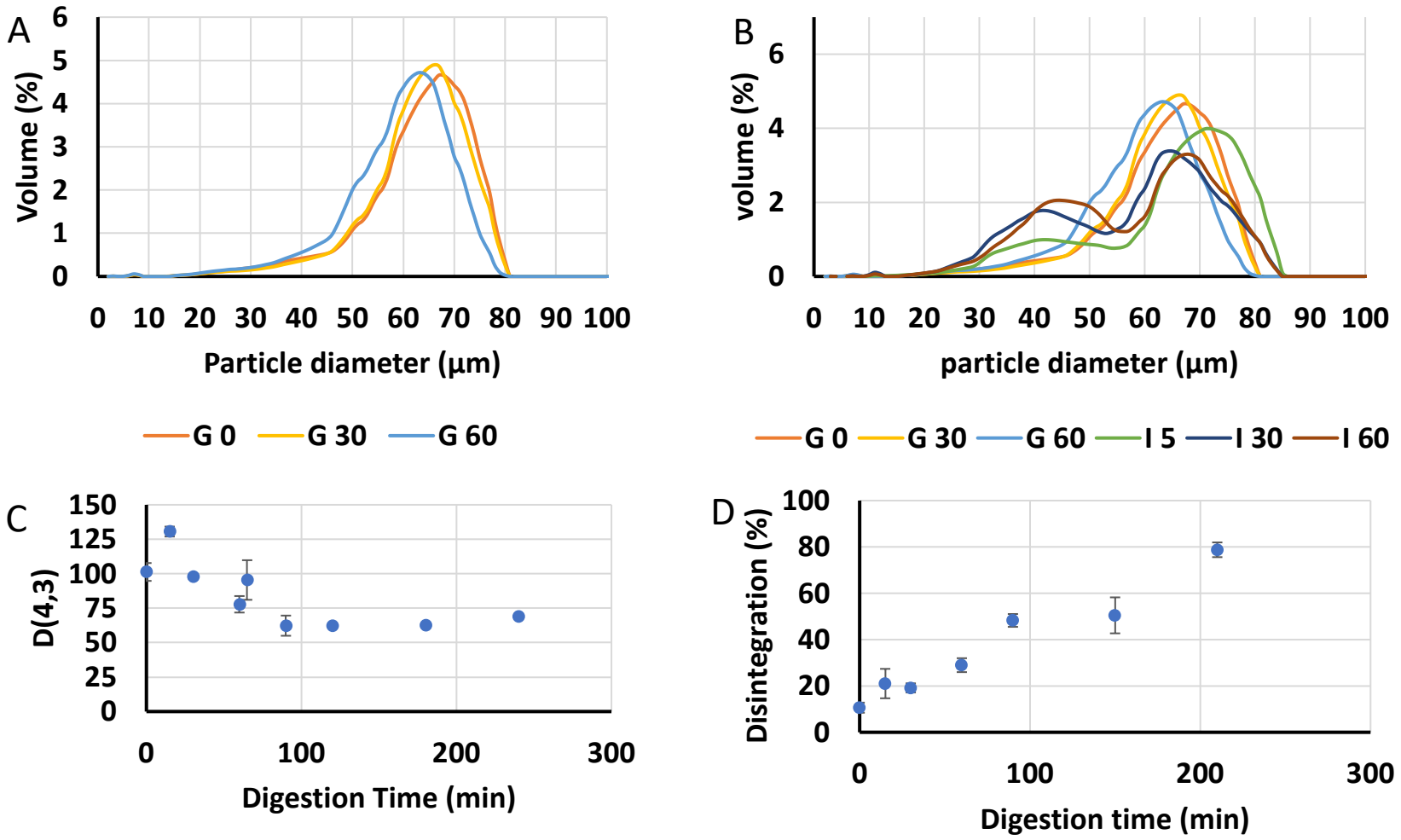

Figure 4.4: Changes in the particle size distribution of Cheddar cheese digesta during gastric (A) and intestinal (B) in-vitro digestion (G0, G30, G60). Volume-weighted mean diameters (d_{43}) for Cheddar cheese (C) during in-vitro gastrointestinal digestion (G0, G15,

G30, G60, I 5, I 30, I60, I120, I180). Disintegration percentage (D) of Cheddar cheese matrix during in-vitro gastrointestinal digestion. Data represents mean ± standard error of mean (n=3).

Overall disintegration rate of liquid milk (80%) was found to be higher than Cheddar (78%), followed by ricotta cheese (60%). Irrespective of the fact that Cheddar cheese started at lower disintegration (10%) rates at the beginning it matched with milk in terms of total disintegration at the end of digestion. This clearly indicates that rate of disintegration of Cheddar cheese was much higher than milk. While milk disintegrated to full extent almost instantly. On the other hand, initial disintegration for ricotta cheese was almost 40% which was increased to 60% after4 hours of digestion, indicating slow rate of digestion.

Protein release in the gastrointestinal tract

The rate of protein release from milk during the digestive process is shown in Figure 4.5. It is clearly evident that protein release for milk increased from 62% to 80% by the end of 4 hours of gastro-intestinal digestion. The digestion in the gastric phase, shows a protein release percentage around 60% at the onset, specifically at 0 min. This initial release phase is important in providing immediate nutritional benefits, allowing for the prompt protein release and absorption by the body.

Following this, the digestion progresses in the intestinal phase continuously increased with the protein release percentage. After a period of three hours, the protein release reached a percentage up to 80. This gradual yet steady increase further reinforces the important role of time in the absorption of proteins, providing sustained nutrient release. The incomplete 80% protein release could be related to the food requiring more time in the intestinal phase or a potential nitrogen loss (Loveday et al., 2022) or availability of enzyme to the substrate. A key factor contributing to this protein release pattern is the physical state of milk and milk proteins. The data in Table 4.1 reveals that milk in its soluble form, which possesses the highest moisture content, demonstrates the most favorable protein release profile. The moisture content likely enhances the digestion and

absorption process, potentially by improving the solubility and accessibility of the proteins in the gastrointestinal tract.

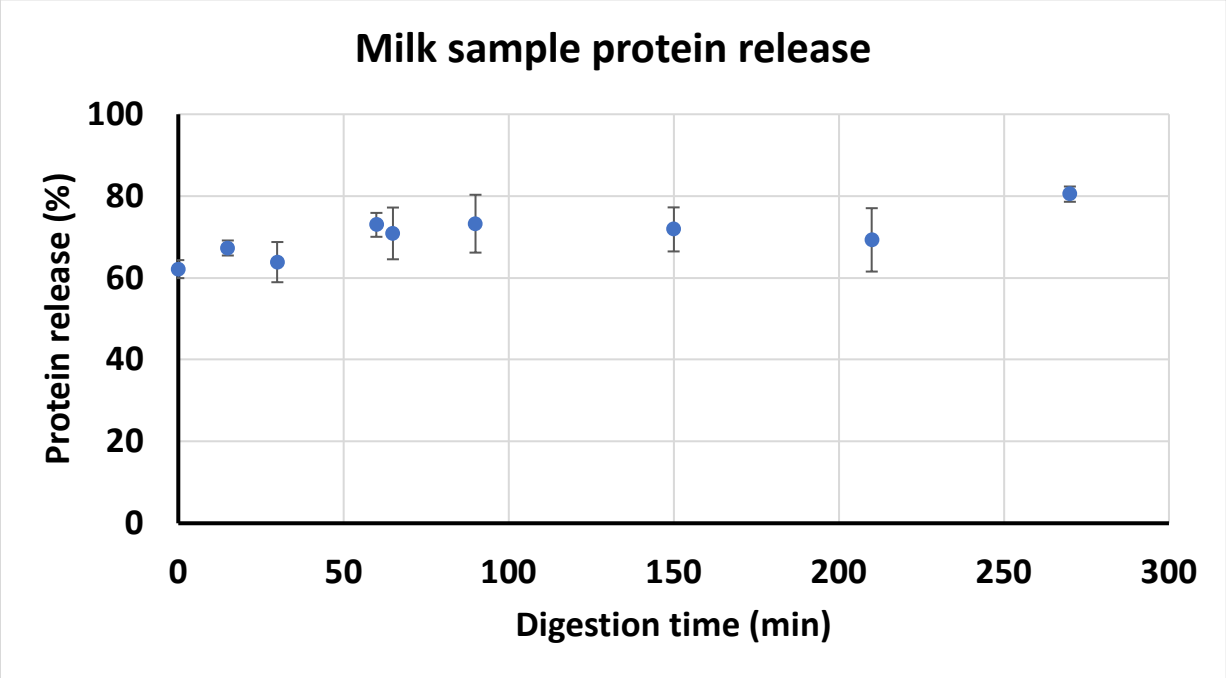

Figure 4.5: Protein release percentage during invitro digestion of milk in the gastric and intestinal stages. The data shown are mean ± SEM.

Figure 4.6, provides a detailed illustration of the progressive protein release in ricotta cheese. As soon as the gastric phase initiates at zero-minute time point, there is a noticeable protein release which constitutes approximately 25% of the total protein. This pattern is continuing consistently through both the gastric and intestinal phases. The explanation behind this progressive protein release lies in the specific properties of ricotta cheese. Primarily, ricotta is a heat and -coagulated

cheese, meaning that the heat treatment it undergoes during processing leads to a change in the structure of its protein particles. This structural change contributes to increased resistance to the action of pepsin (Halabi et al., 2020), a key enzyme involved in protein digestion. Therefore, the protein in ricotta cheese is released gradually and consistently over the course of the gastric and intestinal phases, instead of being rapidly broken down. This provides a clearer understanding of the influence of cheese production methods on the behavior of its proteins during digestion.

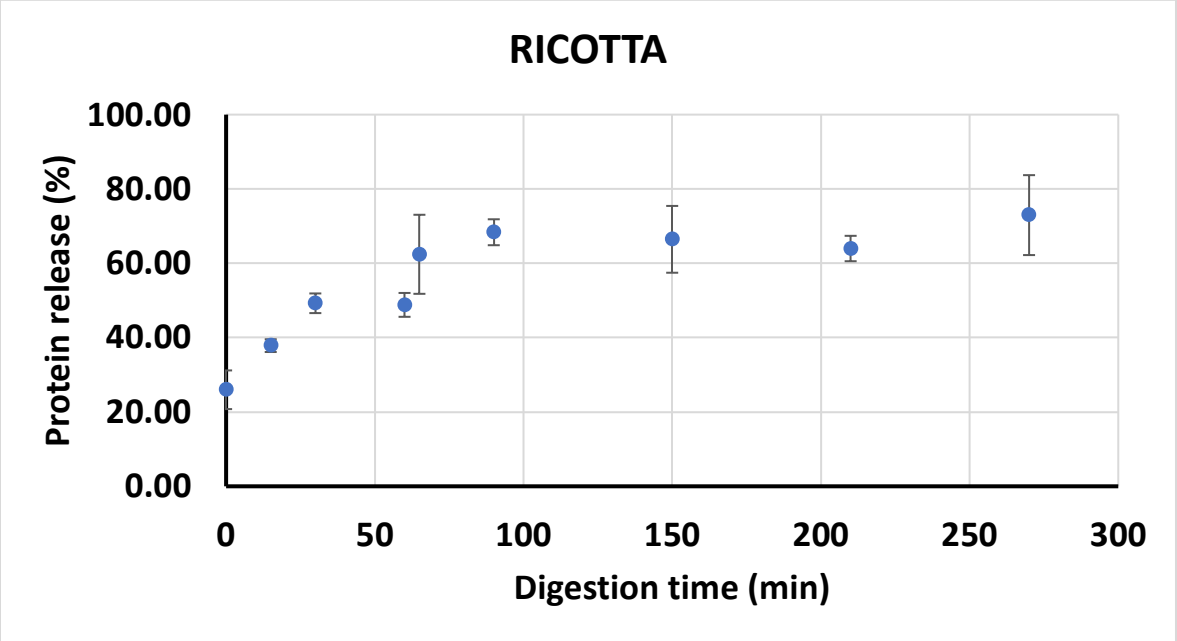

Figure 4.6: Protein release percentage during invitro in both gastric and intestinal phase for ricotta matrixes. The data shown are mean ± SEM.

A comprehensive visualization of protein release from Cheddar cheese during both the gastric and intestinal phases of digestion was provided in Figure 4.7. The protein release initiates

at approximately 40%, with a considerable degree of release during the gastric phase. A slight increase in the protein release percentage continues into the intestinal phase, resulting in about 60% of the cheese's total protein content being released after a span of three hours. This pattern of protein release is markedly faster in Cheddar cheese as compared to Ricotta cheese as shown in Figure 4.6, which could be largely attributed to the composition, structure, and texture in each cheese type (Fang et al., 2016). Moreover, there is always residual rennet activity in the Cheddar cheese which could reduce the molecular chain length of proteins, therefore faster breakdown. In addition to the coagulant type for each product. Cheddar cheese, being rennet-coagulated, is more susceptible to digestion by pepsin during the gastric phase due to the structure of its caseins (Halabi et al., 2020). On the other hand, Ricotta cheese undergoes heat and acid coagulation, a process that potentially results in a delay in the release of protein, as affirmed by the studies of Halabi et al. (2020). This heat-coagulation of ricotta cheese delay the protein release due to the limited access of pepsin on whey protein structures, making them more resistant to digestion (Halabi et al., 2020).

To conclude, the distinct processes of coagulation utilized in the production of Cheddar and Ricotta cheese directly influence their protein release patterns during digestion.

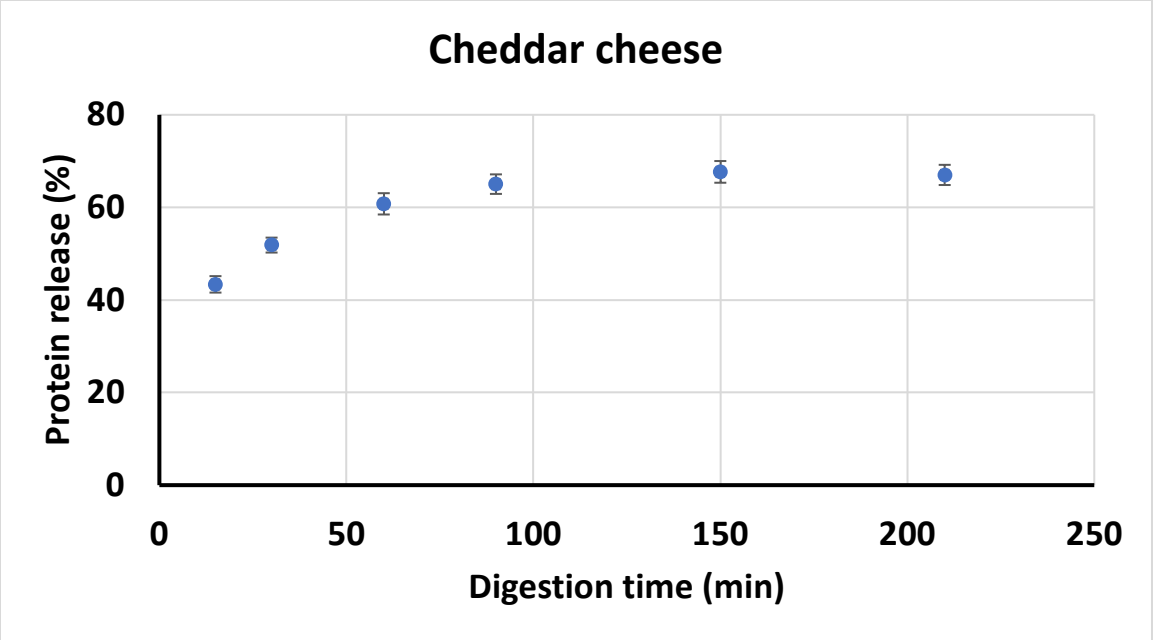

Figure 4.7: Protein release percentage during invitro in both gastric and intestinal phase for cheddar cheese. The data shown are mean ± SEM.

The data shown in Figure 4.8 compares the protein release from three different dairy products, milk, cheddar cheese, and ricotta cheese, across three stages of digestion: oral, gastric, and intestinal. Significant differences ($p<0.05$) were noted in protein release during the oral phase among these three products. Similar trends were observed in the gastric phase, which can be attributed to the enzymatic action of pepsin and its role in disintegrating protein structure. Milk displayed the highest percentage of protein release, attributable to its soluble protein form. This was followed by cheddar and then ricotta cheese, the order of which is likely due to the varying ease of pepsin digestion, with cheddar's structure being more readily digestible. In the intestinal phase, milk continued to exhibit the highest protein release percentage, significantly surpassing ricotta, but not significantly differing from cheddar cheese. Conversely,

cheddar's protein release in the intestinal phase did not significantly differ from either milk or ricotta. These observations can be traced back to the different dairy structures resulting from their manufacturing processes, which dictate their final structure and protein type: soluble form for milk, rennet coagulated structure for cheddar, and heat coagulated structure for ricotta Fang et al. (2016), also noted that different dairy structures, based on their inherent texture and manufacturing process, show unique patterns of protein disintegration and release. For instance, Camembert cheese, with its softer texture, disintegrates faster compared to the more compact, elastic matrix of Mozzarella.

Our findings underline the significant role played by the dairy type and stage of digestion in determining the protein release. Overall, a steady increase in protein release was observed during the digestive process for all three dairy products, with the most pronounced release occurring in the intestine. The observed variances in protein release rates across the dairy products are likely underpinned by their intrinsic characteristics, such as structure, fat content, and solubility.

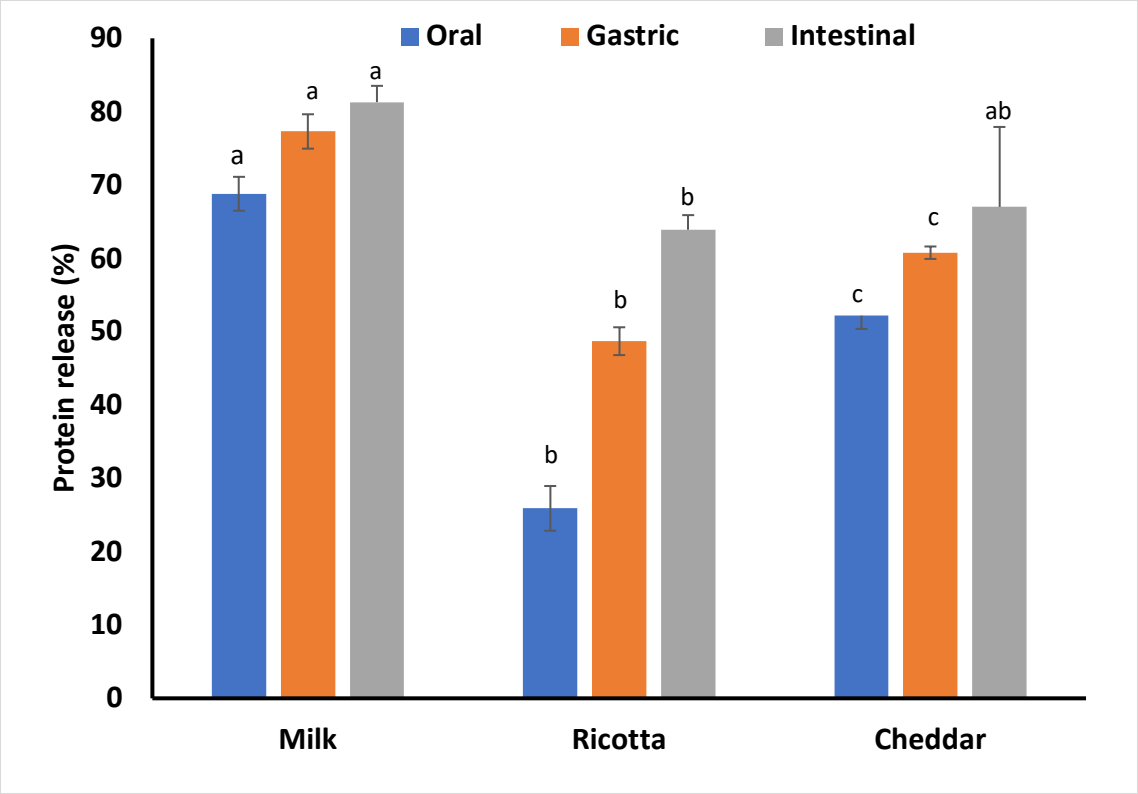

Figure 4.8: Protein release percentage at the end of each digestion stages. Different letters within the same digestion stages were significantly different. Data represent the mean ± SEM of three independent experiments.

Proteolysis during digestion

Figure 4.9, showing the UREA-PAGE analysis of proteins retained in milk during the gastric and intestinal phases, reaffirms that protein present in milk were digested more rapidly in both phases. The soluble nature of milk proteins i.e., casein and whey protein, is largely responsible for this accelerated digestion. Higher amount of water present in the milk can also contribute to the easier mixing of gastric enzyme and their diffusion in the liquid phase and accessibility to the protein molecules. During the gastric phase, only the pellet at the 0-minute exhibited a moderate

band of intact α and β casein. During this phase, β-casein remained detectable in the supernatant. This presence could be related to its incomplete digestion, given its greater resistance to pepsin's action. In the gastric phase, pepsin demonstrates a higher affinity for hydrolyzing κ-casein and α-s_1, with lower activity towards β-casein. The rate of hydrolysis was highest for κ-casein, followed by α-casein and β-casein at the same pH level (Tam et al., 1972). At remaining timepoints in the gastric phase (15 min to 60 min) all protein bands disappeared in the pellet indicating faster degradation of caseins.

In intestinal phase, the breakdown of protein continued with no presence of intact casein bands in both pellet and supernatant at 5 min time point. This indicate that almost complete digestion of protein in milk takes place within the first five min of the intestinal phase.

In summary, the UREA-PAGE analysis, as shown in Figure 4.9, indicates that the soluble form of milk proteins facilitates a faster digestion process, starting with partial breakdown in the gastric phase and culminating in complete breakdown in the intestinal phase. Similar observations were reported in the literature (Barbe et al., 2013).

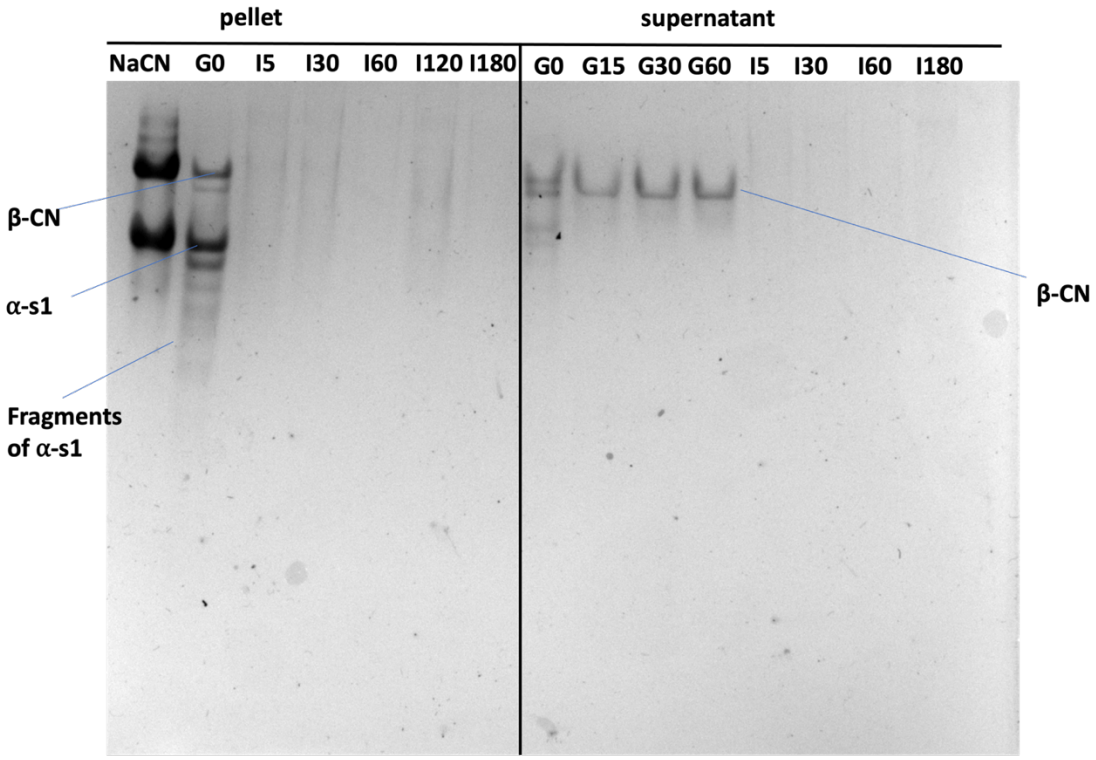

Figure 4.9: UREA-PAGE of supernatant and pellet for digested milk during in-vitro digestion in gastric and intestinal phase. G0 P, I5 P, I30P, I1hr P, I2hrs P, I3hrs P show the retained protein during digestion. GOS, G15 S, G30S, I 5 S, I 30 S, I 1hr S, I 2hrs S release of protein at the GP and IP.

The protein digestion pattern of ricotta cheese during the gastrointestinal stages were shown in Figure 4.10, β and α- casein bands in the supernatant and pellet of digested ricotta cheese in the gastric phase were found darker indicating relatively higher amount of intact caseins as compared to milk samples. As digestion continued in the gastric phase, the intensity of these bands started to disappear in both supernatant and pellet after 60 min in the gastric phase. This indicates that the proteins present in the ricotta cheese matrix were resistant to pepsin proteolysis in gastric

phase, which could be attributed to number of factors. First factors are the structure of the ricotta cheese, which is formed by heat-acid coagulation of milk proteins. Though overall coagulum is soft due to relatively higher moisture content (Table 4.2), the nature of heat acid aggregates different than acid or rennet coagulated proteins (Farkye et al., 2017). Second, due to heating of milk and whey together at higher temperature κ-casein and β-lactoglobulin form heat-induced complexes possibly causing difficulty for pepsin enzyme to reach inside the core of coagulated mass (Halabi et al., 2020). Third reason could be due to increased hydrophobic interactions of both caseins and whey proteins at higher temperature creating more compact structures in order to minimize contact area with aqueous phase (Lorieau et al., 2018). Irrespective of differences in the gastric digestion of milk and ricotta cheese, in the intestinal phase both behaved similarly, i.e., rapid breakdown of proteins within 5 minutes, indicating vulnerability of heat-acid coagulated proteins to the pancreatin enzymes. Generally, cheeses contain reasonable amount of fat which entrapped in the hydrated protein network. Presence of these fat particles in the structure may cause hindrance to mobility of digestive enzymes to the proteins. There may be a possibility that lipases present in the pancreatin was degrading fat too, therefore giving more access to pancreatin for protein breakdown (Fang et al., 2016).

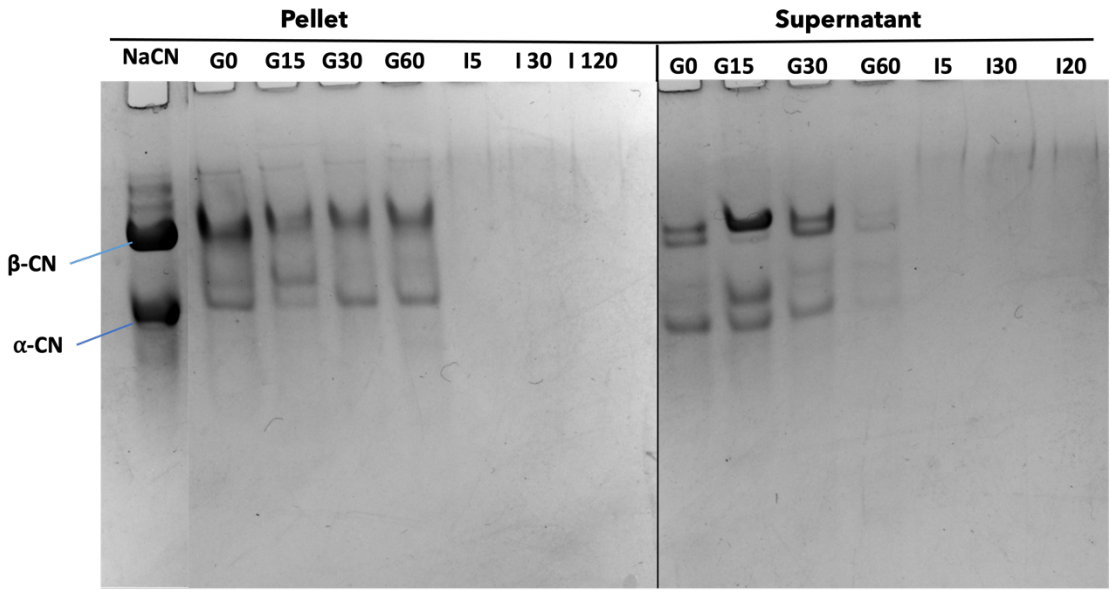

Figure 4.10: UREA-PAGE for ricotta during in-vitro gastro-intestinal digestion in the gastric and intestinal phase. G0 P, G15P, G30P, G60 P, I5 P, I30P, I120 P, show the retained protein during digestion. GOS, G15 S, G30S, I 5 S, I 30 S, I 120hr S, show release of protein at the GP and IP.

The pattern of casein hydrolysis in Cheddar cheese in both pellet and supernatant during in-vitro gastrointestinal digestion at different time intervals was shown in Figure 4.10. As anticipated, the α and β casein bands in Cheddar cheese remained were clearly visible throughout the entirety of the gastric digestion process, particularly in the pellet. The presence of intact casein fractions can be attributed to the dense casein structure found in Cheddar cheese, because of relatively lower moisture content (Table 4.2). However, there was a reduction in the intensity of bands in the supernatant, attributing to low molecular weight soluble components from protein breakdown (Fang et al., 2016).

Once the process transitioned to the intestinal phase, complete degradation was observed in both the pellet and supernatant. This change can be ascribed to the action of pancreatin, as well as other enzymes such as lipase, trypsin, and proteases. This was observed for all three types of dairy products (Fig., 4.9, 4.10, 4.11).

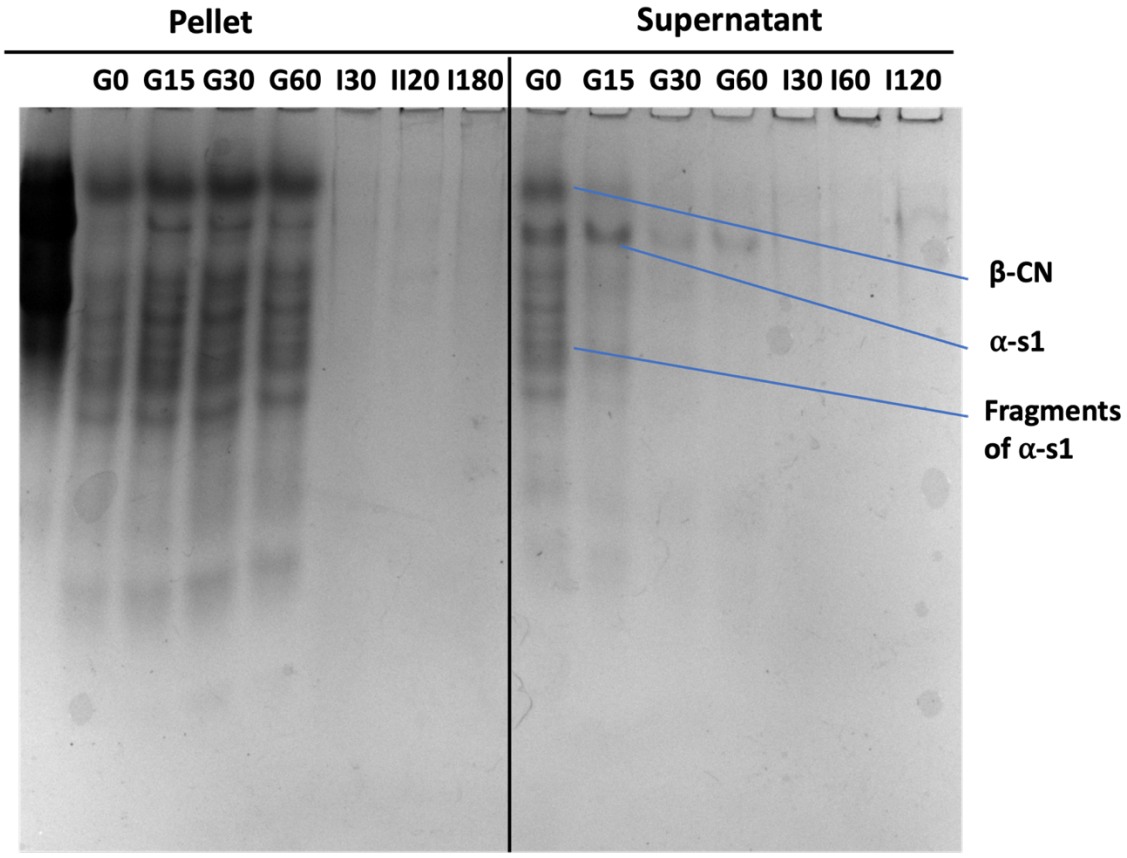

Figure 4.11: UREA-PAGE for Cheddar cheese during in-vitro gastro-intestinal digestion. GOS, G15 S, G30S, G 60 S, I 30 S, I 60 S, I 120 S release of protein at the GP and IP. G0 P, G 15 P, G 30 P, G 60 P, I 30P, I 120 P, I180 P, show the retained protein during digestion.

Overall, UREA page demonstrated the difference in the protein release and disintegration during digestion in the gastric and intestinal phases for supernatant and pellet for the three samples: Cheddar cheese, ricotta, and milk. Differences in the protein release can not

only be attributed to the structural, textural, and compositional differences, but also to the type of protein present in these products e.g., rennet coagulated, heat-acid coagulated, and soluble proteins present in the milk Our results confirm the impact of initial texture on the disintegration of proteins as shown in the figure (3.4). While there is a decrease in particle size distribution beginning in the gastric phase and continuing into the intestinal phase, complete protein hydrolysis is evident in the urea-PAGE during the intestinal phase. This could be due to particle size distribution encompasses various molecule types, including proteins, fats, minerals, etc., whereas urea-PAGE is specific to protein detection. The analysis also reveals distinct variances in the presence of α and β-casein during the process of digestion. Notably, Cheddar cheese demonstrates the most intense α and β-casein bands compared to Ricotta and Milk, attributable to its more compact structure, particularly observable in the pellet at the gastric phase. However, these bands begin to fade progressively in all three samples during the gastric phase and disappear entirely by the intestinal phase, indicating a complete degradation of these proteins. In contrast, Ricotta exhibits less intense bands compared to Cheddar, which could be attributed to its softer texture. Among the three samples, Milk displays the least intensity of bands, likely due to its soluble form and higher moisture content, accelerating digestion and degradation processes.

Finally, these observations offer valuable insights into the role of dairy structure in protein digestion and the potential for leading dairy products to optimize nutritional outcomes.

CONCLUSION

Protein release differed significantly between Cheddar, Ricotta, and Milk samples, notably during the gastric and early intestine digestion phases. Milk had the largest protein release, beginning at 60%, followed by Cheddar at roughly 40%, and Ricotta at 25%. These differences could be attributable to variations in composition, structure, and texture. UREA-PAGE analysis revealed different proteolysis patterns in these products, emphasizing Cheddar's compact structure and resulting in slower and less intense protein breakdown, particularly throughout gastric phase and early intestinal phase. Furthermore, the Milk sample displayed comparable digestive kinetics and protein release patterns to Ricotta, but with higher overall protein release, attributed mostly to its soluble form due to a higher moisture content.

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CHAPTER 5

OVERALL CONCLUSION

The diverse range of dairy products, distinguished by their texture, composition, and their physical state exhibit unique mouthfeel, particle disintegration, and protein digestion patterns during gastrointestinal digestion. This study established those attributes such as texture, composition, and the specific manufacturing process play pivotal roles in determining mouthfeel and digestibility.

SDS Page patterns highlight the diverse protein contents of eight different dairy products, each influenced by its own composition and processing technique. Solid cheeses, which feature dominating casein bands due to rennet coagulation, contrast with semi-solid products with various protein profiles, such as Ricotta and Cottage cheese. The casein and whey protein content in liquid products, such as milk and whey, distinguishes them further. This distinct division of early protein structures has a direct impact on further digestion and nutrient release. For instance, liquid dairy products like milk show a higher friction factor and protein release in the gastrointestinal tract, diverging significantly from their solid counterparts due to their distinct compositions and structures. On the other hand, solid forms like Cheddar cheese have a reduced friction factor and come second in terms of protein release, influenced largely by its protein, fat, and moisture contents.

Semi-solid products, exemplified by Ricotta, showcase distinct tribological behavior and protein release patterns. This underscores the idea that the composition, the physical state of the dairy product, and notably the heat coagulated manufacturing process influence mouthfeel and protein digestion.

Collectively, these findings open doors to the potential for leveraging these insights in devising strategizies to optimize protein availability during digestion. Enhancing dairy compositions based on tribological attributes can be instrumental, presenting new avenues for future dairy science research.

APPENDIX

Invitro digestion protocol (INFOGEST)

Oral phase (30 min)

1. Dilute food with SSF at a ratio of 1:1 (wt/wt) to achieve a swallowable bolus with a paste-like consistency similar to that of tomato paste or mustard at the end of the oral phase. If the consistency of the bolus is thicker than such a paste, add water to achieve the proper consistency. Salivary amylase is needed only to digest starch-containing food. It can be omitted if the food does not contain starch. Do not use lower-purity salivary amylase or pancreatic amylase.
2. Mix food with SSF to achieve a final ratio of 1:1 (w/w), e.g., 5 g of food to 5 g of SSF.
3. Measure the volume of the final digestion mixture of the food + SSF mixture. Record this volume, as it will be used in Step 17.
4. If necessary, simulate mastication by mincing the food in an electric or manual mincer.
5. Depending on the food (e.g., bread), mincing can be done together with the SSF electrolyte (without enzymes).
6. Add SSF electrolyte stock solution to the food, if not done in the previous step.
7. Add $CaCl_2(H2O)_2$ to achieve a total concentration of 1.5 mM in SSF.
8. Add the salivary amylase, if necessary, prepared in water to achieve an activity of 75 U/mL in the final mixture.
9. Add the remaining water to achieve a 1× concentration of the SSF.

10. Incubate while mixing for 2 min at 37 °C.

Gastric phase (2–3 h)

11. Pre-warm the SGF electrolyte stock solution at 37 °C. Add SGF electrolyte stock solution to the oral bolus to achieve a final ratio of 1:1 (vol/vol).

12. Adjust the pH to 3.0 by adding a defined volume of HCl previously determined during a pH-test adjustment experiment, see ‘Experimental design’.

13. Add CaCl2(H2O)2 solution in order to achieve a final concentration of 0.15 mM in SGF.

14. Add the porcine pepsin solution prepared in water to achieve an activity of 2,000 U/mL in the final digestion mixture.

15. Add the gastric lipase solution prepared in water to achieve an activity of 60 U/mL in the final digestion mixture.

16. Verify the pH and adjust to 3.0 if necessary.

17. Add water to achieve a 1× concentration of SGF.

18. Incubate the samples at 37 °C, mixing the digestive mixture sufficiently (e.g., rotating wheel, shaking incubator) for 2 h from the point at which pepsin was added. If there are large precipitates or clogs form, see the ‘Troubleshooting’ section.

Intestinal phase (2–3 h)

19. Pre-warm the SIF electrolyte stock solution in a 37 °C water bath. Add SIF electrolyte to the gastric chyme to achieve a final ratio of 1:1 (vol/vol).

20. Adjust the pH to 7.0 by adding a defined volume of NaOH previously determined during a pH-test adjustment experiment, see ‘Experimental design’.

21. Add the bile solution to the SIF/gastric chime solution to reach a final concentration of 10 mM. Place the solution in a rotating wheel mixer at 37 °C.

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