



Influence of dairy matrices on nutrient release in a simulated gastrointestinal environment



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ABSTRACT

The objective of this study was to compare the kinetics of the release of nutrients (peptides and fatty acids) from different dairy matrices (milks, yogurts, and cheeses) in a simulated gastrointestinal environment. Prior to processing, different heat and homogenization treatments were applied to milks, and different drainage pH levels were used to control calcium concentration in cheeses. The dairy matrices were then subjected to simulated digestion. Matrix degradation, protein hydrolysis, and fat hydrolysis were analyzed during the gastric and intestinal digestion phases. Intense heat treatment of milk induced faster digestion of proteins in the gastric environment. Cheeses were more resistant to protein and lipid digestion than liquid or semi-solid matrices were. No direct relationship could be established between disintegration kinetics and cheese rheological properties. Fatty acid release in the intestinal phase was much faster when matrices were produced from homogenized milk. For cheeses, greater fatty acid release could not be related to faster matrix disintegration, suggesting that the lipid droplet size dispersion was more important than matrix breakdown was for the modulation of lipid digestion kinetics. Calcium soaps were produced in the intestinal environment, and their concentration was higher during the digestion of cheeses in comparison with milks and yogurts. These results suggest that processing-induced modifications to the composition, microstructure, and rheological properties of dairy matrices could be used to control nutrient delivery.

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1. Introduction

The nutritional evaluation of a food is based usually on its composition in terms of carbohydrates, lipids, proteins, vitamins, and minerals. Food products with the same composition do not necessarily have equivalent nutritional value and hence may have different impacts on health. The effect of the structure surrounding the nutrients, also called the “food matrix,” is often neglected in the estimation of the real nutritional value of a food product. The food matrix can modulate the rate and extent of digestion (bioaccessibility) and the absorption (bioavailability) of nutrients (Turgeon & Rioux, 2011).

Various dairy products are available on the market, and each of them requires specific processing steps that influence the structural organization of the matrix, which in turns affects food breakdown and the release of nutrients during digestion. Homogenization is applied to reduce the size of milk lipid droplets and increase the physical stability of products during storage. This treatment induces a considerable increase in the specific surface area of lipid droplets. In vivo studies (Armand et al., 1999; Borel et al., 1994) and in vitro studies (Garcia,

Antona, Robert, Lopez, & Armand, 2014) have shown that the lipid digestion rate was enhanced with emulsions produced with smaller lipid droplets, because the greater surface area provided more sites for the lipase molecules to bind to (McClements & Li, 2010). By creating new interfaces, the homogenization process alters membrane composition at the fat globule interface, resulting in changes that could influence lipid digestion. Garcia et al. (2014) reported that, despite their smaller interfacial area, native fat droplets ($D = 1.7 \mu\text{m}$) present in milk were more easily digested than homogenized fat droplets ($D = 0.3 \mu\text{m}$) were in simulated human gastroduodenal digestion. This result was explained by a major change, induced by homogenization, in the phospholipid and protein composition of the membrane.

In order to extend shelf life, milk homogenization is usually followed by heat treatment (pasteurization or UHT [ultra-high temperature]) that could also modulate nutrient release during digestion. Gallier et al. (2013) observed, in rats, that lipids from homogenized pasteurized cream were more easily digested in comparison with lipids from homogenized raw cream, whereas protein digestion kinetics was similar among the different creams. In another study, caseins were more rapidly digested by mini-pigs than β -lactoglobulin was in unheated milk, but the application of a heat treatment (90 °C, 10 min) increased β -lactoglobulin hydrolysis to the level of that of caseins (Barbé et al., 2013).

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In humans, a higher rate of appearance of plasma amino acids following consumption of UHT milk in comparison with pasteurized milk was also observed (Lacroix et al., 2008).

Processing steps such as coagulation and gelling are applied to produce cheese and yogurt, respectively. In contrast to liquid foods, semi-solid or solid foods need to be disintegrated before nutrients can be released. The disintegration of the matrix is a key factor influencing nutrient release during digestion (Singh, Ye, & Ferrua, 2015). In the gastric environment, the structural characteristics and the properties of the matrix determine how easily digestive enzymes and fluids can diffuse into the food particles (Singh et al., 2015). Several studies have shown the relationship between rheological properties and nutrient release in dairy foods. One study found that the higher viscosity of fermented milk decreased the gastric emptying rate in comparison with milk and delayed the peak in plasma triacylglycerol during the postprandial period in humans (Sanggaard et al., 2004); similar results were observed after consumption of cream cheese in comparison with liquid cream (Fruekilde & Høy, 2004). Barbé et al. (2013) reported that ingestion of a rennet-gelled matrix by mini-pigs slowed down the rate of protein digestion and amino acid absorption in comparison with milk, owing to a reduced stomach emptying rate. Moreover, our team showed that different types of cheeses with similar composition but distinct matrix properties exhibited different kinetics of fatty acid release during *in vitro* digestion (Lamothe, Corbeil, Turgeon, & Britten, 2012): cheeses that exhibited greater cohesiveness and elasticity were more slowly degraded during digestion and resulted in slower rates of fatty acid release. These results highlight the importance of the initial characteristics of the food matrix for explaining the bioaccessibility of a nutrient. However, Barbé et al. (2014) showed that the changes in food matrix properties within the digestive system should also be considered. Those authors reported marked differences in the kinetics of protein digestion and amino acid absorption in mini-pigs following ingestion of milk gels that had the same composition and similar rheological and structural properties but had been produced by two different modes of coagulation (rennet- and acid-gelled curds). Both gels flocculated in the stomach, but only rennet gel particles were subjected to a strong contraction that resulted in delayed gastric emptying.

Calcium is a major constituent of dairy products and has a significant impact on the structural and textural properties of cheese (Lucey & Fox, 1993). The level of calcium associated with casein particles strongly modulates the rheological properties of cheese (Lucey, Johnson, & Horne, 2003). Several factors such as pH values at renneting and draining, the size of curd particles, and cheese ripening time can influence residual calcium in cheese. Reduction of the calcium content has been generally associated with decreased hardness, cohesiveness, and springiness in cheese (Pastorino, Hansen, & McMahon, 2003). These characteristics may have an impact on disintegration of the cheese matrix during digestion and hence on nutrient accessibility. Calcium also plays an important role in the lipid digestion process. Calcium has been found to enhance the rate of triglyceride hydrolysis in emulsions (Hu, Li, Decker, & McClements, 2010). Calcium can form complexes with long-chain free fatty acids (FFA) released from triglyceride hydrolysis, resulting in calcium soaps that are insoluble and precipitate at intestinal pH (Hu et al., 2010; Singh & Ye, 2013). Calcium can enhance the rate of lipolysis, because the formation of calcium soaps prevents fatty acids from accumulating at the lipid droplet surface and limiting the access of lipase to its substrate. Conversely, by promoting insolubility, calcium can limit bioaccessibility and reduce lipid absorption. *In vivo* studies have shown that supplementing a diet rich in fat with calcium from a dairy source decreased blood total and low-density lipoprotein cholesterol concentrations (Lorenzen, Jensen, & Astrup, 2014; Soerensen, Thorning, Astrup, Kristensen, & Lorenzen, 2014). These results are explained in part by an increase in fat excretion in the feces.

The objective of our study was to compare the kinetics of the release of nutrients (peptides and FFA) from different dairy matrices (milk, yogurt, and cheese) in a simulated gastrointestinal environment in

relation to the composition and rheological properties of the matrix. The combined effect of processes such as homogenization and heat treatments and of calcium content on the kinetics of digestion was also studied.

2. Materials and methods

2.1. Materials

Fresh whole milk was obtained from Agropur Cooperative (Saint-Hyacinthe, QC, Canada). Chy-Max Extra chymosin was purchased from Chr. Hansen (Milwaukee, WI, USA). Yogurt was produced with a lyophilized commercial lactic acid culture (Yogotherm; Biena, Saint-Hyacinthe, QC, Canada). Spray-dried milk protein concentrate (MPC-70) was purchased from Idaho Milk Products (Jerome, ID, USA). Alpha-amylase from porcine pancreas Type VI-B 10 U/mg, bovine serum albumin, mucin from porcine stomach Type III, pancreatin from porcine pancreas 8 x USP specifications, pepsin from porcine gastric mucosa 250 U/mg, porcine bile extract, oleic acid, and Triton X-100 were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Ethanol, hexane, diethyl ether, and trichloroacetic acid (TCA) were obtained from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Preparation of dairy matrices

2.2.1. Milk

Whole milk was heated to 50 °C and skimmed using a pilot-scale milk separator (Elecrem 1 model; Fresnes, France). Cream and skimmed milk were mixed in appropriate amounts to obtain 2% milk fat. The protein-to-lipid ratio was 1.65. The standardized raw milk was divided into three parts. One part was batch-pasteurized (65 °C, 30 min). The second part was homogenized twice at 2500 psi and once at 500 psi (50 °C) in a single-stage EmulsiFlex-C5 homogenizer (Avestin, Ottawa, ON, Canada) and then batch-pasteurized (65 °C, 30 min). The third part was homogenized under the same conditions, but the milk was heated at 95 °C for 5 min. The milks were stored overnight at 4 °C before digestion.

2.2.2. Yogurt

Whole milk was heated to 50 °C and skimmed using a pilot-scale milk separator (Elecrem 1 model). Cream, skimmed milk, and reconstituted milk protein concentrate solution (10% in distilled water) were mixed in appropriate amounts to obtain 3% milk fat and 5% protein (protein/lipid ratio = 1.65). The standardized milk was homogenized twice at 2500 psi and once at 500 psi (50 °C) in a single-stage EmulsiFlex-C5 homogenizer and then heated at 95 °C for 5 min. The milk was rapidly cooled on ice and stored overnight at 4 °C before yogurt production. For that process, 1 kg of standardized milk was heated to 43 °C and inoculated with 10 g of lyophilized lactic acid cultures. Fermentation was carried out in a water bath at 43 °C until the pH reached 4.6. The yogurt was then rapidly cooled on ice to approximately 10 °C and forced through a syringe to disrupt the particles. For Greek-type yogurt production, the inoculated milk was placed in 500-mL plastic centrifuge bottles (250 g of milk per bottle) before incubation at 43 °C until the pH reached 4.65. The coagulum was then cut with a spatula and centrifuged at 1000 × g for 15 min (21 °C) to accelerate and control serum drainage. The serum was discarded, and the Greek-type yogurt was cooled on ice to approximately 10 °C and forced through a syringe to disrupt the particles. The regular and Greek-type yogurts were stored for 1 week at 4 °C before digestion and compositional analyses.

2.2.3. Laboratory-scale direct-acidified cheeses

Whole milk was heated to 50 °C and skimmed using a pilot-scale milk separator (Elecrem 1 model). The total protein and lipid contents were standardized to 3.67% and 1.80%, respectively, using appropriate amounts of cream, skimmed milk, and reconstituted milk protein

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