J. Dairy Sci. 100:1–12
https://doi.org/10.3168/jds.2016-11764

Effect of homogenization and heat treatment on the behavior of protein and fat globules during gastric digestion of milk

Aiqian Ye,*†[1] Jian Cui,* Douglas Dalgleish,‡ and Harjinder Singh*†[1]
*Riddet Institute, and
†Massey Institute of Food Science and Technology, Massey University, Palmerston North 4442, New Zealand
‡Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

ABSTRACT

The effects of homogenization and heat treatment on the formation and the breakdown of clots during gastric digestion of whole milk were investigated using a human gastric simulator. Homogenization and heat treatment led to formation of coagula with fragmented and crumbled structures compared with the coagulum formed from raw whole milk, but a larger fraction of the protein and more fat globules were incorporated into the coagula induced by action of the milk-clotting enzyme pepsin. The fat globules in the whole milk appeared to be embedded in the clots as they formed. After formation of the clot, the greater numbers of pores in the structures of the clots formed with homogenized milk and heated whole milk led to greater rates of protein hydrolysis by pepsin, which resulted in faster release of fat globules from the clots into the digesta. Coalescence of fat globules occurred both in the digesta and within the protein clots no matter whether they were in homogenized or heated milk samples. The formation of clots with different structures and hence the changes in the rates of protein hydrolysis and the release of milk fat into the digesta in the stomach provide important information for understanding the gastric emptying of milk and the potential to use this knowledge to manipulate the bioavailability of fat and other fat-soluble nutrients in dairy products.
Key words: milk, homogenization, heat treatment, gastric digestion, protein coagulation

INTRODUCTION

Fresh whole milk is usually processed to be safe for human consumption and to extend its shelf life. Commercially available milk is commonly homogenized and pasteurized. Thermal treatments, such as pasteurization and UHT processing, lead to denaturation of some milk fat globule membrane (**MFGM**) proteins and to interactions between the MFGM and milk serum proteins (Ye et al., 2004). Extensive thermal treatment also leads to the denaturation of whey protein and the association of whey protein with casein micelles by interaction with κ-casein (Anema and Li, 2003). Homogenization increases the stability of milk fat by reducing the size of milk fat globules. This process leads to the casein and whey proteins adsorbing onto the surface of fat globules and reducing the amount of MFGM at the fat globule surface (Ye et al., 2008). Homogenization of whole milk followed by heat treatment causes the denatured whey proteins to attach to the adsorbed casein and MFGM proteins via disulfide bonds (Michalski and Januel, 2006).

Recently, claims have been made that raw milk is more nutritious and more easily digested than heat-treated milk because the proteins are not denatured (Smith, 2010). Therefore, studies on the effect of heat and homogenization processing on the digestibility of milk components have attracted some attention (Tunick et al., 2016). Wada and Lönnerdal (2014) showed that protein bands of SDS-PAGE of in-can sterilized milk underwent rapid decrease during in vitro digestion, and this was true to a lesser extent in UHT milk. Proteomic analysis revealed that the level of lactulosyllysine, which reflects a decrease in protein digestibility, in α-LA, β-LG, and caseins was higher in in-can sterilized milk than in UHT milk samples. Thus, industrial heating may improve the digestibility of milk proteins by denaturation, but the improvement is likely to be offset by heat-derived modifications involved in decrease in protein digestibility, for example, the formation of crosslinks such as lysinoalanine and lanthionine (Wada and Lönnerdal, 2014). Miranda and Pélissier (1987) reported that heat treatment results in accelerated gastric emptying in milk digestion and appears to increase the rate of hydrolysis of the caseins in vivo. Barbé et al. (2013) also reported a significant

Received July 21, 2016.
Accepted September 19, 2016.
[1]Corresponding authors: A.M.Ye@massey.ac.nz and H.Singh@massey.ac.nz

influence of heat treatment on the resistance and sensitivity of both casein and β-LG to hydrolysis in the human stomach in an in vivo experiment. Tunick et al. (2016) recently investigated the effect of heat and homogenization on in vitro digestion of milk protein and fat, showing a faster release in the free fatty acids from homogenized milk in the intestinal fluid. However, these studies used simple in vitro models, and limited in vivo experiments have been done for understanding why differences are found in the digestibility of milk components after different processing treatments.

Recently, we reported that the gastric digestion behavior of the protein in skim milk is influenced by the structure of the coagulum that is induced by the action of the milk-clotting enzyme pepsin on κ-casein in gastric conditions (Ye et al., 2016a). The clot formed from the coagulation of unheated milk showed a tightly knit network with numerous small pores interspersed throughout the matrix, whereas a network structure with larger voids was observed in the coagulation of milk that had been heat treated. With increasing digestion time, as the pH decreased further, the structures of the clots from unheated milk tightened and became less permeable to serum and solutes. These changes apparently affected the hydrolysis of casein by pepsin in the gastric system. In unheated milk, casein hydrolysis was much slower than that in the heated milk. Whey proteins, β-LG, and α-LA remained largely intact during the whole digestion period in unheated milk, but whey proteins in heated milk were readily hydrolyzed by pepsin. In heated milk, both casein and whey protein were involved in the formation of the clot.

For whole milk, milk fat globules seem to be embedded in the coagula formed during gastric digestion. We recently reported that the differences in structures of clots formed with untreated and heated unhomogenized whole milk led to different rates of protein hydrolysis by gastric pepsin, which also resulted in different rates of release of fat globules from the clots (Ye et al., 2016b). In heated whole milk, the release rate of fat globules was much higher than that of untreated whole milk, but it was slower than the rate of loss of fat-free matter during digestion. It has been suggested the interaction between the fat globule membrane and serum proteins in heated milk may hinder the release of fat globules from the protein clots.

Several studies have attempted to understand the lipolysis of milk fat globules in the gastrointestinal tract (Michalski, 2009; Ye et al., 2010, 2011; Gallier et al., 2012, 2013). As lipolysis is an interfacial process, the ultrastructure of the MFGM is likely to play a key role in the digestion and absorption of milk fat. Most studies on the digestion of milk fat globules have used in vitro digestion models with (Gallier et al., 2012) or without (Berton et al., 2009) the gastric digestion step. It has been reported that milk fat globules undergo major changes in the stomach because of the possible action of pepsin on the proteins of the MFGM (Ye et al., 2011; Gallier et al., 2012). Apparent flocculation of milk fat globules occurred in the raw milk incubated in simulated gastric fluid (**SGF**) with pepsin, but no coalescence was observed in both raw milk and cream samples. However, coalescence of fat globules occurred under simulated intestinal conditions, as lipolysis by pancreatic lipase proceeded. The major MFGM proteins were hydrolyzed by pepsin in SGF at different rates, in which the butyrophilin was more resistant to peptic hydrolysis than xanthine oxidase, PAS 6, and PAS 7. This study shows the importance of the MFGM structure in the digestion of the milk fat globules. Therefore, any processing treatment affecting the MFGM structure will influence the way milk fat globules are digested. Changes in the physicochemical properties of fat globules and dispersed hydrolytic products are important in understanding the digestion and absorption of lipids in milk products (Staggers et al., 1990).

This study extends our recent work in which bovine skim milk or whole milk was digested under gastric conditions (Ye et al., 2016a,b). In the present work, the physicochemical behavior and the morphology of milk fat globules and clots formed from homogenized whole milk and heated homogenized whole milk during in vitro gastric digestion in a dynamic human gastric simulator (**HGS**) were investigated. The information obtained from this work will be useful for understanding the digestion and absorption of milk fat and protein from differently processed milks and for the design of products derived from these milks.

MATERIALS AND METHODS

Materials

Bulk whole milk (4.71% fat and 3.51% protein) was collected from the Massey University Dairy Farm, Palmerston North, New Zealand. The homogenized milk was whole milk that had been homogenized by 20/5 MPa at 20°C using a 2-stage valve homogenizer (APV 2000, Copenhagen, Denmark). The average fat globule size of the milk samples was $d_{43} = 3.2 \pm 0.05$ μm and 0.52 ± 0.05 μm for the untreated milk and homogenized milk, respectively. The heated homogenized milk used in the experiments was homogenized milk that had been heated at 90°C for 20 min. Pepsin from porcine gastric mucosa (EC 3.4.23.1; catalog no. P7000, Sigma Chemical Co., St. Louis, MO) had an enzymatic activity of 800 to 2,500 units/mg of protein, as stated by the manufacturer. All other chemicals were obtained