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Gastric digestion of milk protein ingredients: Study using an in vitro dynamic model

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ABSTRACT

The coagulation behavior and the kinetics of protein hydrolysis of skim milk powder, milk protein concentrate (MPC), calcium-depleted MPC, sodium caseinate, whey protein isolate (WPI), and heated (90°C, 20 min) WPI under gastric conditions were examined using an advanced dynamic digestion model (i.e., a human gastric simulator). During gastric digestion, these protein ingredients exhibited various pH profiles as a function of the digestion time. Skim milk powder and MPC, which contained casein micelles, formed cohesive, ball-like curds with a dense structure after 10 min of digestion; these curds did not disintegrate over 220 min of digestion. Partly calcium-depleted MPC and sodium caseinate, which lacked an intact casein micellar structure, formed curds at approximately 40 min, and a loose, fragmented curd structure was observed after 220 min of digestion. In contrast, no curds were formed in either WPI or heated WPI after 220 min of digestion. In addition, the hydrolysis rates and the compositions of the digesta released from the human gastric simulator were different for the various protein ingredients, as detected by SDS-PAGE. Skim milk powder and MPC exhibited slower hydrolysis rates than calcium-depleted MPC and sodium caseinate. The most rapid hydrolysis occurred in the WPI (with and without heating). This was attributed to the formation of different structured curds under gastric conditions. The results offer novel insights about the coagulation kinetics of proteins from different milk protein ingredients, highlighting the critical role of the food matrix in affecting the course of protein digestion.

Key words: milk protein, curd structure, coagulation, gastric digestion, hydrolysis

INTRODUCTION

Milk protein is an important source of nutrients for humans through the different stages of life. A range of milk protein ingredients are used to improve the functional properties and nutritional value of food products. Skim milk powder (**SMP**), milk protein concentrate (**MPC**), sodium caseinate, and whey protein isolate (**WPI**) are the most extensively used protein ingredients in foods and nutritional products.

Skim milk powder is the most widely applied functional dairy ingredient (Singh and Creamer, 1991). Its manufacture involves heat treatment, normally known as preheating, evaporation, and spray drying. The most important effect of the preheating is the induction of the denaturation of the whey proteins to give partially denatured whey proteins, which can simply self-aggregate or can associate with the casein micelles via micellar κ-CN or both (Singh, 2007).

Milk protein concentrate was the first membrane-produced, casein-based product on the market (Carr and Golding, 2016). The caseins and whey proteins are in the same proportions as in milk. The casein is in a micellar form and the whey protein is in its native state because the manufacturing process does not involve preheating. However, a fraction of the colloidal calcium phosphate in MPC may be dissolved during the manufacturing process, leading to loose casein micelle structures and resulting in a smaller fragmented micellar structure (Singh, 2007).

To improve the functional properties of MPC, the casein micelles in some new products have been dissociated to a certain extent by removing the calcium (Ye, 2011). This type of product is referred to as "calcium-depleted MPC" in the present study. The micellar structure of MPC can be converted from the native structure to a structure that is closer to that of sodium caseinate, depending on the level of calcium depletion (Carr and Golding, 2016). However, a significant difference between caseinate and MPC is that MPC contain phosphate, whereas caseinate has reduced phosphate levels because of acidic precipitation and subsequent

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washing. Micellar phosphate increases the buffering capacity because of the formation of dihydrogen phosphate (Ferreira et al., 2003) on the acid-mediated solubilization of colloidal calcium phosphate (Carr and Golding, 2016).

Sodium caseinate, the water-soluble form of casein that is most commonly used in foods, is usually prepared by solubilizing acid casein with NaOH (Mulvihill and Ennis, 2003). The products produced in this manner contain individual casein molecules because the native micellar structure is disrupted during the manufacturing process. The excellent heat stability of caseinates is one of their most important functional benefits; it limits alterations to their properties as a consequence of thermal processing (Carr and Golding, 2016).

Whey is co-product of casein production and cheese making (Carr and Golding, 2016). Whey protein ingredients that contain ≥90% protein are known as WPI. To produce WPI, whey is skimmed by centrifugation or microfiltration and then demineralized by ion exchange, electrodialysis, or nanofiltration. The proteins are concentrated by membrane filtration or ion exchange chromatographic methods (Bansal and Bhandari, 2016). Because the whey protein in WPI is in its native state, its functional properties are largely retained.

Whey protein and casein are usually used as model proteins in studies to investigate the digestion and absorption rate of protein (He and Giuseppin, 2014). Boirie et al. (1997) proposed the concepts of slow casein and fast whey protein according to the different digestion rates of these proteins. Because whey protein is reported to induce a dramatic but short increase in plasma AA after ingestion, it is classified as a fast protein. It has a fast gastric emptying rate, because it stays soluble in the stomach and passes into the small intestine rapidly without being hydrolyzed by pepsin (Boirie et al., 1997; He and Giuseppin, 2014). In contrast, casein is classified as a slow protein for digestion. Casein forms clots in the stomach, which greatly reduces the gastric emptying rate, probably resulting in a slower release of AA (He and Giuseppin, 2014). Casein micelles have been reported to have different digestion behaviors from the individual caseins (Miranda and Pelissier, 1981). In an in vivo gastric digestion study, in a rat stomach, the coagulation of skim milk was much greater than that of a sodium caseinate solution. In addition, the gastric emptying rate and the hydrolysis rate of a mixture of individual caseins were much faster than those of skim milk (Miranda and Pelissier, 1981). This difference in digestion behavior between caseinates and casein micelles occurs primarily because casein micelles can be coagulated by the milk-clotting enzyme pepsin (Tam and Whitaker, 1972) and an acidic pH (Dalgleish and Corredig, 2012), whereas caseinate is coagulated only by low pH and not by an enzyme (Van Slyke and Bosworth, 1913).

In the present work, the physicochemical behavior and the curd formation of different commercial dairy protein ingredients during in vitro gastric digestion were investigated using a dynamic digestion model [i.e., a human gastric simulator (**HGS**)]. The dynamic model allows a more real environment in which to simulate the human gastric digestion process (Kong and Singh, 2010), mimicking gastric contraction, the continuous addition of fresh simulated gastric fluid (**SGF**) that contains pepsin, and simulated gastric emptying. The results offer novel insights about the coagulation kinetics of proteins from different milk protein ingredients, highlighting the critical role of the food matrix in affecting the course of protein digestion. The information obtained from this study will be useful in understanding the digestion of different commercial dairy ingredients and for the design and development of different products derived from these dairy ingredients.

MATERIALS AND METHODS

Materials

In this study, commercial dairy ingredients, including SMP, MPC (4851), calcium-depleted MPC (4861), sodium caseinate (180), and WPI (895), were purchased from Fonterra Co-operative Group Ltd. (Auckland, New Zealand). The compositions of the dairy ingredients, as stated by the manufacturer, are given in Table 1. The MPC (4851) contains 2,160 mg of calcium/100 g, and the partly calcium-depleted MPC (4861) contains 1,260 mg of calcium/100 g. The calcium-depleted MPC was manufactured using cation exchange to replace the divalent ions with monovalent ions and then ultrafiltration and diafiltration (Dybing et al., 2002). Pepsin from porcine gastric mucosa (EC 3.4.23.1; catalog no. 1.07185.0100) was purchased from Merck (Darmstadt, Germany); it had an activity of 0.7 FIP-U/mg, as stated by the manufacturer.

Water was purified by treatment with a Milli-Q apparatus (Millipore Corp., Bedford, MA) and was used for all experiments. All chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO) or BDH Chemicals (BDH Ltd., Poole, England) unless otherwise specified.

Methods

Preparation of Protein Solution. A 200-g 3.0% (wt/wt) protein solution was prepared by dissolving the