LWT - Food Science and Technology 57 (2014) 99–105

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

In vitro gastrointestinal digestion of liquid and semi-liquid dairy matrixes

Laure Rinaldi [a], Sylvie F. Gauthier [a], Michel Britten [b], Sylvie L. Turgeon [a,*]

[a] STELA Dairy Research Centre, Institute of Nutrition and Functional Foods, Laval University, Quebec, QC, Canada
[b] Food Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe, QC, Canada

ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form
16 January 2014
Accepted 20 January 2014

Keywords:
Milk
Yogurt
Free amino acid
In vitro digestion

ABSTRACT

Protein digestion in two liquid dairy matrixes with different heat treatments (pasteurized and sterilized milks) and in one semi-liquid dairy matrix (stirred-yogurt) was investigated using an *in vitro* gastrointestinal digestion model. After buccal digestion, significantly lower amount of soluble proteins were measured in yogurt than in both milks. This difference between dairy matrixes decreased during gastric digestion and disappeared at the end of the duodenal digestion upon the proteolytic action of pepsin and pancreatin. Electrophoresis pattern of digested mixtures showed that casein digestion began at the gastric phase and was slower for pasteurized milk than sterilized milk and yogurt. At the end of duodenal digestion, no more intact caseins were present in all the dairy matrixes while faint bands of whey proteins were still visible for pasteurized milk and yogurt. The release of free amino acids during the duodenal phase varied according to their nature (acid, basic, neutral or hydrophobic) and seems to be governed by the specificity of the enzymes. These results suggest that the severity of milk's heat treatment influences the kinetics of protein digestion, mainly during the gastric phase, and that the impact of processing has to be considered to study protein digestion in dairy products.

1. Introduction

Foods are complex nutrient assemblies subject to various industrial processes. Consequently, food products with same composition have not necessarily the same structural organization and the same nutritional value. Food microstructure generally refers to structural organization of food components to constitute a matrix at the scale level ranging from 10 to 100 μm (Aguilera & Stanley, 1999, p. 432). The microstructure of food is important because nutrients are often located in natural cellular compartments or within assemblies produced during processing and they need to be released during digestion for their further absorption in the gut. Moreover, processing defines food structure and the food matrix structural organization can act as a nutrient-release regulator. Indeed, physical characteristics of food matrix may control the release of the nutrients in the intestinal lumen (bioaccessibility), their transport across intestinal epithelium into the serum (bioavailability), and the induction of metabolic responses. The impact of food matrix on macronutrients nutritional properties was reviewed by Turgeon and Rioux (2011).

Dairy products are used in various forms from liquid milk, gelled fermented products and solid cheese matrixes. The main building blocks in dairy matrix are fat globules, casein micelles and whey proteins, and their different organizations as affected by processing steps such as mechanical, heat, enzyme or acid treatment and result in various structures found in dairy products. Nutritional properties of milk and dairy products have been widely studied through the specific effect of purified dairy nutrients such as proteins or lipids or in the context of nutrient assemblies such as dairy proteins in the milk fat globule membrane (McPherson & Kitchen, 1983). Dairy proteins are interesting because they have a good nutritional value: high content in several essential amino acids (AAs) and good digestibility (Debry, 2005). Moreover, dairy proteins are recognized to contain bioactive peptides which can be released during gastrointestinal (GI) digestion (Korhonen & Pihlanto, 2006).

Some studies in human showed different kinetics of digestion for dairy proteins. Whey proteins were named "fast proteins" because they induce a rapid and important stimulation of postprandial protein synthesis after their ingestion while caseins were considered as "slow protein" as there is a delay in the stimulation of protein synthesis (Boirie et al., 1997). However, using specific or

* Corresponding author. STELA Dairy Research Group, Institute of Nutrition and Functional Food, 2425, rue de l'Agriculture, Pavillon Paul-Comtois, Laval University, Quebec, Canada G1V 0A6. Tel.: +1 418 656 2131x4970; fax: +1 418 656 3353.
E-mail address: sylvie.turgeon@fsaa.ulaval.ca (S.L. Turgeon).

0023-6438/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2014.01.026

purified milk proteins cannot be considered to reflect "real food" and their kinetic of digestion should be different in the presence of other constituents found in dairy products (Dupont, Mandalari, Molle, Jardin, Leonil, et al., 2010; Farnfield, Trenerry, Carey, & Cameron-Smith, 2009; Monogioudi et al., 2011). Human studies have evaluated the effect of milk processing on the kinetics of protein digestion. For example, Lacroix et al. (2008) showed a higher anabolic use of dietary nitrogen (N) after the ingestion of ultra-high temperature (UHT) milk than pasteurized milk, suggesting a different kinetic of protein digestion subsequent to particular treatment of milk. Also in human, the net gastro-jejunal absorption of exogenous N did not differ significantly between milk and a yogurt made with the same milk (Gaudichon et al., 1995). Moreover, the main effect of fermentation was to delay the gastric emptying rate of N (Gaudichon et al., 1995).

Although clinical studies are considered as the gold standard methods to investigate human digestive process, *in vitro* digestion models are useful to screen numerous variables before clinical trials. In the actual context of developing functional foods containing various added nutrients or bioactive components, *in vitro* digestion models enable a first low cost evaluation of their release during the digestion process and their bioaccessibility and potential efficacy. Several *in vitro* GI models have been proposed depending on the food component being analyzed and the nature of food matrix (Hur, Lim, Decker, & McClements, 2010; Kong & Singh, 2008). For example, Sanz and Luyten (2007) studied the *in vitro* release of nutrients to understand the complete digestive process of lipophilic compounds (isoflavone) from enriched custards. Another GI model from mouth to duodenum was developed by Oomen et al. (2003) and further adapted by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005) to study the bioaccessibility of nitrogenous component from solid food matrix as peanut and buckwheat, using digestive solutions with composition closely related to those from human. Using a simulated infant digestion model, Dupont, Mandalari, Molle, Jardin, Rolet-Repecaud, et al. (2010) also showed that the intensity of milk's heat treatment has an impact on the digestion of proteins. The authors demonstrated that gastric digestion of caseins was more rapid from sterilized milk than pasteurized milk or from a yogurt prepared with the same pasteurized milk.

The aim of this study was to evaluate the effect of food matrix on the *in vitro* GI digestion of proteins in commercial liquid (milk) and semi-liquid (yogurt) dairy matrixes. Degradation of proteins was estimated through the analysis of soluble proteins, electrophoresis pattern of dairy proteins and free amino acids (FAAs) release during buccal, gastric and duodenal phases of digestion using an *in vitro* GI digestion model adapted from the one proposed by Versantvoort et al. (2005). Assessing the impact of heat treatment of milk (pasteurization and sterilization) on protein digestion was also an objective of this study.

2. Materials and methods

2.1. Reagents and enzymes

Reagents used for the preparation of digestive fluids were: KCl, NaCl, $NaHCO_3$, $CaCl_2$ and HCl from Fisher (Ottawa, ON, Canada); KSCN, NH_4Cl, monohydrate glucose, glucuronic acid and uric acid from VWR (Mississauga, ON, Canada); NaH_2PO_4 and KH_2PO_4 from JT Baker (Phillipsburg, NJ, USA); $NaSO_4$ from BDH (Mississauga, ON, Canada); urea from EMD (Darmstadt, Germany); bile extract porcine, mucin from porcine stomach (Type III), albumin from bovine serum (purity $\geq$ 98 g/100 g), hydrochloride glucosamine and $MgCl_2$ from Sigma–Aldrich (St. Louis, MO, USA). All the enzymes were purchased from Sigma–Aldrich: alpha-amylase (EC 3.2.1.1) from porcine pancreas type VI-B (28 U/mg solid using starch as substrate), pepsin (EC 3.4.23.1) from porcine gastric mucosa (471–305 U/mg solid using hemoglobin as substrate), pancreatin from porcine pancreas (8× USP specifications) and lipase (EC 3.1.1.3) from porcine pancreas type II (30–90 U/mg protein using triacetin as substrate).

2.2. Commercial milks and yogurt

Milks and yogurt were purchased from a local store (Québec, QC, Canada). Pasteurized milk contained 1 g/100 mL milk fat and 3.6 g/100 mL protein. Sterilized milk contained 1 g/100 mL milk fat and 3.2 g/100 mL protein. Commercial stirred-yogurt, without added polysaccharide (stabilizer), contained 2.5 g/100 g milk fat and 4 g/100 g protein.

2.3. In vitro GI digestion

In vitro GI digestion of matrixes was performed using the model described by Versantvoort et al. (2005) with some modifications to adapt the digestion procedure for liquid and semi-liquid dairy matrixes. This model simulates the digestion process in the human GI tract in a simplified manner by mimicking some physiological conditions such as the chemical composition and pH of digestive fluids, temperature (37 °C) and duration of digestion in buccal, gastric and duodenal phases. According to Versantvoort et al. (2005), the whole digestion process (from mouth to intestine) allowed the evaluation of bioaccessibility of nutrients and toxic compounds.

The composition of artificial juices (saliva, gastric, duodenal and bile) added at each digestion step is reported in Supplement Table 1 as described previously (Versantvoort et al., 2005). However, pancreatin was prepared in duodenal juice, which was centrifuged (9800×*g*, 4 °C, 20 min) as suggested by Sanz and Luyten (2006). The experimental conditions used to digest the different matrixes are summarized in Table 1. The major modifications applied to the digestion procedure were the following: (i) total digestion time in buccal phase was reduced respectively at 20 s and 2 min (vs 5 min) for milks and yogurt, based on the previous work (Sanz & Luyten, 2006); (ii) total digestion time in gastric phase was fixed respectively at 30 and 60 min (vs 2 h) for milks and yogurt, based on preliminary tests and the results obtained by Dupont, Mandalari, Molle, Jardin, Leonil, et al. (2010); (iii) both dairy matrixes were digested during 60 min (vs 2 h) in duodenal phase, also based on preliminary tests; (iv) type of agitation was modified (see Table 1 vs head-over-heels); (v) gastric fluid was added twice (vs once) during gastric phase; (vi) a heat treatment (90 °C, 10 min) was applied to inactivate the enzymes (vs no inactivation treatment) at the end of digestion phases.

Briefly, the experiment was started by adding dairy matrix (9 g) in a beaker (i.d. 4.8 cm) maintained at 37 °C in a water bath then 6 mL of saliva were added. The mixtures were agitated by gentle movements with an overhead stirrer (Caframo, model R2R1-64, Wiarton, ON, Canada) set up with a stainless steel dual-blade paddle (4 × 1.3 × 0.1 cm). For gastric digestion phase, 6 mL of gastric fluid were added to the beaker and the mixture was agitated by orbital movements. After 15 min (milks) or 30 min (yogurt), 6 mL of gastric fluid were added again and the mixture was agitated during an additional 15 min (milks) or 30 min (yogurt). Duodenal phase was started by adding bicarbonate (2 mL), bile (6 mL) and duodenal (12 mL) juices then the mixture was also agitated by orbital movements during 60 min for both milks and yogurt.

Several GI digestions of both milks and yogurt were performed to obtain digested samples at different times during the digestion process: (i) at the end of the buccal phase for both milks and