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Disintegration and nutrients release from cheese with different textural properties during *in vitro* digestion

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ABSTRACT

Recent results showed that solid food disintegration in the stomach may be affected by food texture which was demonstrated to change during digestion. Cheese is complex as, depending on the variety, its composition and texture can be modulated. Cheddar, light Cheddar, Mozzarella and light Mozzarella cheese particles were digested *in vitro*. Cheese disintegration and nutrients release were studied throughout the oral, gastric and duodenal digestion steps in presence or absence of enzymes. Cheese disintegration was significantly affected by the enzymatic treatment (with or without enzymes). The addition of enzymes allowed to reach 72% of cheese disintegration at the end of the duodenal digestion while it has attained 30% when no enzymes were added. Cheddar cheese disintegration was the highest among cheeses. This phenomenon was related to its initial higher fat content which resulted in a higher fat release during digestion. The disintegration at the end of each digestion step was also correlated to cheese composition (proteolysis and fat) and to textural parameters (hardness, resilience, adhesiveness and chewiness). Light Cheddar and Mozzarella exhibited similar disintegration and nutrients release at the end of the digestion due to a relatively small fat reduction (6%) which had limited effect on cheese texture. This study provides quantitative evidence regarding the impact of cheese textural changes during digestion on cheese disintegration and macronutrients release which may further affect nutrients anabolic response and some physiological functions.

1. Introduction

Recently, the food structure and its change during digestion were reported to play an important role on the kinetic of protein digestion. The rate of protein digestion influences amino acids (AAs) release and their subsequent adsorption in plasma. This could affect the postprandial utilizations of proteins for example the protein anabolic response and satiating effect (Boirie & Léger-Guist'hau, 2011; Breen & Phillips, 2011; Dangin et al., 2003; Lacroix et al., 2006). Caseins, for example, coagulate in the stomach acidic environment, while whey proteins remain soluble. Therefore, the postprandial protein anabolism was more important for caseins than whey proteins, since the release of AAs from ingested whey proteins was too fast to sustain the anabolic requirement during the postprandial period (Lacroix et al., 2006). Le Feunteun, Barbé, Rémond, Ménard, Gouar, Dupont et al. (2014) and Barbé et al. (2014) investigated the effect of the dairy matrix structure on the kinetics of milk protein digestion using mini-pig model. Only 25% of the AAs were recovered in plasma after 12 h ingestion of rennet gel (cheese type), while ~90% of the AAs recovered for milk and acid gel (yogurt type). The authors hypothesized that rennet gel may exert the formation of firm aggregates in stomach's acidic environment which subsequently delayed gastric emptying and protein digestion. The rennet gel compact matrix may limit the accessibility of enzymes towards proteins, which should slow protein hydrolysis (Morris & Gunning, 2008). These data suggest that cheese may behave differently during digestion and therefore affect protein degradation.

Cheese is complex as, depending on the variety, its composition and texture can be modulated. Reduced fat Cheddar cheese presents a more compact protein matrix with less space occupied by fat globules increasing cheese hardness and decreasing its cohesiveness compared to regular fat Cheddar (Bryant, Ustunol, & Steffe, 1995; Gunasekaran & Ak, 2003a). Mozzarella cheese presents a compact and fibrous protein matrix texture because of the stretching process during fabrication (Gunasekaran & Ak, 2003a). Fat reduction in Mozzarella cheese usually increases its protein content resulting in a denser protein network and higher hardness, cohesiveness and springiness (Rudan, Barbano, Joseph Yun, & Kindstedt, 1999). These textural and structural changes in cheese with decreasing fat content should affect cheese disintegration and protein digestion during gastro-intestinal (GI) digestion. Kong and Singh (2009a) have studied the disintegration profiles of a variety of solid foods (ex. peanut, carrot), and proposed that the disintegration of solid food in stomach is determined by the forces that hold food matrix together and the forces applied on food in stomach. In

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addition, the disintegration rate was mainly governed by water absorption, textural changes and surface erosion during digestion (Kong & Singh, 2009b). Also, the initial texture of food and its rate of textural softening during digestion have been proposed as good indicators to predict the behavior during *in vitro* gastric digestion (Bornhorst, Ferrua, & Singh, 2015). Therefore, the initial texture of the food matrix influences the disintegration mechanism and subsequently the kinetics of nutrients release.

Few studies were realized on cheese matrix disintegration and nutrients release during *in vitro* digestion (Ayala-Bribiesca, Lussier, Chabot, Turgeon, & Britten, 2016; Fang, Rioux, Labrie, & Turgeon, 2016; Lamothe, Corbeil, Turgeon, & Britten, 2012). However, in these studies the impact of enzymatic treatment (with or without enzymes) on cheese disintegration was not investigated. The role of the cheese composition and texture on cheese disintegration was mentioned but no correlation was ever performed to differentiate their relative influence. This study aimed to compare the disintegration and nutrients release during *in vitro* digestion of cheeses with different fat contents and cheese texture. The factors modulating cheese disintegration were determined using partial least square regression. Cheddar and Mozzarella were chosen as cheese examples because they are the most popular cheeses in Canada. These cheeses were digested under a controlled agitation condition, using a novel *in vitro* model of human digestion developed by our team: the digestion was conducted in a rheometer equipped with a customized geometry. The rotation of the geometry created a fluid flow providing a continuous force to disintegrate cheese particles.

2. Materials and methods

2.1. Chemicals

Glutaradehyde and hexamethyldisilazane were obtained from Sigma Aldrich (Oakville, ON, Canada). Osmium tetroxide and sodium cacodylate were purchased from Canemco and Marivac (Gore, QC, Canada). Enzymes (α-amylase (A3176), pepsin (P7000), lipase (L3126), bile (B8631) and pancreatin (P7545)) and a trypsin-chymotrypsin inhibitor were purchased from Sigma Aldrich (Oakville, ON, Canada). Protein standards of α-casein, β-casein, κ-casein, α-lactalbumin and β-lactoglobulin were purchased at Sigma Aldrich (Oakville, ON, Canada).

2.2. Cheeses studied

Two commercial Cheddar cheeses and 2 commercial Mozzarella cheeses with different fat content were purchased from a local grocery store (Metro, Quebec, QC, Canada). Three different cheese lots were studied for each cheese type. The average fat content was 33% for Cheddar cheese, 21% for light Cheddar cheese, 20% for Mozzarella cheese and 14% for light Mozzarella cheese. All cheeses were analyzed 60 ± 15 days before the expiry date. The fat (ISO/IDF, 2004), moisture (AOAC, 2008), protein (ISO/IDF, 2008) and ash (AOAC, 2000) contents in cheese were analyzed in duplicate. The total nitrogen content was converted to total protein content using 6.38 as a conversion factor. The proteolysis state in cheeses was analyzed (IDF, 1991a). The water soluble proteins fraction in cheese was chosen to represent the degree of cheese ripening. The water soluble fraction contains proteins excluding intact caseins and large peptides. The solids-not fat (% SNF = % total solids − % fat), the moisture in the nonfat substance (% MNFS = (% moisture/(100 − % fat)) × 100)) and the fat in the dry matter (% FDM = (% total solids/% fat) × 100) were also determined.

2.3. Cheese texture

Cheese texture was evaluated by Texture Profile Analysis (TPA) using TA-XT2 Texture Analyzer (Texture Technologies Corp., Hamilton, MA, USA) at room temperature (25 °C) (IDF, 1991b) using an acrylic cylinder geometry (diameter: 2.5 cm). The test conditions were as follows: cylinder samples (1 cm diameter, 1 cm height); compression, 50%; test speed, 1 mm.s^{-1}. The analysis was repeated 11 times for each cheese sample. The 2 distant values were discarded before statistical analysis.

2.4. Cheese microstructure

The central part of the cheese was cut into blocks of approximately 5 × 2 × 2 mm. These blocks were fixed for 1–2 h in a 2.5% glutaradehyde solution (pH 7.3, solubilized in 0.1 M sodium cacodylate) at room temperature. The samples were subsequently washed for 30 min in cacodylate buffer (the buffer was changed 3 times), post-fixed for 90 min in a 1% osmium tetroxide solution, dehydrated using a graded series of ethanol (50%, 75%, 95% and 100%), immersed in 100% ethanol for 50 min, immersed for 60 min in 2 changes of hexamethyldisilazane, and finally air-dried. The dried samples were fractured to expose an uncut plane. The exposed surface was coated using a sputter coater (Nanotech semprep II, Manchester, UK). The obtained samples were examined by scanning electron microscopy (JSM 6360LV, JEOL, Tokyo, Japan).

2.5. In vitro digestion

2.5.1. Cheese particles preparation

Cheeses were cut into pieces weighting 15 ± 0.5 g, then the pieces were milled in a coffee grinder (Hamilton Beach ® Type CM08, 120 V, ON, Canada) to produce cheese particles with median size of 2.4 ± 0.5 mm (Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007). The size of prepared cheese particles was confirmed by sieving and data are presented in Supplementary Table S1 and Fig. S1 as described previously (Fang et al., 2016). Cheese particles were sifted through sieve stacks with apertures of 4.00, 2.36, 2.00, 1.00, 0.50 and 0.30 mm (Canadian Standard Sieve Series, WS Tyler, Saint-Catharines, ON, Canada). The sieve stacks were shook at a speed of 70 (arbitrary units found of the system) for 20 min (model 41314, Retsch Inc., Newtown, PA, USA).

2.5.2. Artificial digestion model

The digestion process involves three steps simulating digestive processes in mouth, stomach and small intestine. Detailed design for the digestion model and the composition of the artificial juices were presented previously (Rinaldi, Gauthier, Britten, & Turgeon, 2014; Rioux & Turgeon, 2012).

The *in vitro* digestion was realized in a rheometer (AR-G2, TA Instruments, New Castle, DE, USA) equipped with a jacketed beaker (52 mm internal diameter, 74 mm internal height, Adams & Chittenden, Berkeley, CA, USA) and a mixing geometry (anchor, 38 mm height and 44 mm diameter, TA Instruments, New Castle, DE, USA, Fig. 1) to ensure good dispersion of cheese particles. The cheese sample (18 ± 0.1 g) was added into the jacketed beaker. 12 mL of saliva was added to start oral digestion that lasted 2 min. Gastric digestion started with the addition of 21 mL gastric juice and lasted 2 h. As opposed to the original protocol proposed by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005), in this study, pepsin was added twice (*vs* once) during the gastric phase (Rinaldi et al., 2014) since preliminary experiment with milk showed that the proteolysis was too fast. Pepsin (30 mg pepsin solubilized in 3 mL gastric solution) was re-added after 1 h gastric digestion. Then, 36 mL of duodenal juice was added to start the duodenal digestion that lasted 1 h. The mechanical disintegration of the cheese matrix during GI digestion was realized by the fluid flow generated by the agitation of an anchor (gap 1000 μm, 30 s^{-1}). The temperature during the whole digestion was maintained at 37 ± 1 °C. The gastric pH was adjusted to pH 2–3 with HCl at the beginning of gastric digestion (Supplementary Table S2). The initial pH of duodenal digestion step was adjusted to

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