International Dairy Journal 97 (2019) 76–85

Contents lists available at ScienceDirect

International Dairy Journal

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

Dynamic in vitro gastric digestion of infant formulae made with goat milk and cow milk: Influence of protein composition

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ARTICLE INFO

Article history:
Received 16 April 2019
Received in revised form
3 June 2019
Accepted 4 June 2019
Available online 15 June 2019

ABSTRACT

The gastric digestion behaviours of infant formulae made with goat milk and cow milk, with different ratios of casein to whey protein, were investigated using an in vitro dynamic infant human gastric simulator. The goat milk infant formulae formed smaller flocs of aggregated protein and oil droplets under gastric conditions, leading to faster protein digestion in goat milk infant formulae than in cow milk infant formulae. The extent of coagulation of protein and the size of flocculated oil droplets were dependent on the protein composition of the formulae. The casein-dominated cow milk infant formula had greater aggregation initially during gastric digestion, but a lower rate of casein digestion. The results suggest that the different composition of the casein micelles in goat milk may play an important role in the lower extent of coagulation and the faster protein gastric digestion in goat milk compared with cow milk.

1. Introduction

Infant formulae provide the human infant with high quality nutrition when breast-feeding is not an option. However, milks obtained from different species have different compositions. For example, cow milk has a higher protein content than human milk (Bosscher et al., 2000; Jensen, 1995; Nguyen, Bhandari, Cichero, & Prakash, 2015); the ratio of whey protein to casein in human milk is 6:4, but is about 2:8 in cow milk (Gurr, 1981; Hernell, 2011). Cow milk contains 80% β-lactoglobulin (β-LG) in the whey protein, but there is no β-LG in human milk (Gurr, 1981).

Most commercial infant formulae are made from cow milk that has been modified to match the composition of human milk. There is increasing consumer demand for milk-protein-based infant formulae made with milks from other species, such as goat milk. Goat milk infant formulae have been well accepted in some markets, especially in Asia, e.g., China. Compared with cow milk, goat milk has a lower level of α_{S1}-casein in the casein (Haenlein, 2004; Jenness, 1980); the milk fat in goat milk has higher levels of medium chain fatty acids and smaller milk fat globules (Attaie & Richter, 2000; Park, Juarez, Ramos, & Haelein, 2007). Goat milk is also considered to be less allergenic and easier to digest because of the formation of softer curds (Bevilacqua et al., 2001; Haenlein, 2004; Hodgkinson et al., 2012; Lara-Villoslada, Olivares, Jiménez, Boza, & Xaus, 2004). Recently, Hodgkinson, Wallace, Boggs, Broadhurst, and Prosser (2018) reported that the casein from goat milk tended to be digested more efficiently than the casein from cow milk and that the peptide profiles from goat milk were distinct from those from cow milk under infant and young child in vitro digestion conditions.

Extensive studies have demonstrated that the kinetics of protein and lipid digestion and the release of peptides and amino acids are strongly influenced by the structure of the original food and the formation of the matrix structure during digestion (Guo, Ye, Bellissimo, Singh, & Rousseau, 2017; Singh, Ye, & Horne, 2009; Turgeon & Rioux, 2011). The formation of the matrix structure is largely dependent on the composition of the protein and the processing history of the food (Gan, Bornhorst, Henrick, & German, 2018; Ye, Cui, Dalgleish, & Singh, 2016a, 2017). The protein composition, such as the amounts of casein and whey protein, also influences the properties of the digestion of infant formulae (Tari et al., 2018). Different protein compositions not only generate different types of peptides and amino acids for absorption and have different influences on the physiological functions, but also lead to different digestion kinetics, gastric emptying rates, satieties and extents of hydrolysis. This is due to differences in resistance to enzyme proteolysis in the gastrointestinal tract and in coagulation behaviour under gastrointestinal conditions

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https://doi.org/10.1016/j.idairyj.2019.06.002
0958-6946/

(Boirie et al., 1997; Bouzerzour et al., 2012; Chatterton, Rasmussen, Heegaard, Sørensen, & Petersen, 2004; Hall, Millward, Long, & Morgan, 2003; Menard et al., 2014; Perrella et al., 2015; Rioux & Turgeon, 2012; Tari et al., 2018; Ye et al., 2016a).

The structured clot formed by casein under gastric conditions is dependent on the original state of the caseins, e.g., the individual caseins in sodium caseinate and the casein micelles in milk and milk protein concentrate (Wang, Ye, Lin, Han, & Singh, 2018). The casein micelles in milk form either a dense clot or a loose curd, because the coagulation induced by pepsin hydrolysis is dependent on previous treatments of the milk such as heat treatment and homogenisation (Ye, Cui, Dalgleish, & Singh, 2017). The caseins in sodium caseinate coagulate only at low pH in the gastric environment (Wang et al., 2018). In contrast, the whey proteins, particularly β-LG, cannot be hydrolysed by pepsin, remain soluble and pass rapidly through the stomach to be digested in the intestine (Peram, Loveday, Ye, & Singh, 2013; Reddy, Kella, & Kinsella, 1988). However, if whey proteins have been denatured by heat treatment, they tend to aggregate to a certain level and are hydrolysed rapidly by pepsin under gastric conditions (Peram et al., 2013; Wang et al., 2018).

Information on the gastric digestion of infant formulae based on goat milk, with its different protein composition compared with cow milk, is limited. An understanding of the differences in the interactions and digestibilities of the proteins and lipids in infant formula emulsions formed with goat milk and cow milk under gastric conditions is important for the development of infant formulae. The objective of this study was to compare the gastric digestion behaviours of commercial infant formulae made with goat milk and cow milk, and of casein-dominated and whey-protein-dominated infant formulae, using a dynamic gastric digestion model.

2. Materials and methods

2.1. Materials

Four infant formulae were provided by the Dairy Goat Co-operative (Hamilton, New Zealand). Their proximate compositions are shown in Table 1. IFG1 and IFG2 were made from goat milk; IFC1 and IFC2 were made from cow milk. IFG1 and IFC1 were whey protein dominant (~60% whey); IFG2 and IFC2 were casein protein dominant (~80% casein).

Table 1
Proximate compositions (w/w%) of the infant formulae.[a]

Component	IFG1	IFG2	IFC1	IFC2
Dry matter	97.1	97.4	97.7	97.2
Protein	10.6	10.9	11.6	11.0
Fat	26.9	26.4	27.9	25.1
Ash	3.0	2.9	3.1	2.4
Lactose	56.6	57.2	55.1	58.7

[a] Abbreviations are: IFG, goat milk infant formula; IFC, cow milk infant formula; 1, whey-protein-dominant infant formula; 2, casein-dominant infant formula.

Pepsin from porcine gastric mucosa (EC 3.4.23.1; catalogue no. 9001-75-6, Merck KGaA, Germany) had a laboratory enzymatic activity of 0.7 FIP-units (43.75 USP units) mg^{-1} protein, as stated by the manufacturer.

All solutions were prepared from analytical-grade chemicals. Deionised water, purified by treatment with a Milli-Q apparatus (Millipore Corp., Bedford, MA, USA), was used for the preparation of all solutions.

Simulated gastric fluid (SGF) was prepared according to a method in a previous study (Minekus et al., 2014) with a slight modification. A fresh mixture of KCl (6.9 mmol L^{-1}), KH_2PO_4 (0.9 mmol L^{-1}), $NaHCO_3$ (25 mmol L^{-1}), NaCl (47.2 mmol L^{-1}), $MgCl_26H_2O$ (0.1 mmol L^{-1}), $CaCl_2$ (0.15 mmol L^{-1}) and $(NH_4)_2CO_3$ (0.5 mmol L^{-1}) was prepared by dissolving these ingredients in water with stirring for 30 min. The SGF (final volume of 1 L) was made up with water to 800 mL, i.e., a 1.25× concentrate. The pH of the SGF was adjusted to 1.5 using 1 M HCl and 1 M NaOH.

2.2. In vitro dynamic gastric digestion in an infant human gastric simulator (HGS)

Each infant formula powder was dissolved in 50 °C water with stirring for 30 min to make up a dispersion sample with 3.0% protein and 7.4% fat. The 100 mL dispersion sample was warmed to 37 °C and then mixed with 9.6 mL of SGF and 2.4 mL of pepsin solution (24 mg L^{-1}) in an infant HGS (Fig. 1), which was originally developed by Kong and Singh (2010) and was constructed at the Riddet Institute (Massey University, New Zealand). The SGF and the pepsin solution were pumped into the HGS separately at flow rates of 0.6 and 0.15 mL min^{-1}, respectively. Digesta samples (15 mL) were removed from the HGS every 20 min to simulate the infant gastric emptying rate. The contraction frequency of the HGS was 3 times min^{-1} to simulate the contraction of the stomach. The temperature of the HGS was maintained at 37 °C by a heater and thermostat. The maximum digestion time was 240 min, but the experiments were terminated at each 20-min interval so that the samples in the HGS could be collected for further analysis. For each of the four milk samples, five experiments were carried out, with time periods of 20, 60, 100, 160 and 240 min. For all infant formula samples, experiments were also conducted with SGF only (without the addition of pepsin) as controls.

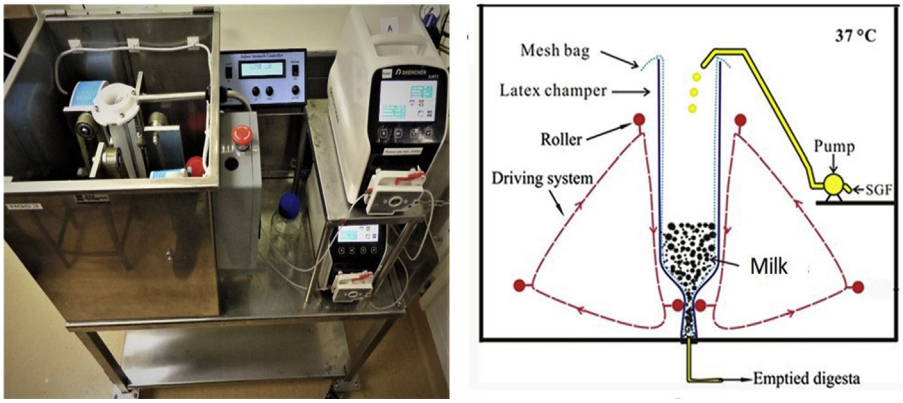

Fig. 1. Image of the infant human gastric simulator (left) and schematic illustration of a latex stomach chamber (right).

2.3. pH measurement

The initial pH in the HGS was defined as the pH of the infant formula samples. With the ingestion of SGF and gastric emptying, the pH in the HGS at different times was assumed to be that of the emptied digesta, because the set-up (roller contraction) prevented easy access into the infant HGS.

2.4. Particle size measurements

The mean particle size and the particle size distribution of the samples obtained from the HGS were measured during the digestion, using a Mastersizer (2000S, Malvern Instruments, Malvern, Worcestershire, England). The particle size of the digested samples was characterised using the Sauter-average diameter [$d_{3,2}$ (μm)] or the volume-surface average diameter [$d_{4,3}$ (μm)], which was calculated according to the following equation (1):

$$d_{32} = \Sigma n_i d_i^3 \Big/ \Sigma n_i d_i^2 \quad \text{or} \quad d_{43} = \Sigma n_i d_i^4 \Big/ \Sigma n_i d_i^3 \tag{1}$$

where n_i is the number of particles with diameter d_i.

2.5. Confocal laser scanning microscopy

The microstructures of the samples and the gastric chymes during the gastric digestion were examined using a confocal laser scanning microscope (Leica SP5 DM6000B, Leica Microsystems, Heidelberg, Germany). The samples for microscopic examination were not pretreated (i.e., heating or pH adjustment); they were placed into an ice bath to arrest the enzymatic action of the pepsin temporarily before proceeding with the analysis. The fluorescent dye Nile Red, dissolved in acetone (0.1%, w/v), was added to the samples to stain the oil phase (argon laser with an excitation line of 488 nm). Fast Green (1.0%, w/v) was used to stain the protein (He–Ne laser with an excitation line at 633 nm). For liquid samples, 200 μL was transferred into an Eppendorf tube and 5 μL 1.0% (w/v) Fast Green and 10 μL 0.1% (w/v) Nile Red were added. The samples were stained for at least 5 min. For solid chyme samples, i.e., flocculated emulsions, an aliquot was taken using a blade and was stained with Fast Green and Nile Red for at least 10 min. The stained samples (liquid or solid) were placed on concave confocal microscope slides (Sail; Sailing Medical-Lab Industries Co. Ltd., Suzhou, China), covered with cover slips and observed using 40× and 100× magnification oil immersion lenses.

2.6. Protein hydrolysis

The time-dependent hydrolysis by pepsin of the proteins in the infant formula samples in the HGS and in the emptied digesta was determined by analysing the protein composition as a function of the digestion time, using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Samples were mixed with sample buffer [0.2 M Tris–HCl buffer, pH 6.8, 40% glycerol, 2% SDS, 0.04% bromophenol blue, β-mercaptoethanol (19:1, v/v)] at a ratio of 1:2 (μL) and 8 μL of each mixture was then heated at 90 °C for 5 min. The mixtures were cooled to room temperature and were loaded (10 μL) on to a resolving gel previously prepared on a Mini-PROTEAN II system (Bio-Rad Laboratories, Richmond, CA, USA). The resolving gel contained 16.0% acrylamide, made up in Tris–HCl buffer, and the stacking gel was made up of 4.0% acrylamide in Tris/HCl buffer. The gel was stained for 60 min with a Coomassie Brilliant Blue R-250 solution (0.003%, w/v, in 10% acetic acid and 20% isopropanol; Merck). The gel was destained with a solution of 10% acetic acid and 10% isopropanol and was scanned using a Molecular Imager Gel Doc XR system (Bio-Rad Laboratories). The protein compositions from SDS-PAGE were scanned and quantified using a Gel Doc™ XR+ imaging system with Image Lab™ software version 5.2 (Bio-Rad Laboratories).

2.7. Statistical analysis

Each experiment was performed at least twice using freshly prepared samples. The results are reported as the calculated means and standard deviations. One-way analysis of variance and the SPSS 19.0 package (IBM, Armonk, NY, USA) were used. Duncan's multiple range tests were used to determine the significant difference of the mean values ($P < 0.05$).

3. Results and discussion

3.1. Characterisation of infant formula samples

The protein composition of goat and cow milk and the infant formula samples was examined by SDS-PAGE under reducing conditions (Fig. 2). Cow milk had almost similar proportions of α_{S1}-casein (27.5%) and β-casein (34.0%), whereas goat milk contained very little α_{S1}-casein and much more β-casein (50.8%) (Table 2). It was interesting to note that goat milk contained relatively more α_{S2}-casein than cow milk and that the α_{S2}-casein band in goat milk had lower mobility than the α_{S2}-casein band in cow milk (Fig. 2). The PAGE results clearly showed that IFG1 and IFG2 made with goat milk and IFC1 and IFC2 made with cow milk had a protein profile similar to that of goat and cow milk, respectively. No α_{S1}-casein band was visible in the goat milk infant formulae. IFG1 contained ~66% whey protein [including both β-LG and α-lactalbumin (α-LA)] and IFC1 contained ~59% whey protein (β-LG and α-LA), which were much higher than the whey protein levels in IFG2 and IFC2 (Fig. 2 and Table 2). These results are in agreement with the manufacturer's claim that IFG1 and IFC1 were whey-protein-dominant infant formulae, and that IFG2 and IFC2 were casein-dominant infant formulae.

The average particle size (d_{43}) values and the particle size distributions of the infant formulae, dissolved either in water or in Tween/EDTA solution, are shown in Table 3 and Fig. 3, respectively. The particle sizes of IFG1 and IFC1 were smaller than those of IFG2

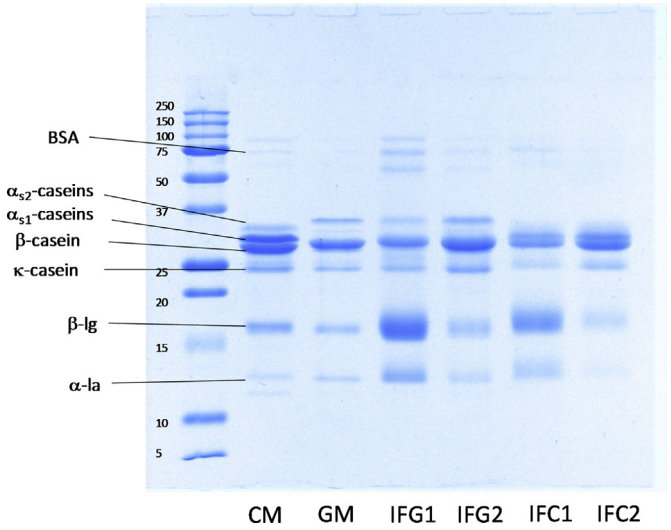

Fig. 2. SDS-PAGE patterns under reducing conditions of the infant formulae. CM, cow milk; GM, goat milk; IFG, goat milk infant formula; IFC, cow milk infant formula; 1, whey-protein-dominant infant formula; 2, casein-dominant infant formula.

Table 2
Protein composition of milks and infant formula samples obtained from quantitative SDS-PAGE under reducing conditions.[a]

Sample	Cow milk (%)	Goat milk (%)	IFG1 (%)	IFG2 (%)	IFC1 (%)	IFC2 (%)
α_{S2}-Casein	7.6	15.8	3.2	12.2		
α_{S1}-Casein	27.5				17.2	39.3
β-Casein	34.0	50.8	17.5	47.9	14.8	34.2
κ-Casein	8.7	8.2	3.9	11.7	4.2	10.3
β-LG	15.9	16.8	52.8	17.4	45.6	12.4
α-LA	3.9	6.0	13.6	6.3	13.0	0.9
BSA	1.0	1.0	4.2	1.7	2.0	0.7
Others	1.0	1.0	4.9	3.0	3.1	1.5

[a] Abbreviations are: IFG, goat milk infant formula; IFC, cow milk infant formula; 1, whey-protein-dominant infant formula; 2, casein-dominant infant formula.

Table 3
Average particle size ($d_{4,3}$) of infant formula samples dispersed in water or in Tween/EDTA solution.[a]

IFG1		IFG2		IFC1		IFC2	
Water	Tween/EDTA	Water	Tween/EDTA	Water	Tween/EDTA	Water	Tween/EDTA
2.05	1.48	3.29	1.46	1.05	0.96	11.26	5.62

[a] Values are in μm. Abbreviations are: IFG, goat milk infant formula; IFC, cow milk infant formula; 1, whey-protein-dominant infant formula; 2, casein-dominant infant formula.

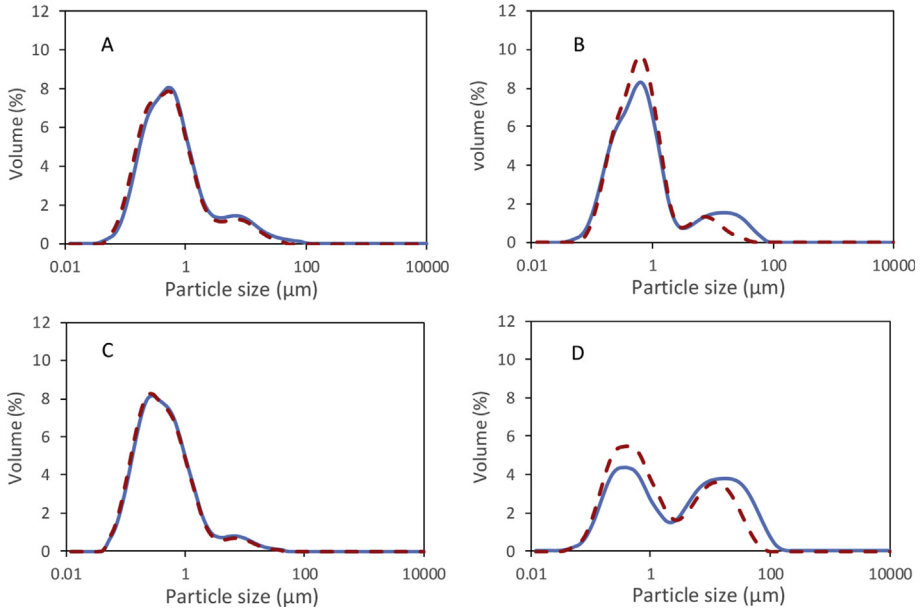

Fig. 3. Particle size distributions of infant formulae (solid line, samples dispersed in water; dashed line, samples dispersed in Tween/ETDA solution): A, IFG1, whey protein-dominant goat milk infant formula; B, IFG2, casein-dominant goat milk infant formula; C, IFC1, whey protein-dominant cow milk infant formula; D, IFC2, casein-dominant cow milk infant formula.

and IFC2. IFC2 had the largest average particle size with a bimodal size distribution. Casein micelles and flocculated oil droplets are dissociated to individual caseins and individual oil droplets in Tween/EDTA solution. After dispersion in Tween/EDTA solution, the average particle sizes of IFG2 and IFC2 were reduced markedly from 3.29 and 11.26 μm to 1.46 and 5.62 μm respectively, whereas there were only slight reductions in particle size for IFG1 and IFC1. The particle size distributions showed an apparent reduction in the peaks in the size range 60–100 μm in IFG2 and IFC2, but almost no change in IFG1 and IFC1 (Fig. 3). This indicated that the oil droplets in IFG2 and IFC2 were flocculated, whereas there was no droplet flocculation in IFG1 and IFC1.

The flocculation of the oil droplets in IFG2 and IFC2 suggested that the particle size may have been related to the protein composition, particularly the casein to whey protein ratio. The presence of a high proportion of casein micelles in IFG2 and IFC2 may have led to bridging flocculation between the oil droplets. Thus, the protein composition and the particle size in the infant formulae might influence their digestion behaviour under gastric conditions.

3.2. *In vitro dynamic gastric digestion*

3.2.1. *pH profiles*

The pH profiles of the infant formula samples during digestion in the HGS are shown in Fig. 4. There was no difference among the four samples in the change in pH with the digestion time; the pH decreased to ~3 after 240 min. The pH of the food in the infant stomach was designed to be 3, which was higher than the pH in the adult stomach (Bourlieu et al., 2014). A slightly lower pH was observed during the digestion of IFG1 probably because of its lower initial pH (about pH 6.4).

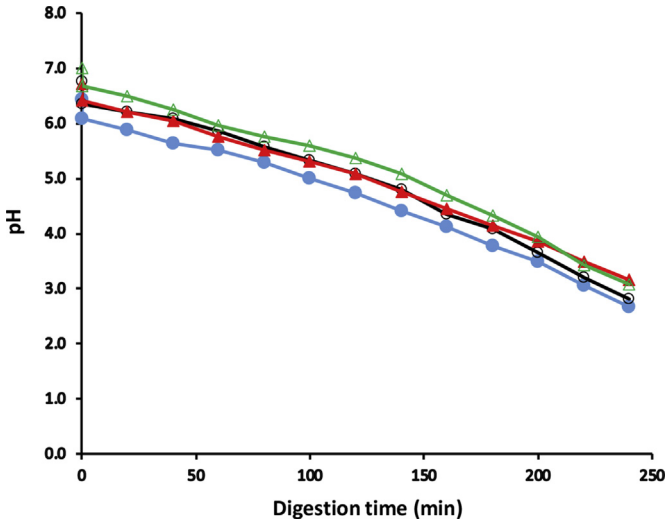

Fig. 4. Change in pH of the infant formulae during gastric digestion in the HGS: ●, IFG1, whey protein-dominant goat milk infant formula; ○, IFG2, casein-dominant goat milk infant formula; ▲, IFC1, whey protein-dominant cow milk infant formula; △, IFC2, casein-dominant cow milk infant formula.

3.2.2. Changes in particle size

The changes in the average particle size (d_{32}) of the infant formula samples under dynamic digestion in the HGS are shown in Fig. 5. In SGF containing no pepsin (Fig. 5A), the particle size of all samples showed no change until 60 min. The size of IFG1 increased dramatically at 80 min and those of the other infant formula samples increased markedly at 120 min. The size of IFC2 increased to about 60 μm, whereas the sizes of the other infant formulae increased to around 20 μm and remained unchanged until the end of digestion. The increase in particle size of IFG1 at an earlier time was probably due to its lower initial pH; the pH of IFG1 was about pH 5.2 at 80 min, whereas the other infant formula samples reached this pH at 120 min (Fig. 4).

The average particle sizes increased at much earlier times for all infant formula samples in SGF with added pepsin (Fig. 5B), compared with the samples without added pepsin. For IFG1, IFG2 and IFC2, some large particles were observed at 0 or 5 min (after mixing with SGF under fasting conditions). The sizes of IFG2 and IFC2 increased to over 30 μm at 50 min and then gradually decreased until the end of digestion. In contrast, the sizes of IFG1 and IFC1 increased to only about 10 μm and then decreased (similar to IFG2 and IFC2).

The increase in the particle size of the infant formula samples indicated the aggregation of protein and the flocculation of oil droplets because of the low pH and/or the action of pepsin. Casein aggregation occurred in SGF containing no pepsin because the pH decreased to about pH 5, which is close to the isoelectric point of casein. However, the aggregation at a much earlier digestion time or at higher pH (pH > 6) in SGF containing pepsin was due to the action of pepsin on casein micelles (Ye et al., 2016a). The greater increase in particle size of IFG2 and IFC2 during digestion suggested that the extent of aggregation in the different infant formulae that was induced by pepsin hydrolysis or low pH was dependent on the composition of the milk proteins in the infant formula samples. The greater extent of aggregation in IFG2 and IFC2 was attributed to their higher proportion of casein micelles, and consequently their greater sensitivity to aggregation via low pH and pepsin hydrolysis.

In contrast, IFG1 and IFC1 had much higher whey protein content than IFG2 and IFC2. Whey protein is less sensitive to pH-induced aggregation and is not aggregated by pepsin. The relatively lower amount of casein in IFG1 and IFC1 was responsible for the reduced extent of aggregation induced by both enzymatic and acidic conditions, compared with IFG2 and IFC2.

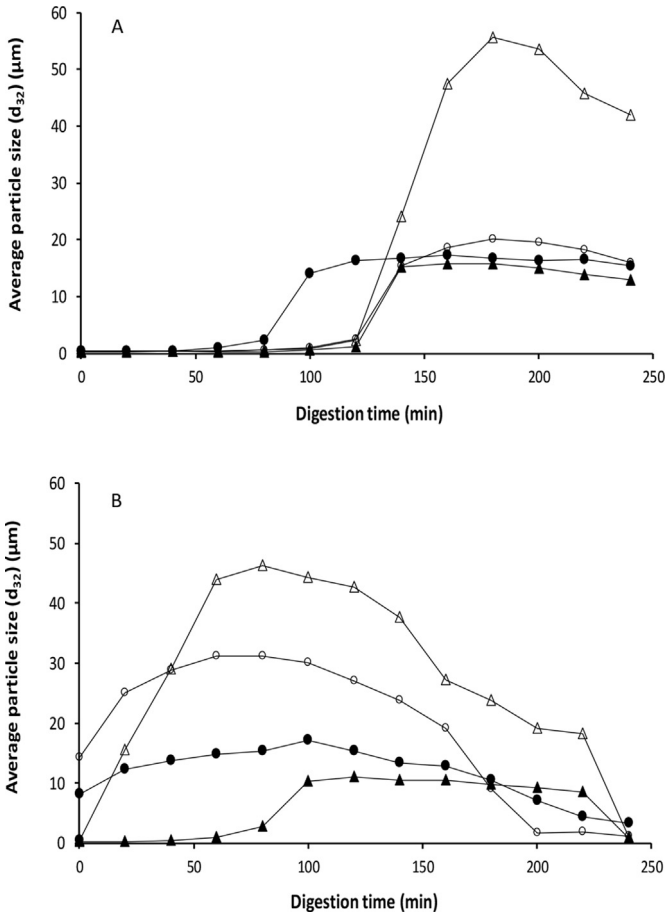

Fig. 5. Change in average particle size (d_{32}) of the infant formulae during gastric digestion in the HGS: (A) SGF containing no pepsin; (B) SGF containing pepsin (●, IFG1, whey protein-dominant goat milk infant formula; ○, IFG2, casein-dominant goat milk infant formula; ▲, IFC1, whey protein-dominant cow milk infant formula; △, IFC2, casein-dominant cow milk infant formula).

3.2.3. Changes in microstructure

The confocal micrographs obtained from the infant formula samples during digestion are shown in Fig. 6. Flocculation was observed at 20 min in IFG1, IFG2 and IFC2, with the largest flocs being seen at 60 min for IFG2 and IFC2. Flocculation in IFC1 was observed at 120 min. At 240 min, the flocs in all infant formula samples were small, were in a similar size range and contained some oil droplets.

Although coagulation of the protein and flocculation of the oil droplets were observed under gastric conditions in these infant formulae, no firm clot was formed. This was in contrast to the structured and firm clot seen in milk under the same conditions in an in vitro dynamic gastric digestion and in an in vivo digestion in the rat stomach (Ye et al., 2017, 2019). It is not clear which factor influenced the structure of the coagulum formed from the digestion of the milk proteins in the IF tested. It has been shown that the composition, the pretreatment during processing, such as heating and homogenisation, and the fat content influence the formation, structure and properties of the milk clots formed during digestion (Mulet-Cabero, Mackie, Wilde, Fenelon, & Brodkorb, 2019; Tari et al., 2018; Wang, Lin, Ye, Han, & Singh, 2019; Ye, Cui, Dalgleish,

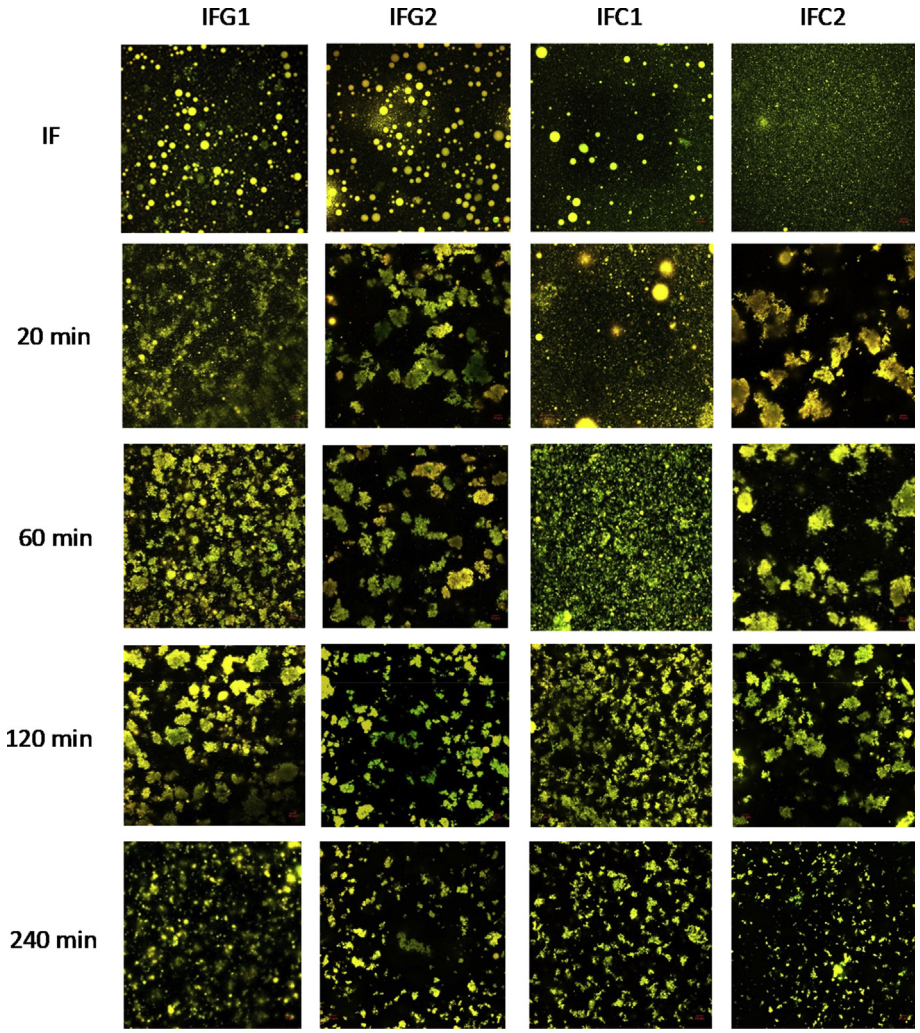

Fig. 6. Confocal micrographs of the infant formulae during gastric digestion (SGF with pepsin) in the HGS at different times. IFG1 and IFG2, whey protein-dominant goat milk infant formula and casein-dominant goat milk infant formula, respectively; IFC1 and IFC2, whey protein-dominant cow milk infant formula and casein-dominant cow milk infant formula, respectively. Scale bars represent 20 μm.

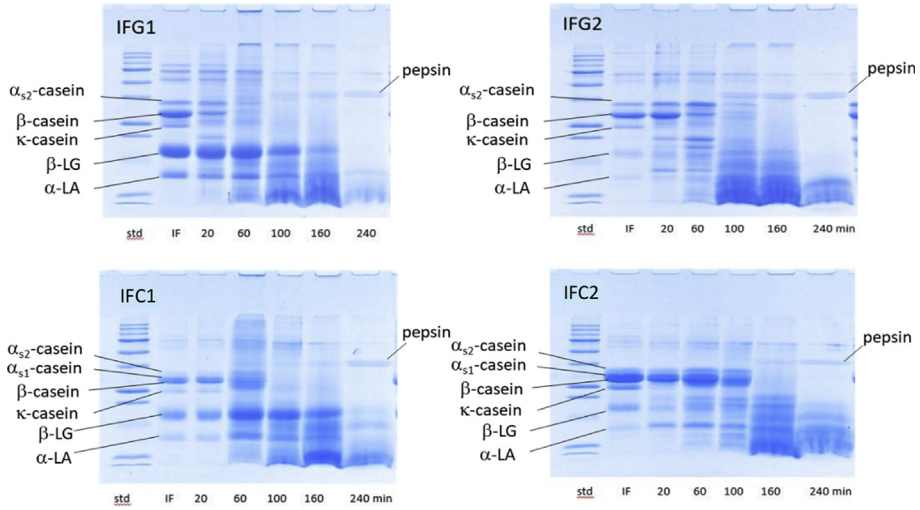

Fig. 7. SDS-PAGE patterns under reducing conditions of the chyme obtained from the infant formulae during gastric digestion (SGF with pepsin) in the HGS at different times: IFG1 and IFG2, whey protein-dominant goat milk infant formula and casein-dominant goat milk infant formula, respectively; IFC1 and IFC2, whey protein-dominant cow milk infant formula and casein-dominant cow milk infant formula, respectively.

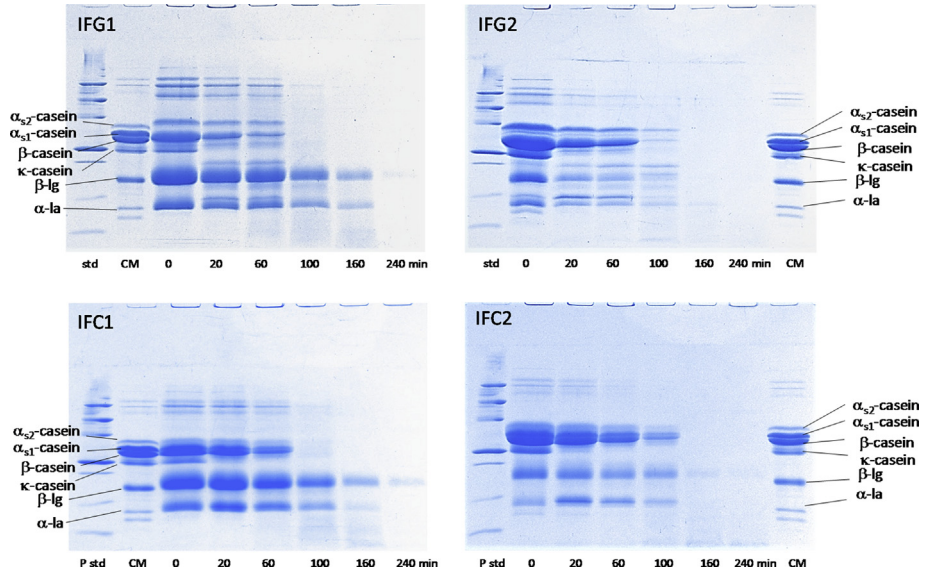

Fig. 8. SDS-PAGE patterns under reducing conditions of the digesta obtained from the infant formulae during gastric digestion (SGF with pepsin) in the HGS at different times (CM, cow milk): IFG1 and IFG2, whey protein-dominant goat milk infant formula and casein-dominant goat milk infant formula, respectively; IFC1 and IFC2, whey protein-dominant cow milk infant formula and casein-dominant cow milk infant formula, respectively.

& Singh, 2016b; Ye et al., 2017, 2019). The high fat content and the homogenisation process used in manufacture of these infant formulae may have contributed to the different structure of the protein coagula formed under gastric conditions.

3.2.4. Hydrolysis of protein

SDS-PAGE patterns of the gastric chyme remaining in the HGS are shown in Fig. 7. In all infant formula samples (except IFC1), the κ-casein band disappeared at 20 min, when the pH was about 6.2 (Fig. 4). This indicated that the pepsin hydrolysed the κ-casein to para- κ-casein at an early digestion time, which initiated the aggregation of protein and the flocculation of oil droplets. This was in line with previous observations on the gastric digestion of milk (Ye et al., 2016b). After 20 min, all protein bands decreased in intensity with increasing digestion time in all infant formula samples. In general, the casein bands decreased in intensity faster than the whey protein bands. Casein bands could not be seen at 100 min in IFG1, IFG2 and IFC1, but β-LG and α-LA were still observed, and

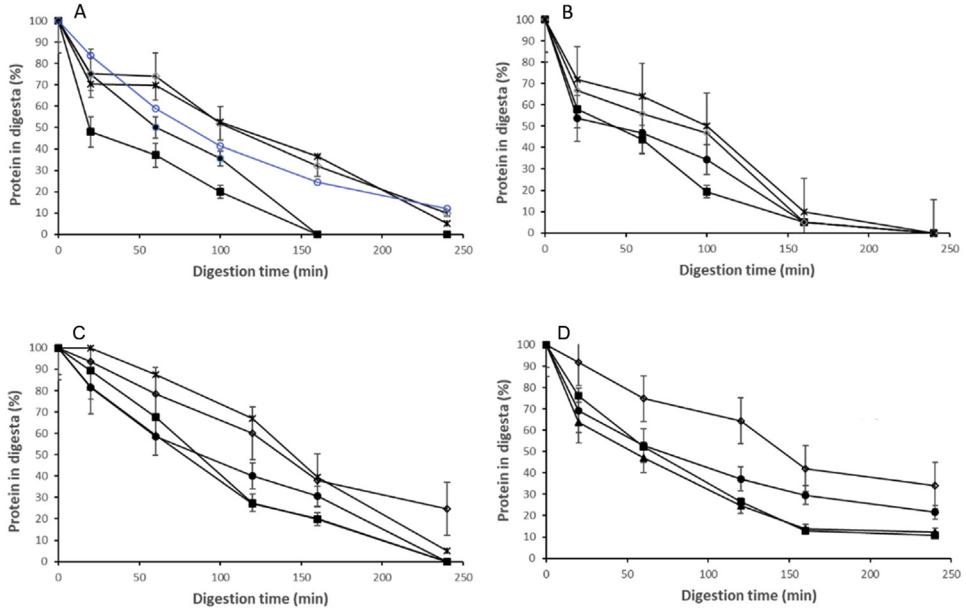

Fig. 9. Change in content of proteins (▲, α_{S1}-casein; ●, α_{S2}-casein; ■, β-casein; ◇, β-lactoglobulin; ∗, α-lactalbumin) in the infant formulae during gastric digestion (SGF with pepsin) in the HGS: A, IFG1, whey protein-dominant goat milk infant formula; B, IFG2, casein-dominant goat milk infant formula; C, IFC1, whey protein-dominant cow milk infant formula; D, IFC2, casein-dominant cow milk infant formula. The dilution of protein by SGF during dynamic digestion in HGS is shown in A (○). The results are reported as the calculated means and standard deviations.

many peptides had appeared. However, in IFC2, casein bands were still observed at 100 min, but had disappeared at 160 min. This indicated that the protein hydrolysis in IFC2 was slower, which could be attributed to the more extensive aggregation of the protein in this sample (Fig. 5). No intact protein was observed at 240 min for all infant formula samples, indicating that almost all of the proteins had been digested in the stomach and that the particles exiting the stomach contained oil droplets and peptides.

Fig. 8 shows SDS-PAGE patterns of the gastric digesta obtained from the different infant formula samples. The changes in the protein bands of the gastric digesta emptied from the HGS at different digestion times displayed similar trends to the chymes remaining in the stomach for all infant formulae (Fig. 7). For IFG1 and IFC1, no casein bands could be observed from 100 min but β-LG and α-LA were still observed until 240 min. At 100 min, casein, β-LG and α-LA bands were observed for IFC2, whereas only very faint casein bands and no whey protein bands were observed for IFG2.

The proteins remaining in the digesta as a function of the digestion time were quantified (Fig. 9). The rate of reduction of the proteins in the digesta was apparently faster in IFG1 and IFG2 than in IFC1 and IFC2 ($P < 0.05$). For example, at 60 min, approximately 40% casein (including α_{S2}-casein and β-casein) remained in the digesta of IFG1 and IFG2, but > 50% casein remained in the digesta of IFC1 (61% casein) and IFC2 (52% casein). At 160 min, the goat milk caseins had reduced to very low levels (0% for IFG1 and ~4% for IFG2), whereas ~30% remained in IFC1 and IFC2 (Fig. 10). In all digesta samples, the amount (relative to input) of whey protein was higher than that of casein during digestion, indicating the resistance of whey proteins to pepsin hydrolysis. Overall, the results indicate that the proteins (especially caseins) were digested faster in the infant formula samples made with goat milk than in those made with cow milk under these dynamic in vitro gastric conditions. This is agreement with previous studies of goat and cow milk under static gastric digestion (Hodgkinson et al., 2018).

Three factors contributed to the rate of protein digestion: (i) hydrolysis by pepsin; (ii) dilution of the digesta by the incoming gastric fluid during digestion; (iii) the formation of large particles, which may have delayed the pepsin proteolysis because of the particles' smaller surface area and hence inhibition of the diffusion of pepsin (Guo, Ye, Lad, Dalgleish, & Singh, 2014; Luo, Borst, Westphal, Boom, & Janssen, 2017; Nyemb et al., 2016). The differences in the extents of hydrolysis among the proteins during digestion occurred because of different resistances of the proteins to proteolysis by pepsin and different levels of protein in the chyme remaining in the stomach. The marked aggregation of the caseins that was induced by pepsin proteolysis or low pH under gastric conditions may have caused retention of the aggregates in the stomach for a longer time (Miranda & Pélissier, 1983; Ye et al., 2017). Of the whey proteins, native β-LG is resistant to some proteases, particularly pepsin, because of its unique structural stability at low pH (Menard et al., 2014; Peram et al., 2013; Reddy et al., 1988). The reduction in β-LG was probably mainly due to dilution by the gastric fluid as the reduction of β-LG was close to or slower than the dilution of protein (Fig. 9A). However, the reduction in α-LA may have resulted from both dilution and pepsin hydrolysis, especially at long digestion times (beyond 160 min), when the pH was lower than pH 4 (Fig. 4). This has been observed previously (Ye et al., 2019); pepsin was shown to be active on α-LA only at pH < 4.

These results suggested that infant formulae made with goat milk may have faster protein digestion than those made with cow milk, probably because they form smaller aggregates under gastric conditions. Infant formulae with high casein to whey protein ratios showed a greater difference in the level of aggregation between the formula made with goat milk and that made with cow milk. It has been reported that the casein in goat milk forms small, soft and

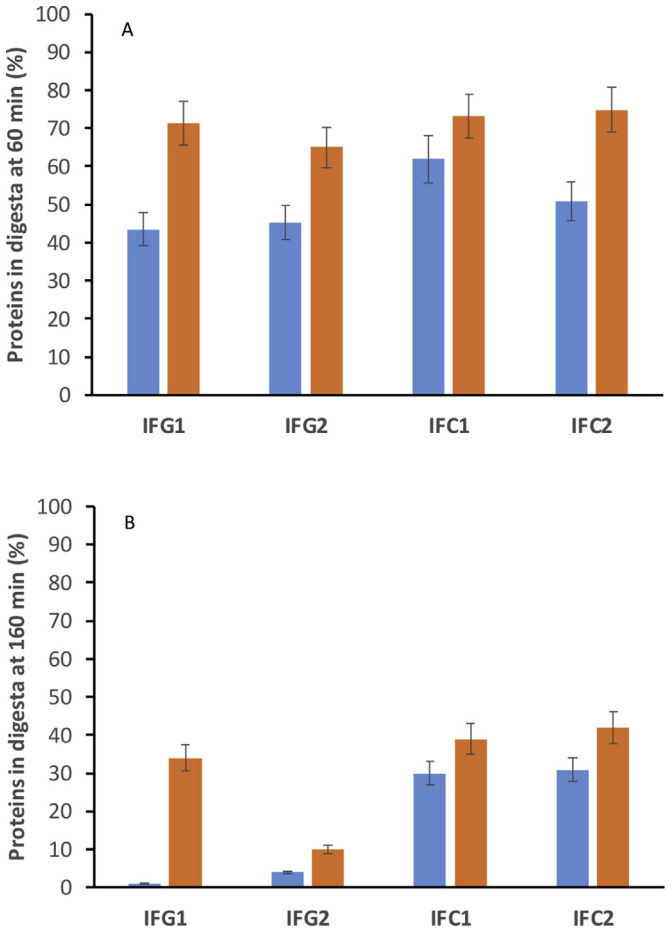

Fig. 10. Casein (■) and whey protein (■) contents of the digesta from the infant formulae at (A) 60 min and (B) 160 min of the gastric digestion (SGF with pepsin) in the HGS: IFG1 and IFG2, whey protein-dominant goat milk infant formula and casein-dominant goat milk infant formula, respectively; IFC1 and IFC2, whey protein-dominant cow milk infant formula and casein-dominant cow milk infant formula, respectively. The results are reported as the calculated means and standard deviations.

friable or loose curds in the human stomach compared with the firm coagulum formed by cow milk (Ceballos, Morales, Martínez, Extremera, & Sampelayo, 2009; Claeys et al., 2014; El-Agamy, 2007; Haenlein, 1996; Jenness, 1980; Maathuis, Havenaar, He, & Bellmann, 2017; Zenebe, Ahmed, Kabeta, & Kebede, 2014); this has been attributed to the lower casein content, specifically lower α_{S1}-casein, the higher proportion of β-casein and the larger casein micelle size in goat milk (Bell & Vlahopouloua, 1995; Clark & Sherbon, 2000; Domagała, 2009; Nguyen, Afsar, & Day, 2018; Ould Eleya, Desobry Banon, & Hardy, 1995). However, the actual mechanism of the coagulation behaviour and the casein micellar structure in goat milk are still not clear. Further investigation is required.

4. Conclusions

The present study showed that a firm clot was not formed during gastric digestion of the infant formulae, unlike that formed during the gastric digestion of the milks. However, coagulation of protein and flocculation of oil droplets, induced by both pepsin proteolysis and low pH under gastric conditions, were observed in the infant formulae and both were influenced by the source and composition of the protein. Infant formulae made with goat milk and cow milk exhibited differences in the extent of protein

coagulation and oil droplet flocculation during gastric digestion. The infant formulae made with goat milk formed smaller flocs of aggregated protein and oil droplets, which may have led to their observed faster protein digestion, than the infant formulae made with cow milk. The extent of the coagulation of protein and the size of the flocculated oil droplets under gastric conditions were also dependent on the protein composition of the infant formulae. The casein-dominated infant formula made with cow milk had greater aggregation in both the original formula and during gastric digestion than the other infant formulae, which led to its lower rate of casein digestion. These results suggest that the different composition of the casein micelles in goat milk may play an important role in its lower extent of coagulation and its faster protein digestion during gastric digestion compared with the casein micelles in cow milk. A more fundamental study of the effect of casein micelle composition on protein coagulation using milks from other species is required.

Acknowledgements

We thank Dr. X. Zhu for technical assistance with the confocal laser scanning microscopy experiments. This study was funded by Dairy Goat Cooperative (DGC) NZ Ltd, (Hamilton, New Zealand).

References

Attaie, R., & Richter, R. L. (2000). Size distribution of fat globules in goat milk. *Journal of Dairy Science, 83*, 940–944.
Bell, A. E., & Vlahopouloua, I. (1995). Preliminary studies on the gelation processes of fermented and GDL-acidified cow and caprine milk systems. *International Journal of Dairy Technology, 48*, 112–116.
Bevilacqua, C., Martin, P., Candalh, C., Fauquant, J., Piot, M., Roucayrol, A. M., et al. (2001). Goats' milk of defective alpha(s1)-casein genotype decreases intestinal and systemic sensitization to beta-lactoglobulin in guinea pigs. *Journal of Dairy Research, 68*, 217–227.
Boirie, Y., Dangin, M., Gachon, P., Vasson, M. P., Maubois, J. L., & Beaufrère, B. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 14930–14935.
Bosscher, D., Van Caillie-Bertrand, M., Van Dyck, K., Robberecht, H., Van Cauwenbergh, R., & Deelstra, H. (2000). Thickening infant formula with digestible and indigestible carbohydrate: Availability of calcium, iron, and zinc in vitro. *Journal of Pediatric Gastroenterology and Nutrition, 30*, 373–378.
Bourlieu, C., Ménard, O., Bouzerzour, K., Mandalari, G., Macierzanka, A., Mackie, A. R., et al. (2014). Specificity of infant digestive conditions: Some clues for developing relevant in vitro models. *Critical Reviews in Food Science and Nutrition, 54*, 1427–1457.
Bouzerzour, K., Morgan, F., Cuinet, I., Bonhomme, C., Jardin, J., Le Huerou-Luron, I., et al. (2012). In vivo digestion of infant formula in piglets: Protein digestion kinetics and release of bioactive peptides. *British Journal of Nutrition, 108*, 2105–2114.
Ceballos, L. S., Morales, E. R., Martínez, L. P., Extremera, F. G., & Sampelayo, M. R. S. (2009). Utilization of nitrogen and energy from diets containing protein and fat derived from either goat milk or cow milk. *Journal of Dairy Research, 76*, 497–504.
Chatterton, D., Rasmussen, J., Heegaard, C., Sørensen, E., & Petersen, T. (2004). In vitro digestion of novel milk protein ingredients for use in infant formulas: Research on biological functions. *Trends in Food Science & Technology, 15*, 373–383.
Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., et al. (2014). Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control, 42*, 188–201.
Clark, S., & Sherbon, J. (2000). α_{S1}-Casein, milk composition and coagulation properties of goat milk. *Small Ruminant Research, 38*, 123–134.
Domagała, J. (2009). Instrumental texture, syneresis and microstructure of yoghurts prepared from goat, cow and sheep milk. *International Journal of Food Properties, 12*, 605–615.
El-Agamy, E. (2007). The challenge of cow milk protein allergy. *Small Ruminant Research, 68*, 64–72.
Gan, J., Bornhorst, G. M., Henrick, B. M., & German, J. B. (2018). Protein digestion of baby foods: Study approaches and implications for infant health. *Molecular Nutrition & Food Research, 62*. Article 1700231.
Guo, Q., Ye, A., Bellissimo, N., Singh, H., & Rousseau, D. (2017). Modulating fat digestion through food structure design. *Progress in Lipid Research, 68*, 109–118.
Guo, Q., Ye, A., Lad, M., Dalgleish, D., & Singh, H. (2014). Effect of gel structure on the gastric digestion of whey protein emulsion gels. *Soft Matter, 10*, 1214–1223.
Gurr, M. I. (1981). Review of the progress of dairy science: Human and artificial milks for infant feeding. *Journal of Dairy Research, 48*, 519–554.
Haenlein, G. F. W. (1996). Nutritional value of dairy products of ewe and goat milk. *International Dairy Federation Special Issue*, 159–178.
Haenlein, G. F. W. (2004). Goat milk in human nutrition. *Small Ruminant Research, 51*, 155–163.
Hall, W. L., Millward, D. J., Long, S. J., & Morgan, L. M. (2003). Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *British Journal of Nutrition, 89*, 239–248.
Hernell, O. (2011). Human milk vs. cow's milk and the evolution of infant formulas. *Nestlé Nutrition Workshop Series. Paediatric Programme, 67*, 17–28.
Hodgkinson, A. J., McDonald, N. A., Kivits, L. J., Hurford, D. R., Fahey, S., & Prosser, C. (2012). Allergic responses induced by goat milk α_{S1}-casein in a murine model of gastrointestinal atopy. *Journal of Dairy Science, 95*, 83–90.
Hodgkinson, A. J., Wallace, O. A. M., Boggs, I., Broadhurst, M., & Prosser, C. G. (2018). Gastric digestion of cow and goat milk: Impact of infant and young child in vitro digestion conditions. *Food Chemistry, 245*, 275–281.
Jenness, R. (1980). Composition and characteristics of goat milk: Review 1968–1979. *Journal of Dairy Science, 63*, 1605–1630.
Jensen, R. G. (1995). *Handbook of milk composition*. New York, NY, USA: Academic Press.
Kong, F., & Singh, R. P. (2010). A human gastric simulator (HGS) to study food digestion in human stomach. *Journal of Food Science, 75*, E627–E635.
Lara-Villoslada, F., Olivares, M., Jiménez, J., Boza, J., & Xaus, J. (2004). Goat milk is less immunogenic than cow milk in a murine model of atopy. *Journal of Pediatric Gastroenterology and Nutrition, 39*, 354–360.
Luo, Q., Borst, J. W., Westphal, A. H., Boom, R. M., & Janssen, A. E. (2017). Pepsin diffusivity in whey protein gels and its effect on gastric digestion. *Food Hydrocolloids, 66*, 318–325.
Maathuis, A., Havenaar, R., He, T., & Bellmann, S. (2017). Protein digestion and quality of goat and cow milk infant formula and human milk under simulated infant conditions. *Journal of Pediatric Gastroenterology and Nutrition, 65*, 661.
Menard, O., Cattenoz, T., Guillemin, H., Souchon, I., Deglaire, A., Dupont, D., et al. (2014). Validation of a new in vitro dynamic system to simulate infant digestion. *Food Chemistry, 145*, 1039–1045.
Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., et al. (2014). A standardised static in vitro digestion method suitable for food—an international consensus. *Food & Function, 5*, 1113–1124.
Miranda, G., & Pélissier, J. P. (1983). Kinetic studies of in vivo digestion of cow unheated skim-milk proteins in the rat stomach. *Journal of Dairy Research, 50*, 27–36.
Mulet-Cabero, A.-I., Mackie, A. R., Wilde, P. J., Fenelon, M. A., & Brodkorb, A. (2019). Structural mechanism and kinetics of in vitro gastric digestion are affected by process-induced changes in cow milk. *Food Hydrocolloids, 86*, 172–183.
Nguyen, H. T., Afsar, S., & Day, L. (2018). Differences in the microstructure and rheological properties of low-fat yoghurts from goat, sheep and cow milk. *Food Research International, 108*, 423–429.
Nguyen, T. T. P., Bhandari, B., Cichero, J., & Prakash, S. (2015). A comprehensive review on in vitro digestion of infant formula. *Food Research International, 76*, 373–386.
Nyemb, K., Guérin-Dubiard, C., Pézennec, S., Jardin, J., Briard-Bion, V., Cauty, C., et al. (2016). The structural properties of egg white gels impact the extent of in vitro protein digestion and the nature of peptides generated. *Food Hydrocolloids, 54*, 315–327.
Ould Eleya, M. M., Desobry Banon, S., & Hardy, J. (1995). A comparative study of pH and temperature effects on the acidic coagulation of milks from cows, goats, and sheep. *Journal of Dairy Science, 78*, 2675–2682.
Park, Y. W., Juarez, M., Ramos, M., & Haenlein, G. F. W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research, 68*, 88–113.
Peram, M. R., Loveday, S. M., Ye, A., & Singh, H. (2013). In vitro gastric digestion of heat-induced aggregates of β-lactoglobulin. *Journal of Dairy Science, 96*, 63–74.
Perrella, S. L., Hepworth, A. R., Gridneva, Z., Simmer, K. N., Hartmann, P. E., & Geddes, D. T. (2015). Gastric emptying and curding of pasteurized donor human milk and mother's own milk in preterm infants. *Journal of Pediatric Gastroenterology and Nutrition, 61*, 125–129.
Reddy, I. M., Kella, N. K., & Kinsella, J. E. (1988). Structural and conformational basis of the resistance of β-lactoglobulin to peptic and chymotryptic digestion. *Journal of Agricultural and Food Chemistry, 36*, 737–741.
Rioux, L. E., & Turgeon, S. L. (2012). The ratio of casein to whey protein impacts yogurt digestion in vitro. *Food Digestion, 3*, 25–35.
Singh, H., Ye, A., & Horne, D. (2009). Structuring food emulsions in the gastrointestinal tract to modify lipid digestion. *Progress in Lipid Research, 48*, 92–100.
Tari, N. R., Fan, N. Z., Archbold, T., Kristo, E., Guri, A., Arranz, E., et al. (2018). Effect of milk protein composition of a model infant formula on the physicochemical properties of in vivo gastric digestates. *Journal of Dairy Science, 101*, 2851–2861.
Turgeon, S. L., & Rioux, L. E. (2011). Food matrix impact on macronutrients nutritional properties. *Food Hydrocolloids, 25*, 1915–1924.
Wang, X., Lin, Q., Ye, A., Han, J., & Singh, H. (2019). Flocculation of oil-in-water emulsions stabilised by milk protein ingredients under gastric conditions: Impact on in vitro intestinal lipid digestion. *Food Hydrocolloids, 88*, 272–282.
Wang, X., Ye, A., Lin, Q., Han, J., & Singh, H. (2018). Gastric digestion of milk protein ingredients: Study using an in vitro dynamic model. *Journal of Dairy Science, 101*, 6842–6852.

Ye, A., Cui, J., Dalgleish, D., & Singh, H. (2016a). The formation and breakdown of structured clots from whole milk during gastric digestion. *Food & Function, 7*, 4259–4266.

Ye, A., Cui, J., Dalgleish, D., & Singh, H. (2016b). Formation of a structured clot during the gastric digestion of milk: Impact on the rate of protein hydrolysis. *Food Hydrocolloids, 52*, 478–486.

Ye, A., Cui, J., Dalgleish, D., & Singh, H. (2017). Effect of homogenization and heat treatment on the behavior of protein and fat globules during gastric digestion of milk. *Journal of Dairy Science, 100*, 36–47.

Ye, A., Liu, W., Cui, J., Kong, X., Roy, D., Kong, Y., et al. (2019). Coagulation behaviour of milk under gastric digestion: Effect of pasteurisation and ultra-high temperature treatment. *Food Chemistry, 286*, 216–225.

Zenebe, T., Ahmed, N., Kabeta, T., & Kebede, G. (2014). Review on medicinal and nutritional values of goat milk. *Academic Journal of Nutrition, 3*, 30–39.