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In vitro studies of the digestion of caprine whey proteins by human gastric and duodenal juice and the effects on selected microorganisms

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The *in vitro* digestion of caprine whey proteins was investigated by a two-step degradation assay, using human gastric juice (HGJ) at pH 2·5 and human duodenal juice (HDJ) at pH 7·5. Different protein and peptide profiles were observed after the first (HGJ) and second (HDJ) enzymatic degradation. The minor whey proteins serum albumin, lactoferrin and Ig were rapidly degraded by HGJ, while α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) were more resistant and survived both 30 and 45 min of the enzymatic treatment. Further digestion with HDJ still showed intact β-LG, and the main part of α-LA also remained unchanged. The protein degradation by HGJ and HDJ was also compared with treatment by commercial enzymes, by using pepsin at pH 2·5, and a mixture of trypsin and chymotrypsin at pH 7·5. The two methods resulted in different caprine protein and peptide profiles. The digests after treatment with HGJ and HDJ were screened for antibacterial effects on some selected microorganisms, *Escherichia coli*, *Bacillus cereus*, *Lactobacillus rhamnosus GG* and *Streptococcus mutans.* Active growing cells of *E. coli* were inhibited by the digestion products from caprine whey obtained after treatment with HGJ and HDJ. Cells of *B. cereus* were inhibited only by whey proteins obtained after reaction with HGJ, while the products after further degradation with HDJ demonstrated no significant effect. Screenings performed on cells of *Lb. rhamnosus GG* and *S. mutans* all showed no signs of inhibition.

Caprine whey proteins: Digestion: Gastric juice: Duodenal juice: Antibacterial

Proteins from milk possess nutritional, functional and biological properties. Milk from different species is known to be a complex mixture of molecular components, containing biologically active molecules, which confers special properties to support infant development and growth beyond basic nutrition. During the last decade, bovine milk proteins and their enzymatically derived peptides have gained increasing attention as precursors of bioactive components, by contributing to specific activities such as antimicrobial, opoid, antihypertensive, immunomodulatory and mineral binding (Meisel & Schlimme, 1996; Pihlanto & Korhonen, 2003).

Due to the increasing production of whey as a by-product of cheese making, the option for processing of whey into a variety of food products is being extensively studied. Current commercial applications are milk-based infant formulas, health supplements, functional foods, drinks etc. Whey contains proteins, lactose, minerals and only minor amounts of fat. The proteins β-lactoglobulin (β-LG), α-lactalbumin (α-LA), lactoferrin (LF), Ig, lactoperoxidase, lysozyme and *N*-acetyl-β-D-glucosaminidase exert specific bioactivity and possess the ability to inhibit or kill a broad spectrum of microorganisms (Tomita *et al.* 1994; Pihlanto-Leppälä, 2000).

Whey from different species contains various amounts of such antimicrobial components. For example, bovine milk has a high concentration of lactoperoxidase, but low concentrations of LF and lysozyme, while human milk has high amounts of LF and lysozyme, and a low concentration of lactoperoxidase (FitzGerald & Meisel, 2003). Colostrum milk is unique and especially rich in many of these antimicrobial components (Korhonen *et al.* 2000*a*,*b*). Peptides derived from milk proteins also exhibit activities against a broad spectra of microorganisms and may thus be able to modulate the gut microflora. Whey proteins are regarded as more resistant to enzymatic attack and undergo hydrolysis much more slowly than caseins. However, enzymatic hydrolysis of bovine whey proteins has been studied and shown to give rise to peptides characterized by lower molecular weights (MW), increased number of ionic groups and an exposure of hydrophobic groups (Panyam & Kilara, 1996). Lactoferricin B, a peptide derived from the N-terminal segment of bovine LF, displays antimicrobial properties against a broad spectrum of both Gram-positive and Gram-negative bacteria (Arnold *et al.* 1977, 1980; Bellamy *et al.* 1992, 1993; Farnaud & Ewans, 2003) and fungi (Kirkpatrick *et al.* 1971; Soukka *et al.* 1992).

Milk proteins, including whey proteins, from other than bovine and human species, such as caprine, ewe and equine species, have also been studied (Miranda *et al.* 2004). Peptides

Abbreviations: HDJ, human duodenal juice; HGJ, human gastric juice; α-LA, α-lactalbumin; LF, lactoferrin; β-LG, β-lactoglobulin; MW, molecular weight; $OD_{600\,nm}$, optical density at 660 nm; WPCG, whey protein concentrate from goat's milk.

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produced by digestion of caprine proteins using commercial enzymes (pepsin, trypsin and chymotrypsin), have been reported to contain antibacterial properties. One of these peptides, lactoferricin C (caprine), was identified as fragment 14–42 of the LF sequence (Recio *et al.* 2000). This peptide also showed strong antimicrobial activity against various types of bacteria (Kimura *et al.* 2000; Recio & Visser, 2000).

In most studies on antibacterial effects of whey proteins, the peptides have been produced by hydrolysis with commercial enzymes such as pepsin and trypsin–chymotrypsin from pig pancreas (Tomita *et al.* 1991). However, if the goal is that these peptides should affect the human gut microflora population, they should rather have been produced by human gastric and duodenal enzymes (Bellamy *et al.* 1992). In addition to the 'standard hydrolysis' due to pepsin, trypsin and chymotrypsin, the gastric and duodenal juice from man also expresses other endo- and exopeptidase activities normally present in the human gut, and is consequently better than commercial enzymes at mimicking 'human digestion'.

The objective of the present work was to compare the degradation of whey proteins from goat's milk by commercial enzymes from pig pancreas, with a method using proteolytic enzymes from human stomach and duodenum. The hydrolysates were then screened for antibacterial effect on some pathogenic bacteria causing human illness, *Escherichia coli* and *Bacillus cereus*, together with *Streptococcus mutans*, responsible for dental caries. The study also included analysis on *Lactobacillus rhamnosus GG,* a probiotic bacteria that is one of the main organisms adhering in the gut.

Materials

Whey proteins

Goat's milk was collected from the university farm. A whey protein concentrate from goat's milk (WPCG) was produced from 570 litres pasteurized (72°C, 15 s) milk. The milk was treated with a starter culture (CHN-11; Christian Hansen, Denmark) for 1 h at 32°C, followed by renneting (25 ml/100 litres milk, rennet type SP175; Christian Hansen). The precipitated casein was removed by centrifugation, and the remaining 450 litres whey were reduced to 50 litres by diafiltration for 3 h (Alfa Laval UFS4, membrane GR-62-6338/48P, cut-off 20 000 MW). WPCG (3 kg) was then obtained after spray drying (Minor; Niro Atomizer A/S, Denmark). The WPCG contained 81 % protein as analysed by Kjeldahl (International Dairy Federation, 1993). Bovine lactoferrin (LF) with a purity of 95 % was supplied by DMV International (Veghel, Netherlands). The Fe content in LF was 121 ppm, giving an Fe saturation of approximately 15 %.

Human gastric and duodenal enzymes

Human proteolytic enzymes were obtained in the activated state by collecting human gastric juice (HGJ) and human duodenal juice (HDJ) according to Holm *et al.* (1988). All gastric and duodenal enzymes used in the present study were obtained from one person. In brief, a three-lumen tube enabled simultaneous instillation of saline in the duodenum and aspiration of HGJ and HDJ. Saline (100 ml/h) was instilled close to the papilla of Vater and HDJ aspirated some 18 cm distally. Aspirates were collected on ice and frozen in aliquots at −20°C. Before further use the individual samples from HGJ and HDJ were mixed into two larger batches to avoid the variations in enzyme activity between the individual samples.

Pepsin activity, with Hb as substrate, was assayed according to Sanchez-Chiang *et al.* (1987). 'Total proteolytic activity' in duodenal juice was assayed with casein as substrate, according to Krogdahl & Holm (1979). One unit of enzyme activity (U) is defined as the amount of enzyme that produces an absorbance of 1·0 at 280 nm in 20 min at 37°C.

Commercial enzymes

Pepsin (EC 3.4.23.1; porcine pepsin A activity 439 U/mg) was obtained from Sigma Chemical Co. (St Louis, MO, USA). Corolase PP (pig pancreas glands, activity 350 PU/mg) was obtained from Röhm GmbH (Germany).

Partial hydrolysis of caprine whey protein concentrate and bovine lactoferrin

The procedure developed to mimic 'normal digestion' in the human gastrointestinal tract consisted of two incubation periods, each lasting 30 min at 37°C. A digestion time of 30 min was selected. First, 10 ml whey solutions (50 g WPCG or bovine LF per litre H_2O) acidified to pH 2·5 with 2 M-HCl was incubated with 50 µl (0·4 U) HGJ. Then pH was adjusted to pH 7·5 with 1 M-NaOH, and 400 µl (13 U) HDJ was added and incubated again with continuous stirring. Aliquots (0·5 ml) were taken at different times during the incubation to follow the degradation. To stop the reaction, samples were put on ice, frozen and freeze-dried. When commercial enzymes were used, 4 mg pepsin (14 U) and 4 mg corolase PP (26 U) were used per g whey protein.

Gel electrophoresis

SDS–PAGE was carried out to evaluate the protein profile (80 000–8000 MW) after each step of hydrolysis (PhastSystem™; Pharmacia Laboratory Separation Division, Sweden). The assay was performed according to standard protocols (Laemmli, 1970), using 20 % acrylamide gels (PhastSystem™ homogeneous 20 gels; Amersham Biosciences AS, Sweden). The molecular mass markers used were the low MW standard kit (LMW Calibration Kit; Amersham Pharmacia, Biotech UK Ltd, UK). Staining was performed according to a standard procedure (Pharmacia Biotech AB, Sweden).

A SDS–PAGE system for small proteins (down to 1000 MW) was also used in order to study the peptide profile after each step of enzymatic treatment. The Tris–Tricine SDS–PAGE method was carried out according to Schägger & von Jagow (1987), using 15 % separating gel and 20 × 20 cm glass plates in the Bio-Rad Protean II System (Bio-Rad Laboratories Ltd, UK). Standard globing MW markers from Pharmacia were used (code no. 80-1129-83; Pharmacia LKB Biotechnology, Sweden).

Bacterial strains and culture conditions

Escherichia coli, strain HMG INF01, was obtained from the departmental culture collection at the Agricultural University of Norway. *Streptococcus mutans*, strain LT11, was provided by the Faculty of Dentistry at the University of Oslo. The bacteria were cultured in Brain Heart Infusion broth (37 g/1; Oxoid Limited, Hampshire, UK), at pH 7·4 and 37°C.

Lactobacillus rhamnosus GG (LGG®), ATCC 53 103, was donated from TINE BA, Oslo, Norway. The *Lb. rhamnosus GG* strain was cultured in MRS broth (52·2 g/l, pH 5·7; VWR International, Oslo, Norway) at 37°C. *Bacillus cereus*, strain RT INF01, was grown at optimum temperature in MRS broth. Active growing cultures (1 %) were used for inoculation.

Assay of antibacterial activity

Freeze-dried samples of WPCG and hydrolysates were solubilized in water and sterile-filtered (0·20 μm; Millipore) before further use. The assay was carried out in sterile honeycomb micro plates (Honeycomb 2; Thermo Labsystems Oy, Finland), and each well contained a total volume of 375 μl. To each well 300 μl freshly inoculated bacteria were added, together with 75 μl protein solution. The final concentration of WPCG or the hydrolysates in the wells varied between 3 and 12 mg/l (0·3–1·2 %) to evaluate minimum inhibition concentration. Growth controls contained 375 μl sterile growth media. The mixtures were incubated at 37°C for 20 h. The absorbance (optical density at 660 nm ($OD_{600\,nm}$)) was measured every 15 min by a plate reader (Bioscreen C; Oy Growth Curves AB Ltd, Finland). For the pathogenic bacteria *B. cereus*, plates containing 250 μl were used instead, and freshly inoculated bacteria and protein solutions were added to give the same concentrations as previous experiments. The plates were incubated at optimum temperature for 10 h, and $OD_{600\,nm}$ was measured (Sunrise Remote Control; TECAN, Austria). The experiments were repeated three times for each bacterial strain, with three parallels of each sample. Data are presented as mean values and standard deviations. To relate absorbance measurements ($OD_{600\,nm}$) to the number of viable cells (colony-forming units), plating on selective agars was performed.

Statistics

Standard deviation was estimated for all the spectrophotometric absorbance measurements on the various bacteria, based on the parallels of each sample. The results obtained from samples containing various sources of whey protein were also analysed in order to investigate the significance of the difference. Student's *t* test (two-sample, assuming unequal variances) was run to compare the different growth curves based on data obtained after 20 h, when equilibrium in the growth curves was reached. For *B. cereus*, however, data obtained after 8 h were used. The differences were considered significant when $P<0{\cdot}05$ (assuming one-tail for comparison between the standard curve of pure bacteria and samples with added whey protein, and assuming two-tail for comparison between two samples with different types of whey protein added).

Results

Degradation of whey proteins from goat's milk by human gastric and duodenal juice in comparison with commercial enzymes

In order to study the degradation of WPCG during *in vitro* digestion, hydrolysis was carried out in two steps; initially with HGJ at low pH (2·5) and second with HDJ at high pH (7·5). Figure 1 shows a time study of the degradation of the major whey proteins with the two enzyme sources after 22, 30 and 45 min of incubation with HGJ, and after 10, 20 and 30 min with HDJ. The time study showed that the main degradation with HGJ took place within the first 22–30 min; thereafter the protein pattern did not change much. Serum albumin, LF and Ig were rapidly and almost 'fully degraded' by HGJ after 22 min, while α-LA and β-LG were more resistant and survived digestion after 30 and 45 min at pH 2·5. The second step of hydrolysis with HDJ at pH 7·5 produced substantial amounts of low-MW peptides after 10 min. Some reduction in α-LA was observed especially after 30 min with HDJ; however, β-LG still seemed to resist digestion by both HGJ and HDJ.

In order to compare the use of HGJ and HDJ with some of the most frequently used commercial enzymes, degradation was also performed by using porcine pepsin and trypsin–chymotrypsin. The protein and peptide profiles of WPCG after stepwise hydrolysis for 30 min at 37°C were analysed (Fig. 2(A, B)). The results revealed a difference between the protein digestion patterns with HGJ and with porcine pepsin. The porcine pepsin seemed to 'fully hydrolyse' the serum albumin, LF, Ig and α-LA, resulting in numerous low-MW peptides. By using the second commercial enzyme mixture, trypsin–chymotrypsin, β-LG still resisted degradation, but the peptide pattern revealed that several of the peptides obtained from the first enzyme degradation were further degraded by the second enzyme treatment.

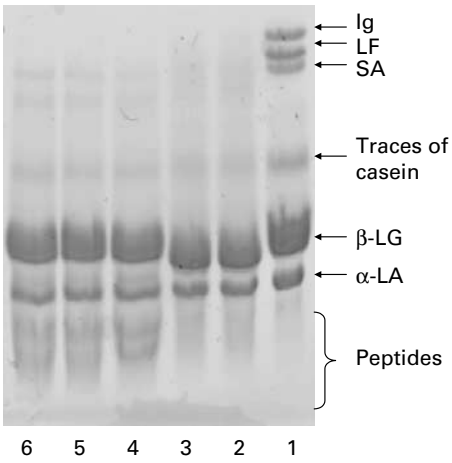

Fig. 1. Time study on the protein profile by SDS–PAGE (20 %) of whey protein concentrate from goat's milk (WPCG) degraded by human gastric juice (HGJ) and human duodenal juice (HDJ). For details of procedures, see p. 563. Arrows indicate the major bands: immunoglobulins (Ig), lactoferrin (LF), serum albumin (SA), casein, β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and peptides. Lanes: 1, native WPCG; 2, WPCG hydrolysed with HGJ (22 min at 37°C); 3, WPCG hydrolysed with HGJ (45 min at 37°C); 4, WPCG hydrolysed with HGJ (45 min at 37°C) and HDJ (10 min at 37°C); 5, WPCG hydrolysed with HGJ (45 min at 37°C) and HDJ (20 min at 37°C); 6, WPCG hydrolysed with HGJ (45 min at 37°C) and HDJ (30 min at 37°C).

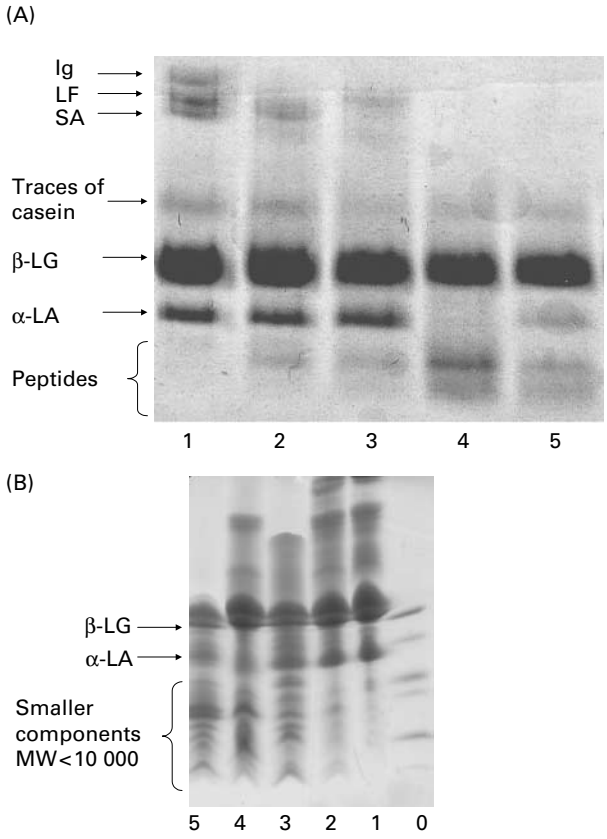

Fig. 2. (A), Protein profile by SDS–PAGE (20 %) of whey protein concentrate from goat's milk (WPCG) degraded by human gastric and duodenal juice, in comparison with commercial enzymes. (B), Peptide profile by SDS–PAGE (4–16 %) of the same samples. For details of procedures, see p. 563. → indicate the major bands: immunoglobulins (Ig), lactoferrin (LF), serum albumin (SA), casein, β-lactoglobulin (β-LG), α-lactalbumin (α-LA) and peptides. The wells contain: 0, molecular weight (MW) marker, range 2512–16 949; 1, native WPCG; 2, WPCG hydrolysed with HGJ (30 min at 37°C); 3, WPCG hydrolysed with HGJ (30 min at 37°C) and HDJ (30 min at 37°C); 4, WPCG hydrolysed with porcine pepsin (30 min at 37°C); 5, WPCG hydrolysed with porcine pepsin (30 min at 37°C) and corolase PP (30 min at 37°C).

In comparison with the commercial enzymes, the initial digestion with HGJ gave rise to only a few peptides. The second digestion with HDJ produced some more peptides in the MW range of 1000–10 000 (Fig. 2(B)). α-LA was degraded to some extent; however, the main part of β-LG remained unhydrolysed. The mixture of HGJ and HDJ produced an entirely different peptide pattern compared with the commercial enzyme digests as seen in Fig. 2. This indicates that the hydrolysates obtained from WPCG after digestion with human and commercial enzymes are very different in composition and content of survived proteins and peptides.

The effect of whey proteins from goat's milk and their degradation products from human gastric and duodenal juice in comparison with commercial enzymes on bacterial growth

Escherichia coli. *E. coli* HMG INF01 was cultured in Brain Heart Infusion broth with the addition of different concentrations (0·3–1·2 %) of WPCG, LF and WPCG hydrolysates. Figure 3 shows the growth of *E. coli* in the standard medium in comparison with growth media containing digested

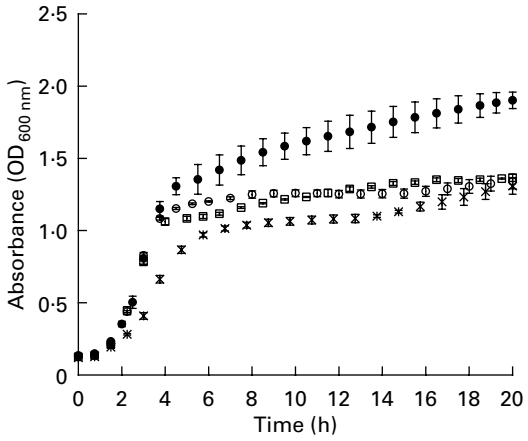

Fig. 3. Growth of *Escherichia coli* HMG INF01 in Brain Heart Infusion broth at 37°C with the addition of hydrolysates of whey protein concentrate from goat's milk (WPCG; 0·4 %) obtained from human or from commercial enzymes and native bovine lactoferrin (LF; 0·4 %). For details of procedures, see p. 563. ●, Control, no added protein; □, WPCG degraded by human gastric juice (30 min) + human duodenal juice (30 min); ○, WPCG degraded by the commercial porcine enzymes pepsin (30 min) + corolase PP (30 min); ⁎, native bovine LF. $OD_{600\,nm}$, optical density at 600 nm.

WPCG from both commercial enzymes and HGJ and HDJ. The effect of bovine LF on growth was also measured for comparison.

Both hydrolysates as well as native bovine LF inhibited the growth of *E. coli* (Fig. 3). No difference in inhibition was observed between the degradation products of WPCG by human enzymes (HGJ and HDJ) or the commercial enzymes (pepsin and corolase PP) after 20 h ($P>0{\cdot}05$), while in the range between 5 and 15 h after inoculation minor differences in inhibition occurred. The growth curves were identical with the control during the first 3–4 h in all cases with the exception of the medium containing LF. After 4 h a clear reduction in growth was registered. Samples containing LF already showed an inhibition at 2 h, and the inhibition during the total time period up to 18 h was much stronger (significant difference, $P<0{\cdot}05$).

In order to detect possible antibacterial effects of WPCG generated early or late during the degradation with human enzymes, samples after the first step by HGJ (30 min) and after the second step by HDJ (30 min) were tested. In addition, unhydrolysed WPCG was included. A reduction in the growth of *E. coli* HMG INF01 was observed ($P<0{\cdot}01$) even at the lowest concentration of the hydrolysed WPCG (0·3 %). The inhibition of *E. coli* cells was maintained during both the first and second steps of hydrolysis by the human enzymes ($P<0{\cdot}01$), as shown in Fig. 4. $OD_{600\,nm}$ for *E. coli* of 1·0 and 1·4 corresponds to $4{\cdot}4\times10^8$ and $8{\cdot}8\times10^8$ colony-forming units, respectively. This shows that approximately 50 % reduction in growth was obtained when the whey protein was added. The results demonstrated no significant difference between the effect of native WPCG, WPCG treated with HGJ, or WPCG hydrolysed with both HGJ and HDJ. An additional experiment with bovine LF (0·3 %) digested with HGJ and HDJ showed that the antibacterial effect was maintained during the digestion process (data not shown).

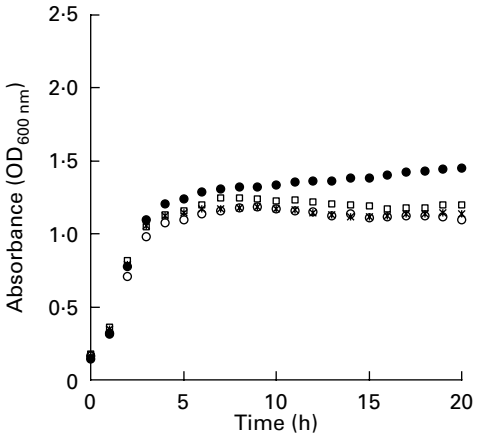

Fig. 4. Growth of *Escherichia coli* HMG INF01 at 37°C with the addition of whey protein concentrate from goat's milk (WPCG 0·3 %) degraded by human gastric juice (HGJ; 30 min) or HGJ (30 min) + human duodenal juice (HDJ; 30 min) and native WPCG (0·3 %). For details of procedures, see p. 563. ●, Control, no protein added; □, native WPCG; ○, WPCG degraded by HGJ (30 min); ⁎, WPCG degraded by HGJ (30 min) + HDJ (30 min). (Standard deviation bars have been omitted for clarity). Standard deviation varied between 0·003 and 0·040 for the control curve, and between 0·001 and 0·060 for the other growth curves. $OD_{600\,nm}$, optical density at 600 nm.

In order to compare the samples with different concentrations of native and hydrolysed WPCG, the growth rate in the logarithmic phase was calculated. Results in Table 1 show a clear reduction in the growth rate when either untreated WPCG, degraded WPCG or bovine LF was added to the growth media. As previously found, there was no significant difference between the inhibitions by diverse types of WPCG, while bovine LF gave a much stronger inhibitory effect in comparison. Table 1 also shows that an increase in the concentrations of native WPCG and hydrolysed WPCG from 0·3 % up to 0·6 % and 1·2 % in the growth media gave no significant change in the inhibitory effect on *E. coli*.

Bacillus cereus. The effect of native WPCG, bovine LF and the degradation products from gastric and duodenal enzymes on the growth of *B. cereus* was also analysed. The protein samples were added in the concentration between 0·3 and 1·2 %. The addition of LF and LF degraded by HGJ and HDJ inhibited the growth of *B. cereus* after 2 h of incubation. The hydrolysed LF seemed to inhibit the growth of *B. cereus* more efficiently in the early log phase than LF. However, the native LF (1·2 %) was slightly more effective in the stationary phase (Fig. 5(A)). Results were similar for 0·3 % LF (data not shown). ⁎

Native and degraded WPCG (0·3 and 1·2 %) were added to active growing cultures of *B. cereus*. The digests of WPCG after the first hydrolysis with HGJ at pH 2·5 showed a high level of cell inhibition. Figure 5(B) shows the results of the hydrolysed WPCG (1·2 %) with HGJ on the inhibition of *B. cereus* ($P=0{\cdot}0006$, time 8 h). Further hydrolysis of WPCG with HDJ at pH 7·5 gave a digest with no detectable inhibition ($P=0{\cdot}36$, time 8 h). A comparative experiment with bovine WPC-80 (native, digested with HGJ (pH 2·5) and HDJ (pH 7·5)) on *B. cereus* was also performed. The results obtained were identical with the studies carried out with caprine whey proteins (data not shown).

Lactobacillus rhamnosus GG *and* Streptococcus mutans *LT11*. Native and digested WPCG by HGJ and HDJ were analysed for the effect on the growth of the probiotic strain, *Lb. rhamnosus GG* and the *S. mutans* LT11 from human oral cavity. The results are shown in Fig. 6(A,B). Neither *Lb. rhamnosus GG* or *S. mutans* was inhibited by the addition of WPCG or any of the degradation products of WPCG in the growth medium. Identical results were obtained when 0·3 % LF and bovine WPC-80 was added. Higher concentrations up to 1·2 % were also tested and the same results were obtained (data not shown).

Discussion

The present *in vitro* study has investigated the difference in the degradation of caprine whey proteins by the use of a combination of HGJ and HDJ in comparison with commercial porcine enzymes. The purpose was to observe if some of the peptides formed could possibly survive the transport through the gastrointestinal tract, and also have an effect on some bacteria.

Gel electrophoresis of the hydrolysates from the two-step enzymatic degradation showed distinct differences due to the use of human *v*. porcine enzymes. Both protein and peptide

Table 1. Growth of *Escherichia coli* (HMG INF01) in Brain Heart Infusion broth at 37°C with varying concentrations of whey protein concentrate from goat's milk (WPCG), digested WPCG and lactoferrin (LF)*

(Mean values and standard deviations for three observations)

Sample/substrate	Concentration of WPCG or LF in the well (%)	Growth rate† ($\Delta OD_{600\,nm}$/h) Mean	SD
Pure culture *E. coli*	–	0·38	0·01
E. coli + WPCG	1·2	0·30	0·02
	0·6	0·32	0·03
	0·3	0·31	0·03
E. coli + WPCG treated with HGJ and HDJ	0·6	0·35	0·01
	0·3	0·32	0·03
E. coli + bovine LF	0·6	0·24	0·01
	0·3	0·21	0·01

HDJ, human duodenal juice; HGJ, human gastric juice; $OD_{600\,nm}$, optical density at 660 nm.
* For details of procedures, see p. 563.
† Growth rate is calculated in the logarithmic growth phase between 1·5 and 4 h after inoculation.

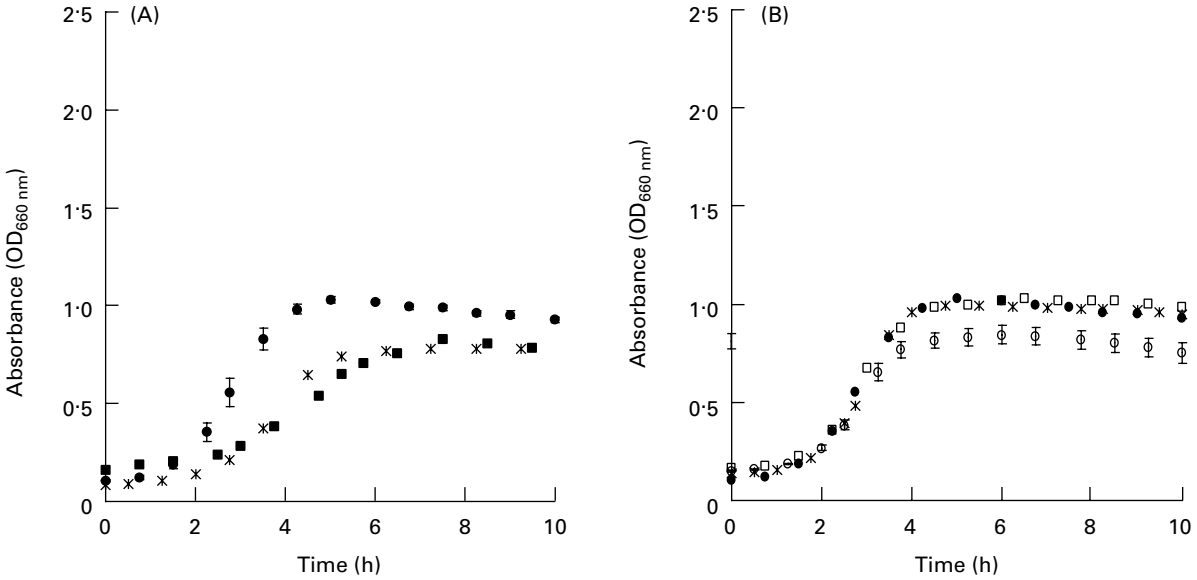

Fig. 5. Growth of *Bacillus cereus* at 30°C with the addition of native whey protein concentrate from goat's milk (WPCG; 1·2 %), native bovine lactoferrin (LF; 1·2 %) and the degradion products by human enzymes. For details of procedures, see p. 563. (A), ●, Control, no protein added; ⁎, addition of native LF; ■ addition of LF degraded by human gastric juice (HGJ; 30 min) + human duodenal juice (HDJ; 30 min). (B), ●, Control, no protein added; □, native WPCG; ○, WPCG degraded by HGJ (30 min); ⁎, WPCG degraded by HGJ (30 min) + HDJ (30 min). $OD_{600\,nm}$, optical density at 600 nm.

profiles (Fig. 2) obtained in this experiment showed that the degradation of the major whey proteins, and the formation of peptides, varied with the type of enzyme. Use of commercial porcine pepsin showed a more pronounced degradation of the proteins, with the formation of a corresponding larger amount of peptides than with HGJ. The major part of the protein degradation by human pepsin appeared to be rapid with little effect due to a prolonged reaction time (Fig. 1). The large number of different peptides obtained with the porcine pepsin indicates rather a species difference, with a broader specificity with this preparation, than just a higher activity. Similar species-dependent results have previously been demonstrated by hydrolysis of β-casein by human pepsin and bovine chymosin (Guillou *et al.* 1991).

The results from the second enzyme digestions using HDJ and the commercial trypsin–chymotrypsin preparations showed that after hydrolysis with HDJ the main part of α-LA remained, while the porcine enzymes degraded this protein very fast. Both types of enzymes left the main part of β-LG undigested. Earlier digestion studies (Chatterton *et al.* 2004) on bovine whey proteins, with HGJ obtained from infants, showed similar results. Only β-LG and small amounts of α-LA remained native after treatment with HGJ at different pH values (2·0–6·5). However, why the purified porcine trypsin and chymotrypsin also caused a more extensive degradation than the more 'complete' human enzyme mixture containing elastases and carboxypeptidases can at present not be explained. In addition to differences in the number

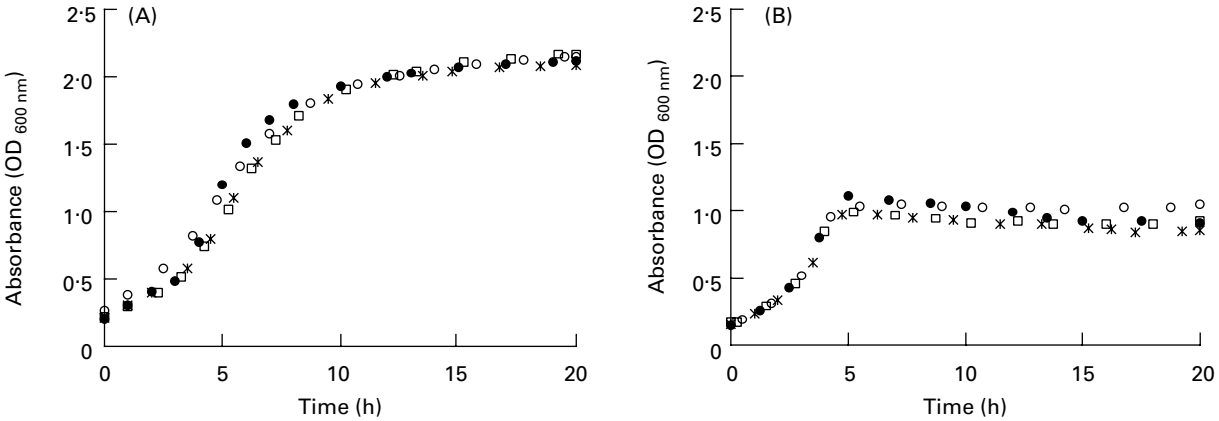

Fig. 6. Growth of *Lactobacillus rhamnosus GG*, ATCC 53 103 (A) and *Streptococcus mutans*, LT11 (B) with the addition of 0·3 % native and hydrolysed whey protein concentrate from goat's milk (WPCG). For details of procedures, see p. 563. ●, Control with no protein added; □ addition of native WPCG; ○, WPCG degraded by human gastric juice (HGJ; 30 min); ⁎, WPCG degraded by HGJ (30 min) and HDJ (30 min). (Standard deviation bars have been omitted for clarity). Standard deviation varied from 0·003 to 0·083 for *Lb. rhamnosus GG*, and between 0·002 and 0·048 for *S. mutans*. $OD_{600\,nm}$, optical density at 600 nm.

of peptidases and proteinases, the presence of various isoforms of pepsin (Dunn, 2002) as well as the pancreatic enzymes (Scheele *et al.* 1981) may play a role. On the other hand, this also clearly demonstrates the need for species-relevant enzymes when *in vitro* systems are used to mimic an *in vivo* situation.

When the digestion products from WPCG were added to active growing cells of *E. coli* strain HMG INF01, significant inhibition of the growth was observed. Other research groups have also observed this effect, however, their focus was mainly on bovine whey proteins, and very few studies are found on caprine whey proteins (Bellamy *et al.* 1992; Tomita *et al.* 1994; Pihlanto-Leppälä, 2000). Some studies on the antibacterial effect of hydrolysates obtained from whey proteins have been reported on lactoferricin, a digestion product from LF (Bellamy *et al.* 1992; Kimura *et al.* 2000; Recio & Visser, 2000). It has been demonstrated that the antibacterial effect is conserved throughout the hydrolysis by pepsin and trypsin–chymotrypsin from pig. *In vivo* experiments by Kuwata *et al.* (1998) showed that antibacterial peptides were released into the HGJ after 10 min of ingestion in human adults. However, no *in vivo* studies have been performed with focus on the influence on these peptides by human duodenal enzymes. The present study shows that WPCG treated with HGJ and HDJ still has the ability to inhibit the growth of *E. coli* HMG INF01, which indicates that the inhibitory peptides probably are resistant to further hydrolysis by human proteolytic enzymes. Another possibility is that the initially active peptides produced could give rise to new and smaller ones, which also possess antibacterial activity. No significant difference in the antibacterial effect was observed when the concentration in the growth medium was increased from 0·3 % to 0·6 % and 1·2 %. This is possibly due to the fact that addition of whey protein hydrolysates always will give both growth stimulants (amino acids etc.) and inhibition to growing bacteria. These two effects have to be considered together.

The inhibition by the two-step digests produced from WPCG on the growth of *B. cereus* RT INF01 varied strongly with the level of degradation by human gastric and duodenal enzymes (Fig. 5). Bellamy *et al.* (1992) demonstrated similar antibacterial effects in whey protein and LF hydrolysates produced with porcine enzymes on different strains of *B. cereus*. Goat whey protein hydrolysate treated with HGJ showed a strong inhibitory effect on *B. cereus*. Identical inhibition was observed in samples with either 1·2 % pure LF or 1·2 % WPCG hydrolysate from HGJ. Since the LF content in bovine milk is only 0·1 g/l, this indicates that other antibacterial components than just LF are present in the sample contributing to the high inhibition. Additional treatment with HDJ totally wipes out the antibacterial effect, indicating a further degradation of the active peptides by enzymes from the human duodenum.

The present study on the effect on the strain *S. mutans* LT11 indicated no signs of inhibition, although earlier findings by Bellamy *et al.* (1992) reported an inhibition of the strain *S. mutans* JCM-5175 by bovine lactoferricin. This is probably due to the low concentration of lactoferricin in the digested samples, caused by the low content of LF in goat's milk. By fractionation of the whey protein hydrolysate this will be investigated further. The antibacterial screenings on *Lb. rhamnosus GG* ATCC-53 103 also indicated no signs of inhibition, which is very positive in the case of the probiotic bacteria.

Conclusion

The present study presents new important information on protein degradation of caprine whey by using *in vitro* digestion utilizing HGJ and HDJ. Since variation in concentration, structure and amino acid content among the milk proteins from different species appear, the caprine peptides produced during digestion may have other nutritional and biological effects than proteins from other species. Most reports on digestion of milk and whey proteins have been performed with commercial enzymes from species other than man. The present results showed very different protein and peptide patterns when human enzymes from the stomach and the duodenal part of the intestine were used. Some of the main proteins, β-LG and α-LA, were more resistant to digestion than the minor protein fractions. The resulting digestion products showed significant antibacterial effects on *E. coli* HMG INF01 and *B. cereus* RT INF01. Interestingly, no effect on *Lb. rhamnosus GG* ATCC-53 103 and *S. mutans* LT 11 was observed. Further studies on characterizing the peptides produced during initial (HGJ) and secondary (HDJ) digestion are being investigated in our laboratory. The results may have an application for food development and reveal a potential for some of these components as ingredients in food for infants, athletes and the elderly.

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