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In Vivo Gastric Digestion of Milk Proteins. Effect of Technological Treatments

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To compare the effects of technological treatments of milk on the gastric emptying of proteins and peptides, three diets were studied: raw milk, pasteurized milk, and yoghurt. In the preruminant calf, all effluents leaving the stomach during 12 h were collected and analyzed for N emptying, NPN level, amino acid composition, and characterization of proteins and peptides by SDS–polyacrylamide gel electrophoresis. With raw milk, casein coagulation is almost immediate in the stomach and casein is evacuated in the form of peptides. With pasteurized milk, casein coagulation is slower. Casein is also evacuated in the form of peptides. With yoghurt diet in which casein was gelified by acidification to pH 4 before ingestion, there is no coagulation in the stomach. Casein is evacuated during the whole digestion process in intact and degraded form. For the three diets, α-lactalbumin was degraded when the pH value was under 3.5. β-Lactoglobulin did not seem to be proteolyzed. Amino acid compositions of the effluents are not so variable in the process of digestion with pasteurized milk as in digestion with raw milk. With yoghurt, amino acid compositions of the effluents are almost identical during the whole digestion process.

Milk covers an important part of the protein contribution in human nutrition. In developed countries, around 20–30% of food proteins come from milk (Hambreus, 1982). Only a small part is consumed as raw milk; most of it undergoes different technological processing, such as heat treatments (pasteurization, UHT), acidification and proteolysis (yoghurt), and cheese-making. The in vivo digestion of milk proteins is initiated in the stomach by pepsins and, in some species, including ruminants, chymosin.

The milk-processing method can have the effect of changing the coagulum structure obtained in the stomach and consequently the manner in which milk proteins are digested (Toullec et al., 1974; Jenkins and Emmons, 1982; Pfeil, 1984). Studies on gastric digestion of treated milk (skim milk, different heated milks) have been made in vivo in pig (Kaufmann, 1984; Pfeil, 1984; Meisel and Hagemeister, 1984), rat (Zebrowska, 1968; Buraczewski et al., 1970, Miranda and Pélissier, 1981, 1983, 1987), and calves (Shillam and Roy, 1963; Stobo and Roy, 1978; Toullec et al., 1971; Guilloteau et al., 1975; Pélissier et al., 1983; Yvon et al., 1984a,b, 1985; Yvon and Pélissier, 1987).

In the present work, we describe for the first time the in vivo gastric digestion of yoghurt in preruminant calf, which is a model of monogastric animals. During the making of yoghurt, bovine skim milk is first pasteurized and then acidified by ferments. To specify the respective roles of pasteurization and acidification on gastric digestion, we also describe the digestion of pasteurized bovine skim milk. These two diets are compared with the reference diet, i.e., raw bovine skim milk.

MATERIALS AND METHODS

Diets. Three types of test meal were prepared from the same fresh bovine skim milk source: (1) raw milk, (2) pasteurized milk, and (3) yoghurt. To obtain diets 2 and 3, milk was heated to 95 °C during 45 s. For diet 3, the milk was then inoculated at 3% final concentration with a preculture of mixed strains *Streptococcus thermophilus* (CNRZ TJ160) and *Lac-*

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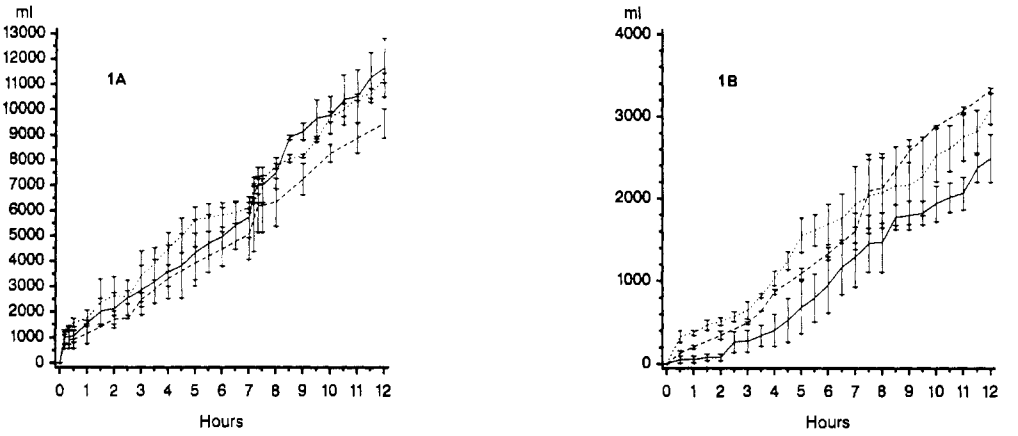


Figure 1. Gastric emptying of fresh matter. (A) Cumulative quantities of fresh matter leaving the stomach (in milliliters) during time of digestion (in hours). (B) Cumulative quantities of endogeneous secretions leaving the stomach (in milliliters) during time of digestion (in hours). (—) Raw milk; (···) pasteurized milk; (- - -) yoghurt.

tobacillus bulgaricus (CNRZ 1B369) on milk. The inoculated milk was incubated at 42 °C during 4 h, brought to 4 °C, and kept at that temperature for 15 days. The two other diets were kept at −20 °C. Each diet was brought to room temperature, and polyethylene glycol (PEG) 4000 was added to a final concentration of 1% just before the animal feeding to estimate the ratio of endogenous secretions in the emptyied fresh matter.

Animals. Preruminant Friesian calves were used. At about 1 month of age, each animal was fitted with an abomasal cannula and a reentrant duodenal cannula. Proximal and distal parts of the duodenal cannula were located around 8 and 15 cm after the pylorus, respectively (Ash, 1962). Animals were allowed to recover from surgery for 7 days before digesta collection was started. Their normal diet was a milk substitute based on skim-milk powder, whey powder, and tallow.

Experimental Procedures. For each experiment, two animals were used simultaneously. The day before experiments, animals received, in the morning, their normal diet to which sodium citrate (11 g/kg) had been added to accelerate gastric emptying (Frantzen et al., 1973), and, in the evening, only water. Under these conditions, the abomasum was empty at the moment of the first meal in the morning of the experimental days.

In the morning of the experiment, the two calves received a test meal (190 g/kg live weight$^{0.75}$) of the same diet which was instilled into the abomasum with a peristaltic pump. The duration of the instillation was between 3 and 9 min. The whole effluent from the abomasum of the first calf (experimental) was then collected over 7 h with fractions taken at 10-min intervals for the first 30 min and at 30-min intervals until to the end of the 7-h period. A second identical test meal was then given, and samples were collected with the same periodicity up to 5 h after this meal. The digesta coming from the second calf (donor) were instilled in the distal part of the cannula of the first calf at its gastric emptying velocity starting as soon as digesta appeared in the proximal part. A milk hydrolysate was instilled in the duodenum of the second calf, in the same manner as that reported by Pélissier et al. (1983). The two calves received, once a week, successively the three experimental diets. With this procedure, each animal was alternatively the experimental and the donor calf for each diet and then was its own reference.

Aliquots were used to measure pH and then returned to samples. Each sample was immediately precipitated with trichloroacetic acid (TCA) to a final concentration of 12%. Samples were centrifuged at 2000*g* for 20 min. The pellet was resuspended in water. Supernatants and sediments were kept at −20 °C until analysis.

Analysis. Endogenous secretions were estimated from the concentration of PEG measured according to the method of Hyden (1955).

Nitrogen emptying was determined by measuring the protein (PN) fraction (insoluble in 12% TCA) by the Kjeldahl method with the colorimetric technique of Koops et al. (1975). The peptidic (NPN) fraction was estimated by amino acid analysis, to eliminate the non-amino-acid fraction. These two measurements were used to determine the amino acid nitrogen (PN + NPN) and the level of hydrolysis of samples [NPN/(PN + NPN)].

Table I. Comparison of pH of Gastric Effluent[a]

	diet		
	1, raw milk	2, heated milk	3, yoghurt
pH of diet before ingestion time	6.4	6.7	4.1
first diet			
30 min	6.4	5.9	4.1
5 h	2.2	3.0	3.0
second diet			
30 min	4.9	6.0	4.1
5 h	2.4	2.8	2.8

[a] Mean obtained with two experiments on each diet.

The nature of products leaving the stomach was analyzed by SDS electrophoresis according to procedures of Trieu-Cuot and Gripon (1981).

Amino acid compositions were determined after acid hydrolysis (HCl 5.7 N, 110 °C, 24 h, under vacuum) by using the method of Spackman et al. (1958) with a Biotronik LC5000 analyzer (Munich, RFA). The amino acid analyses were compared by principal component analysis. With this method, each sample was considered as a point in a 17-dimensional space with 17 variables (the percent of each of the 17 amino acids detected by amino acid analysis). The initial pattern was reduced to a bidimensional space in which the two axes (called factors) were not correlated. The new axes were interpreted in relation to the former variables by computing the loading of each variable on these axes (SAS/STAT, 1985). The smaller the distance between the two points characterizing the two samples, the more similar were the two samples.

RESULTS AND DISCUSSION

Emptying of Fresh Matter. Figure 1A gives the cumulated quantities of fresh matter leaving the stomach with the three diets. After 7 (one meal) and 12 h (two meals), the mean quantities obtained corresponded approximately to the ingested volumes. Very little, if any, difference between the diets was found, although a tendency of a smaller volume emptying was observed with yoghurt. The endogenous secretions were approximately 3 L after 12 h of experiments for 10 L of ingested diet. With raw milk the endogenous secretions tended to be lower than with the two other diets (Figure 1B).

Change of pH. Just before ingestion, the pH values of raw milk, pasteurized milk, and yoghurt are, respectively, 6.4, 6.7, and 4.1. The pH of gastric effluent was roughly 2 before the first meal. Table I gives a comparison of the pH values measured 30 min and 5 h after ingestion of each

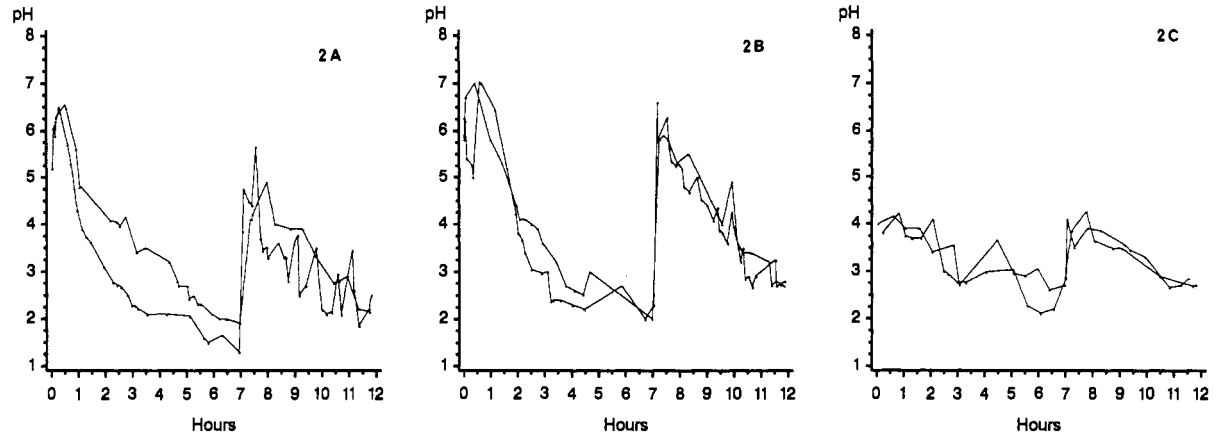


Figure 2. Change in pH of effluent leaving the stomach during time of digestion (in hours). (A) Raw milk; (B) pasteurized milk; (C) yoghurt.

meal. It increased very rapidly to around 6.4 and 5.9 for raw milk and heated milk, respectively, and 4.1 for yoghurt. It decreased slowly to 2 during the first 7 postprandial hours. At the arrival of the second meal of raw milk the pH increased to near 5, a value that was lower than after the first meal. This means that coagulation occurs instantly, and thus casein could not leave the stomach and therefore could not play the same buffer role in the effluent as during the first meal. With the other two diets this phenomenon was not so important. This was probably due to the fact that casein coagulated more slowly with pasteurized milk and did not coagulate with yoghurt. Consequently, stomach contents were more homogenous. The pH of the effluents was similar to that of the original diets (Figure 2). In fact, the three diets appeared to give a different evolution of the pH of the effluents, not only because of their own pH but also because of their coagulation capacity.

Emptying of Nitrogen Products. Total amino acid nitrogen left the abomasum at rates roughly similar to those observed for fresh matter (Figure 3A). After a high value during the first 10 min following the beginning of feeding (12–25% of total intake), the flow rate was very low during the next 50 min and progressively increased until the fifth hour and thereafter decreased until the second meal. The second meal was followed by similar changes that were, however, more rapid. Total cumulative recovery tended to be higher for the yoghurt diet.

Proteic nitrogen emptying occurred very rapidly during the meal (Figure 3B). After that, caseins coagulated and proteic products left the stomach at a flow rate that depended on the structure of the coagulum. With the raw and heated milk diet, coagulation was important and the emptying rate of proteic products was relatively low. With the yoghurt diet, the coagulation did not occur and the flow rate of proteic nitrogen was higher than with the other two diets (Figure 3C). These results show the importance of the coagulum structure on the emptying of nitrogen products from the stomach.

The level of amino acid NPN was higher in yoghurt than in raw or heated milk (1.9%, 0.6%, 1.1%, respectively). In contrast, in the digesta, it varied much less with the yoghurt diet (10–30%) than with the other two diets (5–80%) whose proteins appeared to be much more hydrolyzed before leaving the abomasum (Figure 4). However, the cumulative recovery of amino acid NPN after 12 h was not really lower with the yoghurt diet (Figure 3D). The low NPN level observed with yoghurt was due to a higher PN emptying. After the coagulation, N products of raw and heated milk could easily leave the abomasum only after an important hydrolysis by gastric proteases. With heated milk, the amino acid NPN level increased faster than with raw milk, probably because the coagulation was delayed and the curd was less firm, allowing for a more rapid hydrolysis; i.e., the maximum level was obtained after 3 h compared to 6 h with raw milk. The decrease observed after these maxima times could be due to the emptying of products originating from the hydrolysis of caseins which were not degraded into small peptides and were still insoluble in 12% TCA. In these conditions, the decrease observed was the consequence of the proteolysis.

Characterization of Products Leaving the Stomach. *Electrophoretic Analysis.* The SDS electrophoresis patterns of effluent products from the stomach (Figure 5) confirmed the results obtained by measuring the N emptying. With raw milk (Figure 5A) the caseins appeared only in the first sample collected after the first meal (0–10 min.). Later on, casein coagulated and could not leave the stomach anymore. After some hours, an important number of products were detected by electrophoresis. They corresponded to the hydrolysis of the casein which liberated small peptides, soluble in TCA, and larger peptides still insoluble in TCA. The change of the proportion of these two groups of components explained the development of the NPN level observed. The α-lactalbumin was degraded when the pH decreased under 4, which is in agreement with previous experiments (Yvon et al., 1984b). After the second meal, an almost identical pattern was observed i.e., emptying of a few caseins during a very short time, slow degradation of the caseins with the appearance of degradation products, and disappearance of the α-lactalbumin when the pH decreased.

With heated milk (Figure 5B), the coagulation did not occur before the first hour of digestion: the caseins could be detected in the effluents during a longer time than with raw milk. After 2–3 h, an important number of electrophoretic bands were detected, which corresponded to the emptying of peptidic products originating from the hydrolysis of the caseins. The presence of these products, insoluble in 12% TCA, explained the decrease of the NPN level observed at the same time of digestion. The α-lactalbumin was present during around 3 h after the meal. Degradation products of this protein appeared after that time, which indicates a hydrolysis when the pH decreased. After 7 h, the second meal gave a similar pattern, i.e., emptying of caseins during some hours, rapid appearance of degradation products of caseins, and degradation of α-lactalbumin when the pH decreased.

With yoghurt (Figure 5C), caseins were detected during a longer time than with the other two diets. This confirms

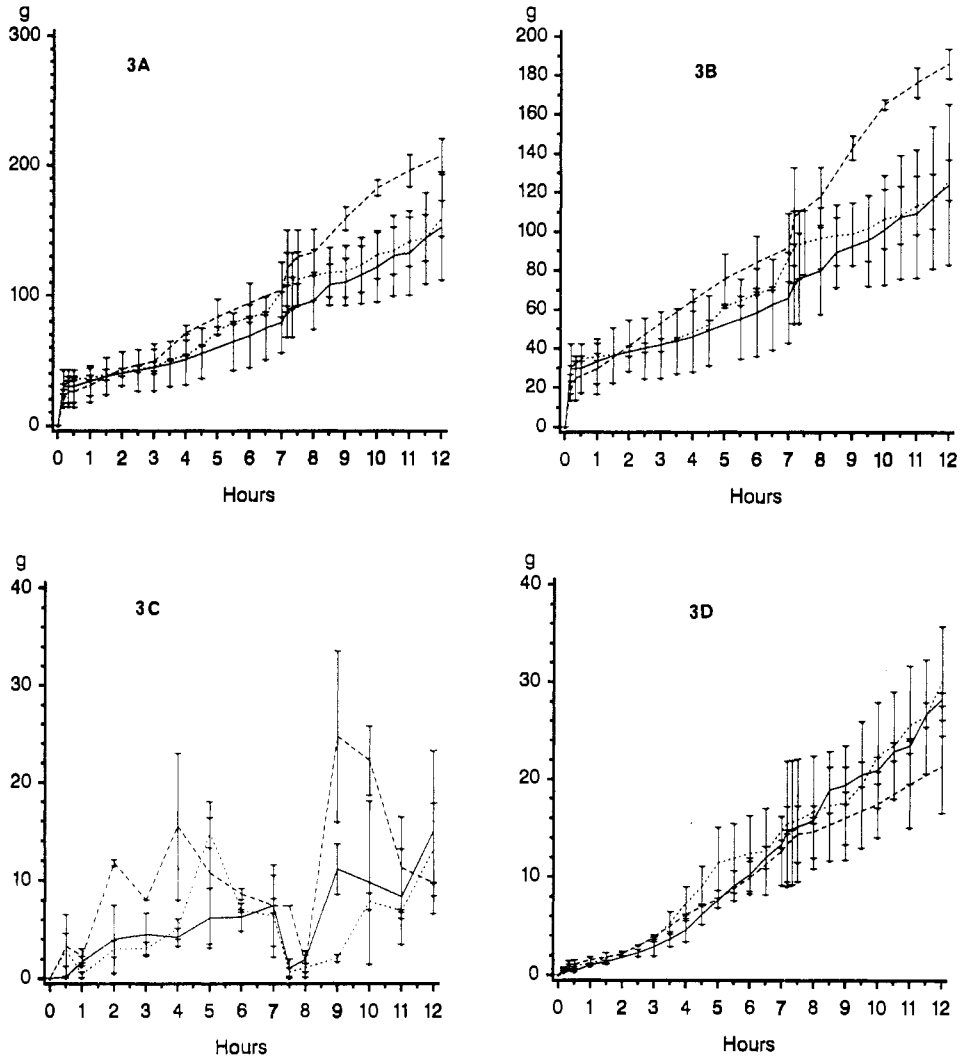


Figure 3. Gastric emptying of nitrogen products. (A) Cumulative quantities of total nitrogen (in grams of protein: N × 6.39) leaving the stomach during time digestion (in hours). (B) Cumulative quantities of proteic nitrogen (in grams of protein: N × 6.39) leaving the stomach during time digestion (in hours). (C) Flow rate of proteic products leaving the stomach (in grams of proteins: N × 6.39/heure) during time digestion. Times 0–10 min and 7 h–7 h 10 min corresponding to the meal are not taken into account. (D) Cumulative quantities of NPN products (in grams of proteins, determined by amino acid analysis) leaving the stomach during time digestion (in hours). (—) Raw milk; (···) pasteurized milk; (- - -) yoghurt.

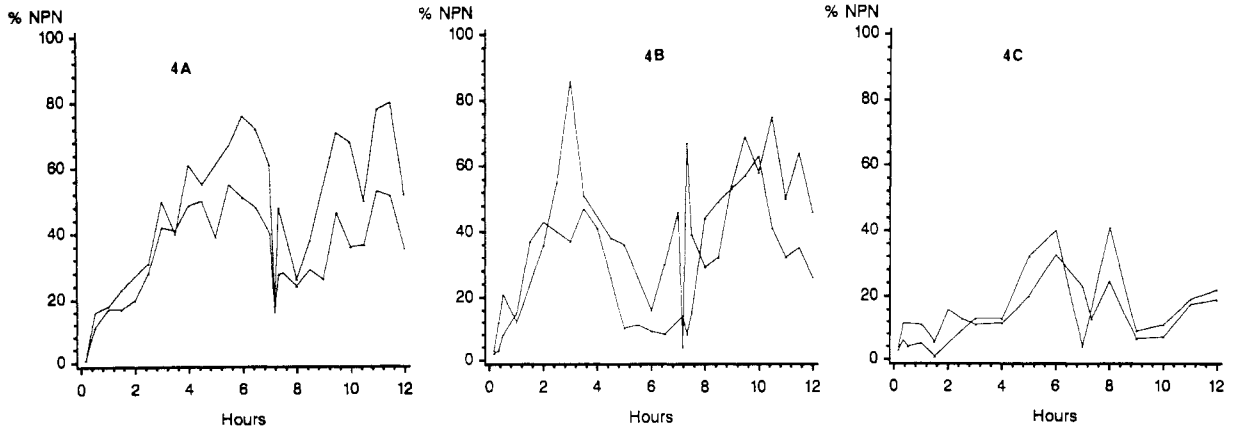
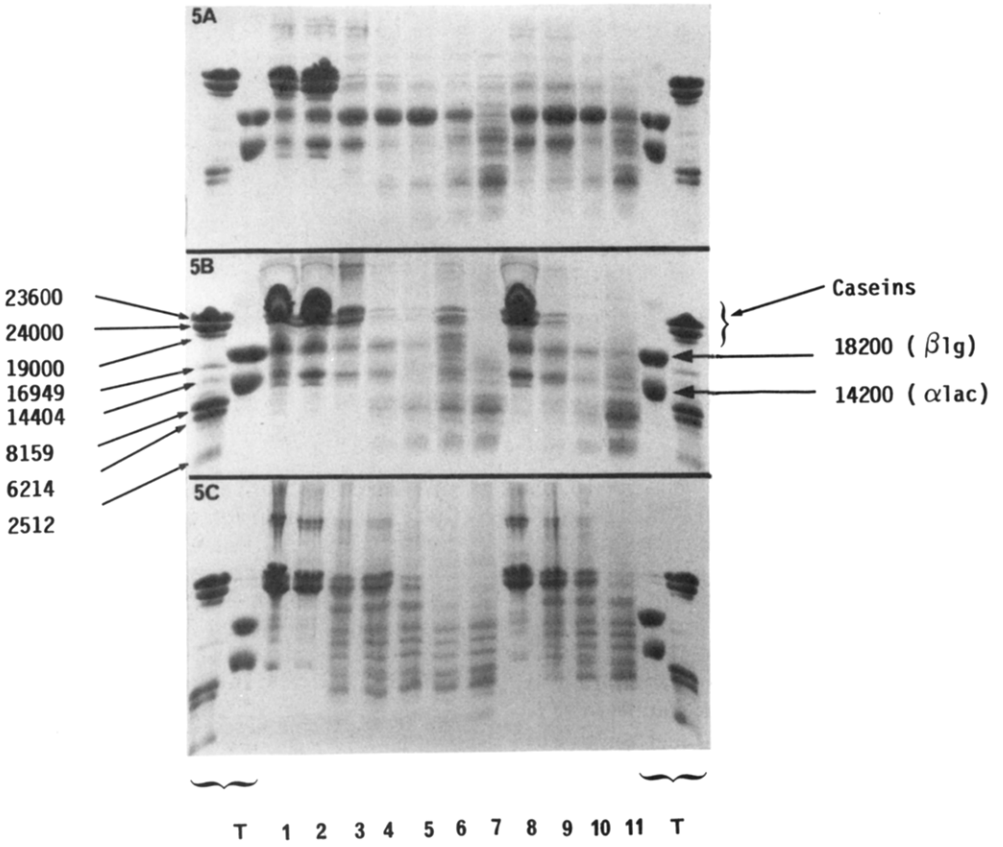


Figure 4. Change of NPN level (in percent) during time of digestion (in hours). (A) Raw milk; (B) pasteurized milk; (C) yoghurt.

that no preferential retention of caseins occurred in the stomach because of the technological treatment of the diet. The emptying of caseins explained the higher level of proteic products and the lower NPN level of the effluents observed with this diet.

β-Lactoglobulin does not seem degraded by gastric enzymes during the 12 h of the experiments with raw milk and pasteurized milk. The large amount of emptied casein products did not permit detection of this protein with yoghurt.

Amino Acid Compositions. Amino acid compositions of the digesta leaving the stomach were determined separately for products soluble and insoluble in 12% TCA. These amino acid compositions have been compared by



T	standards
1	meal
2	0 - 10 min
3	30 - 60 min
4	2h - 2h 30
5	3h - 3h 30
6	5h - 5h 30
7	6h 30 - 7h
8	7h - 7h 10
9	7h 30 - 8h
10	10h - 10h 30
11	11h 30 - 12h

Figure 5. SDS electrophoresis of samples leaving the stomach. (A) Raw milk; (B) pasteurized milk; (C) yoghurt. Standards are a CNBr myoglobin hydrolysate plus caseins; α-lactalbumin plus β-lactoglobulin.

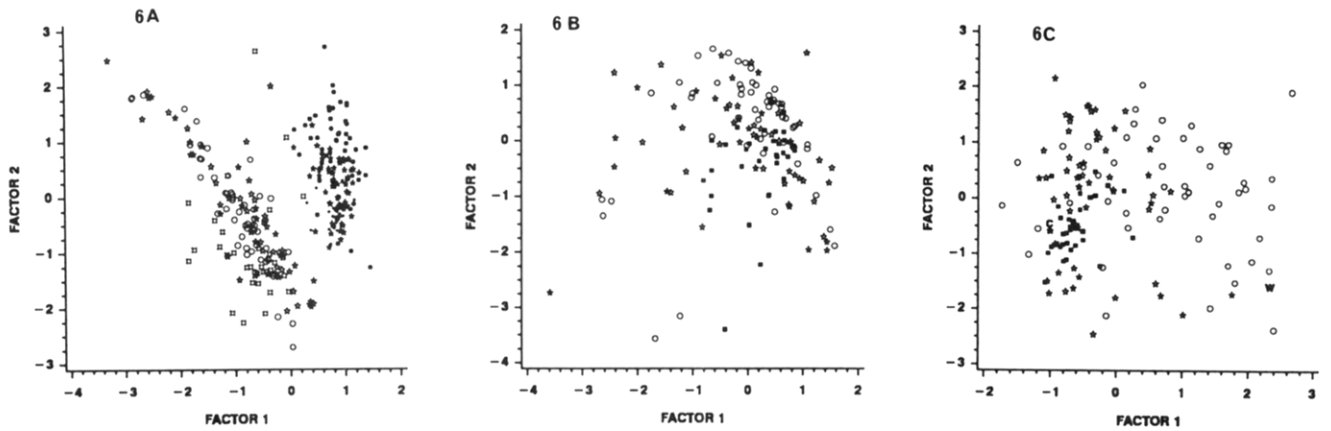


Figure 6. Principal component analysis performed on the relative quantities of the 17 amino acids determined on each sample leaving the stomach. (A) Analysis on whole samples (sediments and supernatants in 12% TCA): raw milk, ● (sediments), ○ (supernatants); pasteurized milk, ★ (sediments), ☆ (supernatants); yoghurt, ■ (sediments), □ (supernatants). (B) Analysis performed on 12% TCA soluble products. (C) Analysis performed on 12% TCA insoluble products. (○) Raw milk; (☆) pasteurized milk; (■) yoghurt.

multivariate analysis. Two main groups appeared (Figure 6A) when all the samples from all the experiments were taken into account (292 experimental points). One corresponded to proteic products, insoluble in 12% TCA, the other to the small peptides, soluble in 12% TCA. These two groups were detected along the first factor, which represents the major part of the observation (55%). This result confirms that the amino acid compositions of small peptides were very different from those of proteic products leaving the stomach. Because of their size, these small pep-

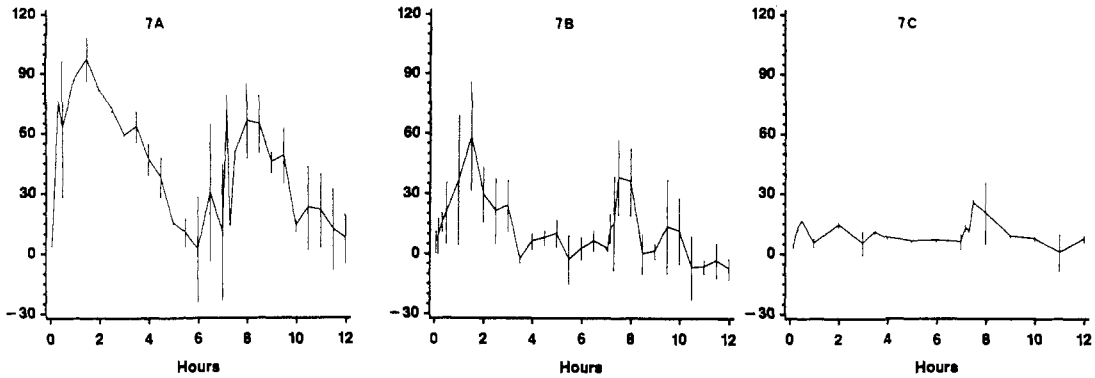


Figure 7. Projection of the principal component analysis during time of digestion. The x axis represents the axis joining the composition of caseins to that of whey proteins. 0 = 100% caseins, 0% whey proteins; 100 = 0% caseins, 100% whey proteins. (A) Raw milk; (B) pasteurized milk; (C) yoghurt.

tides may be considered to be more rapidly absorbed. This showed that the composition of the amino acid of product absorbed by the gut is different from the composition of the whole diet and can vary according to the time elapsed since milk feeding. The amino acid composition only of a protein is not adequate to characterize its nutritional quality.

Principal component analyses were made separately on the amino acid compositions of the fraction soluble in 12% TCA (Figure 6B) and on the proteic fraction, insoluble in 12% TCA (Figure 6C). The amino acid compositions of the effluents were more homogeneous with pasteurized milk than with raw milk. With yoghurt, these compositions were almost identical during the whole digestion process and, for the sediments, very close to that of whole milk. For the proteic fractions of the other two diets, the amino acid compositions were located between the amino acid compositions of caseins and whey proteins.

To estimate the proportion of casein (or of peptides coming from the hydrolysis of casein) in proteic sample, each point was projected, in the multivariate analysis, on the axis joining the composition of casein to that of whey proteins. The relative position of the projection on this axis during digestion time gives an estimation of the change of this proportion (Figure 7). This analysis confirms that the amino acid composition of the products leaving the stomach after ingestion of raw milk became rapidly rather similar to the amino acid composition of whey proteins. Because of their coagulation, caseins stayed in the stomach, whereas whey proteins were emptied. After, caseins were hydrolyzed, and the products leaving the stomach had amino acid compositions closer to that of casein. With heated milk, this phenomenon was not as important because of a weaker coagulation of caseins and a higher rate of casein hydrolysis. With yoghurt, the amino acid composition of the proteic fraction leaving the stomach is relatively constant and close to that of milk. With this diet there is no specific retention of a group of products, opposite to that observed with the two other diets, and the reproducibility of the measurements is better.

CONCLUSION

Technological treatment of milk modifies its coagulation in the stomach. The structure of the coagulum influences the nature of products leaving the stomach. With the firm curd obtained for raw milk, casein was retained for a long time in the stomach and left this organ in the form of small peptides. With the coagulum obtained with a heated milk, gastric emptying of intact casein occurred during a longer period. However, the major difference was a faster proteolysis of the caseins, which resulted more rapidly in a high level of NPN.

When casein was gelified before ingestion as in yoghurt, there was no retention of casein, which left the stomach during the whole time of digestion. The NPN level was not as important, and less variable, than with the other diets, because of the higher quantities of proteic products leaving the stomach.

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