

Stability of bovine immunoglobulins to thermal treatment and processing

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Thermal stability of bovine immunoglobulin (IgG) in model systems and in commercially processed milk products was investigated. Bovine serum IgG dissolved in either phosphate buffered saline (PBS), boiled milk or ultra high temperature (UHT) sterilized milk was heated at temperatures ranging from 62.7°C to 80°C. The *D*-values ranged from 90, 200 and 170 s at 80°C to 25.5, 27.2 and 32.8 min at 72°C for IgG in PBS, boiled milk and UHT milk, respectively. These results suggest slightly greater thermal stability of IgG in milk than in phosphate buffer. IgG content was not changed after 30 min at 62.7°C, nor by holding for 24 h at either ambient temperature or 4°C. Over the temperature range from 72° to 80°C, *z*-values were 6.7, 8.9 and 8.5°C, and energies of activation were 353.5, 258.2 and 298.5 kJ mol⁻¹ for thermal destruction of bovine serum IgG in PBS, boiled milk and UHT milk, respectively. These findings in model systems suggest that commercial pasteurization processes should not result in complete destruction of IgG. Comparison of IgG content in raw milks and corresponding HTST-pasteurized milks of varying fat content indicated 59–76% retention after pasteurization, with no apparent effect by either homogenization, skimming or standardization to 1 or 2% fat levels. The IgG contents and specific antibody activity against lipopolysaccharide fractions of five bacteria were determined for several commercially processed milk products. HTST-pasteurized milks, reconstituted skim milk powder and whey from cheddar cheese production all showed high levels of IgG and specific antibody activity. However, canned evaporated milk and ultra high temperature (UHT) sterilized milk had little or no IgG. This study demonstrates the dependence of bovine IgG stability in milk products on severity of thermal treatment used in various commercial processes.

Keywords: Immunoglobulins IgG, milk, heat, processing, antibody.

INTRODUCTION

Cow's milk immunoglobulins IgG have potential for utilization in the immunological supplementation of infant formulae and other foods (Goldman, 1989; Facon *et al.*, 1993). Specific antibodies in cow's milk have been shown to be effective in human clinical trials against enteropathogenic (Mietens *et al.*, 1979) and enterotoxigenic (Tacket *et al.*, 1988) *Escherichia coli*, cryptosporidium (Tzipori *et al.*, 1986), rotavirus (Ebina *et al.*, 1985; Brüssow *et al.*, 1987; Hilpert *et al.*, 1987) and *Shigella flexneri* (Tacket *et al.*, 1992). The protective effects of antibodies in cow's milk against infection in young animals such as calves, rats, rabbits, piglets,

lambs and foals have also been demonstrated (Menšík *et al.*, 1978; Bridger & Brown, 1981; Saif *et al.*, 1983; Michalek *et al.*, 1987; Stott & Lucas, 1989).

In order to preserve the immunoprophylactic or therapeutic potential of immunoglobulins in cow's milk, it is important to consider Ig stability during storage or following processing methods that include thermal treatments such as pasteurization, sterilization or spray drying. Although studies investigating the thermal destruction of human milk immunoglobulins have been reported (Goldsmith *et al.*, 1983; Morgan *et al.*, 1986), there is little information available for bovine milk IgG.

Yolken *et al.* (1985) measured the levels of anti-rotavirus antibody in raw and pasteurized cow's milk and commercially available infant formulae. Although the level of antibody in the pasteurized milk samples was

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lower than that measured in raw milk samples, it was sufficient to neutralize the *in vitro* replication of rotavirus and to provide protection in mice against rotavirus infection. On the other hand, little or no anti-rotavirus antibody was detected in the infant formulae or other sterile milk preparations. Golay *et al.* (1990) reported that skim milk powder produced using 'normal dairy processing conditions' contained active immunoglobulins; powder processed from milk of immunized cows contained higher specific antibody levels than milk of non-immunized cows. Kobayashi *et al.* (1991) and Murosaki *et al.* (1991) reported that control milk from non-immunized cows had no active IgG compared to immune milk from immunized cows; however, in these two studies it was noted that the immune milk powder was processed by spray drying at low temperatures while the control milk was prepared by a high temperature process.

Recently our laboratory has been conducting research to determine the levels of IgG and specific antibody activity in raw and commercially processed milks. A survey of 254 raw milk samples collected in 1990 and 1991 in the province of British Columbia, indicated levels of IgG in raw milk ranging between 0.030 and 0.71 mg IgG/ml, with mean values of 0.28 and 0.25 mg/ml in 1990 and 1991, respectively (Li-Chan *et al.*, 1994). All of the milk samples had measurable antigen-binding activity in enzyme-linked immunoassay (ELISA) against the lipopolysaccharide fractions of five bacteria tested, *S. flexneri* 1A, *E. coli* O111:B4, *E. coli* O128:B12, *S. typhimurium* and *S. enteritidis*. Fluid milk pasteurized by the HTST (high temperature short time) process by five different dairy processors contained between 0.04 and 0.24 mg IgG/ml (Kummer *et al.*, 1992). Canned evaporated milk and ultra high temperature (UHT) sterilized milk contained little or no detectable IgG, whereas skim milk powder contained 0.17 mg IgG/ml reconstituted milk. Membrane-sterilized IgG prepared from cheddar cheese whey and aseptically added to UHT milk was stable for at least five months when stored at 4, 25 and 35°C (Fukumoto *et al.*, 1994). Although these studies suggest that the retention of Ig in dairy products is dependent on thermal processing conditions, no systematic study on the kinetics of thermal destruction of bovine IgG has been reported.

The objective of this study was to investigate the thermal stability of IgG in model systems and various commercially processed milk products. The first phase of the study determined kinetic parameters for thermal destruction of bovine immunoglobulin G in model systems of phosphate buffered saline, boiled milk and UHT milk. Subsequently studies were conducted to evaluate the effect of commercial processes on the total IgG and specific antibody levels in various dairy products. The changes in IgG content in milk resulting from commercial dairy processing including skimming,

homogenization and HTST pasteurization were monitored. Finally, commercially available milk samples were compared for total IgG content and specific antibody activities against the lipopolysaccharide fractions of five pathogenic bacteria.

MATERIALS AND METHODS

Milk samples

Raw milk collected immediately after milking from the University of British Columbia dairy herd was used as a 'reference' milk in the immunoassays. The IgG content of this milk determined by radial immunodiffusion was 0.306 mg IgG/ml. Milk from vaccinated cows (Stolle low fat milk powder, containing 5.0 mg IgG/g powder) was a gift from Stolle Research & Development Corporation (Cincinnati, OH). The cows had been immunized with a polyvalent vaccine, S-100 antigen, as reported by Golay *et al.* (1990) and Kobayashi *et al.* (1991). Whey from cheddar cheese production was a gift from Dairyland Foods (Burnaby, BC). Commercial HTST pasteurized and UHT sterilized fluid milk, canned evaporated milk and skim milk powders were purchased from local supermarkets. Raw and corresponding milks after homogenization or skimming and HTST pasteurization were donated by Beatrice Foods (Calgary, Alta). Milk powders were reconstituted to 10% total solids using distilled water (100 g powder dissolved in 950 ml water).

Reagents

Rabbit antiserum to bovine IgG, bovine serum IgG, *p*-nitrophenyl phosphate substrate tablets, porcine gelatin type A and lipopolysaccharide (LPS) fractions from five bacteria (*S. flexneri* serotype 1A, *E. coli* serotype O111:B4 and serotype O128:B12, *Salmonella typhimurium* and *Salmonella enteritidis*) were purchased from Sigma Chemical Co. (St Louis, MO). Ovalbumin (99.9% pure) was a gift from Canadian Lysozyme Inc. (Abbotsford, BC). Rabbit anti-bovine IgG alkaline phosphatase conjugate and radial immunodiffusion plates were purchased from ICN Biomedicals Canada Ltd. (Mississauga, ON). Immulon II 96-well microtitre plates were purchased from Fisher Scientific (Ottawa, ON). All other reagents were analytical grade.

Thermal treatment of serum IgG in model systems

Bovine serum IgG was dissolved to give a final concentration of 0.5 mg/ml in phosphate buffered saline (PBS) at pH 7.1 (Kummer *et al.*, 1992), in commercial UHT 2% milk, or in boiled milk (homogenized HTST pasteurized fluid milk which was brought to boiling for 15 min). Previous experiments had indicated undetectable

or only trace quantities of IgG in both UHT and boiled milks, thus indicating these milks to be suitable for investigating the thermal destruction of known quantities of added IgG in milk compared to a phosphate buffer system.

Aliquots (250 μ l) of IgG solution in borosilicate glass disposable culture test tubes (13 $\times$ 100 mm) were covered with parafilm, then incubated in water baths at 62.7, 72, 74, 76, 78 or 80°C. The temperature of the IgG solutions was measured using a Luxtron fluoroptic thermometry system (Mountain View, CA). The time taken for the solutions to equilibrate to the temperature of the water bath ranged between 13–17 s. At specified time intervals, individual test tubes were removed from the water bath and immediately cooled in an ice water bath. Each thermal treatment was conducted in triplicate.

To investigate the possibility of renaturation of IgG during the period between thermal treatment and analysis of IgG concentration by ELISA, preliminary experiments were conducted to compare IgG contents measured in samples when analyzed immediately after thermal treatment and cooling, after 24 h at ambient temperature (approximately 22°C) or after 24 h at 4°C. No effect of storage for 24 h at either temperature was observed.

Thermal destruction of IgG was calculated using the thermal death time method as described by Morgan *et al.* (1986) and Ramaswamy *et al.* (1989), assuming first-order destruction. The *D*-value or decimal reduction time, representing the time required to reduce the IgG concentration by 90%, was calculated for each heating temperature from semi-logarithmic plots of IgG concentration versus heating time. The *z* value, representing the temperature change required to alter rate of destruction by a factor of 10 in the range of temperature (72–80°C) under investigation in this study, was calculated by the negative reciprocal slope of the semi-logarithmic plot of *D* values versus heating temperature. The energy of activation E_a was calculated using the Arrhenius equation, $\ln k = \ln s - (E_a/R)(1/T)$, where *R* is the universal gas constant (8.314 joule deg⁻¹ mole⁻¹), *T* is the temperature in K, *s* is a frequency factor, and *k* is a reaction rate constant calculated from the *D*-value using the relationship $k = 2.303/D$.

Least squares linear regression analyses of the data on semi-logarithmic plots were performed using SYSTAT/SYGRAPH (Wilkinson, 1990*a,b*). In all cases, the models obtained by linear regression analysis were highly significant ($P \leq 0.001$).

Determination of IgG concentration

Quantification of the IgG content was performed using the enzyme-linked immunosorbent assay (ELISA) and radial immunodiffusion (RID) techniques described by Kummer *et al.* (1992). For the analysis of thermal de-

struction of IgG in model systems, standard curves for ELISA were constructed using a stock solution of bovine serum IgG of known concentration as determined by absorbance at 280 nm to prepare standard solutions of IgG in PBS, boiled milk or UHT milk. Triplicate samples of each temperature–time combination were analyzed for IgG by ELISA in duplicate. For analysis of raw and commercially processed milks, the standard curve was constructed using a reference milk, the IgG concentration of which had previously been determined by RID analysis. IgG content of milk samples was analyzed using three dilutions in triplicate, for a total of nine replicates per sample. The ELISA results were confirmed for some samples using RID.

Antigen-binding activity of IgG against bacterial LPS

Specific antibody activities of bovine milk IgG against five pathogenic bacteria were measured using each lipopolysaccharide (LPS) fraction as the antigen in ELISA as described by Li-Chan *et al.* (1994). The specific anti-bacterial LPS binding activity of milk samples was measured using three dilutions in triplicate, for a total of nine replicates per sample. Since no standard antibody preparation with quantified specific IgG activity or titres against these LPS fractions was commercially available, the anti-LPS activities of sample milks are simply compared by using the absorbance values measured in the ELISA.

RESULTS AND DISCUSSION

Thermal destruction of bovine serum IgG in model systems

Semi-logarithmic plots of the IgG content measured by ELISA as a function of heating time at 72, 74, 76, 78 and 80°C are shown in Figure 1. No precipitation was observed during or after any of the heat treatments. It may be suggested that the decreases in IgG concentration measured using ELISA and RID were due to denaturation or unfolding of IgG molecules as a result of heat treatment, leading to loss of antigenicity. Although aggregation reactions subsequent to denaturation may have occurred, detection of precipitation would be difficult given the low levels of IgG (approximately 0.125 mg in 250 μ l).

Table 1 shows the decimal reduction times or *D*-values (time for a 90% reduction in IgG activity) calculated for each heating temperature. The higher *D*-values for IgG destruction in both UHT and boiled milks, compared to PBS, suggest higher stability of IgG in milk than in buffer over this temperature range. Also shown in Table 1 are *D*-values for thermal destruction of bovine milk IgG added to UHT milk reported by Fukumoto *et al.* (1994) and of human milk IgA reported by Morgan *et al.* (1986) over a similar temperature range.

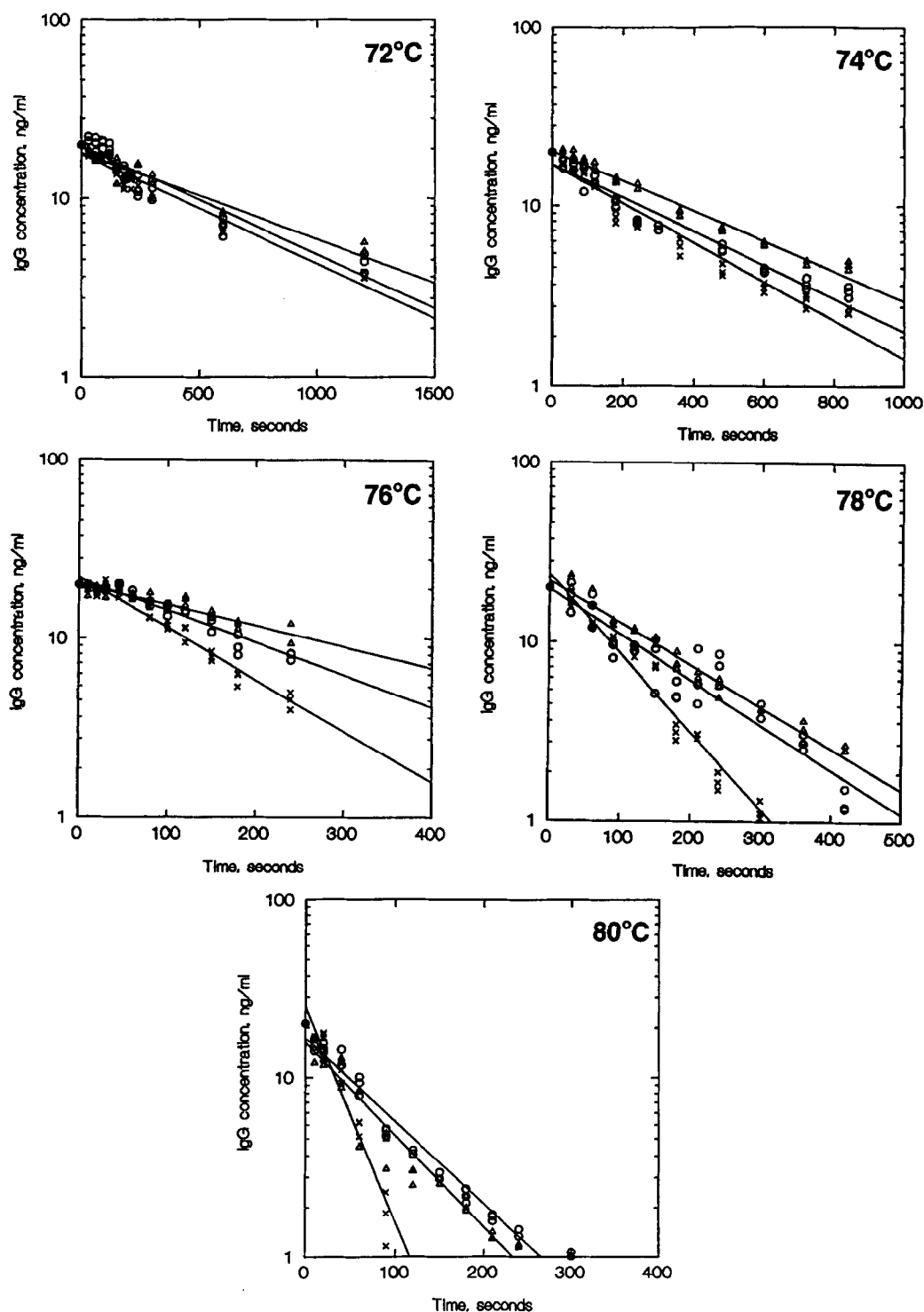


Fig. 1. Semi-logarithmic plots of IgG concentration in PBS (X), boiled milk (O) and UHT milk (Δ) as a function of time of heating at 72, 74, 76, 78 and 80°C. (Concentration of IgG determined by ELISA before heating was 20 ng/ml, representing 25 000-fold dilution of the samples containing 0.5 mg IgG/ml.)

No loss in IgG activity was observed after 30 min incubation at 62.7°C for bovine serum IgG in any of the three model systems in this study. Similar findings have been observed for milk IgG; no change in IgG content of raw bovine milk was detected after 30 min incubation at temperatures of 60°C or below (unpublished observations, D. D. Kitts and J. Ng). Similarly, IgG in human

milk has been reported to be stable to heating at 62.5°C for 30 min, although IgA and IgM contents were substantially reduced (Goldsmith *et al.*, 1983).

Calculation of the energies of activation E_a using the Arrhenius relationship yielded values of 353.5, 258.2 and 298.5 kJ mol⁻¹ for IgG destruction in PBS, boiled milk and UHT milk, respectively. These values are of

Table 1. *D*-Values for thermal destruction of bovine serum IgG in model systems in the present study, compared to *D*-values reported for bovine milk IgG^a and human milk IgA^b

Temperature (°C)	<i>D</i> -value (s)				
	Bovine serum IgG in			Bovine milk IgG in UHT milk ^a	Human milk IgA in human milk ^b
	PBS	Boiled milk	UHT milk		
72	1530	1630	1970	—	319
74	860	990	1200	2330	—
76	365	580	845	—	59.6
78	230	400	440	470	25.7
80	90	200	170	260	—

^aFukamoto *et al.* (1994).^bMorgan *et al.* (1986).

the same general magnitude as those reported for denaturation of other whey protein components over a similar temperature range, including lactoferrin (Sanchez *et al.*, 1992), β -lactoglobulin A and B and α -lactalbumin (Dannenberg & Kessler, 1988).

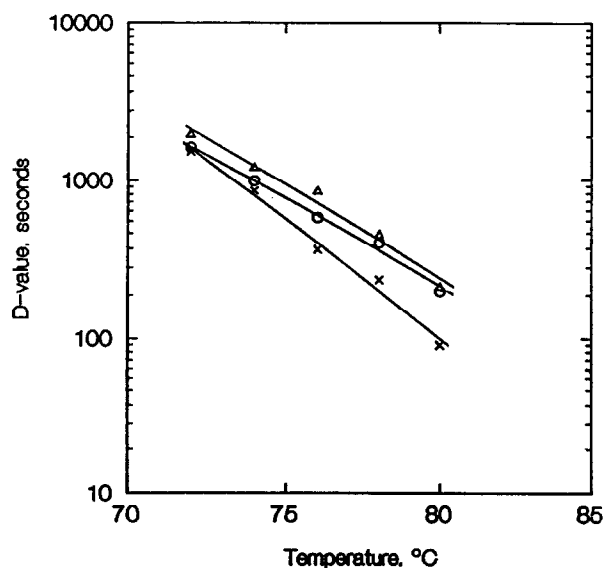
Semi-logarithmic plots of the *D*-values versus temperature (Fig. 2) yielded the following *z*-values for bovine serum IgG: 6.7°C in PBS, 8.9°C in boiled milk, and 8.5°C in UHT milk. Fukamoto *et al.* (1994) reported a *z*-value of 4.35°C for bovine milk IgG added to UHT milk, but pointed out possible inaccuracy of this value due to the limited sensitivity of the radial immunodiffusion (RID) method used to analyze low IgG concentration. Morgan *et al.* (1986) reported a *z*-value of 5.5°C for destruction of IgA in human milk. The slight discrepancy in *z*-values may be due in part to the lower limits of detection possible using ELISA as the assay technique. In the present study, this technique enabled a more accurate determination of decreasing IgG concentrations at increasingly severe conditions of

heating temperature or time, compared to the RID method used in the other two studies.

Differences should also be considered in the methodology for thermal treatment when comparing the results of this study to those of Fukamoto *et al.* (1994) and Morgan *et al.* (1986). In the present study, come-up times for 250 μ l aliquots of the milk samples to equilibrate to the temperatures (62.7–80°C) of the heating water baths ranged between 13–17 s. Fukamoto *et al.* (1994) heated their samples in 100 μ l sealed glass micropipettes in constant temperature water baths, with a come-up time of 4 s for temperatures between 62 and 80°C. Morgan *et al.* (1986) used a HTST pasteurizer designed and built specifically for treatment of small quantities of milk, with come-up times of 0.2–1.0 s for temperatures up to 90°C; however, for lower temperature heat treatments, 2 ml aliquots of milk in pre-heated test tubes were immersed in constant temperature water baths, resulting in long come-up times of 2 min at 58°C and 3 min at 62 and 68°C.

Finally, it is also possible that inherent differences exist in the thermal stability of bovine serum IgG (this study), bovine milk IgG (Fukamoto *et al.*, 1994) and human milk IgA (Morgan *et al.*, 1986). Bovine milk and serum contain two subclasses of IgG. Bovine serum IgG is composed of about 55% IgG₁ and 45% IgG₂, while bovine milk IgG is composed of about 90% IgG₁ and 10% IgG₂, although the levels of the latter are elevated by conditions such as mastitis (Butler, 1981). It is not known whether these subclasses show different denaturation behavior.

Generally, higher thermal stability of bovine serum IgG in milk than in phosphate buffer was indicated by the larger *D*-values (Table 1) for thermal destruction in both boiled and UHT milks than in PBS at temperatures ranging from 72 to 80°C. The higher *z*-values and lower *E_a* values suggest less temperature dependence of the rate of IgG destruction in milk than in buffer, over this temperature range. This is in contrast to results reported by Sánchez *et al.* (1992) for the destruction of lactoferrin. Both apolactoferrin and iron-saturated lactoferrin had smaller *D*-values when heated in milk,

**Fig. 2.** Semi-logarithmic plots of *D*-values for thermal destruction of IgG in PBS (X), boiled milk (O) and UHT milk (Δ) as a function of temperature of heating.

indicating greater thermal lability of lactoferrin in milk than in phosphate buffer. The binding of iron increased thermal stability in both milk and phosphate buffer, as suggested by larger *D*-values for the iron-saturated lactoferrin than the apo-form of the protein; however the iron-saturated form showed a stronger temperature dependence in its rate of destruction, as indicated by its smaller *z*-value and higher *E_a*. Sánchez *et al.* (1992) stated that bovine milk showed a greater change in pH with an increase of temperature than phosphate buffer, especially above 90°C, but over the temperature range of 72–85°C, the change of pH was slight. In our present study, no changes in pH were observed after heating, the measured pH values being 6.6 for both boiled and UHT milks, and 7.1 for PBS. In order to determine if the higher rate of destruction occurring in PBS compared to in the milks was due to these differences in pH, the rates of thermal destruction of IgG at 78°C were measured for solutions of IgG in PBS, boiled and UHT milks adjusted either to pH 6.6 or 7.1. The *D*-values at 78°C for IgG in PBS, boiled milk and UHT milk were 230, 400 and 440 s, respectively, at pH 6.6, and 230, 535 and 465 s, respectively, at pH 7.1. Thus, at both pH 6.6 and 7.1, IgG was more stable to heat treatment when dissolved in either boiled milk or UHT milk than in phosphate buffer.

Effects of HTST pasteurization, skimming and homogenization on IgG content of fluid milks

Table 2 shows the IgG contents in raw milk samples and the corresponding milks after HTST pasteurization, homogenization, skimming and standardization to 2% and 1% fat content. Generally, the raw milks contained between 0.256 and 0.386 mg IgG/ml milk, while pasteurized milks contained between 0.195 and 0.261 mg IgG/ml milk. The % retention of IgG after pasteurization ranged from 59% to 76%. No effect of skimming or homogenization on the IgG content was observed. These results demonstrate that a large proportion of the IgG in milk should be retained after commercial processing to produce HTST pasteurized fluid milk products with varying fat content. In our previous work (Kummer *et al.*, 1992), we reported similar values of IgG concentration in five samples of homogenized HTST pasteurized milks from four different dairy processors, ranging from 0.14 to 0.24 mg/ml. However, two samples from a fifth processor contained much lower contents of 0.04 and 0.07 mg/ml and it was speculated that this reflected more severe thermal treatment of those milks.

IgG concentrations and specific antibody activity in commercially processed milks

Figure 3 shows the total or non-specific IgG concentrations in various commercially processed fluid milks and

Table 2. IgG contents in raw milks and corresponding milks after various processes^a

Type of milk product ^b	IgG concentration (mg/ml)	
	Raw	Pasteurized ^c
skim (A)	0.386	0.241 (62%)
skim (B)	0.356	0.261 (73%)
skim (C)	0.305	0.216 (71%)
homogenized (A)	0.256	0.195 (76%)
homogenized (B)	0.322	0.200 (62%)
homogenized (C)	0.340	0.232 (68%)
2% fat	0.311	0.230 (74%)
1% fat	0.368	0.217 (59%)

^aSamples were collected on-line during processing by Beatrice Foods (Calgary, AB).

^bThe different letters in parentheses after skim and homogenized milks represent samples obtained on three different days of processing.

^cNumbers in parentheses are the % retention of IgG after pasteurization, calculated from the contents of IgG in raw and pasteurized milk samples.

skim milk powder obtained from dairy processors or retail outlets in the Greater Vancouver or Lower Mainland area. Also included for comparison are the IgG concentrations in raw milk from a local dairy processor, whey from cheddar cheese production, as well as a low fat milk powder processed from the milk of immunized cows in Cincinnati (referred to as hyperimmune milk). These latter 3 samples contained between 0.43–0.48 mg IgG/ml, whereas pasteurized fluid milks and

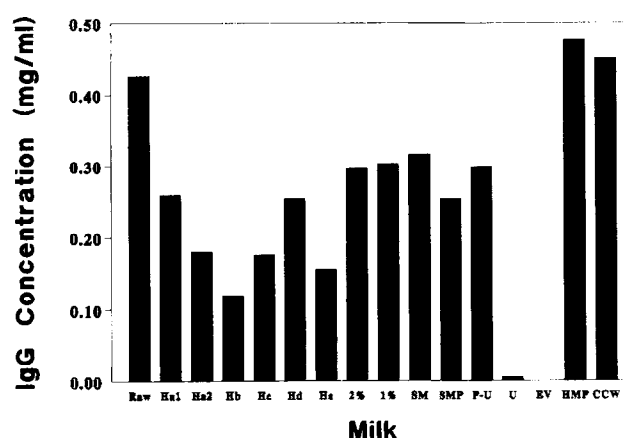


Fig. 3. Concentration of IgG (mg/ml) of various commercial dairy products determined by radial immunodiffusion. Sample Identification: Raw = unprocessed milk; H, 2%, 1% and SM = high temperature short time or HTST pasteurized milks after homogenization, skimming or adjustment of fat content (samples Ha–He represent homogenized samples obtained from five different dairy processors; Ha1 and Ha2 were two samples from processor a); SMP = skim milk powder; P-U = milk receiving sub-pasteurization heat treatment prior to UHT process; U = ultra high temperature or UHT sterilized milk; EV = canned evaporated milk; HMP = hyperimmune milk powder; CCW = whey from cheddar cheese production.

reconstituted skim milk powder contained between 0.12–0.32 mg IgG/ml. The results for IgG contents in the HTST-pasteurized fluid milks as well as skim milk powder are consistent with the values of % retention reported in Table 2. On the other hand, of particular interest was the fact that little or no IgG could be detected in the canned evaporated milk and in UHT-sterilized milk.

Figure 4 shows the results of ELISA for determination of specific antibody activity in these milks against the lipopolysaccharide (LPS) fractions of five bacteria. With the exception of canned evaporated milk and UHT-sterilized milk, all the samples showed measurable antibody activity against the five LPS. Although the highest activities were observed for the hyperimmune milk sample, it can be seen that the commercially available milks obtained from regular, non-immunized herds also contained antigen-binding antibody activity against all five LPS antigens tested. These results are consistent with our previous survey showing specific

antibody activity in 254 raw milks collected in 1990 and 1991 from various farms across the province of British Columbia. The present results indicate that a substantial proportion of the antibody activity is retained in the milks after commercial processing, with the exception of severe thermal treatment processes such as those encountered during production of canned evaporated milk and UHT-sterilized milk.

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REFERENCES

- Bridger, J. C. & Brown, J. F. (1981). Development of immunity to porcine rotavirus in piglets protected from disease by bovine colostrum. *Infect. Immunol.*, **31**, 906–10.
- Brüssow, H., Hilpert, H., Walther, J., Sidoti, J., Mientens, C. & Bachmann, P. (1987). Bovine milk immunoglobulins for passive immunity to infantile rotavirus gastroenteritis. *J. Microbiol.*, **25**, 982–6.
- Butler, J. (1981). A concept of humoral immunity among ruminants and an approach to its investigation. In *The Ruminant Immune System*, ed. J. E. Butler, J. R. Duncan & K. Nielson. Plenum Press, NY, pp. 3–55.
- Dannenberg, F. & Kessler, H.-G. (1988). Reaction kinetics of the denaturation of whey proteins in milk. *J. Food Sci.*, **53**, 258–63.
- Ebina, T., Sato, A., Umez, K., Ishida, N., Ohyama, S., Oizumi, A., Aikawa, K., Katagiri, S., Katsushima, N., Imai, A., Kitaoka, S., Suzuki, H. & Konno, T. (1985). Prevention of rotavirus infection by oral administration of cow colostrum containing antihuman rotavirus antibody. *Med. Microbiol. Immunol.*, **174**, 177–85.
- Facon, M., Skura, B. J. & Nakai, S. (1993). Potential for immunological supplementation of foods. *Food Agric. Immunol.*, **5**, 85–91.
- Fukamoto, L. R., Skura, B. J. & Nakai, S. (1994). Stability of membrane sterilized bovine immunoglobulins aseptically added to UHT milk. *J. Food Sci.*, **59**, in press.
- Golay, A., Ferrara, J.-M., Felber, J.-P. & Schneider, H. (1990). Cholesterol-lowering effect of skim milk from immunized cows in hypercholesterolemic patients. *Amer. J. Clin. Nutr.*, **52**, 1014–9.
- Goldman, A. S. (1989). Immunologic supplementation of cow's milk formulations. *Bull. IDF*, **244**, 38–43.
- Goldsmith, S. J., Dickson, J. S., Barnhart, H. M., Toledo, R. & Eitenmiller, R. R. (1983). IgA, IgG, IgM and lactoferrin contents of human milk during early lactation and the effect of processing and storage. *J. Food Protect.*, **46**, 4–7.
- Hilpert, H., Brüssow, H., Mientens, C., Sidoti, J., Lerner, L. & Werchau, H. (1987). Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. *J. Infect. Dis.*, **156**, 158–66.
- Kobayashi, T., Ohmori, T., Yanai, M., Kawanishi, G., Yoshikai, Y. & Nomoto, K. (1991). Protective effect of orally administering immune milk on endogenous infection in X-irradiated mice. *Agric. Biol. Chem.*, **55**, 2265–72.

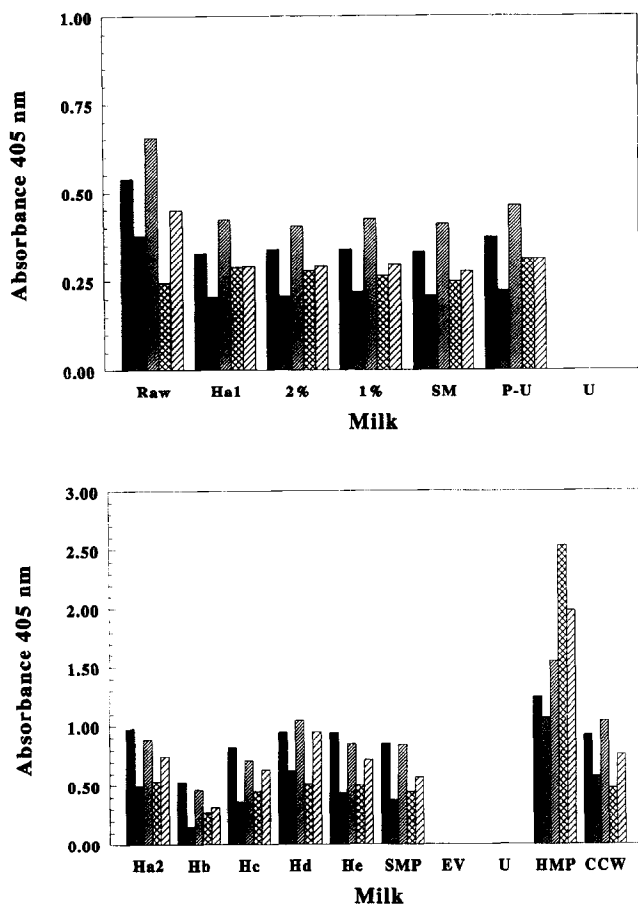


Fig 4. Absorbance values in ELISA of antigen-binding activity against lipopolysaccharide fractions from five bacteria. The two panels are results of ELISA performed on two days and show the absorbance values for milk samples diluted 1:3 with PBS in ELISA. Identification of the milk samples is given in the legend to Figure 3. The bacterial lipopolysaccharides were as follows: $\blacksquare$ *E. coli* O128B:12; $\blacksquare$ *E. coli* O111:B4; ▨ *S. flexneri* 1A; ▩ *S. enteritidis*; ▬ *S. typhimurium*.

- Kummer, A., Kitts, D. D., Li-Chan, E., Losso, J. N., Skura, B. J. & Nakai, S. (1992). Quantification of bovine IgG in milk using enzyme-linked immunosorbent assay. *Food Agric. Immunol.*, **4**, 93–102.
- Li-Chan, E., Kummer, A., Losso, J. N. & Nakai, S. (1994). Survey of immunoglobulin G content and antibody specificity in cow's milk from British Columbia. *Food Agric. Immunol.*, **6**, in press.
- Menšík, J., Salajka, E., Štěpánek, J., Ulmann, L., Procházka, A. & Dressler, J. (1978). Use of polyvalent cow colostrum in the prevention of enteric infections in calves and piglets. *Ann. Rech. Vet.*, **9**, 255–8.
- Michalek, S. M., Gregory, R. L., Harmon, C. C., Katz, J., Richardson, G. J., Hilton, T., Filler, S. J. & McGhee, J. R. (1987). Protection of gnotobiotic rats against dental caries by passive immunization with bovine milk antibodies to *Streptococcus mutans*. *Infect. Immunol.*, **55**, 2341–7.
- Mietens, C., Keinhorst, H., Hilpert, H., Gerber, H., Amster, H. & Pahud, J. J. (1979). Treatment of infantile *E. coli* gastroenteritis with specific bovine anti-*E. coli* milk immunoglobulins. *Eur. J. Ped.*, **132**, 239–52.
- Morgan, J. N., Toledo, R. T., Eitenmiller, R. R., Barnhart, H. M. & Maddox, F. (1986). Thermal destruction of immunoglobulin A, lactoferrin, thiamin and folic acid in human milk. *J. Food Sci.*, **51**, 348–51.
- Murosaki, S., Yoshiki, Y., Kubo, C., Ishida, A., Matsuzaki, G., Sato, T., Endo, K. & Nomoto, K. (1991). Influence of intake of skim milk from cows immunized with intestinal bacterial antigens on onset of renal disease in (NZB × NZW)_{F1} mice fed ad libitum or restricted in energy intake. *J. Nutr.*, **121**, 1860–8.
- Ramaswamy, H. S., Van de Voort, F. R. & Ghazala, S. (1989). An analysis of TDT and Arrhenius methods for handling process and kinetic data. *J. Food Sci.*, **54**, 1322–6.
- Saif, L. J., Redman, D. R., Smith, K. L. & Theil, K. W. (1983). Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from immunized or non-immunized cows. *Infect. Immunol.*, **41**, 1118–31.
- Sánchez, L., Peiró, J. M., Castillo, H., Pérez, M. D., Ena, J. M. & Calvo, M. (1992). Kinetic parameters for denaturation of bovine milk lactoferrin. *J. Food Sci.*, **57**, 873–9.
- Stott, G. H. & Lucas, D. O. (1989). Immunologically active whey fraction and recovery process. *US Patent* 4 834 974, May 30.
- Tacket, C. O., Losonsky, G., Link, H., Hoang, Y., Guesry, P., Hilpert, H. & Levine, M. M. (1988). Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. *New Engl. J. Med.*, **318**, 1240–1.
- Tacket, C. O., Binion, S. B., Bostwick, E., Losonsky, G., Roy, M. J. & Edelman, R. (1992). Efficacy of bovine milk immunoglobulin concentrate in preventing illness after *Shigella flexneri* challenge. *Amer. J. Trop. Med. Hyg.*, **47**, 276–83.
- Tzipori, S., Robertson, D. & Chapman, C. (1986). Remission of diarrhoea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. *Brit. Med. J.*, **293**, 1276–7.
- Wilkinson, L. (1990a). *SYSTAT: The System for Statistics*. SYSTAT, Inc., Evanston, IL.
- Wilkinson, L. (1990b). *SYGRAPH: The System for Graphics*. SYSTAT, Inc., Evanston, IL.
- Yolken, R. H., Losonsky, G. A., Vonderfecht, S., Leister, F. & Wee, S. (1985). Antibody to human rotavirus in cow's milk. *New Engl. J. Med.*, **312**, 605–10.

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