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Effect of heat treatment of bovine colostrum on bacterial counts, viscosity, and immunoglobulin G concentration

J. A. Elizondo-Salazar,* B. M. Jayarao,† and A. J. Heinrichs*[1]
*Department of Dairy and Animal Science, and
†Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park 16802

ABSTRACT

A study was conducted to identify the optimal temperature and time at which heat treatment of bovine colostrum would least change viscosity and IgG concentrations yet reduce bacterial count. First-milking colostrum with >50 g of immunoglobulins/L (measured by colostrometer) was collected from 30 Holstein cows. Aliquots of colostrum were heated for 0, 30, 60, or 90 min at 57, 60, or 63°C in a water bath. Samples were examined for viscosity, IgG_1, and IgG_2 concentrations, standard plate count, coagulase-negative staphylococci, environmental streptococci, coliform, gram-negative noncoliform, *Streptococcus agalactiae*, and *Staphylococcus aureus* counts. All heat treatments reduced counts of all bacteria groups measured compared with untreated colostrum samples. Heat treatment at ≥60°C denatured IgG_1 compared with untreated colostrum; however, colostral IgG_2 levels were not reduced when temperature was held at 60°C for <60 min. Viscosity was not affected when temperature was held at 60°C for <60 min. In this study, heat treatment of bovine colostrum at 60°C for 30 or 60 min reduced bacterial count, slightly reduced IgG concentration, and did not affect viscosity.
Key words: calf, immunoglobulin G, pasteurized colostrum

INTRODUCTION

The bovine neonate is born agammaglobulinemic and depends on colostral Ig intake to obtain adequate passive immunity (Besser and Gay, 1994; Weaver et al., 2000). Thus, early ingestion of colostrum by the newborn is critical for its survival. Failure of passive transfer of colostral Ig is associated with increased morbidity and mortality from neonatal diseases. It has been estimated that more than 40% of United States dairy heifer calves had serum IgG concentrations <10 g/L in a USDA survey (USDA:APHIS, 1993).

Disease-causing bacteria, including *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**) (Streeter et al., 1995), *Listeria monocytogenes* (Doyle et al., 1987), *Campylobacter jejuni* (Lovett et al., 1983), *Salmonella* spp. (Spier et al., 1991), and *Escherichia coli* (Steele, 1997) can be transferred to newborns through colostral and milk secretions, either by direct shedding from the mammary gland or from fecal contamination of colostrum during collection, handling, or storage. Most programs for controlling the spread of infectious disease within a herd recommend feeding colostrum from noninfected dams only. However, this recommendation can limit colostrum availability if a high percentage of the herd is infected, and it creates additional expense for producers who must dispose of colostrum and purchase commercial colostrum substitutes (Stabel et al., 2004).

Pasteurization is one possible measure to reduce transfer of potential pathogens. However, some constituents of colostrum are thermolabile (Meylan et al., 1996; Godden et al., 2003). In addition, decreased serum IgG, lactoferrin concentration, and neutrophil function, which could compromise immunological status, have been observed in calves fed pasteurized bovine colostrum (Meylan et al., 1996; Godden et al., 2003). Another potential obstacle to colostrum pasteurization is increased viscosity, which can cause some colostrum to congeal into a thick, pudding-like consistency that is unacceptable for feeding and can make cleaning of equipment difficult (McMartin et al., 2006).

The focus of the present study was to identify the ideal time and temperature range for heat treatment of colostrum that would result in significant reduction of bacterial count while having minimal effects on viscosity and IgG concentration.

MATERIALS AND METHODS

Colostrum Collection and Heat Treatment

First-milking colostrum with ≥50 g of IgG/L as measured by colostrometer (Biogenics, Mapleton, OR) and

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[1]Corresponding author: ajh@psu.edu

Table 1. Least squares means for bacterial load of bovine colostrum after heat treatment at 3 different temperatures for 0, 30, 60, or 90 min[1]

		Bacterial count[2] ($\log_{10}$ cfu/mL)						
Temperature (°C)	Time, min	SPC	ES	SAG	CNS	SA	CC	NC
Ambient	0	4.60^{a}	4.23^{a}	1.33^{a}	4.31^{a}	4.31^{a}	4.32^{a}	4.58^{a}
57	30	3.85^{b}	3.86^{bc}	-0.52^{b}	3.85^{b}	1.15^{b}	3.87^{b}	3.85^{b}
	60	4.02^{b}	4.01^{b}	0.00^{b}	3.84^{b}	0.48^{b}	3.84^{b}	3.53^{bc}
	90	3.58^{b}	3.53^{bc}	0.00^{b}	3.53^{c}	0.00^{b}	1.46^{c}	0.00^{c}
60	30	3.63^{bcd}	3.85^{bc}	0.00^{b}	3.54^{c}	0.00^{b}	0.48^{c}	0.00^{c}
	60	3.55^{bc}	3.63^{bc}	0.00^{b}	1.60^{c}	0.00^{b}	0.00^{c}	0.00^{c}
	90	2.63^{de}	3.53^{bc}	0.00^{b}	0.90^{c}	0.00^{b}	0.00^{c}	0.00^{c}
63	30	2.83^{cde}	3.53^{bc}	0.00^{b}	1.46^{c}	0.00^{b}	0.00^{c}	0.00^{c}
	60	2.31^{e}	3.52^{bc}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}
	90	0.30^{e}	0.70^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}

[a–e]Means with different superscripts within a column indicate a significant difference ($P < 0.05$).

[1]n = 3/sample point.

[2]ES = environmental streptococci; SAG = *Streptococcus agalactiae*; SA = *Staphylococcus aureus*; CC = coliform count; NC = noncoliform count.

naturally contaminated with several bacteria (see Table 1) was collected from 30 Holstein cows housed at the Pennsylvania State University dairy herd. Colostrum from each cow was thoroughly mixed by shaking the container vigorously and frozen at −20°C. At the start of the experiment, colostrum samples were thawed overnight at 4°C and then thoroughly mixed for 10 min. From this thoroughly mixed colostrum, ten 15-mL sterile screw-cap centrifuge tubes were filled with 10-mL aliquots from each of the 30 colostrum samples (total n = 300). Samples were heated for 0, 30, 60, or 90 min at 57, 60, or 63°C in a preheated water bath, as described by Meylan et al. (1996). Samples were placed in the water bath, brought to temperature, and held for the prescribed time. Temperature was continuously monitored by 2 thermometers, 1 in the water bath and the other in a 10-mL sample of colostrum that served as an indicator. After heat treatment, samples were placed in an ice bath until they cooled to 30°C.

Colostrum Analysis

Heat-treated and untreated samples were examined for SPC, CNS, environmental streptococci (**ES**), coliform (**CC**), gram-negative noncoliform (**NC**), *Streptococcus agalactiae* (**SAG**), and *Staphylococcus aureus* counts (**SA**; Jayarao et al., 2004). Colostrum samples were thoroughly mixed by inverting tubes 20 to 25 times, and then 50 μL of sample was placed on selective and nonselective media using an inoculating loop. Plate count agar was used for enumeration of SPC. The ES and SAG in colostrum samples were estimated using modified Edward's agar supplemented with colistin sulfate and oxolinic acid (Sawant et al., 2002). MacConkey's agar no. 3 (Oxoid, Basingstoke, UK) was used to determine CC and NC. Baird Parker's agar (Becton Dickinson, Cockeysville, MD) was used to determine CNS and SA. Plates for enumeration of SPC were incubated at 32°C for 48 h. Plates for enumeration of CNS, ES, SA, CC, SAG, and NC were incubated at 37°C for 48 h. The ES, CNS, SA, SAG, CC, and NC bacterial counts were enumerated on media. These media are selective for a particular group of bacteria.

There are no previous guidelines for evaluating bacteriological quality of colostrum. However, Jayarao et al. (2004) reported the following guidelines for evaluating bacteriological quality of raw bulk tank milk: SPC ≤5,000 cfu/mL, ES ≤500 cfu/mL, CNS ≤500 cfu/mL, CC ≤50 cfu/mL, NC ≤200 cfu/mL; SA and SAG should not be detected. Even though these guidelines are primarily for bulk tank milk, they will be used to draw inferences on bacteriological quality of colostrum.

The concentrations of IgG_1 and IgG_2 in colostrum were determined by immunoprecipitation using single radial immunodiffusion (**RID**; VMRD, Pullman, WA) as described by Hadorn and Blum (1997). A monocular comparator (VMRD) was used to read precipitin rings. Viscosity was measured with a digital viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA) using parallel plate geometry (plate diameter = 50 mm). The gap between the 2 plates was set at 0.50 mm to allow good contact between sample and plates. Shear rate was set at 1.0 rpm and temperature was set at 39°C.

Decimal Reduction Time

Decimal reduction time (**D-value**) is the interval of time required, under a defined set of conditions, to provide a 1 decimal logarithm (1 $\log_{10}$) or 90% reduction in the initial viable bacterial population (Jay, 2000). The determination of D-value involved heating the

colostrum samples for 30, 60, or 90 min at 57, 60, or 63°C in a preheated water bath, as described by Meylan et al. (1996). Samples were placed in a water bath, brought to temperature, and held for the prescribed time. Higher D-values for bacterial destruction suggest higher resistance to thermal treatment.

Statistical Analysis

Concentration of IgG (g/L), bacterial counts (cfu/mL), and viscosity [($\log_{10}$(Pa·s)] were analyzed using the MIXED procedure of SAS 9.1 (SAS Institute, 2006). In this model, cow was considered a random effect. Viscosity measurements and bacteriology data were log-transformed to normalize residuals and obtain P-values. A value of $P < 0.05$ was used as the test of significance. The statistical model used for the analysis was

$$Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + cow_k + e_{ijk},$$

where Y_{ijk} = dependent variables, μ = overall mean, T_i = temperature effect i, W_j = time effect j, $(TW)_{ij}$ = effect of temperature by time interaction, cow_k = random effect of cow k, and e_{ijk} = residual.

RESULTS AND DISCUSSION

Bacterial Load

Bacterial counts of colostrum after various time and temperature treatments are presented in Table 1. Counts for all bacteria categories declined as time and temperature increased compared with bacterial counts in untreated colostrum.

Standard plate count estimates the total number of aerobic bacteria; it is an accepted standard for estimating quality of food products including milk and milk products and is a good indicator of hygienic milk production practices. In colostrum, SPC provides a useful estimation of total bacterial load. A decline ($P < 0.05$) in SPC was observed at the lowest time and temperature combination (57°C for 30 min). Treatment at 57°C for 90 min or 60°C for 30 or 60 min resulted in almost 1 $\log_{10}$ reduction in SPC, and treatment at 60°C for 90 min or 63°C for 60 min resulted in approximately 2 $\log_{10}$ reductions in SPC (Table 1).

Environmental streptococci consist of a large heterogeneous group of gram-positive, catalase-negative cocci that inhabit the dairy environment. Environmental streptococci have been documented to cause mastitis and elevate SCC in milk and can considerably increase SPC. In this study, all treatment combinations reduced ES (Table 1), but heat treatment was less effective at reducing ES than any other bacteria group.

Both SAG and SA are mastitis pathogens, and SA is a potential calf pathogen. These organisms should not be detected in high quality milk (Jayarao et al., 2004). In the present study, colostrum samples were contaminated with SAG and SA; however, all heat treatment combinations reduced the number of these organisms (Table 1) compared with the control treatment, making the treated colostrum a safer feedstuff for the newborn calf.

High quality bulk tank milk should have CNS <500 cfu/mL. In the current study colostrum samples had CNS exceeding this standard value, but CNS are not common calf pathogens and there was almost 1 $\log_{10}$ reduction after heating at 57°C for 90 min or at 60 or 63°C at all times tested (Table 1).

Coliform organisms include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., whereas gram-negative, noncoliform organisms include *Pseudomonas* spp. and *Serratia* spp. Jayarao et al. (2004) reported that CC and NC should not exceed 50 cfu/mL and 200 cfu/mL, respectively, in high quality raw milk. Coliforms are important calf pathogens and should be kept as low as possible in colostrum. In this study untreated colostrum samples exceeded CC and NC standards (Table 1). However, CC organisms were not detected after heating colostrum at 60°C for 60 min, and NC were not detected after heating at 57°C for 90 min. These results corroborate previous findings that common pasteurization temperatures are sufficient to destroy gram-negative and many gram-positive bacteria (Jay, 2000), thereby making the colostrum a safer nutrient for the newborn calf.

It is important to note that the ES, CNS, SA, CC, and NC counts shown in Table 1 are counts observed on selective enrichment media. These media allow growth of a specific group of bacteria, not differential counts of the baseline count; therefore, the sum of ES, CNS, SA, and CC will not be equal to or near the baseline count. Similar observations have been reported by Jayarao et al. (2004) with bulk tank milk samples.

To better understand thermal destruction of microorganisms in colostrum, D-values, the time required to destroy 90% of the organisms at a given temperature (Jay, 2000), are presented in Table 2 for bacterial groups present in colostrum samples. As temperature increased, the time required to destroy pathogenic organisms was reduced. Higher D-values for bacterial destruction suggest higher resistance to thermal treatment. In this case, ES were more resistant than other groups of bacteria, and NC organisms were least resistant to thermal treatment. It is important to note that

Table 2. Time required to destroy 90% of organisms (D-values, min) at each heating temperature for bacterial groups[1] present in bovine colostrum samples

	D-value (min)				
Temperature (°C)	SPC	ES	CNS	CC	NC
57	34.09	65.22	40.00	37.04	30.93
60	25.64	54.55	36.14	32.26	10.00
63	23.50	45.92	23.08	10.75	10.00

[1]ES = environmental streptococci; CC = coliform count; NC = noncoliform count.

D-values at 60°C for most bacterial groups studied were approximately 30 min or less, suggesting that this time and temperature could be used for treating colostrum with heat. Some heat-resistant bacteria remained after treatment at 60°C for 30 min, but at significantly lower levels.

Despite the fact that a large number of studies have investigated the effectiveness of pasteurization in reducing the number of pathogens in milk, fewer studies have reported on heat treatment of colostrum, which is much more viscous and has higher protein and fat levels (Kehoe et al., 2007). Early studies on pasteurization of bovine colostrum using the same times and temperatures recommended for milk (63°C for 30 min) reduced or eliminated important bacterial pathogens. Stabel et al. (2004) showed that heat treatment of colostrum in a low temperature range (63.9 to 66.7°C) did not immediately destroy MAP. However, recovery of MAP from colostrum was reduced by 2 $\log_{10}$ after 10 min of pasteurization and achieved a low point of <3 cfu/mL after 30 min. Increasing the temperature range (68.3 to 70.8°C) completely abrogated recovery of viable MAP from colostrum. The authors concluded that pasteurization in that temperature range effectively destroys MAP in colostrum, providing dairy producers with an alternative to purchasing commercial colostrum replacement products.

Godden et al. (2006) inoculated batches (30 L) of first-milking bovine colostrum with *Mycoplasma bovis* (108 cfu/mL), *L. monocytogenes* (106 cfu/mL), *E. coli* O157:H7 (106 cfu/mL), *Salmonella enteritidis* (106 cfu/mL), and MAP (103 cfu/mL). Colostrum batches were then heated at 60°C for 120 min in a commercial on-farm batch pasteurizer, and 50-mL subsamples were collected at 15-min intervals throughout the heat treatment process for bacterial culture. All pathogens were reduced to undetectable levels after colostrum was heated at 60°C for 30 min, with the exception of MAP, which was undetectable after 60 min of treatment at 60°C. In an on-farm experiment, Johnson et al. (2007) reduced total bacteria and total coliform counts by heat treating colostrum at 60°C for 60 min. The present study confirms previous research that has demonstrated that treating colostrum with heat can significantly reduce pathogenic bacteria. In addition, the current study shows that pasteurization also reduces the number of environmental bacteria in colostrum.

Colostrum Viscosity and IgG Concentration

Results of RID analysis for IgG_1 and IgG_2 concentrations and viscosity of colostrum are shown in Table 3. Total IgG concentration decreased as temperature and holding time increased (Figure 1). The concentration of IgG_1 ranged from 71.6 to 12.9 g/L, and that of IgG_2 ranged from 3.2 to 1.5 g/L, showing a significant decline as temperature and time increased. Least squares means for IgG_1 and IgG_2 concentrations were not different between the control and colostrum heated at 57°C, regardless of treatment duration. However, when colostrum was heated at 60°C there was a reduction in IgG, especially in IgG_1, even when colostrum

Table 3. Least squares means of IgG_1 and IgG_2 concentrations and viscosity of bovine colostrum after heat treatment at 3 different temperatures for 0, 30, 60, or 90 min[1]

Temperature (°C)	Time (min)	IgG_1 (g/L)	SEM	IgG_2 (g/L)	SEM	Viscosity [$\log_{10}$(Pa·s)[2]]	SEM
Ambient	0	71.6^{a}	4.64	3.2^{a}	0.22	2.59^{de}	0.15
57	30	66.8^{ab}	4.48	3.0^{ab}	0.22	1.98^{e}	0.09
57	60	66.7^{ab}	4.25	3.0^{ab}	0.23	1.83^{e}	0.08
57	90	62.6^{ab}	4.26	2.9^{ab}	0.22	2.23^{e}	0.11
60	30	56.7^{bc}	4.01	2.7^{abc}	0.21	2.03^{e}	0.08
60	60	47.9^{dc}	2.86	2.6^{abc}	0.20	2.82^{d}	0.15
60	90	40.5^{d}	2.40	2.4^{bcd}	0.19	3.85^{c}	0.20
63	30	27.7^{e}	1.79	2.2^{cd}	0.19	3.80^{c}	0.19
63	60	22.8^{e}	1.55	1.9^{de}	0.18	4.61^{b}	0.24
63	90	12.9^{f}	1.14	1.5^{e}	0.17	4.92^{a}	0.19

[a–f]Means with different superscripts within a column indicate a significant difference ($P < 0.05$).

[1]n = 3/sample point.

[2]Pa·s = kg·m^{-1}·s^{-1}.

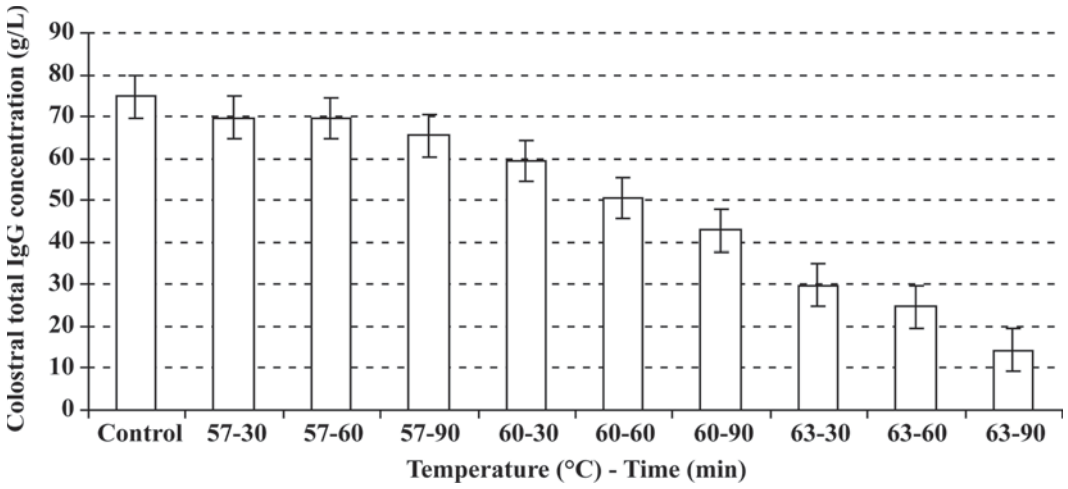

Figure 1. Changes in total IgG concentration in bovine colostrum samples after heat treatment at various time and temperature combinations (top of the bars represent SEM).

was heated for just 30 min. The greatest reduction in IgG concentration was observed when colostrum was heated at 63°C. There were no differences in viscosity [($\log_{10}$(Pa·s)] following treatment between the control and samples heat-treated at 60°C for 30 or 60 min (Table 3). However, there was an increase in viscosity when colostrum samples were heated at 60°C for 90 min and at 63°C regardless of time (Table 3). Combined effects of heat treatment on SPC and viscosity are presented in Figure 2.

In the animal science literature, several studies have been published on the effects of heat treatment of colostrum on denaturation of antibodies and viscosity. Meylan et al. (1996) indicated a 12.3% loss of IgG after pasteurizing 5-mL colostrum samples at 63°C for 30 min. Godden et al. (2003) investigated the effect of on-farm commercial batch pasteurization (63°C for 30 min) on IgG concentrations and the fluid and feeding characteristics of colostrum, meaning a suitable consistency so that it could be easily fed to calves using either bottle or esophageal feeder. They observed 58.5 and 23.6% reductions in IgG concentration for 95-L and 57-L batches, respectively. They also reported that pasteurization did not change the consistency or feeding characteristics of colostrum. One on-farm, batch heat treatment experiment reported no differences in colostral IgG concentration compared with the control samples when 8-L batches of colostrum were heated at 60°C for 60 min (Johnson et al., 2007).

Because bovine IgG in colostrum has the potential to be used as an immunological supplement for infant formulas and other hyperimmune foods, its stability

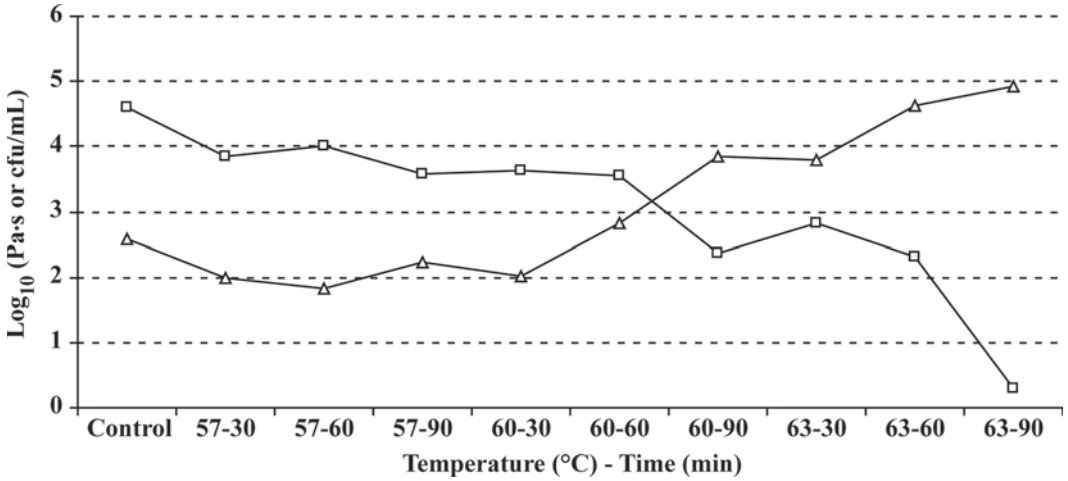

Figure 2. Changes in viscosity (Δ) and standard plate count (□) in bovine colostrum samples after heat treatment at various time and temperature combinations.

to thermal treatment has been widely studied in the food sciences using different experimental techniques (Dominguez et al., 1997; Chen et al., 2000; Cao et al., 2007). In general, these studies suggest that IgG denaturation involves an initial reversible unfolding of native structure, with loss of globular configuration, which can proceed further to irreversible denaturation and aggregation via hydrophobic and disulfide interactions (Indyk et al., 2008). deWit and Klarenbeek (1984) studied the effects of heat treatment on structure and solubility of the Ig fraction of whey. They showed that Ig are among the most heat-stable whey proteins, which was attributed to the high content of disulfide bonds and whey components such as fats, lactose, carbohydrates, salts, and other proteins that help stabilize antibodies during thermal treatment (Chen et al., 2000; Indyk et al., 2008). Moreover, the immune-reactivity of IgG is the most thermoresistant among the Ig (Mainer et al., 1997).

For a protein to display its biological activity, it must adopt its correct 3-dimensional structure (Price, 2000). Consequently, changes in secondary or tertiary structures may be responsible for changes in biological activity upon heating (Li et al., 2005). However, it is important to note that at the low temperatures used in the present study, some unfolding of the 3-dimensional structure may occur, but this unfolding is reversible and native structure can be regained (Goto and Hamaguchi, 1982; Price, 2000). Another important aspect is that denaturation of a multi-domain protein (such as IgG) can be described as a 2-state process in which individual domains can be affected independently and in different orders, depending on conditions (Vermeer and Norde, 2000). In this case, when IgG is subjected to thermal treatment, the antigen binding site in the Fab fragment denatures more quickly or at a lower temperature than the Fc region (Vermeer and Norde, 2000; Cao et al., 2007). This agrees with Dominguez et al. (1997) who indicated that structural alterations in heated IgG are mainly located in Fab fragments, where the antigen-binding site is located, rather than in the Fc fragment. Dominguez et al. (1997) showed that the ability of IgG to bind an antigen, and thus to maintain its immunological activity, was maintained after a heat treatment of 63 to 65°C for 60 min. This is in agreement with the results of Li-Chan et al. (1995), who found that heating IgG at 62 ± 7°C for 30 min had no effect on its binding activity against bacterial lipopolysaccharides. Mainer et al. (1997) concluded that low temperature–long time pasteurization (63°C for 30 min) did not have any effect on IgG concentration in colostrum. Furthermore, Ustunol and Sypien (1997) showed that at 70°C IgG was the most heat stable Ig (compared with IgM and IgA) and heat treatment for 40 min did not reduce its activity. Lindstron et al. (1994) reported on the thermally induced unfolding of bovine milk Ig using differential scanning calorimetry in the temperature range from 25 to 100°C and demonstrated that thermal unfolding of Ig at pH 6.6 took place at 80.9°C. Among the individual Ig fractions, IgG_1 unfolded at 79.4°C and IgG_2 at 76.7°C. Li et al. (2005) showed a decrease in bovine IgG immunoactivity with changes in its secondary structure. They indicated that 72°C is the critical temperature point for IgG molecules to change their secondary structure, which is in agreement with Li-Chan et al. (1995), who reported that 73°C is the critical temperature for bovine IgG to lose immunoactivity. In general, food science research results have indicated that a substantial proportion of the antibody activity of IgG is retained 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 (Li-Chan et al., 1995).

CONCLUSIONS

Heat treatment of colostrum significantly reduced bacterial load in a variety of colostrum samples, indicating that heat treatment of colostrum could serve as an effective method for reducing pathogen exposure to newborn calves. Heat treatment of 10 mL of bovine colostrum at 60°C and above resulted in significant denaturation of colostral IgG_1 as measured by RID; however, colostral IgG_2 concentrations were not reduced when the temperature was held at 60°C for 30 or 60 min. Viscosity was not affected when the temperature was held at 60°C for 30 or 60 min. The findings of this study suggest that heat treatment of bovine colostrum at 60°C for 30 to 60 min may be used as an optimal temperature and timing, at which heat treatment would produce no significant changes in viscosity, a small reduction in measured IgG concentration, and a significant reduction in bacterial count.

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