Effect of Heat and High-Pressure Treatments on Microbiological Quality and Immunoglobulin G Stability of Caprine Colostrum

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ABSTRACT

Caprine colostrums (6 batches) were subjected to heat (56°C for 60 min and 63°C for 30 min) and high-pressure (400 and 500 MPa for 10 min at 20°C) treatments at laboratory scale, and analyses of the main microbial groups and the extent of IgG denaturation (determined by immunodiffusion) were performed. Overall mean microbial values in raw colostrums were: total count, 5.55 log cfu/mL; Enterobacteriaceae, 2.64 log cfu/mL; lactococci, 5.41 log cfu/mL; lactobacilli, 2.34 log cfu/mL; and enterococci, 4.06 log cfu/mL. Neither Salmonella spp. nor Listeria monocytogenes were detected, whereas coagulase-positive staphylococci were found in various colostrum samples with an overall mean of 1.02 log cfu/mL. Heat and high-pressure treatments significantly reduced total count (1.47 log), lactococci (1.45 log), enterococci (2.47 log), and Enterobacteriaceae, whereas lactobacilli and coagulase-positive staphylococci counts were reduced to undetectable levels, but differences between technological treatments were not statistically significant. High-pressure treatments were as efficient in reducing the bacterial population as were heat pasteurization treatments: 95.50 and 96.93% for pressure treatments of 400 and 500 MPa, and 91.61 and 97.59% for heat treatments of 56°C for 60 min and 63°C for 30 min, respectively. All treatments assayed produced a reduction in colostrum IgG concentration (27.53, 23.58, 23.33, 22.09, and 17.06 mg/mL for raw, heat-treated at 56°C for 60 min or 63°C for 30 min, and pressure-treated at 400 and 500 MPa, respectively), but differences were only observed between raw colostrums and those pressure-treated at 500 MPa. This laboratory-scale study indicated that 20- to 30-mL volumes of goat colostrum could be heated and pressure-treated (400 MPa) to produce hygienic colostrum without affecting IgG concentration.

Key words: heat and pressure treatment, microorganism, immunoglobulin G, caprine colostrums

INTRODUCTION

Colostrum is a nutrient-rich fluid produced by female mammals immediately after giving birth. Due to its high content in Ig, mainly IgG, colostrum provides the major antimicrobial protection and confers passive immunity preventing diseases caused by microbial infections in the newborn (Foley and Otterby, 1978). Thus, it is generally recommended that newborns be fed with fresh high-quality colostrum as soon as possible after birth. However, one potential method of transmission of infectious diseases to dairy animals is through feeding infected colostrum. Pathogens that may be transmitted within colostrums, either by direct sucking of the mammary gland or postharvest contamination, include bacteria such as Mycobacterium avium ssp. paratuberculosis, Salmonella spp., Listeria monocytogenes, and Escherichia coli (Steele et al., 1997), or viruses such as caprine arthritis-encephalitis (Guerrault, 1990) and bovine leukemia (Perrin and Polack, 1988). One effective method to prevent transmission of infectious diseases to newborns is pasteurization. Pasteurization for the destruction of pathogenic microorganisms and the reduction of endogenous milk microbiota has been traditionally conducted by heat treatment. Adams et al. (1983) demonstrated the inactivation of caprine arthritis-encephalitis virus in colostrum by applying a heat treatment of 56°C for 60 min. Moore et al. (1996) successfully inactivated the bovine immunodeficiency virus with heat treatment of 47°C for 30 min. Stabel (2001) achieved total destruction of Mycobacterium avium ssp. paratuberculosis in bovine colostrum after pasteurization treatment of 65.5°C for 30 min. Godden et al. (2006) found no viable Mycoplasma bovis, L. monocytogenes, E. coli O157:H7, and Salmonella enteritidis (inoculated to colostrum) after heat treatment of 60°C for 30 min, and inoculated M. avium ssp. paratuberculosis were not recovered after colostrum was heat-treated at 60°C for 60 min. How-
ever, IgG are thermolabile compounds, and severe heat denaturation has been observed after treatment above 75°C (Li-Chan et al., 1995; Chen et al., 2000). The effects of pasteurization by heat treatment on IgG in bovine colostrum have been analyzed by Meylan et al. (1996) and Tyler et al. (2000), with both studies showing a slight reduction in IgG levels in pasteurized (63°C, 30 min) bovine colostrum, whereas Steinbach et al. (1981) did not observe differences in IgG concentration in bovine colostrum after treatment at 55°C for 30 min, with the subsequent positive effect on the transfer of passive immunity to calves. More recently, McMartin et al. (2006) heated bovine colostrum at 59, 60, 61, 62, and 63°C and suggested that colostrum could be heated to 60°C for up to 120 min without changing the viscosity or IgG concentration, but heating colostrum to 63°C resulted in an estimated 34% decrease in IgG concentration and 33% increase in viscosity. Although studies investigating the thermal destruction of human and bovine IgG in colostrum and milk have been reported, little information is available on caprine colostrum decontamination while maintaining IgG content (Argüello et al., 2003). On the other hand, heating experiments performed with IgG in bovine colostrum have shown higher IgG activity retention than that obtained with IgG in PBS buffer at similar pH (Domínguez et al., 2001). This difference suggests a protective effect of other milk components such as proteins, fat, and salts that cause a delay in denaturation and prevent aggregation of IgG during heat treatment. Goat milk and colostrum differ from their bovine counterparts in composition (CN, whey protein, fat, and salts), so research is required to verify the operating pasteurization conditions required to hygienize colostrum and retain IgG activity in goat colostrum.

Over the last decade, high-pressure (HP) treatment of foods and food components has gained increasing interest as a nonthermal method of modifying the structure and functional properties of food macromolecules, such as proteins, without affecting the nutritional value, flavor, color, and vitamin content. Effectiveness of HP in microbial inactivation has been reported for different foodborne pathogens and spoilage microorganisms (Smelt, 1998) with the purpose of enhancing product safety and improving the shelf life of the food. High pressure has been proposed as an alternative technology to thermal processing with various possible applications in the dairy industry (Trujillo et al., 2002) including sanitization of milk for making yogurt and fresh or ripened cheeses, fresh cheese sanitization, and cheese ripening acceleration.

The aim of this work was to determine the effect of heat (temperature and time) and high-pressure treatments on microbiological quality and immunoglobulin G stability of caprine colostrum.

**MATERIALS AND METHODS**

**Colostrum Treatments**

Six batches of Majorera goat colostrum taken from different herds were used. The samples were obtained after birth (first milking colostrum) and were refrigerated (−4°C) until treated (<24 h). Aliquots of colostrums (20 mL) in duplicate were heated in glass tubes (12 × 200 mm) and immersed in a thermostatically controlled water bath with agitation (60 oscillations/min) at a constant temperature of either 56 or 63°C for 60 or 30 min, respectively (24 samples for analysis). The samples were allowed to equilibrate and reach the water bath temperature for 5 min before initiation of measuring time.

For pressure treatments, colostrum samples in duplicate were packed into flexible tubes of 30 mL (Azlon, Bibby Sterilin Ltd., Stone, Staffordshire, UK), vacuum-sealed, and pressurized in a batch isostatic press (GEC Alsthom ACB, Nantes, France) at 400 and 500 MPa for 10 min (24 samples for analysis). Pressure was built up using a standard pressurization rate of about 200 MPa/min. During pressure buildup, temperature increase due to adiabatic heating was controlled with a heating/cooling system and was maintained at 20°C. After heat and pressure treatments, the samples were transferred to ice water for rapid cooling, and stored at 4°C until microbiological analyses were performed; samples for IgG determination were frozen at −20°C (<3 mo). In this study, we considered the global treatment including the initial phase of variable temperature or pressure from the experiment. Untreated colostrum samples in duplicate were used as a control (12 samples for analysis).

**IgG Quantification**

Colostrum IgG concentration was determined using an immunodiffusion method (Mancini et al., 1965). The standard curve was prepared in accordance with Catty and Raykundalia (1988) using pure goat IgG (Sigma-Aldrich, St. Louis, MO).

**Microbiological Analyses**

The microbiological quality of the colostrum samples was assessed by enumerating the following microorganisms. Total counts (TC) were enumerated on plate count agar medium (Oxoid Ltd., Basingstoke, UK) incubated for 72 h at 30°C. Enterobacteriaceae were enumerated on violet red bile glucose agar medium (Bio-
HEAT AND PRESSURE TREATMENT OF COLOSTRUM 835

of Raw Goat Colostrums

Microbiological Quality and IgG Concentration

60 min, 63°C

Statistical Analysis

bacterial population before treatment.

where f = bacterial population after treatment, and i =

categorical as separate results, and a posthoc Tukey analysis

was performed using SPSS (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Microbiological Quality and IgG Concentration of Raw Goat Colostrums

There are a limited number of publications describing

the microbiological quality of colostrum (Stewart et al., 2005; Godden et al., 2006; McMartin et al., 2006),

and very limited data about the microbial quality of
goat Colostrums

Microbiological Quality and IgG Concentration

of Raw Caprine Colostrums

In agreement with the literature, all raw colostrums

were contaminated with a TC that varied from 1.46 ×

\[ \text{TC} = \text{Total count}; \ E = \text{Enterobacteriaceae}; \ LC = \text{lactococci}; \ LB = \text{lactobacilli}; \ EC = \text{enterococci}; \ S = \text{coagulase-positive staphylococci}. \]

\[ \text{ND} = \text{Not detected}. \]

Table 1. Counts (log_{10} cfu/mL) of the main microbial groups and IgG concentration (mg/mL) in raw caprine colostrums

<table>
<thead>
<tr>
<th>Colostrum</th>
<th>TC</th>
<th>E</th>
<th>LC</th>
<th>LB</th>
<th>EC</th>
<th>S</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.46</td>
<td>2.30</td>
<td>5.42</td>
<td>2.04</td>
<td>3.60</td>
<td>ND</td>
<td>29.64</td>
</tr>
<tr>
<td>B</td>
<td>5.19</td>
<td>3.06</td>
<td>5.10</td>
<td>2.00</td>
<td>3.90</td>
<td>1.40</td>
<td>23.94</td>
</tr>
<tr>
<td>C</td>
<td>6.14</td>
<td>2.78</td>
<td>5.86</td>
<td>2.19</td>
<td>4.64</td>
<td>1.00</td>
<td>36.71</td>
</tr>
<tr>
<td>D</td>
<td>5.16</td>
<td>2.98</td>
<td>5.10</td>
<td>2.80</td>
<td>3.95</td>
<td>2.10</td>
<td>21.51</td>
</tr>
<tr>
<td>E</td>
<td>5.57</td>
<td>2.54</td>
<td>5.43</td>
<td>2.98</td>
<td>4.22</td>
<td>ND</td>
<td>25.20</td>
</tr>
<tr>
<td>F</td>
<td>5.76</td>
<td>2.18</td>
<td>5.55</td>
<td>2.02</td>
<td>4.06</td>
<td>1.60</td>
<td>28.18</td>
</tr>
</tbody>
</table>

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\[ \text{ND} = \text{Not detected}. \]

Table 1 shows bacterial log counts of microorganisms and IgG in raw caprine colostrums. The IgG concentrations at the first milking were 21.51 to 36.71 mg/mL. Results agree with previous experiments in Majorera goats (Argüello et al., 2003, 2006) and are consistent with those reported by Ubellarte et al. (1987) in Saanen and Camosciata goats, and by Le-vieux et al. (2002) in Saanen and Alpine goats.

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\[ \text{ND} = \text{Not detected}. \]
Overall, colostrum TC were below the limit set by European regulations (European Union, 1992) for the microbiological quality of raw goat milk, and only one sample exceeded $5 \times 10^5$ cfu/mL.

An elevated number of Enterobacteriaceae in milk or colostrums indicates deficient handling during milking, collection, or manipulation. In the present study, Enterobacteriaceae were a constant component of the microbiota, with counts that ranged from $1.5 \times 10^2$ to $1.15 \times 10^3$ cfu/mL. Enterococci were found at a high level with notable variations between samples (from $4.0 \times 10^2$ to $4.35 \times 10^4$ cfu/mL). Lactococci and lactobacilli were 2 permanent groups of the colostrum microflora but with significantly higher counts for lactococci ($1.25 \times 10^5$ to $7.2 \times 10^5$ cfu/mL) compared with lactobacilli ($1.0 \times 10^2$ to $9.5 \times 10^2$ cfu/mL).

Coagulase-positive staphylococci were detected in 4 of the 6 samples analyzed, with counts that varied from $1.0 \times 10^1$ to $1.25 \times 10^5$ cfu/mL, and only one sample exceeded the threshold value for the number of bacteria ($m = 10^2$ cfu/mL) but without prejudice to compliance with the limits established in Europe (European Union, 1992) for this group in raw goat milk: $n = 5$, $C = 2$, $m = 10^2$ cfu/mL, $M = 5 \times 10^5$ cfu/mL, where $n =$ number of sample units comprising the sample; $m =$ threshold value for the number of bacteria, and the result is considered satisfactory if the number of bacteria in all sample units does not exceed “m”; $M =$ maximum value for the number of bacteria, and the result is considered unsatisfactory if the number of bacteria in one or more sample units is “M” or more; $c =$ number of sample units where the bacteria count may be between “m” and “M”, the sample being considered acceptable if the bacteria count of the other sample units is “m” or less.

Neither Salmonella spp. nor Listeria monocytogenes were detected in any colostrum sample using the protocols described above.

The results obtained for caprine colostrums confirm the studies made by Poulsen et al. (2002) in bovine colostrums, who reported that 82% of samples analyzed presented poor microbiological quality, and more of these colostrum samples had close to or even greater than $10^6$ cfu/mL with a high content of coliforms. However, these authors also found some pathogens such as Escherichia coli and Salmonella spp. According to Stewart et al. (2005), bacterial counts are very low ($<10^5$ cfu/mL) in bovine colostrum samples collected directly from the udder if disinfection of cow teats before milking and postmilking is carried out. However, counts significantly increase in relation to the colostrum production method, harvest equipment, and storage processes used on-farm.

### Effect of Heat (Temperature and Time) and Pressure Treatments on Microbiological Quality and IgG Concentration of Colostrums

Table 2 shows bacterial log counts of microorganisms and IgG in raw, heat-, and pressure-treated colostrums. Heat and HP treatments significantly reduced TC (1.5 to 1.32 log) and there were no statistical differences among the treatments assayed. Overall, HP treatments were as efficient in reducing the total bacterial population as were heat pasteurization treatments: reductions of 95.50 and 96.93% for pressure treatments of 400 and 500 MPa, and 91.61 and 97.59% for heat treatments of 56°C for 60 min and 63°C for 30 min, respectively. These results are similar to those reported by Buffa et al. (2001) studying the effect of pasteurization (72°C, 15 s) and HP (500 MPa, 15 min, 20°C) treatments on TC in caprine milk.

Enterobacteriaceae and lactobacilli counts were reduced by heat and HP treatments up to undetectable levels regardless of the initial microbial level, temperature, and pressure applied in the treatments. These results agree with those of Buffa et al. (2001) who observed total inactivation of Enterobacteriaceae and lactobacilli in caprine milk submitted to HP (500 MPa, 15 min, 20°C).

Reductions in lactococci counts by the technological treatments were similar to those described for TC (1.45 log), and enterococci were significantly reduced in either pasteurized or HP-treated colostrums (2.47 log).

Coagulase-positive staphylococci were not detected in heat or pressure-treated samples. Gervilla et al. (1999) studying HP inactivation of Staphylococcus aureus 534 CECT in ewe's milk at different temperatures for 10 min and pressure treatments from 200 to 500 MPa found that this microorganism is very resistant to pressure treatment, although at 500 MPa and 25°C, reductions between 1.9 to 2.4 log were achieved.

Because no Salmonella spp. nor Listeria monocytogenes were detected in any raw colostrums, we cannot draw conclusions about the efficacy of heat and HP treatments on the destruction of these pathogenic microorganisms. However, there are numerous studies in the literature about the destruction of foodborne pathogens by both heat and pressure treatments. Effectiveness of HP in microbial inactivation has been reported using E. coli, L. monocytogenes, Salmonella spp., Staphylococcus aureus, and Bacillus cereus in milk (Patterson and Kilpatrick, 1998; Gervilla et al., 1999; McClements et al., 2001; Wuytack et al., 2003).

The IgG values in the colostrums after heat and pressure treatments are shown in Table 2. A reduction in colostrum IgG concentration was observed in all treatments assayed: $-14$ and $15\%$ for heat treatments.
of 56°C for 60 min and 63°C for 30 min, and –20 and 38% for pressure treatments of 400 and 500 MPa, respectively, but statistical differences were only observed between raw colostrums and those HP-treated at 500 MPa. Overall, treatments of 56°C for 60 min and 63°C for 30 min would be considered equivalent heat treatments because they diminished bacterial counts to the same degree, and no significant differences in IgG contents were observed between treatments.

The reduction observed on the IgG concentration after heat treatments was similar to that observed in bovine colostrum by Meylan et al. (1996) and Tyler et al. (2000) at 63°C for 30 min (~12% IgG reduction) and McMartin et al. (2006) at 63°C for 120 min (~34% IgG reduction), but was lower than that reported by Argüello et al. (2003) in the same goat breed. These authors applied heat treatments of 57°C for 10 min to the colostrums and then transferred them to a thermos bottle preheated with boiling water for 1 h, obtaining an IgG reduction of ~38%. By contrast, Steinbach et al. (1981) found no reduction in bovine colostrum IgG concentration after a heat treatment of 55°C for 30 min.

The reduction in IgG concentration observed after pressure treatments agrees with the results obtained by Felipe et al. (1997), who did not observe differences in the levels of Ig in goat milk (determined from their loss of solubility at pH 4.6 by gel permeation fast protein liquid chromatography and SDS-PAGE) with pressure treatments up to 300 MPa, but some aggregation occurred between 300 and 500 MPa. According to Howlett et al. (1992), bovine IgG does not undergo conformational changes below 210 MPa, but when pressure is increased to 820 MPa, some conformational changes and aggregation appear to occur, the rate of change being faster between 210 and 460 MPa.

Although rheological measurements were not conducted on raw and treated colostrums, some samples of HP-treated colostrums, especially those treated at 500 MPa, presented higher viscosity (visually) compared with their raw and heat-treated homologues. This observation could be explained by the denaturation of β-LG produced by HP treatment (Felipe et al., 1997), which could produce large protein aggregates increasing colostrum viscosity.

**CONCLUSIONS**

In conclusion, the results of this study indicate that heat treatments of 56°C for 60 min and 63°C for 30 min could help us to obtain hygienic goat colostrum of good microbiological quality while preserving the IgG concentration. Furthermore, HP treatments at 400 and 500 MPa produced satisfactory sanitation of goat colostrums to a similar extent as heat treatments, but treatment of 500 MPa caused significant losses of IgG in goat colostrums.
Although these results are promising because they suggest the possibility of hygienizing goat colostrum by heat or pressure (400 MPa) treatments while maintaining Ig content, the findings are preliminary and should be interpreted with caution. The study has been performed with 6 batches of goat colostrum using small volumes (20 to 30 mL) and under laboratory conditions to simulate heat or pressure pasteurization conditions. Further research is needed to study the use of commercial on-farm batch pasteurization equipment to treat larger volumes of caprine colostrum, as would be the situation under farm conditions. On the other hand, the results have shown that HP processing at 400 MPa could be an alternative to heat treatment of colostrums. High-pressure processing, in a batch or semi-continuous process, is gaining commercial acceptance, and some medium capacity (500 kg/h) industrial applications are currently in operation. The process can result in excellent product quality but remains relatively expensive and so is restricted to high added-value products.

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