J. Dairy Sci. 96:2011–2019
http://dx.doi.org/10.3168/jds.2012-6006
© American Dairy Science Association®, 2013.

# Gravity separation of fat, somatic cells, and bacteria in raw and pasteurized milks[1]

**Z. Caplan, C. Melilli, and D. M. Barbano[2]**
Northeast Dairy Food Research Center, Department of Food Science, Cornell University, Ithaca, NY 14853

## ABSTRACT

The objective of experiment 1 was to determine if the extent of gravity separation of milk fat, bacteria, and somatic cells is influenced by the time and temperature of gravity separation or the level of contaminating bacteria present in the raw milk. The objective of experiment 2 was to determine if different temperatures of milk heat treatment affected the gravity separation of milk fat, bacteria, and somatic cells. In raw milk, fat, bacteria, and somatic cells rose to the top of columns during gravity separation. About 50 to 80% of the fat and bacteria were present in the top 8% of the milk after gravity separation of raw milk. Gravity separation for 7 h at 12°C or for 22 h at 4°C produced equivalent separation of fat, bacteria, and somatic cells. The completeness of gravity separation of fat was influenced by the level of bacteria in the milk before separation. Milk with a high bacterial count had less (about 50 to 55%) gravity separation of fat than milk with low bacteria count (about 80%) in 22 h at 4°C. Gravity separation caused fat, bacteria, and somatic cells to rise to the top of columns for raw whole milk and high temperature, short-time pasteurized (72.6°C, 25 s) whole milk. Pasteurization at ≥76.9°C for 25 s prevented all 3 components from rising, possibly due to denaturation of native bovine immunoglobulins that normally associate with fat, bacteria, and somatic cells during gravity separation. Gravity separation can be used to produce reduced-fat milk with decreased bacterial and somatic cell counts, and may be a critical factor in the history of safe and unique traditional Italian hard cheeses produced from gravity-separated raw milk. A better understanding of the mechanism of this natural process could lead to the development of new nonthermal thermal technology (that does not involve heating the milk to high temperatures) to remove bacteria and spores from milk or other liquids.

Received July 31, 2012.
Accepted October 21, 2012.
[1]Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors or Cornell University.
[2]Corresponding author: dmb37@cornell.edu

**Key words:** gravity separation, fat, bacteria, somatic cells

## INTRODUCTION

Gravity separation of raw milk before cheese making (McSweeney et al., 2004) is part of the traditional method of production of Grana Padano and Parmigiano Reggiano cheeses, and it is still used today in Italy. Gravity separation is the traditional method for removal of a portion of fat from milk before cheese making to control the level of fat in the dry matter content of the final cheese. Typically, the temperature of raw milk received at traditional Grana-type cheese factories can be in the range of 7 to 15°C, and gravity separation is faster when the milk temperature is at the higher end of this range. For these cheeses, approximately 40 to 60% of the fat is removed by gravity separation. Mechanical cream separators have not been used for milk standardization for these cheese varieties because cheese makers believe that the sensory characteristics of the cheeses are not the same as when gravity separation is used. The effect of gravity separation on the particle size of fat globules that reside in different layers of milk after gravity separation has been reported (Ma and Barbano, 2000). Possible reasons for the perception by traditional cheese makers that flavor development differs due to gravity separation of milk may be related to the fact that when gravity separation versus a centrifugal cream separator is used to standardize milk fat content, the 2 methods produce standardized milks with different milk fat globule size distribution and total bacteria, spore, and somatic cell counts. Gravity separation produces reduced-fat milk for cheese making that has smaller average particle size and more native milk fat globule membrane per unit weight of milk fat. Gravity-separated milk contains a higher proportion of native milk enzymes associated with the milk fat globule membrane and lower bacteria and spore content, which may contribute, in part, to the superior fat-derived flavor development in cheeses made from gravity-separated milk.

In addition to their unique flavors and aromas (Moio and Addeo, 1998), these Grana cheese varieties have had

an excellent safety record (Johnson et al., 1990a,b,c), even though they are produced from raw milk. Previous work has shown some evidence of partitioning of bacteria and somatic cells during gravity separation of raw milk. Rossi (1964) reported that the number of total bacteria, coliforms, thermophilic bacteria, and spores in partially skim milk is decreased to 5 to 10% of that in the raw milk. Dellagio et al. (1969) showed that gravity separation of raw milk with addition of pure bacterial cultures caused *Clostridium tyrobutyricum* BZ15, *Streptococcus cremoris* 760 and 803, *Acinetobacter* 12-2 and R66, *Escherichia coli* NCDO 1246, *Pseudomonas fluorescens* P442, and *Flavobacterium* 8-9 to rise to the top. These studies suggest that gravity separation can be used to physically remove bacteria and spores from raw milk and to produce raw milk with reduced fat content and reduced bacterial count. The fact that Grana-type cheeses made from raw milk reduced in fat content by gravity separation are perceived to develop better flavor might be due in part to the removal of bacteria and spores in the gravity-separated cream. No data were found in the literature characterizing the effect of gravity separation on milk somatic cells.

Gravity separation of fat, somatic cells, and bacteria may be due to their interactions with native bovine immunoglobulins. Euber and Brunner (1984) reported a clear indication that native immunoglobulins in milk are involved in the agglutination and clustering of milk fat globules that occurs during gravity separation of milk fat. Immunoglobulins may also associate with viable bacteria, spores, and somatic cells, and cause these components to rise to the top as milk fat does.

Processes by which heat-resistant bacteria, especially spore-forming microbes, and somatic cells could be physically removed from fluid milk instead of using very high heat to kill them could improve the sensory quality and shelf life of dairy products. The objective of experiment 1 was to determine whether the extent of gravity separation of milk fat, bacteria, and somatic cells is influenced by time and temperature of gravity separation or the level of contaminating bacteria in the raw milk. The objective of experiment 2 was to determine the effect of different temperatures of milk heat treatment on the gravity separation of milk fat, bacteria, and somatic cells.

## MATERIALS AND METHODS

### Experiment 1: Gravity Separation of Raw Milk

***Milk Processing.*** Raw, uncooled milk was collected from approximately 100 individual Holstein cows at the Cornell University Teaching and Research Center (Ithaca, NY) and commingled. The milk was cooled to 12°C and split into two 14-kg portions. One portion was inoculated with environmental bacteria from the farm and the other portion remained uninoculated. This step was done to create the same milk with high ($\sim 2 \times 10^6$ cfu/mL) and low ($\sim 1 \times 10^4$ cfu/mL) total bacterial counts. The mean SCC of the raw milks used in this study was 199,500 ± 25,000 cells/mL. Approximately 1,000 to 1,200 g of milk was poured into each of 3 sanitized glass gravity separation columns (cat no. 03-789-5C, Fisher Scientific, Hampton, NH), one column per treatment. The top of each column was closed with a rubber stopper, the stopcock at the bottom of each column (68.6 cm tall × 5.1 cm diameter) was closed, and the outlet of the column was capped with a Pasteur pipette bulb (cat no. 03-448-22, Fisher Scientific) filled with 200 ppm of chlorine sanitizer. The columns plus milk were moved to a 4°C or 12°C cooler, depending on the treatment. We observed some increase in bacterial count in the milk from the beginning to the end of gravity separation, approximately a 10 to 30% increase in bacteria count, depending on the treatment. This was taken into account in the calculation of the separation of the cells in the different fractions. The 3 treatments were as follows: (1) raw milk with high bacteria count gravity separated at 12°C for 7 h, (2) raw milk with high bacterial count separated at 4°C for 22 h, and (3) raw milk with low bacterial count separated at 4°C for 22 h. After 7 h for the first column and 22 h for the second and third columns, fractions were removed for determination of SPC, SCC, and fat content. This experiment was replicated 3 times starting with a different batch of milk each time.

***Sampling and Analysis.*** Samples of the uninoculated raw milk and the inoculated milk before gravity separation were taken for SPC, SCC, and fat content determination. After 7 h at 12°C for the first gravity separation column, and after 22 h at 4°C for the second and third gravity separation columns, a cream layer was visible at the top of each column. The cream layer represented approximately the top 100 to 120 mL of volume of milk in the column. Milk was collected (20 fractions) sequentially from the bottom of each gravity separation column. Starting from the bottom of the column, milk was removed by weight in approximately 100-g amounts directly into 120-mL sterile plastic snap-top vials (Capital Vial Inc., Fultonville, NY) until about 100 g of milk remained in the column. The last 100 g (i.e., the material originally at the top of the column) was removed in ten 10-g fractions directly into 60-mL sterile plastic snap-top vials (Capital Vial Inc.). Each fraction removed from the column was tested for SCC and fat. The top 10 fractions removed from the gravity separation columns were very high in bacteria, SCC, and fat, and had to be diluted before analysis

with electronic testing methods. The dilution was done (wt/wt) using autoclaved permeate from ultrafiltration of skim milk that was free of fat and somatic cells. This dilution reduced the bacteria, SCC, and fat content of the samples so that they would be within the proper working range of the instrument calibration. A dilution factor was used to calculate the original content of bacteria, SCC, and fat from the instrument results for each of the top 10 fractions.

Milk fat content was determined (AOAC International, 2000; method 972.160) using a mid-infrared milk analyzer (Milkoscan 605, Foss Electric, Hillerød, Denmark), and SCC (Fossomatic 360, Foss Electric) was determined by an optical fluorescent method (AOAC International, 2000; method 17.13.01; 978.26). The SPC of all samples (Suhren et al., 2001) was determined using a Bactoscan FC (Foss Electric).

### *Experiment 2: Gravity Separation of Pasteurized Milk*

***Milk Processing.*** The raw milk handling was different in experiment 2 compared with experiment 1. In experiment 2, the raw milk was handled according to typical practice in the United States. The warm milk was pumped from the milking parlor through a plate cooler into the bulk tank. The milk in the bulk tank represented the milk from 2 milkings of about 400 cows. The milk in the bulk tank is collected once a day and held at 4°C with agitation every 20 min at the farm. The milk was then pumped into the bulk tank truck and transported to the university dairy. The raw milk was held for an additional 12 h at 4°C with agitation every 20 min before experiment 2. Raw whole milk was heated from 4 to 50°C in a stainless steel–jacketed steam kettle (Groen DN 30, Chicago, IL) and immediately pasteurized using a bench-top shell-and-tube HTST pasteurizer, as described previously (Ma and Barbano, 2003). Milk was pasteurized at 4 temperatures: 72.6, 76.9, 81.0, or 85.0°C. Milk was pumped through the HTST system at 460 mL/min. The milk was heated to 72.6°C, held for 25 s, and then cooled to 4 to 5°C before exiting the HTST system. Approximately 5 kg of pasteurized milk was allowed to exit the HTST system before sample collection began. The pasteurized milk exiting the system was collected directly into 2-L plastic containers, capped, and stored on ice. After collection of the 72.6°C pasteurized milk, the temperature of the milk in the holding tube of the HTST system was increased to 76.9°C while continually processing milk. Once the HTST system was stabilized and the milk in the holding tube had reached 76.9°C, 5 kg of milk was allowed to exit the HTST system. This process was repeated in a stepwise fashion to increase the temperature of milk in the holding tube to 81.0 and 85.0°C, and milk was processed and collected for each temperature.

Raw milk and milk pasteurized at each of the 4 temperatures described above were poured into gravity separation columns, as described for experiment 1, in a room where the air was HEPA-filtered. Glass gravity separation columns, similar to those used in experiment 1, were sanitized with 200 ppm chlorine solution before pasteurization began. Approximately 1,000 to 1,200 g of milk was poured into each of 5 separate columns, one per treatment. Each 2-L container of milk was weighed before and after pouring the milk into the column to determine the weight of milk in each column. The columns plus milk were moved to a 4°C cooler. After 22 h of gravity separation, the columns plus milk were moved back to the 25°C room one at a time, and fractions were collected for determination of SPC, SCC, and fat content. This experiment was replicated 3 times starting with a different batch of raw whole milk each time.

***Sampling and Analysis.*** Before heating the raw whole milk, samples were collected for determination of SPC, SCC, and fat content, and one 2-L container was filled from the steam kettle to be gravity separated. After milk was poured into each column, the remaining milk from each treatment was sampled for SPC, SCC, and fat content determination. After 22 h of gravity separation at 4°C, a cream layer was visible at the top of the raw milk and 72.6°C pasteurized milk separation columns. This layer represented approximately the top 70 and 105 mL of milk in the raw and 72.6°C pasteurized columns, respectively. No cream layer was visible at the top of the other 3 columns of milk that were pasteurized at 76.9, 81.0, and 85.0°C for 25 s. Milk was removed in 20 fractions from each column and collected for SCC and fat content determination, as described for experiment 1.

For determination of bacterial counts, 4 samples from each gravity separation column were made from the 20 fractions collected from each gravity column and analyzed. All fractions were mixed thoroughly by inversion before sample removal, and all samples were removed in a UV-light sanitized, HEPA-filtered air transfer hood (Labconco Purifier class II Safety Cabinet, Labconco Corp., Kansas City, MO). A 1-mL sample from each of the bottom 9 fractions was removed from each 120-mL vial and commingled in a sterile 60-mL vial to make a composite for bacteria testing, representing approximately the lower 900 mL of milk in the column. A 10-mL sample of the next highest 100-g fraction was removed from its 120-mL vial into a sterile 60-mL vial for bacteria testing. A 1-mL sample from each of the next 5 highest 10-g fractions was removed from each 60-mL vial and commingled in another sterile 60-mL vial to

make a composite for bacteria testing. A 1-mL sample from the 5 uppermost 10-g fractions was removed from each 60-mL vial and commingled in another sterile 60-mL vial to make a composite for bacteria testing. The SPC of all composite samples was determined using the pour plate method (Wehr and Frank, 2004). The milk remaining in each of the 120- and 60-mL vials, after sampling for bacterial count, was used to determine SCC and fat content.

### *Statistical Analysis*

The cumulative percentages of total fat, bacterial count (i.e., cfu), and SCC were plotted as a function of the cumulative weight of milk in the gravity separation column for each of the treatments and averaged across 3 replicates. Visual inspection of the graphs showed one rate of change for total fat, bacterial count, and somatic cells with increasing cumulative weight of milk in the gravity column from 0 to about 90% (lower 90% of milk in the column) and a very different rate of change from about 90 to 100% (upper 10% of milk in the column) of the milk in the column. A quadratic regression was used to characterize the rate of change in total fat, bacterial count, and somatic cells in the upper 10% of the weight of milk in each column. These equations were used to calculate the total fat, bacterial count, and somatic cells remaining in the upper 8% of the milk in the column for each treatment in each replicate at the end of gravity separation. The means were compared by ANOVA using a model with treatment, replicate, and column by treatment interaction with Proc GLM of SAS (version 8.02, SAS Institute Inc., Cary, NC) using a *t*-test.

## RESULTS AND DISCUSSION

### *Experiment 1: Gravity Separation of Raw Milk*

Fat, bacteria (i.e., cfu), and somatic cells rose to the top of all columns during gravity separation, and all 3 components were concentrated in the upper 10% of milk in the column. The mean cumulative amount of each component as a function of the cumulative amount of milk weight in the column for each treatment is shown in Figures 1, 2, and 3. The gravity separation of the high bacteria count milk at 12°C for 7 h (Figure 1) achieved a similar separation of fat, bacteria, and somatic cells to that at 4°C for 22 h (Figure 2). No difference ($P > 0.05$) was detected in gravity separation of somatic cells and fat at the 2 different times and temperatures of the high bacteria count milk; however, a greater proportion ($P < 0.05$) of the bacteria were present in the upper 8% of the milk weight in the gravity separation done

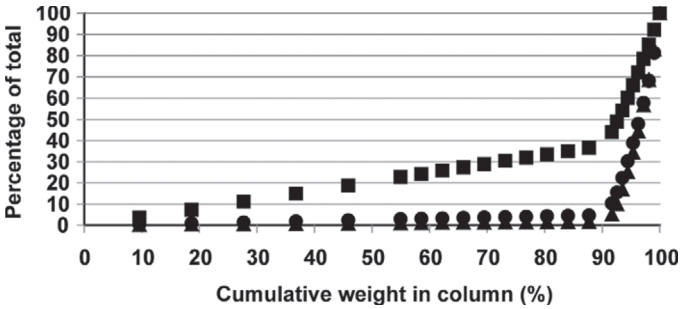


**Figure 1.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for high bacteria count raw milk (~2 × $10^6$ cfu/mL) separated at 12°C for 7 h in experiment 1.

for 7 h at 12°C than that for 22 h at 4°C (Table 1). Therefore, the rate of gravity separation of fat, bacteria, and somatic cells was faster at a milk temperature of 12°C than of 4°C. However, even though a high relative percentage of bacteria in the high bacteria count milk (i.e., 2 × $10^6$ cfu/mL) was removed in the upper layer after gravity separation, the count in the lower layer was still very high (~3 × $10^5$ cfu/mL). Thus, it is always important to start with good quality milk before gravity separation. Essentially, gravity separation will make the quality of good milk better, but it will not make bad quality milk into good quality milk.

The distributions of fat, bacteria, and somatic cells in the gravity-separated inoculated (high bacteria count) milk and the same milk uninoculated (low bacteria count), separated at 4°C for 22 h, are shown in Figures 2 and 3. The overall pattern of separation was similar, but the rising of the bacteria in the low bacteria count milk (Figure 3) did not appear to be as complete, on a relative basis, as that in the high bacteria count milk

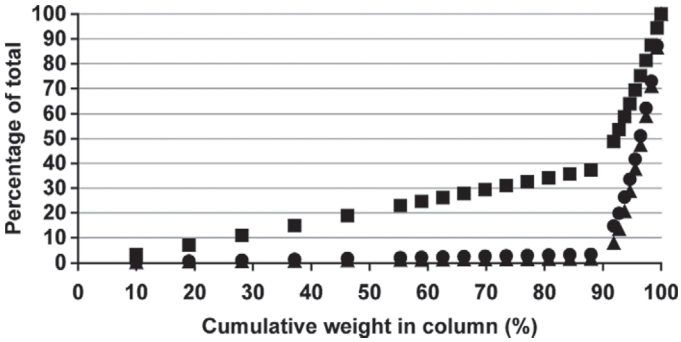


**Figure 2.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for high bacteria count raw milk (~2 × $10^6$ cfu/mL) separated at 4°C for 22 h in experiment 1.

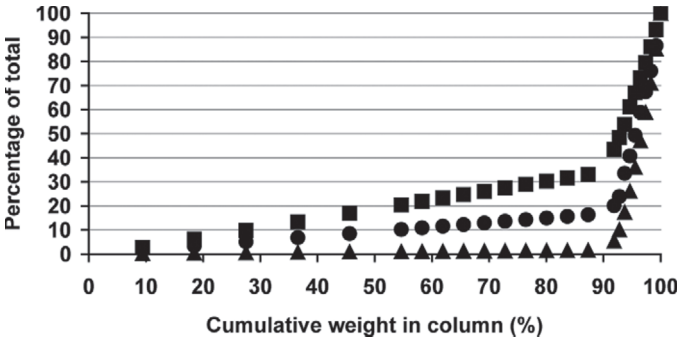


**Figure 3.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for low bacteria count raw milk (~1 × $10^4$ cfu/mL) separated at 4°C for 22 h in experiment 1.

(Figure 2). This difference in bacteria separation on a relative basis for high and low count milks may be due to a difference in the types of bacteria in the inoculated and uninoculated milks or to the smaller average distance between bacterial cells and immunoglobulins in the milks with the higher bacteria count. Some samples were test for spore count and it appears that spores also concentrated at the top during gravity separation (data not reported). No difference ($P > 0.05$) was detected in the relative portion of somatic cells in the upper 8% of the milk weight in the gravity column (Table 1) in the high and low bacteria count milks. However, the rising of somatic cells to the top of the gravity column was very efficient for all treatments, with about 90 to 95% of the somatic cells rising to the upper 8% of the milk weight in the gravity columns for all treatments (Table 1).

The rising of the fat was not as complete as the separation of somatic cells; however, we observed a significant difference in the separation of fat between the high and low bacteria count milks. The rising of fat to the upper 8% of the column (Table 1) was much more complete ($P < 0.05$) in the low bacteria count milk (about 80%) than in the high bacteria count milk (about 50 to 55%). In contrast, the rising of bacteria to the upper 8% of the column (Table 1) was more complete ($P < 0.05$) in the high bacteria count milk (about 84 to 88%) than in the low bacteria count milk (about 56%). Therefore, competition between bacteria and fat may exist for interaction with immunoglobulins in the gravity separation process.

Euber and Brunner (1984) reported that native bovine immunoglobulins in milk are involved in the agglutination and clustering of milk fat globules that occur during gravity separation of milk fat. These immunoglobulins may also play a role in the gravity separation of bacteria and somatic cells. Bovine milk contains a variety of immunoglobulins whose primary role is to transfer passive immunity from mother to neonate (Hurley, 2003). The immunoglobulins present in the highest concentrations in bovine milk are $IgG_1$, $IgG_2$, IgA, and IgM, with approximate concentrations of 0.60, 0.12, 0.13, and 0.04 mg/mL, respectively (Lindstrom et al., 1994).

Euber and Brunner (1984) reported that IgM is a heat-labile component necessary for fat globule clustering and that approximately 7% of IgM in normal milk participates in a single creaming. It is not known if IgM interacts with bacteria. Removal of IgG and IgA did not affect fat creaming (Euber and Brunner, 1984). Frenyo et al. (1986) reported that immunoglobulins are not randomly distributed among fractions of bovine milk, and that IgM and IgA tend to associate with the fat or cream fraction. Frenyo et al. (1986) indicated that when labeled immunoglobulins were incubated with milk leukocytes (i.e., somatic cells) alone, secretory (s)IgA and IgM became significantly associated with them. Frenyo et al. (1986) also reported competition between somatic cells and fat or between casein and fat, for sIgA and IgM, but no significant association between fat and $IgG_1$ and $IgG_2$.

**Table 1.** The mean relative percentages (n = 3) of total starting bacteria (cfu), fat, and somatic cells in the original milk that was in the upper 8% of the milk weight in the gravity separation column after gravity separation at time-temperature combination

| Bacteria count[1] | Gravity separation | | | | |
|---|---|---|---|---|---|
| | Time (h) | Temperature (°C) | Bacteria | Fat | Somatic cells |
| High | 7 | 12 | 88.2[a] | 54.6[b] | 93.8[a] |
| High | 22 | 4 | 84.4[b] | 50.6[b] | 90.8[a] |
| Low | 22 | 4 | 55.8[c] | 80.4[a] | 94.3[a] |
| LSD[2] | | | 1.1 | 18.5 | 5.9 |

[a–c]Means in the same column not sharing a common superscript are significantly different ($P < 0.05$).

[1]High = milk inoculated with environmental bacteria to a count of ~2 × $10^6$ cfu/mL; low = uninoculated milk with a count of ~1 × $10^4$ cfu/mL.

[2]LSD = least significant difference at $P < 0.05$.

One functional role of IgG is its ability to inhibit adherence of bacteria to the intestinal epithelium (Ohnuki and Otani, 2005). Xu et al. (2006) showed that after treatment of enteroinvasive *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae* with specific IgG, agglutination reaction with agglutinated masses and irregular structure with destruction of cell walls were found. These results imply that specific IgG may be capable of agglutinating bacteria and degrading or destroying cells walls, thereby inhibiting the growth of certain bacteria.

Our results (Table 1) indicate that the presence of very different levels of bacteria in milk influences the gravity separation of milk fat but not that of somatic cells. When the bacteria count of the milk was high, a much lower percentage of the fat (50 to 55% vs. 80%) rose to the top of the gravity separation column in 22 h at 4°C. The bacteria may compete for interaction with the same immunoglobulin that is important for the gravity separation of the fat, and high bacteria counts in milk may reduce the extent of gravity separation of fat. We do not know if the distribution of bacteria types in the upper layers of the gravity separation column is different from that of the residual bacteria present in the lower layers. When cows are challenged with invasive species of bacteria, their immunological response to that challenge may produce a profile of immunoglobulin in milk that has more affinity for specific types of bacteria. It would be interesting to see if the rate and completeness of gravity separation of different types of bacteria are different when cows are induced to produce milk with a different immunoglobulin profile. The fact that the gravity separation of somatic cells was not influenced by the bacteria count in the milk may indicate that a different immunoglobulin or different mechanism could be involved in the gravity separation of somatic cells compared with separation of fat and bacteria. It has been reported that IgG is predominantly involved with interactions with bacteria (Xu et al., 2006), and IgM is predominantly involved with interactions with fat globules (Frenyo et al., 1986).

### *Experiment 2: Gravity Separation of Pasteurized Milk*

The mean cumulative amount of fat, bacteria, and somatic cells as a function of the cumulative amount of milk weight in the column for each treatment is shown in Figures 4 through 8. Fat, bacteria (i.e., cfu), and somatic cells rose to the top of the raw and 72.6°C columns during gravity separation (Figures 4 and 5, respectively), and all 3 components were concentrated in the upper 10% of milk in the column. We found no evidence of gravity separation of fat, bacteria, or somatic cells in milk that was pasteurized at 76.9, 81.0, and 85.0°C (Figures 6, 7, and 8, respectively). The mean amount of fat, bacteria, and somatic cells in the upper 8% of the weight of the milk in the column is shown in Table 2 for each treatment. When comparing gravity separation of the raw milk and milk pasteurized at 72.6°C for 25 s, separation of the fat and somatic cells was more complete ($P < 0.05$) in the milk pasteurized at 72.6°C than in the raw milk, with the relative effect being much greater for fat than for somatic cells. It is interesting to note in the comparison of the data for the gravity separation of fat in raw milk in experiment 1 (Table 1) and experiment 2. The percentage of the total milk fat that was recovered in the upper 8% of the gravity column was much lower in experiment 2 (35.3%) than in experiment 1 (80.4%), whereas the separation of somatic cells and bacteria was similar in the 2 experiments. The lesser degree of gravity separation of fat in experiment 2 may have resulted from the effect of a long agitation time (about 36 h) or intermittent agitation on the milk fat globule and milk fat globule membrane. This may indicate that the surface characteristics of the fat globules have a large effect on their interactions with immunoglobulins in the gravity separation process. Although no difference ($P > 0.05$) was detected in bacterial count in the upper 8% of the weight of milk in the gravity columns for the raw and milk pasteurized at 72.6°C, we found a trend for more efficient separation of bacteria in the milk pasteurized at 72.6°C than in raw milk. The reason for the enhancement of gravity separation of these components by the 72.6°C heat treatment is not known. Heat can influence the intramolecular disulfide bonds in heat-sensitive milk components and immunoglobulins, with the potential for heat-denatured components to interact with each other and the surfaces of native milk fat globules, casein micelles, bacteria, and somatic cells. We observed a very large effect ($P < 0.05$) of pasteurization at 76.9, 81.0, and 85.0°C for 25 s on gravity separation of all components (Table 2) compared with pasteurization at 72.6°C and raw milk. Gravity separation of fat, bacteria, and somatic cells was completely stopped by heat treatment of the milk at 76.9, 81.0, and 85.0°C (Figures 6, 7, and 8, and Table 2). It is not known if heat treatment of milk at temperatures <72.6°C or between 72.6 and 76.9°C would enhance gravity separation of fat, bacteria, or somatic cells, and this should be investigated. These results suggest that the general mechanism of gravity separation is similar for all of these components. In general, immunoglobulins are involved in gravity separation of fat and, specifically, IgM is the heat-labile component necessary for fat globule clustering (Euber and Brunner, 1984). It is likely that immunoglobulins are involved in gravity separation of bacteria and somatic cells. Each immuno-

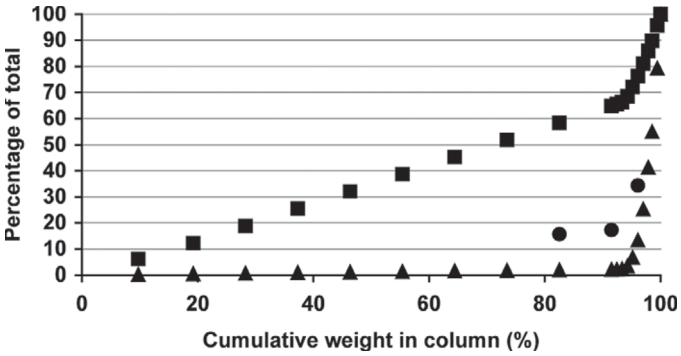


**Figure 4.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for raw milk separated at 4°C for 22 h in experiment 2.

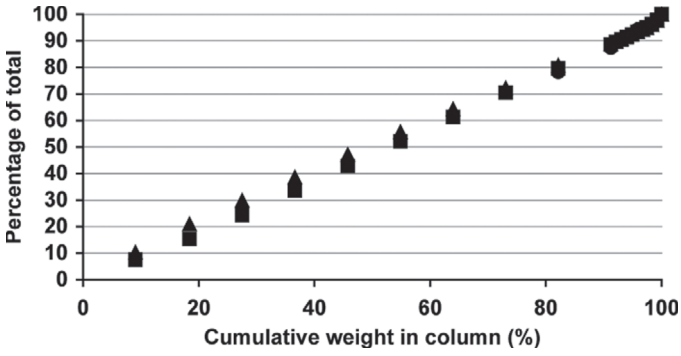


**Figure 6.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for 76.9°C for 25 s pasteurized milk separated at 4°C for 22 h in experiment 2.

globulin molecule contains 2 identical heavy chains and 2 identical light chains, both of which are composed of variable and constant regions (Korhonen et al., 2000). Disulfide bonds link each heavy and light chain pair, as well as linking the 2 heavy chains to form a Y-shaped molecule with 2 antigen-binding sites (Hurley, 2003). The number and position of disulfide bonds linking the heavy chains varies with each immunoglobulin isotype (Hurley, 2003). Each immunoglobulin molecule contains 4 disulfide bonds; however, each isotype is found in a different form, leading to different numbers of disulfide bonds. Immunoglobulin G is found only in the monomeric form, but IgA can be mono-, di-, or tetrameric, and IgM is pentameric (Hurley, 2003). Disulfide bonds within immunoglobulins may be broken by high heat treatment, resulting in a change in their functional behavior and biological function.

The results of experiment 2 indicate that heat-labile components in milk (possibly various immunoglobulins) are responsible for gravity separation of fat, bacteria, and somatic cells. When milk was pasteurized at ≥72.6°C, we found no evidence of separation for any of the components. Li-Chan et al. (1995) reported that the D-values (time for 90% inactivation) for IgG decreased as pasteurization temperature increased from 72 to 80°C. Other immunoglobulins may also denature with increasing temperature, preventing their association with fat, bacteria, and somatic cells. At temperatures ≥76.9°C, intramolecular disulfide bonds within the immunoglobulins may denature, or other milk proteins may denature and associate with immunoglobulins through the formation of intermolecular disulfide bonds, preventing the association between the immunoglobulin and fat, bacteria, and somatic cells. Further work in our laboratory, at pasteurization temperatures intermediate to those reported here, indicates that

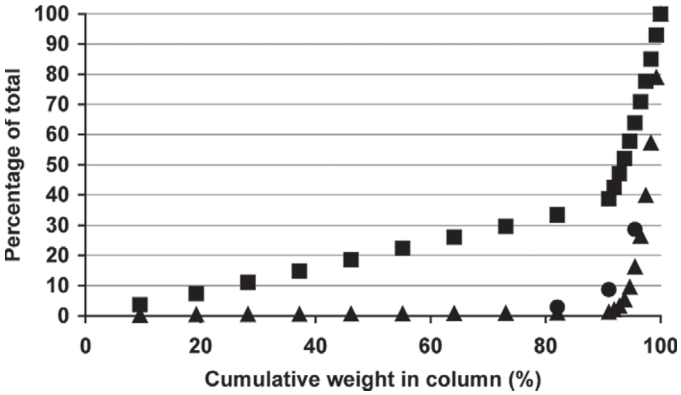


**Figure 5.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for 72.6°C for 25 s pasteurized milk separated at 4°C for 22 h in experiment 2.

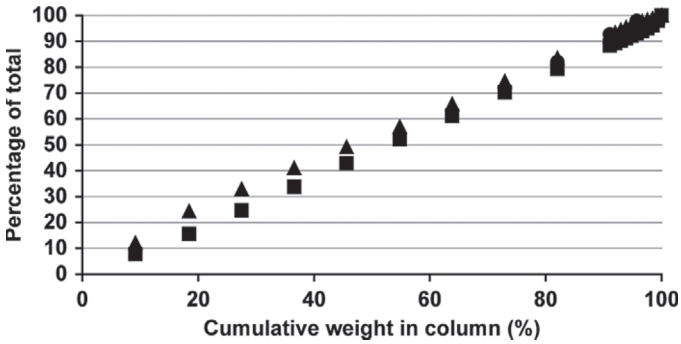


**Figure 7.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for 81.0°C for 25 s pasteurized milk separated at 4°C for 22 h in experiment 2.

**Table 2.** The mean relative percentages (n = 3) of the total starting bacteria (cfu), fat, and somatic cells in the original milk that was in the upper 8% of the milk weight in the gravity separation column after gravity separation at 4°C for 22 h

| Pasteurization treatment (25 s) | Bacteria | Fat | Somatic cells |
|---|---|---|---|
| Raw | 83.2[a] | 35.3[b] | 93.1[b] |
| 72.6°C | 91.4[a] | 57.5[a] | 96.4[a] |
| 76.9°C | 11.1[b] | 10.4[c] | 9.8[c] |
| 81.0°C | 6.0[b] | 10.5[c] | 6.9[d] |
| 85.0°C | 6.9[b] | 10.4[c] | 6.0[d] |
| LSD[1] | 15.3 | 3.9 | 1.0 |

[a–d]Means in the same column not sharing a common superscript are significantly different ($P < 0.05$).

[1]LSD = least significant difference at $P < 0.05$.

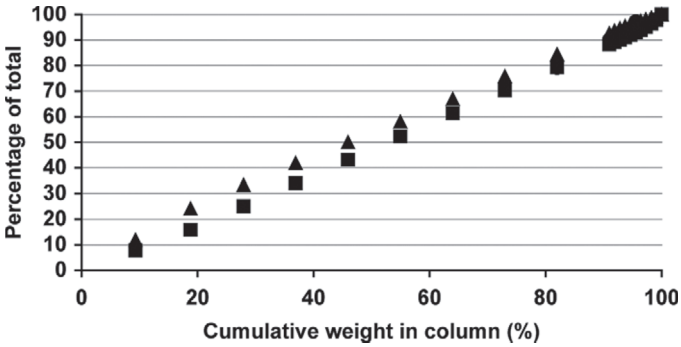


**Figure 8.** Average (n = 3) cumulative percentage of total fat (■), somatic cells (▲), and total bacteria (cfu; ●) as a function of the percentage of the cumulative amount of total milk in the gravity separation column for 85.0°C for 25 s pasteurized milk separated at 4°C for 22 h in experiment 2.

the rate of gravity separation decreases very quickly at about 74.5°C for 25 s (data not shown). It is not clear why bacteria and somatic cells rise to the top of the gravity separation column instead of settling to the bottom of the gravity column because of their apparent greater density than fat. However, in follow-up preliminary experiments (data not reported), in the gravity separation of raw skim milk, we found that the somatic cells separated and rose to the top of the column in the absence of fat. Therefore, the rising of somatic cells does not appear to depend on the presence of native milk fat globules.

## CONCLUSIONS

Fat, bacteria, and somatic cells rose to the top during gravity separation of raw milk. About 50 to 80% of the fat and bacteria were present in the top 8% of the milk at the end of gravity separation. Gravity separation for 7 h at 12°C produced equivalent separation of fat, bacteria, and somatic cells as did separation for 22 h at 4°C. The completeness of gravity separation of fat was influenced by the level of bacteria in the milk before gravity separation. Milk with a high bacteria count had less (about 50 to 55%) gravity separation of fat than milk with a low bacteria count (about 80%) in 22 h at 4°C. Gravity separation caused fat, bacteria, and somatic cells to rise to the top of columns for raw whole milk and HTST-pasteurized (72.6°C, 25 s) whole milk. Pasteurization at ≥76.9°C for 25 s prevented all 3 components from rising, possibly because of denaturation of native bovine immunoglobulins that normally associate with fat, bacteria, and somatic cells during gravity separation. Gravity separation can be used to produce reduced-fat milk with low bacteria and somatic cell counts and may be a critical factor in the long history of safe and unique traditional Italian hard cheeses produced from gravity-separated raw milk. A better understanding of the mechanism of this natural process could lead to the development of new nonthermal thermal technology (that does not involve heating the milk to high temperatures) to remove bacteria from fluid milk or other liquids.

## ACKNOWLEDGMENTS

The authors thank the following Cornell University staff members for technical assistance: Esref Dogan, Bob Kaltaler, Jessica Mallozzi, Mark Newbold, Karen Wojciechowski, and the staff at the Cornell Teaching and Research Center (Ithaca, NY), the Dairy One laboratory (Ithaca, NY) for the Bactoscan analysis, and staff of CoRFiLaC (Ragusa, Italy) for their collaboration.

## REFERENCES

AOAC International. 2000. Official Methods of Analysis. 17th ed. AOAC International, Arlington, VA.

Dellagio, F., J. Stadhouders, and G. Hup. 1969. Distribution of bacteria between the bottom, middle, and cream layers of creamed raw milk. Neth. Milk Dairy J. 23:140–145.

Euber, J. R., and J. R. Brunner. 1984. Reexamination of fat globule clustering and creaming in cow milk. J. Dairy Sci. 67:2821–2832.

Frenyo, V. L., J. E. Butler, and A. J. Guidry. 1986. The association of extrinsic bovine $IgG_1$, $IgG_2$, SIgA and IgM with the major fractions and cells of milk. Vet. Immunol. Immunopathol. 13:239–254.

Hurley, W. L. 2003. Immunoglobulins in mammary secretions. Pages 421–447 in Advanced Dairy Chemistry. Vol. 1: Proteins. 3rd ed. P. F. Fox and P. L. H. McSweeney, ed. Kluwer Academic/Plenum Publisher, New York, NY.

Johnson, E. A., J. H. Nelson, and M. Johnson. 1990a. Microbiological safety of cheese made from heat-treated milk. Part I. Executive summary, introduction and history. J. Food Prot. 53:441–452.

Johnson, E. A., J. H. Nelson, and M. Johnson. 1990b. Microbiological safety of cheese made from heat-treated milk. Part II. Microbiology. J. Food Prot. 53:519–540.

Johnson, E. A., J. H. Nelson, and M. Johnson. 1990c. Microbiological safety of cheese made from heat-treated milk. Part III. Technology, discussion, recommendations, bibliography. J. Food Prot. 53:610–623.

Korhonen, H., P. Marnila, and H. S. Gil. 2000. Milk immunoglobulins and complement factors. Br. J. Nutr. 84:S75–S80.

Li-Chan, E., A. Kummer, J. N. Losso, D. D. Kitts, and S. Nakai. 1995. Stability of bovine immunoglobulins to thermal treatment and processing. Food Res. Int. 28:9–16.

Lindstrom, P., M. Paulsson, T. Nylander, U. Elofsson, and H. Lindmark-Mansson. 1994. The effect of heat treatment on bovine immunoglobulins. Milchwissenschaft 49:67–71.

Ma, Y., and D. M. Barbano. 2000. Gravity separation of raw bovine milk: Fat globule size distribution and fat content of milk fat fractions. J. Dairy Sci. 83:1719–1727.

Ma, Y., and D. M. Barbano. 2003. Milk pH as a function of $CO_2$ concentration, temperature, and pressure in a heat exchanger. J. Dairy Sci. 86:3822–3830.

McSweeney, P. L. H., G. Ottogalli, and P. F. Fox. 2004. Diversity in cheese varieties: An overview. Pages 1 to 23 in Cheese Chemistry, Physics, and Microbiology. Vol. 2: Major Cheese Groups. 3rd ed. Elsevier Academic Press, London, UK.

Moio, L., and F. Addeo. 1998. Grana Padano cheese aroma. J. Dairy Res. 65:317–333.

Ohnuki, H., and H. Otani. 2005. Antigen-binding and protein G-binding abilities of immunoglobulin G in hyperimmunized cow's milk treated under various conditions. Anim. Sci. J. 76:283–290.

Rossi, J. 1964. Sul processo di caseificazione del formaggio grana. Il Latte 38:301–305.

Suhren, G., J. Reichmuth, and H. G. Walte. 2001. Bacteriological quality of raw milk: Conversion of Bactoscan-FC counts onto the scale of the official method. Milchwissenschaft 56:380–384.

Wehr, H. M., and J. F. Frank, ed. 2004. Standard Methods for the Examination of Dairy Products. 17th ed. Am. Publ. Health Assoc. Inc., Washington, DC.

Xu, L. B., L. Chen, W. Gao, and K. H. Du. 2006. Bovine immune colostrum against 17 strains of diarrhea bacteria and in vitro and in vivo effects of its specific IgG. Vaccine 24:2131–2140.