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

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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.

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[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.
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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