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The effect of immunoglobulins and somatic cells on the gravity separation of fat, bacteria, and spores in pasteurized whole milk[1]

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ABSTRACT

Our objective was to determine the role that immunoglobulins and somatic cells (SC) play in the gravity separation of milk. The experiment comprised 9 treatments: (1) low-temperature pasteurized (LTP; 72°C for 17.31 s) whole milk; (2) LTP (72°C for 17.31 s) whole milk with added bacteria and spores; (3) recombined LTP (72°C for 17.31 s) whole milk with added bacteria and spores; (4) high-temperature pasteurized (HTP; 76°C for 7 min) whole milk with added bacteria and spores; (5) HTP (76°C for 7 min) whole milk with added bacteria and spores and added colostrum; (6) HTP (76°C for 7 min) centrifugally separated, gravity-separated (CS GS) skim milk with HTP (76°C for 7 min) low-SC cream with added bacteria and spores; (7) HTP (76°C for 7 min) CS GS skim milk with HTP (76°C for 7 min) high-SC cream with added bacteria and spores; (8) HTP (76°C for 7 min) CS GS skim milk with HTP (76°C for 7 min) low-SC cream with added bacteria and spores and added colostrum; and (9) HTP (76°C for 7 min) CS GS skim milk with HTP (76°C for 7 min) high-SC cream with added bacteria and spores and added colostrum. The milks in the 9 treatments were gravity separated at 4°C for 23 h in glass columns. Five fractions were collected by weight from each of the column treatments, starting from the bottom of the glass column: 0 to 5%, 5 to 90%, 90 to 96%, 96 to 98%, and 98 to 100%. The SC, fat, bacteria, and spores were measured in each of the fractions. The experiment was replicated 3 times in different weeks using a different batch of milk and different colostrum. Portions of the same batch of the frozen bacteria and spore solutions were used for all 3 replicates. The presence of both SC and immunoglobulins were necessary for normal gravity separation (i.e., rising to the top) of fat, bacteria, and spores in whole milk. The presence of immunoglobulins alone without SC was not sufficient to cause bacteria, fat, and spores to rise to the top. The interaction between SC and immunoglobulins was necessary to cause aggregates of fat, SC, bacteria, and spores to rise during gravity separation. The SC may provide the buoyancy required for the aggregates to rise to the top due to gas within the SC. More research is needed to understand the mechanism of the gravity-separation process.
Key words: gravity separation, somatic cells, immunoglobulin, bacteria, spores

INTRODUCTION

The process of gravity separation has been used for traditional cheese making of Grana and Parmigiano-Reggiano raw milk cheeses in the north of Italy (Fox et al., 2004). The primary purpose of the gravity-separation step in the manufacture of these cheeses is to decrease the fat content of the milk before cheese making by removal of the upper cream layer (Fox et al., 2004). Traditional Parmigiano-Reggiano cheeses are made by allowing raw milk to gravity separate overnight at 20°C, removing some of the cream layer, and mixing in fresh raw whole milk to obtain a 2.4 to 2.5% fat milk. The milk for traditional Grana Padano cheeses is prepared by gravity separating raw whole milk at 12 to 15°C for 12 h and then removing the fat layer to get a 2.1 to 2.2% fat milk (Fox et al., 2004).

Euber and Brunner (1984) reported that IgM is involved in the aggregation and gravity separation of milk fat globules. Two types of lymphocytes exist in blood: B cells and T cells. Immunoglobulins are produced by B cells (Parham, 2009). The B cells produce immunoglobulins that are specific to a particular pathogen, based on an encounter of the B cell with that pathogen (Parham, 2009). The B cells have immunoglobulins bound to the cell membrane exterior and when the bound immunoglobulin binds to a pathogen, the B cell is stimulated to produce free immunoglobulin molecules to bind to that pathogen. Those immunoglobulins produced can only bind to that specific antigen (Parham, 2009).

Immunoglobulins bind to bacteria and spores by recognizing specific carbohydrates or proteins on the outside layer of the pathogen (Parham, 2009). By

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attaching to the surface of the pathogen, the immunoglobulins help signal to other white blood cells the presence of the pathogen (Parham, 2009). Other white blood cells (neutrophils and macrophages) can then destroy the pathogen or activate the complement system (Parham, 2009). The complement system is a series of proteins that are generally proteolytic in character (i.e., serine proteases) in the blood that bind to the surface of bacteria to mark them as targets for phagocytosis by neutrophils and macrophages. This interaction between the complement, immunoglobulins, bacteria and spores, and white blood cells in the body may be involved in the aggregation of bacteria and spores in milk during gravity separation.

Colostrum has a much higher concentration of immunoglobulins than milk. Total immunoglobulins are present at an average concentration of 61.4 mg/mL in colostrum and 0.8 mg/mL in milk (Butler, 1983). Vaccination of cows can change the immunoglobulin composition of the milk. Vaccination against *Escherichia coli* O111:B4, also known as J5 vaccination, caused the milk to contain IgM, IgG_1, and IgG_2 specific toward J5 (Wilson et al., 2009). Cows that were immunized with inactivated *Staphylococcus aureus* strains had IgA present in milk that was specific for *Staphylococcus aureus* (Tempelmans Plat-Sinnige et al., 2009).

It appears that gravity separation may play an important role in reducing the bacteria and spore content of the milk before manufacture of grana-style cheeses. Traditional cheese makers have stated that they continue to use gravity separation as opposed to more modern centrifugal separation due to better cheese flavor and fewer gas defects (Caplan et al., 2013). Caplan et al. (2013) reported that fat, somatic cells (**SC**), and bacteria gravity separate in raw and pasteurized (72.6°C for 25 s) whole milk by rising to the top. Both Dellaglio et al., (1969) and Rossi (1964) reported that spores rise to the top during the gravity-separation process. The flavor difference when using gravity separation to produce these cheeses may be due to the removal of undesirable bacteria and spores in the cream layer.

The gravity separation of fat, bacteria, and SC in whole milk was stopped when the milk was pasteurized at temperatures >76.9°C for 25 s (Caplan et al., 2013). When colostrum was added to pasteurized (76°C for 7 min) skim milk, it restored the gravity separation of SC (Geer and Barbano, 2014). Thus, SC gravity separates even when the fat has been removed from milk. The gravity separation of fat was restored when IgM was added back to whole milk subjected to high heat treatment and when IgM was removed using an IgM-specific antiserum, the gravity separation of fat did not occur (Euber and Brunner, 1984). Immunoglobulins are a good candidate for the heat-labile component of the gravity separation of fat based on the fact that they are found in bovine blood serum, colostrum, and milk and they have the capability to form agglutinations of bacteria (Hurley and Theil, 2011). Our objective was to determine the role that immunoglobulins and SC play in the gravity separation of bacteria and spores in milk.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

An experiment was designed to determine if the presence of SC or immunoglobulins, or both, is necessary for gravity separation to occur in milks. The experiment comprised 9 treatments (shown in Table 1): (1) low-temperature pasteurized (**LTP**; 72°C for 17.31 s) whole milk; (2) LTP (72°C for 17.31 s) whole milk with added bacteria and spores; (3) recombined LTP (72°C for 17.31 s) whole milk with added bacteria and spores; (4) high-temperature pasteurized (**HTP**; 76°C for 7 min) whole milk with added bacteria and spores; (5) HTP whole milk (76°C for 7 min) with added bacteria and spores and added colostrum; (6) HTP (76°C for 7 min) centrifugally separated, gravity-separated (**CS GS**) skim milk with HTP (76°C for 7 min) low-SC cream with added bacteria and spores; (7) HTP (76°C for 7 min) CS GS skim milk with HTP (76°C for 7 min) high-SC cream with added bacteria and spores; (8) HTP (76°C for 7 min) CS GS skim milk with HTP (76°C for 7 min) low-SC cream with added bacteria and spores and added colostrum; and (9) HTP (76°C for 7 min) CS GS skim milk with HTP (76°C for 7 min) high-SC cream with added bacteria and spores and added colostrum.

The milks in 9 treatments were gravity separated at 4°C for 23 h in glass columns. Five fractions were collected by weight from each of the column treatments, starting from the bottom of the glass column: 0 to 5%, 5 to 90%, 90 to 96%, 96 to 98%, and 98 to 100%. The SC, fat, bacteria, and spores were measured in each of the fractions. The experiment was replicated 3 times in different weeks using a different batch of milk and different colostrum. Portions of the same batch of the frozen bacteria and spore solutions were used for all 3 replicates.

The GLM procedure of SAS (9.3; SAS Institute Inc., Cary, NC) was used to determine if the presence or absence of immunoglobulins or SC, or both, caused gravity separation of fat, SC, bacteria, and spores in whole milk. The ANOVA model had terms for treatment and replicate as categorical variables. If the F-test for the model was <0.05, then the percentage of SC, fat, bacteria, and spores in the different treatments were