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Effect of colostrum on gravity separation of milk somatic cells in skim milk[1]

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ABSTRACT

Our objective was to determine if immunoglobulins play a role in the gravity separation (rising to the top) of somatic cells (SC) in skim milk. Other researchers have shown that gravity separation of milk fat globules is enhanced by IgM. Our recent research found that bacteria and SC gravity separate in both raw whole and skim milk and that heating milk to >76.9°C for 25 s stopped gravity separation of milk fat, SC, and bacteria. Bovine colostrum is a good natural source of immunoglobulins. An experiment was designed where skim milk was heated at high temperatures (76°C for 7 min) to stop the gravity separation of SC and then colostrum was added back to try to restore the gravity separation of SC in increments to achieve 0, 0.4, 0.8, 2.0, and 4.0 g/L of added immunoglobulins. The milk was allowed to gravity separate for 22 h at 4°C. The heat treatment of skim milk was sufficient to stop the gravity separation of SC. The treatment of 4.0 g/L of added immunoglobulins was successful in restoring the gravity separation of SC as compared with raw skim milk. Preliminary spore data on the third replicate suggested that bacterial spores gravity separate the same way as the SC in heated skim milk and heated skim milk with 4.0 g/L of added immunoglobulins. Strong evidence exists that immunoglobulins are at least one of the factors necessary for the gravity separation of SC and bacterial spores. It is uncertain at this time whether SC are a necessary component for gravity separation of fat, bacteria, and spores to occur. Further research is needed to determine separately the role of immunoglobulins and SC in gravity separation of bacteria and spores. Understanding the mechanism of gravity separation may allow the development of a continuous flow technology to remove SC, bacteria, and spores from milk.

Key words: gravity separation, somatic cell, colostrum, immunoglobulin

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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 the authors or Cornell University.
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INTRODUCTION

A well-known phenomenon in bovine milk is the gravity separation, or rising to the top, of fat globules when milk is allowed to stand unagitated. It was determined that the fat globules rose quicker than predicted by Stokes' Law (Troy and Sharp, 1928). If the fat globules clustered together, the faster rising could be explained (Troy and Sharp, 1928). Fat globules in milk aggregate together in a similar fashion to aggregation of components in blood (Babcock 1889).

Bacteria rise to the top during gravity separation along with the fat. Several researchers looked at the composition of the bacteria species before and after gravity separation and found that some species of bacteria rose to the top more than others (Dellaglio et al., 1969; Abo-Elnaga et al., 1981; Franciosi et al., 2011). It has been estimated that between 67 to 99% of the initial bacteria rises to the cream layer after gravity separation (Abo-Elnaga et al., 1981).

Competition also exists between bacteria and fat, as milk that is higher in bacteria counts will have a higher percentage of total bacteria at the top but a lower percentage of total fat at the top compared with milk with a lower starting bacteria count (Caplan et al., 2013). Stadhouders and Hup (1970) showed that bacteria agglutinate in milk and become attached to the fat globules, suggesting a similar agglutinating process for fat and bacteria. Evidence also exists that spores rise to the top during gravity separation (Rossi, 1964; Dellaglio et al., 1969)

Very little research has looked at the gravity separation of somatic cells (**SC**) in milk. Similar to the rising of bacteria and fat, SC would concentrate in the top portion of the milk after gravity separation (Caplan et al., 2013). Caplan et al. (2013) also found a similar trend among the gravity separation of fat, bacteria, and SC; however, SC and bacteria were more concentrated after 22 h at 4°C than the fat. The role of SC in the gravity separation of fat and bacteria is uncertain, as its unknown at this time if it is a necessary component.

What still is not well understood is the actual mechanism behind the gravity separation. In a series of research experiments by Mertens (1933a,b), he came up with 2 main points regarding the gravity separation of fat. The first is that a factor that affects gravity separation exists in skim milk that can be heat denatured. The second factor is that something in colostrum enhances gravity separation of milk. Previous work showed that pasteurization at temperatures >76.9°C for 25 s prevented gravity separation of fat, bacteria, and SC in whole milk (Caplan et al., 2013), which agrees with results from Mertens (1933a). Furthermore, the fact that fat, bacteria, and SC all stop their gravity separation with heat is further evidence of a common mechanism.

Immunoglobulins are good candidates for the heat-labile component of the gravity separation of fat based on the fact that they are found in blood serum, colostrum, and milk of bovines and they have the capability to form agglutinations (Hurley and Theil, 2011). Colostrum is a rich source of immunoglobulins, which could explain why Mertens (1933b) found that the addition of colostrum increased the rate of gravity separation. Euber and Brunner (1984) showed that IgM was a necessary component for the gravity separation of fat. The addition of IgM to whole milk heated at 75°C for 30 min restored the creaming of fat, whereas the addition of IgG did not (Euber and Brunner, 1984). They also found that when they removed IgA and IgG from raw milk, no effect occurred on the creaming, but removal of IgM did reduce creaming (Euber and Brunner, 1984).

Immunoglobulins are a type of B-cell receptor that binds to antigens. The basic structure of an immunoglobulin consists of a Y-like structure that has 2 identical heavy chains and 2 identical light chains (Parham, 2009). The immunoglobulin molecule is held together by 2 disulfide bonds in the hinge. A disulfide bond also links together the heavy and light chain (Parham, 2009). It is possible that disulfide bonds in the immunoglobulin molecule are destroyed by heat, leading to conformational changes within the immunoglobulin molecule, causing it to lose its activity. Bovine milk and colostrum immunoglobulin are composed primarily of IgA, IgG_1, IgG_2, and IgM.

Two identical antigen-binding sites are found on each Y structure of the immunoglobulin molecule (Parham, 2009). The antigen-binding sites recognize carbohydrates or proteins on the surface of a pathogen (Parham, 2009). Variability exists between different antigen-binding sites, which determines which type of antigens it can bind to (Parham, 2009). Immunoglobulin M is involved in the first immune response when an antigen is detected and, therefore, has lower specificity (Hurley and Theil, 2011). Immunoglobulin G is involved in the secondary immune response and has more specificity toward particular antigens (Hurley and Theil, 2011). Immunoglobulin A is the primary antigen found in mucosal secretions (Hurley and Theil, 2011).

Research in our laboratory has shown that SC will gravity separate in skim milk, in the absence of fat globules. Evidence exists (Euber and Brunner, 1984) that milk fat globule membrane material may be important in the gravity separation process, but not the actual fat globules themselves. It has been observed that homogenization will stop the gravity separation of fat milk. Euber and Brunner (1984) added IgM and skim milk membrane separately to homogenized milk. The addition of IgM had no effect on the gravity separation of fat, but the addition of the skim milk membrane restored the gravity separation. Milk fat globule membrane material is found in both whole milk and skim milk, which could explain why SC gravity separate in both skim and whole milks.

Little research was found comparing the concentrations of immunoglobulins in whole versus skim milk, but it appears that, at least for IgG, no change in concentration occurred after the cream was removed (Li-Chan et al., 1995). Although no literature found compares IgM and IgA in whole versus skim milk, a higher association of IgM and IgA with fat exists than for IgG_1 and IgG_2 (Frenyo et al., 1986). It is possible, therefore, that the levels of IgM and IgA would be lower in skim milk. However, Euber and Brunner (1984) found that only 7% of the total amount of IgM present in whole milk is needed for gravity separation of fat. It is likely then that even if the immunoglobulin levels are lower in skim compared with whole milk, it is not enough to prevent the gravity separation of SC in skim milk.

More research is needed to understand how SC gravity separate in skim milk and whether SC play an important part in the gravity separation process. Our objective was to determine if immunoglobulins play a role in the gravity separation of SC in skim milk. Bovine colostrum served as a source of immunoglobulins, due to its high concentration of immunoglobulins compared with milk.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

An experiment was designed to determine if addition of raw colostrum (a crude source of bovine immunoglobulins) could restore gravity separation of SC for pasteurized (>74.5°C for 25s) skim milk. The experiment comprised 6 treatments: raw skim, pasteurized skim, and pasteurized skim milk with 4 levels of added immunoglobulins from colostrum (0.4, 0.8, 2.0, and 4.0 g/L of added immunoglobulins). Six fractions were collected by weight, starting from the bottom of each gravity separation column (0 to 90%, 90 to 92%, 92 to 94%, 94 to 96%, 96 to 98%, and 98 to 100%) after 22