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ORIGINAL PAPER

Cold agglutination: mechanism and role

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INTRODUCTION

Cow milk contain 3.2 % milk fat and more than 95% of the total milk lipid is in the form of a globule ranging in size from 0.1 to 15 µm in diameter. These liquid fat droplets are covered by a thin membrane, 8 to 10 nm in thickness, whose properties are completely different from both milk fat and plasma. The native fat globule membrane (FGM) is comprised of apical plasma membrane of the secretary cell which continually envelopes the lipid droplets as they pass into the lumen. The major components of the native FGM, therefore, are protein and phospholipids. The lipids in bovine milk are present in microscopic globules as an oil in-water emulsion. Both the fat content of the milk and the fatty acid composition of the lipids can vary considerably as a result of changes in factors like breed of cow, diet and stage of lactation. The fat content can vary from about 3.0 to 6.0%. Milk fat is present in spherical droplets, which range from about 0.2 to 15 mm in diameter, with the bulk of the fat being in globules 1.0 to 8.0mm diameter. Milk contains about 15×10^9 globules per ml. Fat globules in milk are naturally emulsified by a complex layer of surface material, the milk fat globule membrane (MFGM), which accounts for 2–6% of the mass of the fat globules (MacGibbon 2020; Singh and Gallier, 2017).

The stability of an emulsion denotes its ability to resist changes in its properties over time (i.e., higher emulsion stability implies slower change in emulsion properties). Instability of an emulsion may be physical or chemical in nature. Chemical instability, which results in an alteration in the chemical structure of the lipid molecules due to oxidation or hydrolysis. Physical instability results in an alteration in the spatial distribution or structural organization of the globules (i.e., the dispersed phase of the emulsion). The important mechanisms responsible for the physical instability of emulsions can be divided into two categories: gravitational separation and droplet aggregation.

Gravitational separation involves the movement of emulsion droplets due to the fact that they differ in density from the surrounding liquid. If the droplets have a lower density than the surrounding medium, they tend to move upwards, a process referred to as creaming. Droplet aggregation is said to occur when droplets stay together for a time much longer than they would in the absence of colloidal interactions, (i.e., than can be accounted for by collisions due to Brownian motion). Flocculation is one of the main mechanism responsible for the physical instability of droplets through aggregation. Flocculation of droplets is defined as the aggregation of droplets to give three-dimensional floccules, wherein the droplets remain as individual entities. Flocculation occurs as a result of collisions; the extent of flocculation is determined by both the total number of droplet collisions per unit time per unit emulsion volume.

Kinetics aspects of agglutination

- Bovine milk is stored in the cold under quiescent conditions, a cream layer will
- form due to the rise of milk fat globules
- Rate of rise of the milk fat globules is accounted for by stokes' law
- Milk fat globules tend to rise in large clusters, which rise at a higher rate than individual globules
- Collision of globules is a prerequisite for both flocculation and clumping and for coalescence

- Flocculation rate of fat globules by agglutinin is predicted by

$$J= 4/3kTN^2/\eta$$

Where j is no of collision per unit time and unit volume, K is Boltzmann constant, T is absolute temperature, N is no of partials per unit volume and η is viscosity.

CREAMING OF MILK

Because milk fat has a lower density than milk plasma, it tends to rise under the influence of a gravitational or centrifugal force. For perfect spheres, the rate of rise, v, is given by Stokes' Law:

$$V= 2G /9\eta\ (\rho_s-\rho_f)\ r^2$$

Where G - Acceleration due to gravitational, ρ_s - Density of the skim milk, ρ_f - Density of the fat, r- Radius of the fat globule, η - Viscosity of the skim milk.

At 16^0C the average density of milk fat is 0.93 and skim milk is 1.036. Larger molecules rise several times faster than smaller ones and consequently collide with the slowly moving small globules, forming clusters which rise at an increased rate, pick up more globules and continue to rise at a rate with the effect of increased radius.

COLD AGGLUTINATION

When bovine milk is stored in the cold under quiescent conditions, a cream layer will form due to the rise of milk fat globules. However, the rate of rise of the milk fat

globules is considerably faster (30min) than can be accounted for by Stokes' law for individual globules.
This is due to the fact that milk fat globules tend to rise in large clusters, which rise at a considerably higher rate than individual globules. The clustering of milk fat globules during cold storage markedly resembles the agglutination of bacteria or red blood cells, due to the action of the immunoglobulin IgM. Hence, the clustering of milk fat globules in the cold is referred to as cold agglutination. Cryoglobulins are complexes of immunoglobulins (predominantly IgM) and possibly other proteins like lipoproteins present on fat globule membrane. They are less soluble below 37^0C and precipitate to an increasing extent the more the temperature is lowered and redisperse on warming (Keenan and Mather, 2006).

Coalescence: An irreversible increase in the size of fat globules and a loss of identity of the coalescing globules.

Flocculation: A reversible agglomeration/clustering of fat globules with no loss of identity of the globules in the floc; the fat globules that flocculate.

Cryoglobulin precipitates: An immunoglobulin in milk forms a complex with lipoproteins. This complex, known as cryoglobulin precipitates.

Agglutinin: Protein that function as part of the immune mechanism of body, Milk contains immunoglobulin IgM, acts as agglutinin Agglutinin is a substance that causes particles to coagulate to form a thickened mass, this phenomenon known as agglutination.

Cryoglobulins: IgM forms a complex with lipoproteins known as cryoglobulins.

Euglobulin: Simple proteins that are soluble in dilute salt solutions and insoluble in distilled water.

MECHANISM OF COLD AGGLUTINATION

When aggregation of the cryoglobulins occurs in the cold they may precipitate on to the surfaces of large fat globules causing them to agglutinate probably through a reduction in surface potential. The cryoprecipitate globulins may also form a network in which the fat globules are entrapped. This is main mechanism of creaming trough cold agglutination.

Two most important phenomena related with the mechanism for cold agglutination of milk fat globules are 'Merthens effect and the 'Samuelson effect'. Merthens (1933) observed that milk reconstituted from homogenized skim milk and unhomogenized cream has poor creaming ability (''Merthens effect''). It was proposed initially that this is due to denaturation of the agglutinin on homogenization. The mixture of pasteurised-unhomogenized and homogenized –unpasteurized skim milk can induce cold agglutination and Samuelson et al. (1954) explained the reason that two components are required for cold agglutination: a homogenization-labile component and a heat-labile component ("Samuelson effect").

Mechanism for cold agglutination which involves three components: (1) the milk fat globules, (2) IgM, the heat-labile component, which functions as a cold agglutinin; and (3) the skim milk membrane (SMM), the homogenization-labile component, consisting of lipoprotein particles present in the aqueous phase of milk. These components interact through specific carbohydrate moieties. IgM can interact with both SMM and the fat globules, whereas SMM interacts with IgM only. Fat globules can be clustered to a limited extent by IgM alone, but clustering is considerably more extensive in the presence of SMM, which acts as a cross-linking agent.

FACTORS AFFECTING COLD AGGLUTINATION

1. **Concentration of cryoglobulins in milk:** It influences the rate creaming. Colostrum (rich in Ig) creams well and late lactation milk (deficient in Ig) creams poorly. And addition of blood serum and colostrum's enhances the creaming of milk.
2. **Fat Content:** In high fat content milk, the proportion of larger fat globules is high so collision between globules is greater and floccules formation is quicker and also the depth of cream layer is high because of the greater interstices of aggregates formed from large globules contain only 20% fat.
3. **Temperature:** Creaming is faster and complete at lower temperature (<20^0C) because of of the temperature dependent precipitation of cryoglobulins. After 37^0C there is a complete denaturation of cryoglobulins.
4. **pH:** Optimum pH is in the range of 5.5- 7. Acidification of milk decreases the cold agglutination behaviour because of the denaturation of cryoglobulins.
5. **Ionic strength:** Optimal concentration of monovalent captions for agglutination is about 0.04M.
6. **Size of fat globules:** Cold agglutination behaviour is less in small fat globules size because it need more amount of agglutinin to cover large surface area.
7. **Species:** Buffaloe , goat, sheep do not exhibit cold agglutination behaviour.

PROCESSING FACTORS

1. **Homogenization:** Homogenization of milk prevents this creaming by decreasing the diameter and size distribution of the fat globules, causing the speed of rise to be similar for the majority of globules. As well, homogenization causes the formation of a recombined membrane which is much similar in density to the continuous phase. It prevents creaming because of two reasons 1) Decrease in fat globule size so need more agglutinin for covering over all surface of smaller fat globules 2) Inactivation of some cryoglobulins during homogenization.

2. **Agitation:** Agitation during the initial stages of creaming promotes and enhances cluster formation and creaming possibly because of the increased probability of collisions and also lead to the deposition of all the cryoglobulin on to fat globule

surfaces. If cold. creamed milk is agitated gently , the clusters are dispersed and do not reform unless the milk is rewarmed to 40⁰C and then recooled. Violent agitation is detrimental to creaming possibly due to denaturation of the cryoglobulins and alteration to the fat globule surfaces.

3. **Heating:** Agglutination and creaming are impaired or prevented by heating owing to the denaturation of cryoglobulins. Addition of Igs to heated milk restores creaming (except after very severe heat treatment)

ROLE

Cold agglutination increases the speed of natural creaming. Raw milk and cream exhibit non-Newtonian rheological properties when they held under the conditions which favour cold agglutination of fat globules. Cold agglutination of fat globules concentrates bacteria in cream that is partly nonspecific flocculation.

CONCLUSIONS

Agglutination is partly achieved by cryoglobulins, which precipitate onto fat globules during cooling. Agglutination is inactivated by heating and homogenization. More research is needed to elucidate the agglutination process

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