Effects of Heat Treatment and Subsequant Storage on the Sedimentation of Milk Proteins

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1. Introduction

In 1953, Ramsdell and Whittier reported that heating milk for 10 minutes at 212°F. produce no measurable aggregation or coagulation of whey proteins. These results were based on centrifugation experiments in which the supernatant were anallysed for acid-coagulable nitrogen Edmendson and Tarassuk (1956) obtained similar results, but pointed out that some criteria were needed for differentiating casein from denatured whey proteins before such a concolusion could be drawn from these observations. Such confirmation would appear particularly desirabe in view of contradictory observations. Fer example, Patton and Josephson (1949), found evidence of an associations between the whey proteins and casein based on analysis for sulfhydryl groups. In 1954, Mc Gugan et al., showed that, when a mixture of sasein and B-lactoglobulin was heated at 85°C for 30 min. ot pH 6.9, the B-lactoglobulin was converted into a form which moved with α -casein during moving- boundary electrophoresis. They postulated a complex between the two proteins.

Since that time many investigators have alse suggested a complex between k-casein and B-lactoglobulin. Zittle et al. (1962), demonstrated by means of moving-boundary electrophoresis, Ultra centrifuge stabilization by calcium chloride and clotting by rennin that mixtures of k-casein and B-lactoglobulin complex upon heating.. Morr et al. (1962), used polarization of fluorescence technique to demonstrate the interaction. Trautman & Swanson (1958), Sawyer et al. (1963) and Sawyer (1969) used sulfhydryl blocking agents and moving - boundary electrophoresis to provide additional evidence for the interaction between B-lactoglobulin and k-casein. Aurand et al. (1963), used gel and immune-electrophoresis to show that a complex formed on heating between B-lactoglobulin and casein and between casein and serum albumin. Hartman and Swanson, (1965) demonstrated by means of polyacrylamide disc electrophoresis, a complex beings to occur between k-casein and B-lactglobulin when they are mixed in equal parts by weight in a solution of milk salts and heated to 70°C for 30 min. They interact completel when heated to 85°C under the same conditions.

Raw skimmilk contains an appreciable amount of soluble casein, Von Hippel & Waugh (1955), which under the influence of heat may become centrifugable. However, any increase in centrifugable nitrogen beyend 78% of the total nitrogen indicates that denatured whey ptoteins either are sedimenting separately or are being carried down with the casein. In considering this approach, Hilgeman and Jenness (1951) observed a rapid change in soluble calcium and phosphorus immediately following the heat treatment of milk. Christianson et al. (1954) and Tessier & Rose (1958) reported decreases in the concentration of ionized calcium when milk was subjected to heat. Demott, (1969) reported that B-lactoglobulin, heated at 65 to 75°C for 30 min. did not bind any more calcium than that unheated.

Christianson et al. (1954) and Demott (1968) further reported that decreased ionized calcium from heating, apperaed to be reversible after 24 hr. of storage. Similar changes in centrifugable nitrogen might occur.

Therefore, the purpose of this investigation was to determine the effect of different heat treatments and subsequant storage on the changes in centrifugable nitrogen as a function of time following several heat treatment.

2. Experimental Procedure

Portions of skimmilk were heated in a tubu-

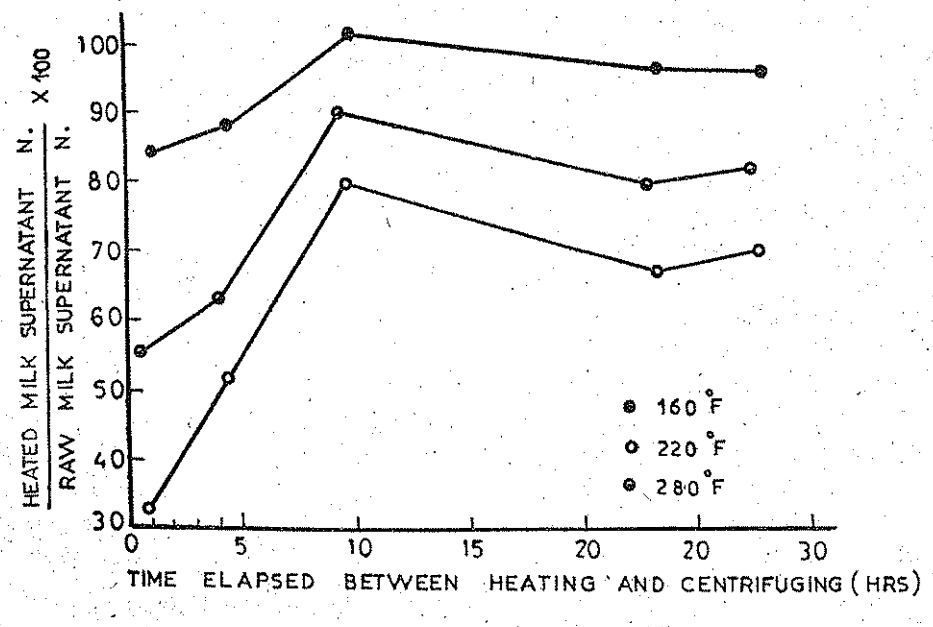
lar heater for one min. at 160, 220, or 280°F. and immediately colled to 36°F. Samples of each milk, plus the raw skimmilk control, were centrifuged at intervals overa a 46 hr. period in the K roter of the Spince Model E Ultracentifuge. Centrifugation was carried out under refrigeration for two hours. using an average centrifugal force of 113.000 xG. Under these conditions, essentialyy all of the centrifugable casein will be removed. However, analysis of the supernatant liquids from the raw skimmilk revealed variations related to the actual temperature of centrifugation, which varied in different runs between 40° and 50°F. Therfore, the analysis of the serums from the heated samples of skimmilk have in each case been referred to the corresponding values obtained with the unheated sample.

Supernatants and gels were analysed for nitrogen by the method of Kjeldahl.

3. Results and Discussion

Results of the experiment are presented in Fig. 1. The supernatant nitrogens in each of the heat -treated samples are plotted as percent of those found in the raw skimmilk control. immediately following the heat treatment, all samples show a marked decrease in protein content. The decrease is roughly proportional to the heat treatment in two cases. The analytical data suggest that some protein breakdown may have occurred in the sample heated to 280°F. Since the treatment at 220°F. produced the maximum effect, calculations will be based on this sample. A reduction in supernatant nitrogen to 33% of that found in raw skimmilk supernatant corresponds, in this case, to the centrifugation of 92% of the total nitrogen. Thus there can be no question that whey proteins are being centrifuged immediately after this heat treatment.

All three samples showed a rapid shift in supernatant nitrogen following the heat treatments. The maximum observed at ten hours. The nitrogen values may indicate a preferential solubilization of B-casein (Sullivan et al, 1955). Results obtained at 46 hours are not included in fig. 1. since they reveal no significant change from those at 28 hours. In approximately 24 hours, each sample shows a reversion to what appears to be an equilibrium value characteristic of the previous heat treatment. It. is not clear at present whether this increase in soluble nitrogen is largely due to a re-establishment of the equilibrium between soluble and micellar casein or to a resolubilization of the whey proteins.



Summary

The effect of different heat treatments and subsequent storage on the sedimentation of milk proteins was studied.

Samples of skimmilk were heated at three different temperatures (160°, 22° and 280°F) for one minute. Heat treated samples and raw skimmilk samples were centrifuged at intervales and supernatants nitrogen was determined.

Immediately following the heat treatment such a marked drop in the supernatant nitrogen was observed. Thus it must be concluded that whey proteins are being sedimented. A rapid shifts in supernatant nitrogen was found during the subsequent period of storage, and the maximum values were obtained afterten hours of storage. Later on an equilibrium occured at the nitrogen values dependent on the severity of the heat treatment.

It is not clear at present whether this increase in soluble nitrogen is largely due to re-establishment of the equilibrium between soluble and micellar casein or to a re-solubilization of the whey proteins.

ÖZET

Süt proteinlerinin sedimentasyonu üzerine ısı işleminin ve sonraki depolamanın etkileri.

Bu çalışmada farklı ısı işlemlerinin ve sonraki depolamanın süt proteinlerinin sedimentasyonu üzerine etkileri araştırılmıştır.

Denemede materyal olarak kullanılan yağsız süt üç farklı sıcaklık derecesinde (160°, 220° ve 280°F) 1 dakika süreyle tutulmuştur. Isı işleminden geçmiş örnekler ve yağsız çiğ süt örneği belirli periyodlarda santrifüj edilerek supernatant nitrojenleri tayin edilmiştir.

Isı işleminden hemen sonra örneklerin supernatant nitrojenlerinde önemli bir düşüş saptanmıştır. Bunun serum proteinlerinin sedimentasyonundan ileri geldiği anlaşılmaktadır. Ancak daha sonraki depolama sürecinde supernatant nitrojeninde hızlı bir değişim izlenmiş ve 10 saat sonra maksimum değere ulaşmışlardır. Daha sonra nitrojen değerlerinde ısı işlemine bağlı olarak bir denge oluşmuştur.

Eriyebilir nitrojendeki bu artışın daha ziyade eriyebilir kazein ile misel halindeki kazein arasında dengenin tekrar kurulmasından mı yoksa serum proteinlerinin tekrar erir hale geçmelerinden mi kaynaklandığı henüz açıklığa kavuşmamıştır.

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