EFFECT OF CASEIN TYPE ON STABILITY AND BROWNING OF RECOMBINED EVAPORATED MILK

By

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ABSTRACT

EFFECT OF CASEIN TYPE ON STABILITY AND BROWNING OF RECOMBINED EVAPORATED MILK

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The effects of casein type on heat stability and browning of recombined evaporated milk were investigated to provide data essential for improvements in quality. Three types of recombined evaporated milk were developed utilizing sodium-caseinate, calcium-caseinate, and nonfat dry milk (micellar-casein) as casein sources. Whey protein concentrates, lactose, unsalted butter, water, and artificial evaporated milk flavor were used to provide the other components.

Sodium-caseinate milk exhibited the highest heat stability and browning reaction, whereas calcium-caseinate milk showed the lowest heat stability and browning.

Forewarming, addition of salts such as sodium citrate, and readjusting the pH to its original value resulted in a significant increase in heat stability.

Addition of sodium hexametaphosphate (0.13%) was found to reduce the browning reaction significantly for all three types of milk.

Heating was found to reduce the available lysine and increased the size of casein micelles, whereas sodium hexametaphosphate showed an increase in available lysine and reduced the size of casein micelles.

Sensory evaluation by a consumer panel showed no significant differences in acceptability when 10%, 20%, and 30% of micellar-casein milk were mixed with Na-caseinate milk or Ca-caseinate milk.

IN THE NAME OF ALLAH
THE MERCIFUL THE COMPASSIONATE

DEDICATION

To my wife Huda, our children Abdullah
and Munerah, and my entire family

ACKNOWLEDGMENTS

I would like to express my appreciation and gratitude to my major professor, Dr. John A. Partridge, for his guidance and encouragement throughout my graduate program. Deep appreciation and gratitude are expressed to Dr. J.R. Brunner for his valuable advice and constructive comments.

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INTRODUCTION

Evaporated milk is a commercially sterile, concentrated milk containing not less than 7.9% (w/w) fat and not less than 25.9% (w/w) total solids. The primary advantages of evaporated milk are convenience, low cost as compared to fresh whole milk, and relatively long shelf life. It is widely used for infant feeding, in cooking, for drinking when diluted or for adding to coffee or tea as whitener. The production of evaporated milk serves a twofold purpose. First, the milk can be preserved from a time of abundance to a time of scarcity. Second, evaporated milk can be easily transported from a locality of heavy production to an area where the production of milk is low.

Although domestic sales of evaporated milk have declined in most Western countries, export sales to the Third World are significant. Thus, there remains commercial pressure to solve outstanding difficulties (Hardy et al., 1984).

Production of evaporated milk has shown a remarkable fluctuation over the years (see Table 1). The cause of the decline in consumption and production of evaporated milk may be attributed to the economics of

Table 1--World Production of Evaporated Milk[a]

Area	1979-81[c]	1982	1983	1984	1985	1986
World	4,587,970[b]	4,811,139	4,499,250	4,614,310	4,683,919	4,605,506
N.America	1,336,912	1,340,682	1,299,213	1,373,300	1,378,802	1,274,116
S.America	182,528	172,850	200,904	182,949	184,759	189,700
Asia	572,341	658,600	599,881	623,413	653,937	675,560
Australia	81,493	67,000	67,201	68,659	66,274	65,170
Europe	1,835,308	1,995,252	1,745,611	1,835,939	1,853,260	1,794,101

[a]From FAO Production Yearbook, 1982, v. 36; 1985, v. 39; and 1986, v. 40.

[b]metric ton

[c]average annual production of evaporated milk

production, competition from new products, and/or decrease in exports.

Physical changes in improperly heated evaporated milk may prevent it from being placed on the market. Since the evaporating process may involve thousands of pounds of milk and many hours of labor, the financial loss to the manufacturer is great. It is important to maintain careful control over the processing of the product which would keep defects at a minimum.

Recombining can be used extensively in many developing countries with a limited indigenous dairy industry, in countries which have a marked seasonal shortfall in the milk supply, and in isolated areas. By utilizing material such as sodium caseinate, calcium caseinate, whey protein concentrates, milk fat and lactose, formulation of evaporated milk should be possible. The following advantages would be achieved: 1) reduced transport costs due to the elimination of transporting water, 2) lower packing costs due to availability of lower cost labor and materials, especially in developing countries, and 3) eliminating refrigeration in transport and storage of raw materials.

Sweetsur and Muir (1982a) stated that there are still a significant number of intermittent problems which cause difficulty within the manufacturing sector of the dairy industry. Most of these problems are associated with changes in protein stability in products

such as evaporated milk. They indicated that comparatively little research applicable to this product has been carried out.

Evaporated milk is a complex system containing as its major components protein, lipids, carbohydrates, and minerals. For evaporated milk to be commercially successful, the consumer must receive a product free of defects.

This present work represents a study of heat stability and browning (discoloration) of evaporated milk. The purposes of this project were:

- to study the effect of casein type on browning and heat stability of recombined evaporated milk.

- to investigate the possibilities of overcoming browning and heat coagulation problems.

The applications of this study are:

- to control or inhibit the brown color in recombined evaporated milk.

- to provide recombined evaporated milk having improved properties such as improved color, stability and flavor.

LITERATURE REVIEW

Milk Proteins

The proteins of milk are of great importance in human nutrition and influence the behavior and properties of the dairy products containing them. The major milk proteins can be classified into two broad categories: casein and serum (whey) proteins. Casein has been described by Brunner (1976) as "those phosphoproteins precipitated from raw skim milk at pH 4.6 at 20° C." Approximately 80% of the protein in bovine milk consists of four phosphoproteins, the α_{S1}-caseins, α_{S2}-caseins, B-caseins, and K-caseins (Holt, 1986).

α-caseins

α_{S1}-caseins, along with the α_{S2} caseins, make up the calcium-sensitive α_S fraction. Brunner (1977) defined α_S-caseins as all phosphoproteins that are precipitated by low concentrations of calcium and accounting for 50-55% of whole casein. Whitney et al. (1976) listed some of these chemical and physical properties. They can be precipitated in 0.4M $CaCl_2$ at

pH 7.0 and at a temperature of 4°C, and K-casein is able to stabilize them against precipitation.

B-caseins

B-caseins account for 30-35% of whole casein. Although sensitive to calcium, B-caseins are not precipitated by concentrations sufficient to precipitate α_{S1}-caseins. When sensitized, they form colloidal suspensions rather than the copious precipitate encountered with α_{S1}-caseins (Brunner, 1977). Swaisgood (1973) stated that there are two distinguishing characteristics of B-casein: a strongly temperature-dependent association and the temperature dependence of its solubility in the presence of calcium. Brunner (1977) indicated that B-caseins possess temperature-, concentration-, and pH-dependent association-dissociation equilibria. At a temperature below 8°C or at high values of pH, dissociation into monomers occurs. At high temperatures and near neutrality, association into threadlike polymers takes place. The kinetic association is much lower than those of α_{S1}-caseins.

K-caseins

K-caseins constitute about 15% of whole casein and were first recognized as the calcium-insensitive fraction in micellar casein (Waugh et al., 1970). In

the presence of calcium ions, K-caseins interact with calcium-sensitive α_{S1}- and B-caseins to form thermodynamically stable micelles (Waugh, 1971). Vreeman et al. (1977) stated that K-caseins occur as a mixture of polymers held together by disulfide bonds, and an equilibrium is established between the polymers and monomers in a few hours. The authors indicated that when the K-caseins are converted completely to monomers by reduction of the disulfide bond by a suitable disulfide reducing agent, they possess considerable heterogeneity, consisting of a major carbohydrate-free component and at least six minor components.

Swaisgood (1985) summarized several unique features of K-caseins: 1) They remain soluble in calcium solutions under conditions that precipitate all other casein components. 2) They are the only major caseins which have some forms containing carbohydrate side chains. 3) They have the capacity to stabilize other caseins against precipitation by calcium through formation of colloidal micelles.

γ-caseins

γ-caseins constitute about 5% of whole casein. A relationship between the components of whole γ-casein and B-caseins has been established. The γ-caseins apparently are the result of limited proteolysis of B-caseins by endogenous casein-associated proteases.

The γ-caseins behave similarly to B-caseins with regard to temperature concentration and pH-dependent association- dissociation equilibria (Brunner, 1977). Whitney et al. (1976) noted that all components of γ-caseins have been shown by amino-acid analysis, molecular weight, peptide maps, and partial amino-acid sequence to be identical with fragments of B-caseins.

Whey proteins

After the precipitation of caseins, the remaining proteins are found in the serum or whey and include bovine serum albumin, α-lactalbumin, β-lactoglobulin, immunoglobulin, and proteose-peptone fraction. The two proteins of this fraction present in the greatest concentration are α-lactalbumin and β-lactoglobulin.

α-lactalbumin. Broadbeck et al. (1967) identified this protein as one of the two subunits of lactose synthetase. Ebner (1971) stated that α-lactalbumin is necessary for the synthesis of lactose by its interaction with galactosyltransferase, an enzyme which catalyzes the transfer of galactose from uridine diphosphate galactose to N-acetylglucose.

β-lactoglobulin. β-lactoglobulin is the major serum protein, accounting for up to 50% of the non-casein protein of skim milk (Cerbulis and Farrell, 1975). This protein was the first protein to be crystallized (Palmer, 1934). McKenzie et al. (1972)

noted the presence of a free sulfhydryl group and two disulfide groups. Whitney et al. (1976) deduced the position of the two disulfide bonds.

Brunner (1977) summarized some features of this protein. Like other whey proteins, β-lactoglobulin undergoes time-and temperature-dependent thermo-denaturation at temperatures in excess of 65°C. Extensive conformontional transitions occur, exposing highly reactive groups, i.e., -SH, ϵ-NH_2, and hydrophobic areas. In the absence of casein micelles, as in whey, they participate in homogeneous and heterogeneous, irreversible association interactions, culminating in aggregation and precipitation, especially, in the isoelectric pH range. In the presence of casein, as in skim milk, the interaction is somewhat more specific.

McKenzie et al. (1971) indicated the heat-induced interaction of β-lactoglobulin and K-casein which proceeds through the mechanism of disulfide interaction involving the exposed β-lactoglobulin thiol group and the disulfides of K-casein. The presence of K-casein during the thermo-denaturation retards the complete aggregation of β-lactoglobulin. Also, the association of β-lactoglobulin with K-casein increases the heat stability of colloidal caseinate.

Casein Micelles

The casein in cows' milk occurs in the form of a colloidal dispersion which is responsible for the high turbidity of skim milk. The particles of this dispersion range in size from 20 to 600 nm and are referred to as casein micelles. The dry matter consists of about 93% casein; the remainder is inorganic material, of which calcium and phosphate are the main components and which is called colloidal calcium phosphate (Schmidt, 1982).

Brunner (1977) stated that about 80-90% of the caseins in normal milk are in the form of colloidally dispersed, nearly spherical micelles, and micelles are assembled from subunits of uniform size of about 10-20 nm in diameter, containing from 25-30 casein monomers. The remaining portion (10-20%) constitutes the "soluble" or non-micellized caseins. Brunner also indicated that the amount and composition of the soluble fraction varies with environmental factors such as pH, temperature, and calcium ion concentration. At pH 4.6, for example, the soluble caseins participate concomitantly with micelle dissociation and precipitation, and at temperatures below 8°C, a portion of micellar components, particularly B-caseins and K-caseins, dissociate into the serum, thus increasing the proportion of soluble caseins.

Casein Micelle Models

Several models have been proposed for the structure of casein micelles. The three general classes of models that have been reported are the coat-core models, internal structure models, and submicellar models.

Coat-Core Models. Waugh and his associates (1970) proposed a model of micelle structure characterized by formation of core polymers of α_{S1}- and B-caseinates upon addition of calcium ions. Colloidal stabilization takes place by the formation of a surface coat of K-casein which limits the growth of the micelle (Figure 1). Slattery (1976) suggested that the only K-casein in the micelle interior would come from "being trapped during rapid micelle formation." Sullivan et al. (1959) reported correlation between the concentration of K-casein and particle size of casein micelles. They found that smaller micelles contained a higher percentage of K-casein.

Parry and Carrol (1969) suggested that K-casein might serve as a "nucleation center" of the micelle with remaining parts of the micelle, including the surface, composed of α_{S1}- and B-casein (Figure 2). However, Slattery (1976) indicated that this micelle model should have surface properties similar to colloidal α_{S1}-casein, so that to change the method of interaction would require K-casein at the center to alter the

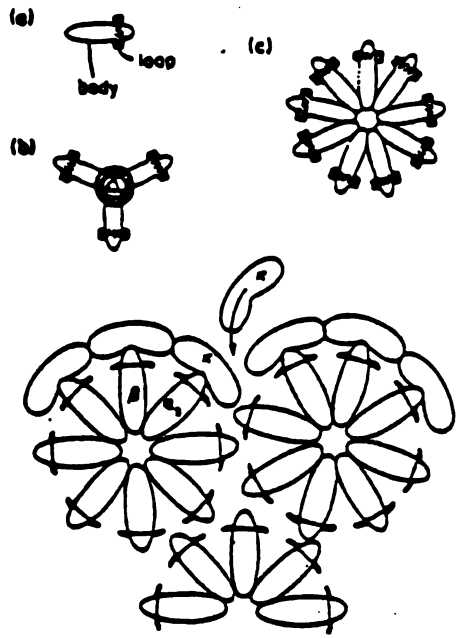


Figure 1. Waugh's proposed model for the casein micelle. (a) Monomer model of α_{s1}- or B-casein with charged loop. (b) a tetramer of α_{s1}-casein monomers. (c) planar model of a core polymer of α_{s1}- and B-caseins. The lower portion shows how K-casein might coat core polymers (Waugh et al., 1970).

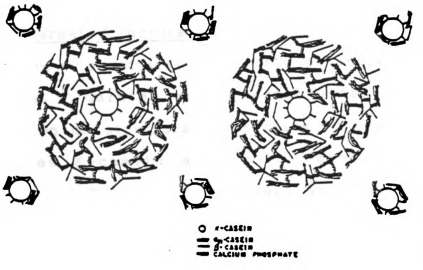


Figure 2. Casein micelle model proposed by Parry and Carrol (1969), showing the location of K-casein in the micelle.

properties of α_{s1}- and B-caseins at the micelle surface. Slattery calculated that there would be a "minimum of 90 molecular layers between the K-casein core and micelle surface" and indicated that it would be "impossible for the core to cause the surface to be noncoalescent." Ashoor et al. (1971), using a large cross-linked polymer of papain, concluded that all three casein proteins (α_{s1}-, B-, and K-caseins) occupy surface positions in approximately the same percentage as they are present in milk.

Internal Structure Models. These models are based on the known structural properties of individual casein proteins. Garnier and Ribadeau-Dumas (1970) used information on structural properties to propose a model that uses K-casein as the "keystone" of micelle structure. Trimers of K-casein are linked to three chains of α_{s1}- and B-casein which extend from the K-casein similar to a Y-like structure. The chains of α_{s1}- and B-casein may connect with other structures to form a loosely packed network (Figure 3). This model provides the demonstrated porosity, but calls for a uniform distribution of K-casein regardless of micelle size.

Rose (1969) proposed a model based on the known endothermic polymerization of B-casein (Figure 4). B-casein monomers begin to self-associate into chain-like polymers to which α_{s1}-monomers become

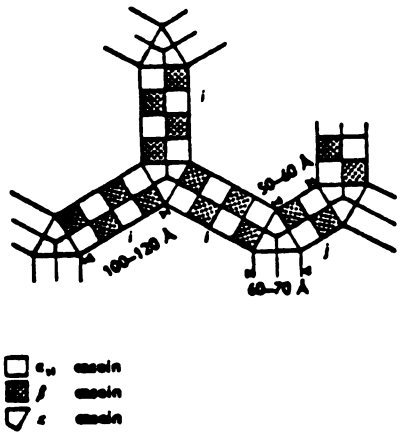


Figure 3. Structure of the repeating unit of the casein micelle (from Garnier and Ribadeau-Dumas, 1970).

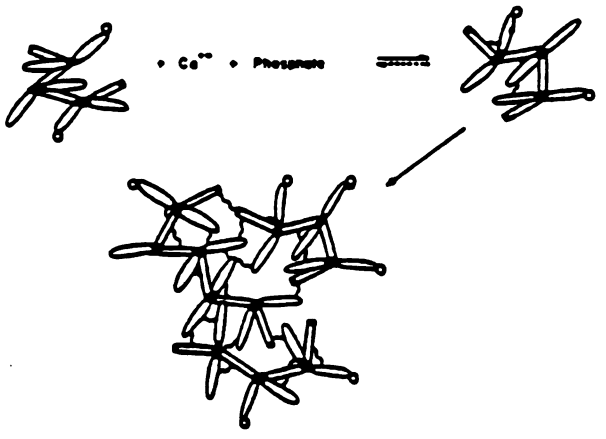


Figure 4. Schematic representation of the formation of a small casein micelle. The rectangular rods represent B-casein, the more elliptical rods represent α_s-casein, and the S-shaped lines show apatite chain formation. The circles represent K-casein (from Rose, 1969).

attached, and B-casein interacts with α_{S1}-monomers. B-casein is located internally and K-casein externally, but following coalescence a small amount of K-casein is located in an internal position. As the micelle is formed, colloidal calcium phosphate is added into the network as a stabilizing agent. This model demonstrated the role of colloidal calcium phosphate in micelle stabilization.

Submicellar Models. Shimmin and Hill (1964) were the first researchers to propose a submicellar model based on their study of casein micelle structure using electron microscopy. Morr (1967) suggested that α_{S1}-, B-, and K-casein monomers were aggregated by calcium into subunits stabilized by hydrophobic bonding and calcium caseinate bridges. Micelles are formed subsequently by aggregation of subunits by colloidal calcium phosphate (Figure 5).

Slattery and Evard (1973) proposed a model composed of submicelles of "spherical soap-micelle-like aggregates of all three of the major casein subunits" (Figure 6). The amount of α_{S1}-, B-, and K-casein present in each micelle could vary but the K-casein subunits are associated by lateral interactions. A partially hydrophilic (K-casein and attached carbohydrate) and hydrophobic (α_{S1}- and B-caseins with hydrophobic bonds and bound calcium) submicelle could be formed. Casein micelle formation could possibly then

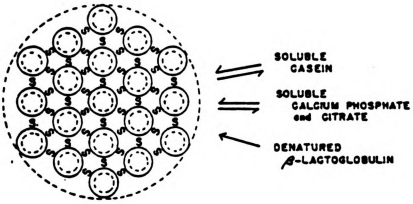


Figure 5. Structure of the casein micelle proposed by Morr (1967). The S-shaped lines represent calcium phosphate linkage between small spherical complexes of the α_{s1}-, B-, and K-casein.

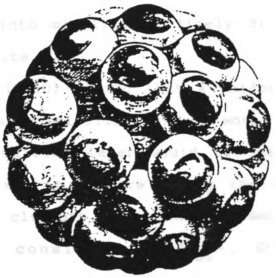


Figure 6. Casein micelle model proposed by Slattery and Evard (1973). The micelle is composed of about 40 submicelles. The lighter portions of the submicelles represent α_{s1}- and B-casein, while the darker portions represent the K-casein.

occur by aggregation and stabilization by calcium and phosphate salt bridges between submicelles.

Carroll and Farrell (1983) reported that the location of the K-casein is indeed related to casein micelle size. Casein micelles with the K-casein located predominately at the periphery of the micelle were found to have diameters of 142 ± 42 nm, while those with a diameter of 92 ± 22 nm had a more uniform distribution of K-casein. These conclusions are more in accord with the model of Slattery and Evard (1973). Schmidt (1980) adapted Slattery and Evard's idea of the uneven K-casein distribution and postulated submicelles with a hydrophobic core, covered by a hydrophilic coat in which the polar moieties of the K-casein molecules were accumulated in one area. The submicelles were assumed to aggregate into micelles by finely divided colloidal calcium phosphate (CCP).

Schmidt (1980) stated that a uniform distribution of CCP over the submicellar surface would be unrealistic since the resulting layer would be less than 0.1 nm thick; therefore, it is more likely that binding occurs via $Ca_9(PO_4)_6$ clusters. In Schmidt's model (Figure 7), submicelles consisted of α_{S1}-, α_{S2}-, B-, and K-casein, and the submicelles are linked together by $Ca_9(PO_4)_6$ clusters to form micelles. Almost all submicelles containing K-casein occur in the surface shell of the micelles. As the micelles grow, the

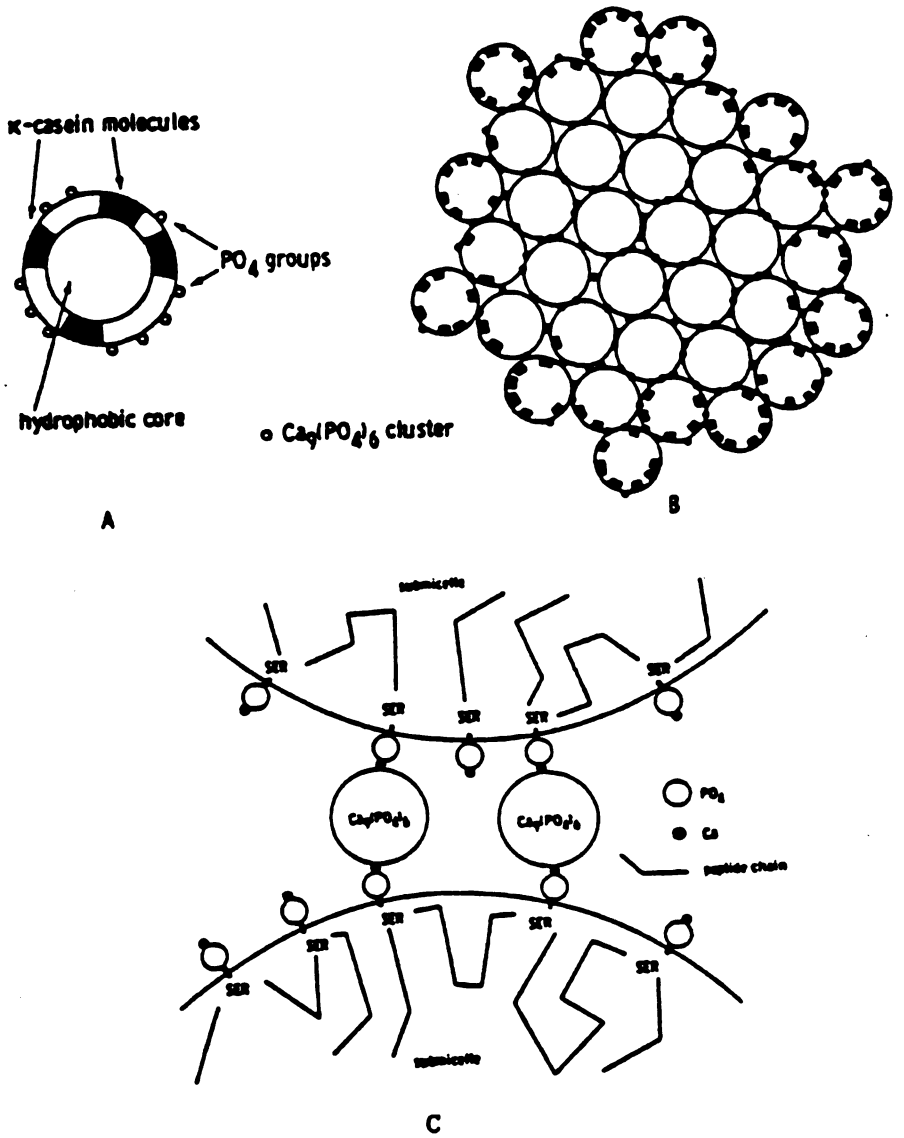


Figure 7. Casein micelle model proposed by Schmdit (1980). Schematic representation of a submicelle (A), a casein micelle composed of submicelles (B), and binding two submicelles via a $Ca_9(PO_4)_6$ cluster (C).

K-casein content of the surface shell increases, with an accompanying decrease in the number of phosphoserine residues. Big micelles have a higher surface K-casein content than small ones, so that micellar growth will ultimately come to an end.

Manufacture of Caseinates

Two basic types of casein are produced, and they are named in accordance with the coagulation agent employed. They are acid casein and rennet casein. Three types of acid casein are produced commerically: lactic, hydrochloric, and sulphuric casein. The other type of casein manufactured commerically is rennet casein, which is produced from skim milk that has been clotted by the action of rennin (Muller, 1982; Southward and Walker, 1980).

Casein is extracted from milk by the process outlined in Figure 8. All casein manufactured by the three processes described above are insoluble in water. However, addition of alkali (such as sodium or calcium) will cause the fresh curd or rehydrated casein to dissolve. Acid casein can be dissolved fully, or at least nearly completely, by reaction with alkali to pH 6.7-7.5, while rennet casein cannot be dissolved unless sufficient alkali is added to increase pH to above 9.0. These latter products can be effectively dispersed, however, at a pH of about 7.5 by using various complex

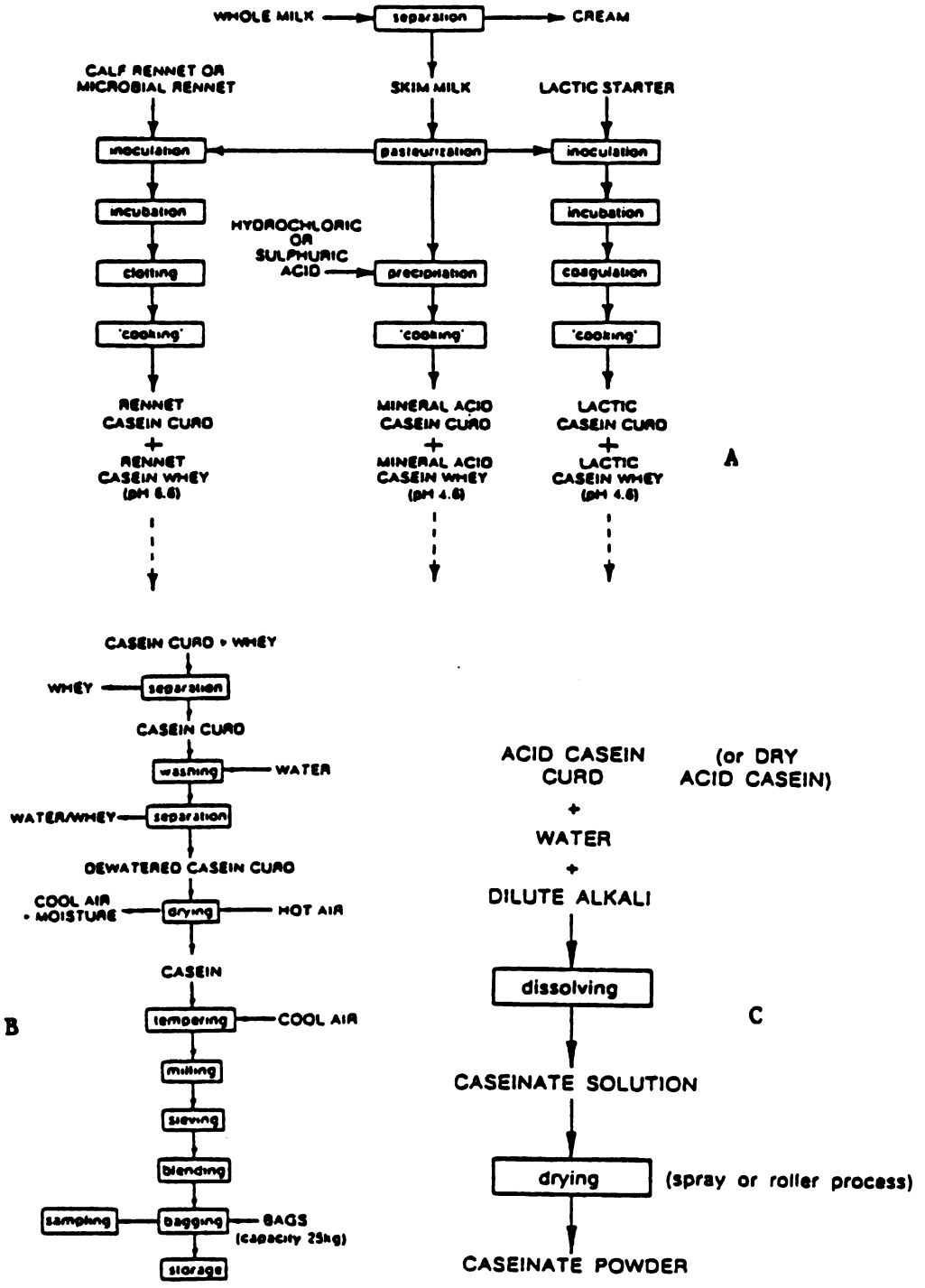


Figure 8. Processing involved in the precipitation of acid and rennet caseins from milk (A). Washing and drying of casein (B). Manufacture of dried caseinates (C) (from Southward and Walker, 1980).

phosphates. The phosphates sequester the calcium that was associated with the casein in the form of a stable casein-phosphate-calcium complex. The soluble products formed by the above reaction are known as caseinates (Southward, 1985).

Effects of Heat on Milk

The process of manufacturing recombined evaporated milk is now well-established, but it is not without problems. Mann (1976) found that the main manufacturing problem is the possibility of heat coagulation during sterilization, which depends on the heat stability of the milk. Another problem which takes place when milk is subjected to prolonged heating is a browning reaction. Among dairy products, browning is the most noticeable defect of sterilized evaporated milk. Lactose and casein are the two major constituents involved in the browning of evaporated milk. These problems of heat coagulation and browning in evaporated milk are especially of importance from an economic point of view. All milks are not similar with respect to the quantitative makeup of their components. This suggests that the intensity of heat required to initiate heat coagulation and browning will vary for different milks depending upon their composition.

Influence of Milk Composition on Heat Stability

Hunziker (1949a) and Rose (1963) reviewed the factors affecting the heat stability of evaporated milk and identified two main types of problems: 1) those which affect the properties of the milk, and 2) those which influence the efficiency of manufacturing. The first group includes: a) pH of milk; b) the milk proteins; and c) the salt-balance which includes the cations (calcium and magnesium) and the anions (phosphate and citrate). The second group includes: a) forewarming; b) concentration; and c) homogenization pressure.

Influence of pH. Many investigators have confirmed the important relation between the pH and heat stability of milk as measured by coagulation time. Fox and Hearn (1978a) concluded that the surface charge is important in determining the heat stability of milk and the pH change could result in low stability. Rose (1961a, b) found that the heat stability of milk at 140°C is very strongly affected by pH in the range 6.4-7.0. Pyne and McHenry (1955) found that the decrease in the pH of milk heated at 130°C was proportional to coagulation time. Nearly half of the total heat-induced acidity was derived from decomposition of lactose. The remainder was derived from the formation of tricalcium phosphate from phosphate liberated from casein and from

soluble phosphate initially present. They concluded that heat-induced acidity is normally a factor in the heat coagulation time.

Fox (1981) stated that three principal reactions account for the decline in pH: 1) production of organic acids from lactose, 2) precipitation of primary and secondary calcium phosphate as tertiary phosphate with concomitant release of H^+, and 3) hydrolysis of organic (casein) phosphate and its subsequent precipitation as $Ca_3(PO_4)_2$ with release of H^+. These reactions contribute 50%, 20%, and 30%, respectively, to the pH decline.

Singh and Fox (1987) reported that the complexing of β-lactoglobulin with K-casein on micelles at pH < 6.9 increased surface charge and hydration and prevented the dissociation of K-casein, thus stabilizing casein micelles in the pH range of 6.5-6.7. However, Singh and Fox (1985) showed that K-casein, possibly after complexing with whey proteins, dissociated from the micelle surface when milk was heated at 140°C for 1 minute at pH values > 6.9. The residual K-casein-deficient micelles are sensitive to precipitation in the presence of Ca^{2+}. However, stability increases at higher pH values (> 7.1) owing to increased negative charge on the K-casein-depleted micelles, thereby reducing the maximum and minimum in the heat coagulation time (HCT)-pH profile.

Rose (1962) suggested that the interaction between K-casein and β-lactoglobulin is implicated in the shape of the HCT/pH curve and that the failure of this interaction to occur at pH $\sim$ 6.9 may be responsible for the heat stability minimum. However, Morrissey (1969a) concluded that the maximum in the HCT/pH curve is a consequence of low stability at pH $\sim$ 6.9 which is attributed to heat-induced precipitation of Ca-phosphate on casein micelles sensitized by complexation with heat-denatured whey proteins. Creamer and Matheson (1980) showed that denatured whey proteins do not complex with casein micelles on heating milk at pH $>$ 6.85. Kudo (1980) suggested that the minimum in the HCT/pH curve of milk occurs because K-casein, with complexed whey proteins, dissociates from the micelles at pH 6.9, thereby reducing the heat stability of residual micelles. Singh and Fox (1986) found that whey proteins complexed with casein micelles after heating milk at $\geq$ 90 °C for 10 minutes at pH $\leq$ 6.9 while at higher pH values (e.g., 7.3) whey proteins and K-casein-rich protein dissociated from the micelles on heating.

Sweetsur and White (1975) observed that the lower the heating temperature, the lower the pH of milk at coagulation time (CT), and there was no general quantitative relation between the heat stability of the milk as measured by CT and either the rate of decrease

of pH or the pH of milk at CT. They also showed that increasing the protein content of milk by up to 50% only slightly increased the rate of acid production. Davis and White (1959) indicated that the source of protein-derived acidity is the formation of tricalcium phosphate following the libration of casein-ester phosphate, which becomes pronounced after a time-temperature treatment in excess of 30 minutes at 110°C. Sweetsur and White (1975) suggested that the acidity derived from the primary and secondary effects of a Maillard reaction between lactose and the ϵ-amino group of lysine residues will also contribute to the pH decrease with continued heating. Darling (1980) and Sweetsur and White (1975) reported that lactose-free milk also coagulates, at a higher pH.

Sweetsur and White (1974) showed that the shape of the HCT/pH curve was temperature-dependent and coagulation in the minimum region of the curve occurred prematurely due to precipitation of the larger, K-casein-deficient micelles.

Dalgleish et al. (1987) found that in milks at pH 6.9 and 7.2, heating to 130°C caused loss of protein from the micelles to the serum. As the heating time increased, more protein was released into the serum, until a maximum occurred at all pH values after about 20 minutes of heating. After this, the protein concentration in the serum decreased, so that the

non-sedimental protein became sedimentable either by self-aggregation or by binding to the micellar protein.

Fox and Hoynes (1975) showed that the addition of β-lactoglobulin (B-lg) to serum protein-free casein micelles (SPFCM) in milk had two effects on heat stability: 1) an increase in stability in the pH range of 6.4-6.8, apparently due to a gradual shift of the HCT/pH curve of SPFCM to more acidic values (an effect for which no explanation was offered) and 2) the introduction of a minimum at pH values > 6.8 which extended over an increasingly wide range as the level of added B-lg increased. In this range B-lg was considered to sensitize the casein micelles (presumably through interaction with K-casein) to heat-induced precipitation of Ca-phosphate. Greater phosphate precipitation is likely to occur at high pH values, which may offset the stabilizing influence of the increased protein charge.

Influence of Milk Salts. The salts are important from a nutritional point of view and because they determine the stability of milk proteins. Not all of the salts are dissolved. The casein micelles contain undissolved calcium phosphate which is called "colloidal calcium phosphate" (CCP).

Knoop et al. (1979) suggested that CCP is tricalcium phosphate, $Ca_3(PO_4)_2$, which is not very stable and might be transformed into a hydroxyapetite, $Ca_5OH(PO_4)_3$, as reported by Schmidt (1979).

Dickson and Perkins (1971) concluded that the amount of calcium bound to the casein is equivalent to the number of ester phosphate groups present. Rose (1962) confirmed that removal of colloidal calcium phosphate (CCP) increases stability at all values throughout the pH range 6.3-7.0 and showed that the minimum in the heat coagulation time (HCT)/pH curve disappears when more than 30% of the CCP is removed. Fox and Hoynes (1975) found that progressive removal of CCP, which results in an increase in non-micellar casein, increases stability at or below the pH of maximum stability, but removal of more than 40% of the CCP renders the caseinate system unstable at all pH values above 7.1.

Fox and Hearn (1978b) found that deposition of calcium phosphate on the casein micelles or on the casein-β-lactoglobulin complex results in an increase in sensitivity to calcium ions. Sweetsur and White (1974) concluded that the larger micelles are rendered more labile by deposition of calcium phosphate than the smaller micelles and consequently precipitates more rapidly. Dalgleish et al. (1987) noted that through heating the caseins became extensively dephosphorylated, and dephosphorylated protein was dissociated from the casein micelles during the first 20 minutes of heating. They found that dephosphorylated caseins were more susceptible to precipitation by Ca^{2+} than were the native proteins.

Aoki and Kako (1983), Kudo (1980), and Creamer et al. (1978) concluded that heating milk at 130°C and a pH of 6.9 caused loss of protein from micelles to the serum.

Horne and Davidson (1986) showed that higher levels of Ca^{2+} increased the size of the micellar core, thinned the stabilizing layer, and destabilized the system. Conversely, lowering the Ca^{2+} level increased the thickness of the stabilizing layer and made the system more stable. Horne and Parker (1981) reported that the addition of Ca would lead to a higher concentration of colloidal calcium phosphate. They also noted that the addition of phosphate would increase colloidal phosphate, or calcium phosphate itself, but did not have an equivalent effect on stability of milk to ethanol.

Singh and Fox (1986) showed that K-casein-deficient micelles were more sensitive to precipitation in the presence of Ca^{2+} than native micelles or whey-protein coated micelles. Probably the loss of K-casein from the micelle surface exposes the Ca^{2+}-sensitive caseins, resulting in their aggregation in the presence of Ca^{2+}.

Zittle et al. (1957) found that both heated and unheated β-lactoglobulin in the presence of calcium bound the same amount of calcium in the pH range of 6-8. Subsequently, Dellamonica et al. (1958) observed

that a dilute solution of β-lactoglobulin (1%) was not precipitated by calcium chloride when heated in the presence of casein. The reason for this, they postulated, was that the casein reduced the concentration of calcium chloride available to β-lactoglobulin.

Throughout processing of milk there was a gradual reduction of soluble calcium phosphate with a corresponding increase in calcium and phosphate bound to the colloidal phase. Sterilization of the concentrate resulted in a further reduction of soluble calcium phosphate and there was a transfer of calcium and phosphate from the serum to the colloidal phase (Hardy et al., 1984).

Sweetsur and Muir (1982a) found that the heat stability of concentrated skim milk is significantly correlated with soluble calcium in raw milk. Fox and Hearn (1978b) found that the stability of calcium phosphate decreases with increasing temperature and increasing pH. At 120 °C there appears to be a sharp decrease in ion solubility at ~ pH 6.8. Fox et al. (1967) reported that heat-precipitated calcium phosphate is much less soluble on cooling than indigenous colloidal calcium phosphate, especially if the temperature is > 110 °C. Visser (1962) stated that heat-precipitated calcium phosphate in milk is protected against sedimentation probably through association with

casein micelles. Kudo (1980) noted that the loss of calcium and phosphate from the milk serum was approximately the same at all pH values. However, variation in the amounts of calcium phosphate deposited on casein micelles could be another factor which might affect heat stability.

Pyne (1958) concluded that soluble salts were the principal factors influencing heat stability, but Rose (1962) maintained that they did so only in the presence of β-lactoglobulin. Rose (1961a) and Mettwally et al. (1978) observed that by increasing the calcium content, the maximum heat stability was decreased, and by increasing the phosphate content there was an increase in the maximum heat stability. This effect, however, was reversed at high levels of phosphate.

DeMan and Batra (1964) showed that when 60 mg/100 ml of calcium was added to skim milk, the ratio of ionic to soluble calcium shifted from 0.56 to 0.64, and the ratio of soluble to total calcium shifted from 0.33 to 0.42. This indicated that only one quarter of the calcium ions remained in ionic form. They also found that the destabilizing effect of adding 10 mg/100 ml of calcium ions was counteracted by approximately 60 mg of added citrate.

Dalgleish et al. (1987) agreed with Evenhuis and de Vries (1956) that heating had little effect on citrate in milk. Sweetsur and Muir (1982a) found that

the heat stability of concentrated homogenized milk could be altered markedly by including small changes in the levels of soluble calcium or phosphate ions.

Marshall and Green (1980) stated that ion binding by the various additives may be a contributory factor in the stability of casein micelles. Sweetsur and Muir (1980) found that the most effective, permitted additive to improve heat stability was a mixture of NaH_2PO_4 and Na_2HPO_4. This mixture (ratio 1:1) was added to milk before homogenization and concentration to a final level of 200 mg/100 ml concentrate.

Forewarming

Hunziker (1949b) stated that forewarming fluid milk was the most common method for inducing increased heat stability in evaporated milk. Tessier and Rose (1964) indicated that when milk is heated, the denaturing β-lactoglobulin can interact with itself, with soluble casein, and with K-casein exposed on the micellar surface. Feagan et al. (1972) suggested that the heat stability of the milk is dependent upon heat induced interactions involving the proteins, particularly β-lactoglobulin.

Muir (1984) reported that the effectiveness of the forewarming treatment is related to the extent of whey protein denaturation. He found that forewarming at 90°C for 10 minutes causes major denaturation of whey

proteins and results in a marked improvement of the initial heat stability of concentrate. Newstead and Bucke (1983) noted that the greatest heat stability of raw skim milk was obtained by using forewarming treatments of 110-120°C for 120-240 seconds. Morrissey (1969b) reported that preheating reduces the stability of milk not subsequently concentrated, with a minimum at 80-90°C for 10 minutes.

Newstead et al. (1979) stated that when forewarming was carried out following both homogenization and evaporation, no increase in heat stability was induced by the forewarming treatment. When milk was forewarmed after either homogenization or evaporation, the evaporated milk was considerably less heat-stable than normally processed milk in which the forewarming treatment preceeded homogenization.

Newstead et al. (1977) showed that evaporated milk from which the whey proteins had been largely removed was heat-stable whether the milk was preheated or not. Evaporated milk made from the original milk was heat-stable only if the milk had been preheated.

Morrissey and O'Mahony (1976) and Koops and Westerbeck (1970) suggested that variation in the response of individual cow milks to preheating is probably due to the variation in the β-lactoglobulin/K-casein ratio. Newstead et al. (1977) noted that the presence of native undenatured whey protein in

concentrated milks had a detrimental effect on heat stability which preheating helped to eliminate.

Sweetsur and Muir (1981) observed that forewarming at 150°C for 1.5 minutes increases the stability of unconcentrated milk throughout the pH range 6.6-7.3, particularly in the region of minimum stability. Webb and Bell (1942) found that preheating at ultra-high temperatures for short times produced a concentrate which is very stable to subsequent heat processing, but the product had a low viscosity.

Griffin et al. (1976) reported that preheating tended to increase the heat stability of milk when the pH of the milk was lower than the pH of maximum heat stability. When the pH of the milk was greater than that of the maximum heat stability, forewarming tended to reduce the heat stability. Pearce (1979) concluded that the only effect of forewarming was to change the pH of maximum heat stability from about 6.6 to 6.5. Rose (1962) found that forewarming milk at 120°C for 10 minutes decreased milk pH by about 0.09 and lowered the pH at which maximum heat stability occurs by about 0.16. He also found that the behavior of an individual milk after forewarming depended upon its original pH relative to the pH at maximum heat stability.

Vujicic et al. (1968) showed that forewarming caused a gradual reduction of soluble salts with a corresponding increase in calcium and phosphate bound to

the colloidal phase. Belec and Jenness (1960) indicated that the levels of soluble salts, especially Ca^{2+}, influence the response of milk to preheating.

Browning Reactions

Non-enzymatic browning reaction (Maillard reaction) occurs widely during processing and storage of food materials. The colors produced range from pale yellow to dark brown, depending on the type of food and/or the extent of the reaction.

Various types of browning reaction have been extensively reviewed by Hodge (1953) and Patton (1955). Jenness and Patton (1959) stated that lactose and casein are the two principal reacting materials for browning within a milk system. These substances appeared to "brown" readily when heated together, but did not "brown" when heated separately. The authors (1959) found that reaction in the "dry" state appeared to be on a 1:1 basis of glucose and ϵ-amino group of lysine. Other basic amino acids such as arginine and histidine may be involved secondarily. Adrian (1974) mentioned that the amino acid in proteins which appears to react most readily is the N-terminal residue of the chain. Basic amino acids, especially lysine, appear to be next in reactivity. Adrian further indicated that lysine is 5 to 15 times more often damaged than other amino acids; next in reactivity are sulfur-containing amino acids or,

sometimes, tryptophan. Patton and Flipse (1953) reported that the linkage between carbon one of lactose and the protein was at least 67% disrupted by reheating, illustrating that a portion of the complex was not heat stable. They also noted that the binding reaction started before color development.

Despite a number of publications on the subject, the procedure developed by Keeney and Bassette (1959) for determining hydroxymethylfufural has been used by several research groups (e.g., Zadow, 1970; Konietzko and Reuter, 1980, 1986; and Fink and Kessler, 1986). In this method, differentiation was made between free HMF and potential (total) HMF. Klostermeyer et al. (1981) reviewed the formation of HMF and its use as an indicator of the extent of the Maillard browning reactions and indicated that HMF concentration correlated directly with the degree of heat treatment. The authors (1981) found that galactose and glucose, the breakdown products of lactose, are much more reactive with protein than lactose.

Van Boekel and Rehman (1987) reviewed the formation of HMF, as schematically shown in Figure 9. After condensation of lactose [1] with lysine residue [2], a Schiff base is formed [3] which via Amadori rearrangement is converted to lactulosylamine [4] (1-amino-1-deoxy-ketose). The Amadori compound can be converted via enolization [5], [6] into compound [7].

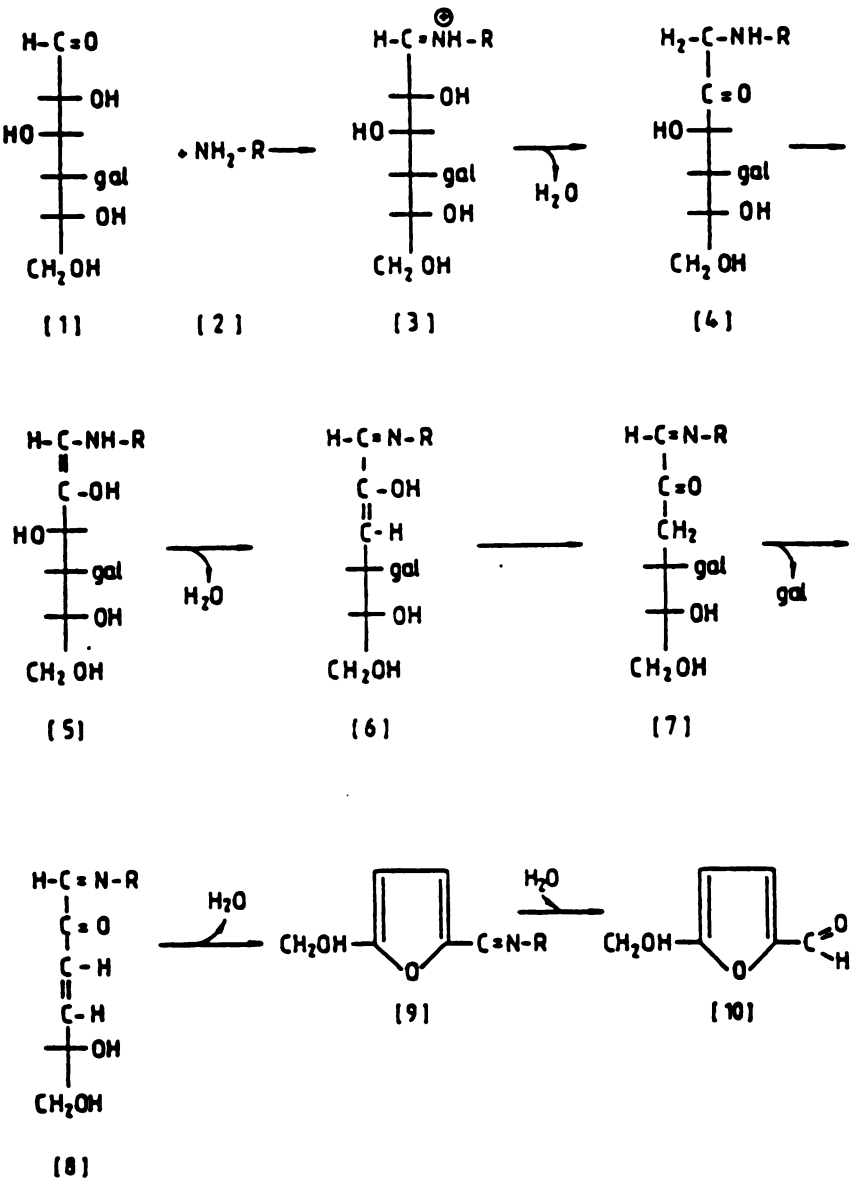


Figure 9. Schematic presentation of the formation of HMF [10] from lactose [1] and lysine residue [2]. gal= galactose. (from Van Boekel and Rehman, 1987).

Upon further decomposition, HMF [10] is eventually formed. Hydroxymethylfurfural may react further in the Maillard reaction.

Hodge (1953) indicated that the formation of HMF was one of a number of pathways whereby the nonenzymatic browning reaction proceeds. Keeney and Bassette (1959) showed that HMF formed a compound with 2-thiobarbituric acid which had a maximum absorption at 443 nm, and this absorption was proportional to the concentration of HMF formed. The authors (1959) also suggested a sensitive digestive procedure involving the heating of milk with added oxalic acid to convert early intermediates of the browning reaction to HMF.

Ellis (1959) demonstrated that spectroscopic examination revealed the presence of 5-HMF whose concentration was positively related to the intensity of browning. The author (1959) reported that the browning of D-glucose alone was due to the effect of heat and alkalinity, whereas in the Maillard reaction there was a loss of amino groups.

Patton (1955) stated that browning in heated milk resulted from the interaction of lactose with casein. He also reported that ϵ-amino groups of lysine were key reactants. An autoclaved system containing casein and a reducing sugar consistently showed the greatest amino acid losses in lysine. Significant losses of arginine,

histidine, and tryptophane were accompanied by lysine destruction.

Ellis (1959) indicated that the Amadori rearrangement mechanism was applicable only to N-substituted aldosyl amine but a reversed Amadori rearrangement was shown to occur for D-fructosyl-amine. The author (1959) reported that the enol may be converted into a Schiff base of a furaldehyde, or to a reductone by loss of water. He indicated that when D-glucose and glycine were heated at 96°C in a citric acid solution, an insoluble melanoidin was produced resulting in a decrease in total nitrogen and amino nitrogen. The Schiff base of 5-HMF-aldehyde, which had been found in the D-glucose-lysine and D-glucose-phenylalanine reactions, was polymerized to brown products.

A wide variety of flavor compounds have been isolated as products from the Maillard browning reaction. The Strecker degradation reaction is thought to be a source for producing characteristics of brown flavors (Reynolds, 1965). Ferretti et al. (1970) isolated and identified forty compounds from a lactose-casein browning system in the dry state.

Hodge (1953) demonstrated that browning, of whatever type, was caused by the formation of unsaturated, colored polymers of various composition. Moller et al. (1977) identified lactulselysine and

fructose-lysine by enzymatic hydrolysates of casein in stored (UHT) milk. Patton (1950) indicated that glycine-casein or degradation products of casein were essential to the conversion of lactose to HMF.

Reaction Conditions and Their Effects

Jenness and Patton (1959) reported that the principal factors affecting browning in fluid milk systems are: heat treatment, pH, total solids concentration, storage time and temperature, oxygen and various added compounds.

Temperature and Duration of Heating. Jenness and Patton (1959) indicated that heat treatment was the most important factor in the browning of fluid milk. Groux (1974), Lea and Hannan (1949), and Overby et al. (1959) all found that the intensity of the reaction increased as the temperature increased. Lea and Hannan (1949) showed that in the casein-glucose reaction the velocity of the loss in amino-nitrogen increases 40,000-fold when the temperature moves from 0°to 80°C. Duration is also an important factor controlling the extent of the browning reaction. Hurrell and Carpenter (1974) noted that almost as many ϵ-aminolysine groups had reacted in an albumin-glucose mixture after 30 days of storage at 37°C as had reacted when the same mixture was heated for 15 minutes at 121°C.

Ellis (1959) stated that the rate of the Maillard reaction appeared to increase with temperature. The reaction temperature has an effect on the reversibility of the intermediate stage of browning. The reaction is reversible at 25°C while at 100°C it is not, especially when glucose and glycine are in diluted solution.

pH. Adrian (1974) and Patton (1955) reported that acidification tends to inhibit the Maillard reaction and alkalinity increases the rate of reaction. Lea (1950) found that the rate of reaction increased approximately linearly with increasing pH values from 3 to 8 and possibly up to 10. Adrian (1974) reported that free amino acids have the same reactivity as those in proteins with the exception of tryptophan, which showed a reverse sensitivity, being more sensitive in acidic than alkaline media.

Ellis (1959) mentioned that browning occurred both in alkaline and acidic media, but the amino-carbonyl reaction was very weak in acid. He mentioned that the extent of reaction decreased with a rise in hydrogen-ion concentration, but the rate of reaction increased almost linearly with the hydrogen ion concentration. The intensity of the Maillard reaction generally increases with the elevation of pH. Most authors (Lea and Hannan, 1949; Tannenbaum, 1966) limit it to an interval between pH values of 3 and 9 or 10. In more acid or alkaline media, sugar degradation without interference from amino

acids tends to dominate, reducing the possibility of Maillard reactions.

Powell and Spark (1971) stated that the reactivity of amino groups is stronger when the amino acid is in anionic form, i.e, when the pH is superior to the pH 1 value. This depends on the amino acid character; it is low for the diamino acids (pH 3 for the aspartic and glutamic acids) and high for the basic amino acids (pH 10 for lysine and arginine). Ellis (1959) indicated that since the basic amino group disappears in the Maillard reaction, the pH of an aqueous solution of the reactants will decrease. Therefore, the initial pH of the solution, or the presence of a buffer, should have an important effect on the progress of the reaction.

Lea and Rhodes (1952) reported that 2-deoxy-galactose and glucos-amine (2-amino-glocose) both produced much more rapid browning with casein at a pH of 6.3 and a temperature of 37°C than did galactose or glucose separately. The glucosamine amino groups disappeared rapidly and discoloration and insolubility of protein developed.

Total Solids Concentration. Jenness and Patton (1959) reported that an increase in browning occurred when there was an increase in the concentration of milk solids. Patton (1952) showed that an increase in the color development (browning) in an aqueous casein

suspension occurred with increasing amounts of lactose following autoclaving.

Storage Time and Temperature. Data of Patton (1952) showed an increase of color intensity (browning) with both an increase in temperature and storage time. Very little color change was noted in samples stored at refrigeration temperature (4°C). Moller et al. (1977) reported that browning was very evident in UHT milk stored at 37°C for 3 years, and in this sample part of the lysine residue was accounted for by lactulose-lysine and fructose-lysine, which indicated that lysine residues could be engaged in sugar linkage. Ellis (1959) found that when dried mixtures, such as D-glucose and glycine were ground together and stored at 50°C there was no apparent change in color, but when small proportions (2, 5, and 10%, respectively) of water were added, the mixtures turned brown in 24 hours at 50°C. Kliman and Palansch (1968) reported that the HMF of spray-dried powders increased during storage at a temperature of 37° to 60°C; the powder was not immediately cooled after drying. The authors (1968) noticed a five-fold increase in HMF before a noticeable change in color of the powder occurred. Patton (1955) stated that concentrated products which were stored at room temperature for appreciable time were by far more susceptible to browning when sterilized than the unconcentrated milks.

Water. Water is absolutely necessary for the initial reaction to take place, but on the other hand it inhibits the browning reaction which comprises a series of dehydrations (Mauron, 1981). Wolfrom and Rooney (1953) showed that when the moisture content was either zero, or above 90%, no browning was observed at 65°C, and the maximum browning was reached when the water content was about 30%.

Lea and Hannan (1949) reported that maximum loss of ϵ-aminolysine groups was at a moisture level of 15-18%. Ellis (1959) indicated that the Maillard reaction is sensitive to the relative humidity of the air in contact with reactants, if the latter do not contain water.

Adrian (1974) reported a decrease of approximately 21% of available lysine in milk powder containing 10% moisture compared to the control with 4% moisture. Jenness and Patton (1959) indicated that milk dried to moisture levels below 5% displayed essentially no change in color following two years of storage at 37°C.

Oxygen. Browning intensity is often affected by the atmosphere but this action depends on complex factors in which amino acids do not necessarily interfere (Adrian, 1982). Hodge (1953) concluded that neither carbonyl-amino reaction nor caramelization were dependent upon the presence of oxygen to produce browning.

Ellis (1959) stated that in acidic solution, atmospheric oxygen favors both the interaction of amino groups and color formation. Jenness and Patton (1959) reported that oxygen is a factor which favors browning of milk. At most, its role is secondary since exclusion of oxygen is not completely preventive.

Various Added Compounds. Jenness and Patton (1959) stated that reducing sugars favor browning to a greater extent than nonreducing sugars. Burton et al. (1962) and Ellis (1959) reported that the presence of sulfites retarded nonenzymatic browning reactions.

Burton et al. (1963) reported that phosphates tend to increase coloration. Adrian (1982) mentioned that in acid media, copper, iron, and zinc reduce both browning and blocking of amino acids. In a weak alkaline medium, copper increases browning without affecting the loss rate of amino acids.

Nutritional Aspects

Rolls (1982) stated that the proteins of raw milk are readily digestible and of high biological value. Whey proteins are superior to casein, as the latter have a slight deficiency of the sulfur amino acids (methionine and cysteine). Ford et al. (1966) indicated that the more conservative treatments have relatively little effect on the nutritive value of milk proteins,

but the more severe procedure, such as in-bottle sterilization, result in a lowering of biological value.

Milk proteins may be affected by heat in two ways. The first is when proteins (particularly β-lactoglobulin) are denatured to some degree by any heating (in pasteurization, 10%; in-bottle sterilization, 100%). This does not affect biological value (Rolls, 1982). Secondly, the availability of amino acids, particularly of sulfur amino acids, may be reduced by protein-protein interactions. The availability of lysine may be reduced by protein-carbohydrate interactions, involving the formation of a Schiff base by the Maillard reaction (Finot, 1973).

Adrian (1982) stated that amino acids are subject to different types of degradation and destruction which can be classified as follows: 1) Destruction of amino acids may occur without any intervention of exterior agents. Losses are provoked by decarboxylation and condensation phenomena. These reactions have a nonenzymatic character because they are produced at high temperatures. 2) Amino acids are easily combined with reducing sugars at room temperature and even more easily combined during heating. The Maillard reaction is a complex phenomenon that makes the amino acids unavailable. It is responsible for a selective destruction of lysine and basic amino acids that is very detrimental to protein efficiency. 3) During the

development of the Maillard reaction, amino acids can be directly destroyed without previous blocking because of a reaction between reductones and amino acids.

Mauron (1981) indicated that heating milk under not too extreme conditions produces an almost pure early Maillard reaction, and lysine is the amino acid involved in the first place and the reaction takes place between the ϵ-amino group and the carbonyl group of lactose. Finot (1983), using a series of milk samples submitted to different heat treatments, showed that the only product formed during heating of milk is the Amadori rearrangement product, namely, ϵ-N-deoxylactosyl-lysine.

Adrian (1982) stated that the first compounds of the Maillard reaction are characterized by enzyme resistant linkages. The amino acids thus linked to the sugar cannot be enzymatically hydrolyzed. However, a chemical hydrolysis can break the link and regenerate the initial components, sugar and amino acids. These amino acids are said to be "blocked" or "unavailable," to distinguish them from those further involved in the reaction and irrecoverable by means of chemical hydrolysis that are "destroyed" or "lost."

Mauron (1981) mentioned that at least three mechanisms were responsible for the decrease in amino acid availability: 1) the involvement of an amino acid side-chain in the Maillard reaction, 2) the formation of cross-links between peptide chains through aldol or

internal condensations, and 3) the decrease in overall digestibility of the protein.

Brown et al. (1972) found that when a casein-glucose mixture loses 8% of its amino N by heating, the destruction of histidine reaches 17%; that of arginine, 22%; and that of lysine, 46%. The latter is essentially due to the amount of the free ϵ-amino groups of lysine. Adrian (1982) indicated that among the three basic amino acids only lysine is essential for the organism. Mauron (1981) found that the protein efficiency ratio of evaporated milk was the same as that of sweetened condensed milk, yet 20% of the lysine was made unavailable by a Maillard reaction in evaporated milk.

Dye-Binding Method for Estimating Available Lysine

Dye-binding procedures have been used for many years as a rapid indicator of the total protein content of milk samples and of other foods of almost constant amino-acid composition (Ashworth, 1966; Udy, 1971). Such procedures are thought to depend on the attraction between negatively charged dyes and the positively charged basic amino groups of lysine, arginine, and histidine (Figure 10).

Dye-binding capacity (DBC) may also be useful as an indicator of nutritional damage during heat treatment, as lysine can combine through its ϵ-amino group to form nutritionally unavailable derivatives

(Hurrell and Carpenter, 1975). A high concentration of milk fat or lactose does not interfere with binding capacity (Ashworth, 1966; Haga and Sugano, 1986).

Hurrell and Carpenter (1975) found that for many food materials, the dye-binding capacity (DBC) with Acid Orange 12 was equivalent to the sum of histidine, arginine, and lysine amino acids. Hurrell and Carpenter (1976) noted that only the lysine groups appeared to be propionylated (react with propionic anhydride), after which they no longer reacted with the dye (Figure 11). Therefore, measuring the difference in the dye-binding capacity of protein before and after masking the lysine group by proprionylation could form the basis of a procedure specific for lysine.

The propionylation procedure was accomplished by shaking for 15 minutes with 0.2 ml propionic anhydride and 2 ml sodium acetate solution (16% W/V). The lysine group was propionylated and propionic acid was released. The dye was added and the dye-binding capacity was measured. Dye-binding lysine was calculated by subtracting the DBC of the propionylated material, measuring "arginine + histidine," from that of untreated material, measuring "available lysine + arginine + histidine" (Hurrell et al., 1979).

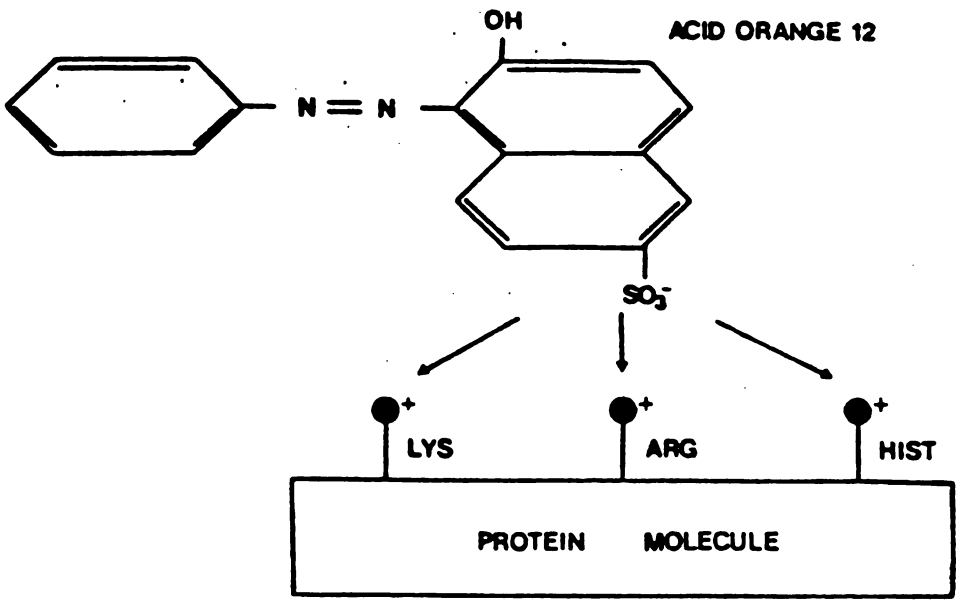


Figure 10--Reaction of Acid Orange 12 with Proteins.

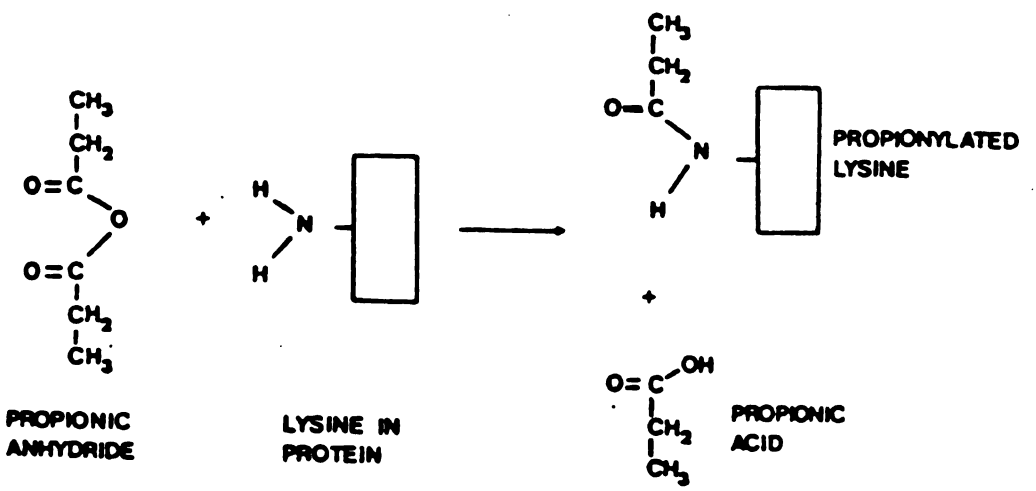


Figure 11--Reaction of Lysine with Propionic Anhydride.

ANALYTICAL PROCEDURES

Determination of Total Calcium

The standard titration solution was prepared by dissolving 10 grams of disodium ethylenediamine tetraacetate (Fisher Scientific Co., Fairlawn, NJ) and 2 grams of sodium hydroxide pellets in water which was made to one liter. The solution was standardized against a standard solution of $CaCl_2$ to be equivalent to approximately 1.0 mg of calcium per milliliter. Calcium indicator was prepared by grinding 100 grams of sodium chloride and 0.2 grams of ammonium purpurate (Sigma Chemical Co., St. Louis, MO) into intimate mixture.

An anion exchange column was prepared by placing 3 grams of the resin Duolite A-4 (Chemical Process Co., Redwood City, CA) in a column 8 X 150 mm. A short length of rubber tubing was attached to the capillary with a pinch clamp. The column was prepared for use by backwashing with water to stratify the resin particles and eliminate air, passing several portions of 1 N sodium acetate, and rinsing with distilled water.

Ten milliliters of milk were placed in a 100 ml volumetric flask and diluted with 20 ml of distilled

water. The sample was allowed to set for 10 minutes, after which 2.5 ml of 0.5 N sodium hydroxide was added. The acid dissolved the colloidal calcium salts and dispersed the casein on the acid side of its isoelectric point.

Addition of alkali brought the pH to 4.0 whereupon the casein was precipitated and the calcium remained in solution. The contents of the flask were made with water to volume and thoroughly mixed. The precipitate was filtered off, leaving a clear water filtrate. To eliminate the interference of phosphate, ten ml of the filtrate was passed through an anion exchange column, followed by two 10 ml portions of distilled water to rinse the column. The entire effluent was collected.

To determine total calcium, to 30 ml of ion-exchanged solution, 1 to 2 ml of 1.5N sodium hydroxide was added to bring the pH above 10 as determined with universal indicator paper. Then one scoop (0.2 gram) of prepared calcium indicator was added. The solution was titrated, with stirring, with standardized ethylenediamine tetraacetate solution to purple color that did not change on addition of another drop of titrant (Jenness and Patton, 1953).

Ionic Calcium Determination

A super centrifuge, Serval Type SS-1, was used to obtain ultrafiltrate product for ionic calcium

determination. Centrifuge tubes (50 ml) were fitted with a perforated plexiglass platform and support ring which served as a support for specimens placed in dialysis membrane bags (approximately ten centimeters long). One end of the dialysis membrane (cutoff > 10,000 daltons) was twisted and a small knot was tied and then filled with 15 ml of evaporated milk. The membrane was closed with a small knot and tied. The tube was wrapped with cheese cloth and placed into a 50 ml centrifuge tube and centrifuged for 15 minutes at approximately 5000 rpm (Figure 12). Four ml of distilled water was added to approximately 1 milliliter of centrifugal ultrafiltrate. One milliliter of 1.5 N sodium hydroxide and 0.1 gram of the prepared calcium indicator were added, and the solution was titrated with ethylenediamine tetraacetate solution to a purple color that did not change on addition of another drop of titrant. The titer was converted to calcium concentration (J.R. Brunner, personal communication).

Viscosity, Total Solids, Fat, Protein, and Lactose Determination

A Brookfield viscometer was used to measure viscosity. Total solids determination was accomplished by using the Mojonnier Milk Tester. The Babcock method was used to determine fat content. Protein determination was done according to the Lowery method (Lowery et al., 1951) as modified by Cooper (1977).

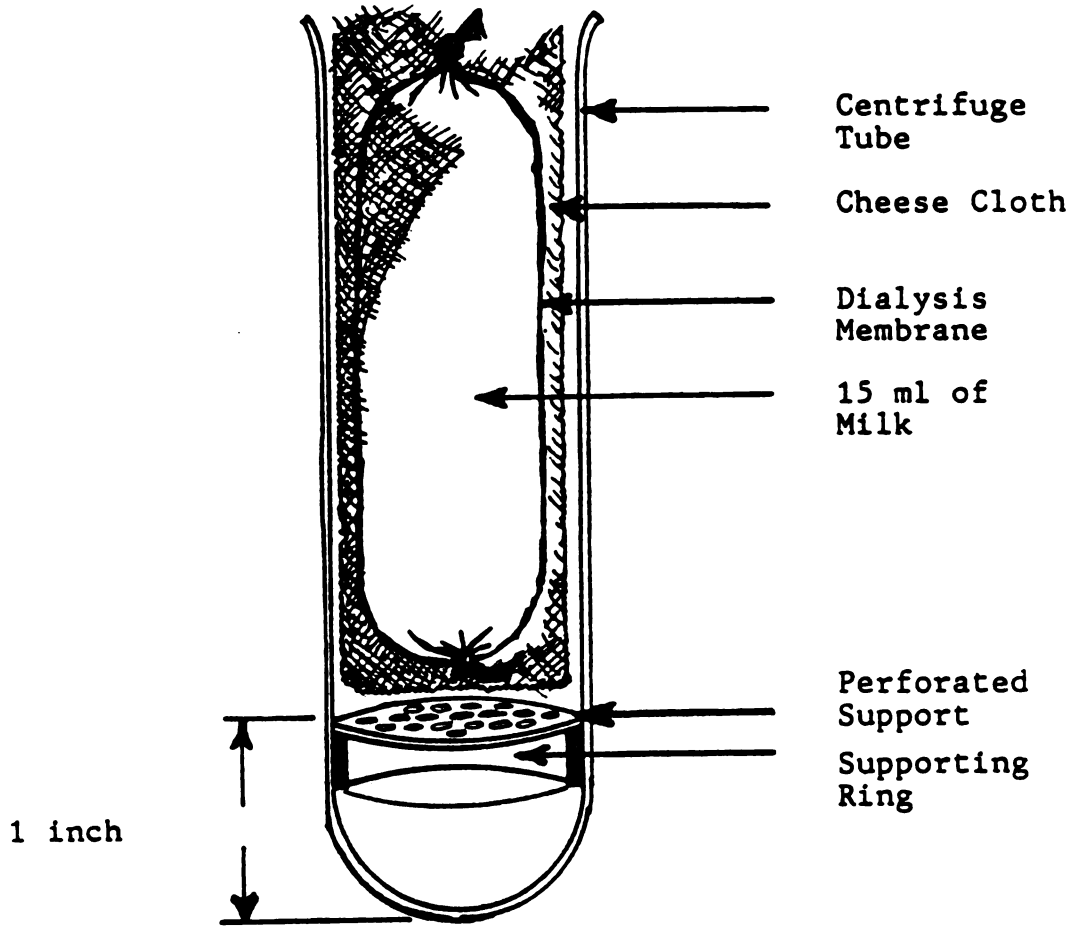


Figure 12. Device used to collect ultrafiltrate for ionic calcium determination.

Lactose was determined by the method of Dubois et al. (1956). Total nitrogen was determined using the microKjeldahl method (AOAC, 1985). The automated Kjeldahl unit consisted of a Büchi 332 distillation unit, a Büchi 342 control unit, a Metrohm 655 Dosimat, a Metrohm 614 Impulsomat, and a Metrohm 632 pH-meter (Brinkmann).

EXPERIMENTAL PROCEDURES

Formulation and Manufacturing Procedures

Materials used in the manufacture of evaporated milk and their sources included calcium caseinate, sodium caseinate, and milk calcium minerals (New Zealand Milk Products, Inc., Petaluma, CA); whey protein concentrate and lactose (Ridgeview, LaCrosse, WI); Carrageenan (FMC Corporation, Philadelphia, PA); and artificial evapoarted milk flavor (Haarmann and Reimer Corp., Springfield, NJ), nonfat dry milk (Valley Lea Dairies, Inc., Sebewaing, MI), and unsalted butter (Land-O'-Lakes, Inc., Arden Hills, MN).

The formulation of the components used in the manufacture of recombined skim evaporated milk (RSEM) (caseinate type) consisted of 3% (W/W) caseinate (Na-caseinate or Ca-caseinate), 1.5% (W/W) whey protein concentrate, and 4.0% (W/W) lactose. Two types of RSEM were prepared: Na-caseinate and Ca-caseinate. Micellar casein (M-CN), the third type, was prepared by dispersing 8.5% (W/W) of nonfat milk powder in water at 24°C (75°F).

Caseinates were very difficult to disperse in water. While Na caseinate was relatively easier to

disperse than Ca caseinate, both may form lumps with a tough, hydrated outer layer enclosing a dry core. Once these lumps had formed they were not easily broken up, even under relatively severe agitation. There are, however, several things which can be done to facilitate obtaining a satisfactory dispersion of caseinates.

Proper attention to temperature would noticeably reduce dispersion problems, particularly in situations where less than adequate agitation is available. In general, the following temperatures were used:

Calcium caseinate optimum temperatures were 21°-26°C (70°-80°F) and after initial dispersion, the temperature was raised to aid in complete dispersibility. Na-caseinate optimum temperatures were 60°-70°C (140°-160°F).

Dry blending other ingredients used in a formulation with the caseinates prior to dispersion in water was helpful in reducing the time of dispersion. Additionally, adequate agitation was effective in reducing the caseinates' tendency to float on the surface.

After dispersion, the mixture was forewarmed to 93.3 C (200 F) for ten minutes. Then the mixture was concentrated by evaporative condensation at 55°C (131°F) and 28 inches of vacuum until a total solid nonfat (TSNF) content of 18% was attained. Processing sequence

for the manufacture of recombined evaporated milk is shown in Table 2.

Immediately after the sterilization holding time, the tubes were cooled with cold water to 22°C (70°F). Hunziker (1949c) recommended that it should not take more than 15 minutes to cool the finished product and that cooling should be uniform which would require the even distribution of water around the tubes. Failure to do so causes a lack of uniformity in smoothness and color. The resulting milk was cooled in ice water and stored at ≃5°C (40°F) for subsequent analysis.

A shortage of calcium in Na-caseinate and Ca-caeinate milks compared to micellar-casein (M-CN) milk was found through total calcium assay. By calculating the shortage of calcium, 0.323% (W/V) and 0.457% (W/V) calcium salts were added to Ca-caseinate and Na-caseinate milks, respectively, to achieve nearly the same amount of calcium in the three types of milk. Tricalcium phosphate, a calcium source found naturally in milk, was dispersed in distilled water and then added to milk before forewarming.

After manufacturing of skim evaporated milk, Na-caseinate and Ca-caseinate milk, to which calcium salts were added, showed settling or precipitation after sterilization. To hold calcium in suspension and eliminate settling, carrageenan (0.02% W/V) was added. Carrageenan was dry-blended with calcium salts, then

Table 2--Processing sequence for the manufacture of recombined evaporated milk.

Production Step	Parameter Monitored	Standard Process
Standardization	TMSNF	TMSNF[a] 8.5% (W/W)
Forewarming	Temperature, time	93.3°C / 10 min
Concentration	Temperature, TSNF	55°C, TMSNF 18% (W/W)
Fat addition	Fat	MF[b] 7.9% (W/W)
Homogenization	Temperature, pressure	62.7°C, 2000 / 500 psi
Sterilization	Temperature, time	118.3°C, 15 min

[a]total milk solid nonfat

[b]milk fat

added to milk before forewarming. Additionally, 0.10% (W/V) of artificial evaporated milk flavor was added to improve the flavor of caseinate milk.

Evaluation of Forewarming

To determine the effect of forewarming on heat stability, samples were taken from the three types of skim evaporated milk which were prepared: Ca-caseinate skim evaporated milk, Na-caseinate skim evaporated milk, and micellar-casein skim evaporated milk. The first group of samples was not forewarmed. The second group of samples was heated to 93.3°C (200°F) for 5 minutes. The third group of samples was heated to 93.3°C for 10 minutes. The remaining samples were heated to 93.3°C for 15 minutes. Following the forewarming, the heat coagulation times (HCT) (in minutes) at 140°C (284°F) were determined.

Heat Stability

An oil bath equipped with a thermoregulator (+ 1C) and mixer was used for temperature stability evaluations and sterilization of milk samples. Temperatures ranging from 118.3°C (245°F) to 140°C (284°F) were used. Capped pyrex tubes (15 ml) were employed as sample holders and fitted with Spectrum-Cap Telfon discs having an inside diameter of 13 mm.

Heat stability was measured as the heat coagulation time (HCT), which is "the length of time

from the immersion of a sample of milk tube in an oil bath at 140°C (284°F) until the onset of coagulation as indicated by the appearance of coagulated particles."

Effect of Salts on Heat Coagulation Time (HCT) of Skim Evaporated Milk

To determine the effect of calcium, sodium citrate, disodium phosphate, and sodium hexametaphosphate (SHMP) on the heat coagulation time (HCT), a solution of 10% was made and 0.05 ml, 0.10 ml, 0.15 ml, and 0.2 ml of each salt solution were added to 15 ml of skim evapoarted milk. Then to each portion enough distilled water was added to eliminate the dilution factor. The entire mixture was well shaken. Control samples containing no salt were always run at the same time. To control pH change as a result of adding salts, adjustment of pH to 6.6 was made by slow addition of 1N HCl and 1N NaOH solutions.

pH

To determine the influence of salts on the HCT/pH profile of skim evaporated milk, 0.2 ml of 10% solution of calcium chloride, sodium citrate, disodium phosphate, and sodium hexametaphosphate were added to 15 ml portion of skim evaporated milk with continuous stirring. The pH of the milk samples, after adding the salts solution, was adjusted by slow addition, with constant stirring, of 1N HCl or 1N NaOH to various pH values between 6.2

and 7.0. All adjusted samples were held for at least one hour before the heat coagulation time (HCT) assay was determined at 140°C (284°F). Control samples containing 15 ml of skim evaporated milk and 0.2 ml of distilled water and no salt were run at the same time.

Effect of Intermittent Neutralization on the Heat Coagulation Time (HCT) of Skim Evaporated Milk

Twenty-five ml of skim evaporated milk at pH 6.6 was heated at 140°C (284°F) for 2.5 minutes. The pH of heated samples was determined after cooling the samples to 20°C (68°F). A subsample was then taken and the remainder was readjusted to pH 6.60 and heated at 140°C (284°F) for 2.5 minutes, after which the pH was again readjusted to 6.60 at 20°C (68°F). This heating and pH adjustment cycle was repeated every 2.5 minutes, following which 2.5 ml of each subsample was heated at 140°C (284°F) until coagulation.

Determination of Browning in Skim Evaporated Milk

The hydroxymethylfurfural (HMF) content was used as a chemical parameter in judging the browning reaction. Keeney and Bassett's (1959) method was used to measure HMF. This method is based on the digestion of intermediate amino-sugar compounds by oxalic acid and then reacting the filtrate with thiobarbituric acid (TBA).

HMF values were determined as follows:

- Skim evaporated milk samples were reconstituted with distilled water to a fluid milk basis (~9% TS) before testing. The milk samples were tempered to room temperature.
- Ten grams of milk were transferred into a 50 ml test tube. Five ml of oxalic acid solution (0.3 N) was added to the tube contents and then mixed thoroughly.
- The tubes were covered with parafilm and aluminum foil, then placed in a boiling water bath for one hour, after which they were removed and cooled in ice water to room temperature.
- 5 ml of 40% trichloracetic acid (TCA) was added to the tube contents and mixed, then the contents of the tubes were filtered through Whatman No. 42 paper.
- 4 ml of the filtrate was transferred into a 25 ml test tube, and 1 ml of 0.05 M thiobarbituric acid (TBA) was added and mixed.
- The tubes were placed in 40°C (104°F) water bath for 35 minutes, then removed from the water bath and cooled in ice water to room temperature.
- A blank consisting of distilled water, oxalic acid solution, TCA, and TBA was run along with the tested samples.
- Absorbance of solution was measured in the Spectronic 21 spectrophotometer at 443 nm which

measured free and potential HMF from browning intermediates. The results were expressed in terms of fluid milk equivalent (micromoles HMF per liter of milk).

Effect of Organic Acids

To investigate the effect of adding selected organic acids on browning reaction, 0.05 ml, 0.10 ml, 0.15 ml, and 0.2 ml of 10% solutions of lactic acid, acetic acid, tartaric acid, citric acid, gluconic acid, and propionic acid were added to 15 ml of skim evaporated milk. Distilled water was added to give a total volume of 15.2 ml and the pH was adjusted to pH 6.6. A heating temperature of 118.3°C (245°F) was carried out for 15 minutes. The HMF values were determined as described above. Control samples containing 15 ml of skim evaporated milk and distilled water and no acids were run at the same time.

Effect of Alkali Chemicals

To determine the effect of adding alkali chemicals on the browning reaction of skim evaporated milk, 0.05 ml, 0.10 ml, 0.15 ml, and 0.2 ml of 10% urea and 10% ammonium hydroxide were added to 15 ml of milk. Distilled water was added and the pH was adjusted to 6.6. Heating and HMF determination were monitored as before.

Effect of Salts

To study the effect of adding different types of salts on the browning reaction, 0.05 ml, 0.10 ml, 0.15 ml, and 0.2 ml of 10% solutions of sodium sulfite, sodium bisulfite, calcium chloride, disodium phosphate, sodium citrate, and sodium hexametaphosphate were added to 15 ml of skim evaporated milk. Distilled water was added and the pH was adjusted to 6.6. Heating and HMF determination were monitored as before.

Effect of pH

To determine the effect of pH values on the browning reaction of skim evaporated milk, milk samples were adjusted to different pH values ranging from 6.2 to 7.0 by gradually adding 1N HCl and 1N NaOH with constant mixing. Milk samples were equilibrated at 5°C for one hour. Heating and HMF determination were monitored as before.

Color Measurement

To determine the influence of adding different substances on the color of skim evaporated milk, 0.2 ml of 10% solutions of citric acid, urea, disodium phosphate, sodium citrate, calcium chloride, sodium hexametaphosphate, sodium sulfite, and sodium bisulfite were added to 15 ml of milk. After the sterilization process was completed (118.3°C for 15 minutes) and the

samples were cooled, the Hunter Color Difference Meter was used to measure the yellowness of the color (+b value).

Determination of Available Lysine

Hurrell and Carpenter's (1979) method was used to determine available lysine. In a centrifugal bottle, a 1.0 g sample (B) was shaken for 15 minutes on a laboratory shaker with 0.2 ml propionic anhydride and 2 ml sodium acetate solution (16% W/V). Then 40 ml of dye solution was added to the same flask and the mixture was shaken vigorously for 60 minutes. A sample (A) was treated in exactly the same way but without the addition of propionic anhydride.

Approximately 10 ml of each reaction mixture was centrifuged for 10 minutes at 5,000 rpm. An aliquot was diluted 50-fold with a buffer solution and the absorbance was measured at 475 nm. Dye concentration was determined from a standard curve and the amount of bound dye was calculated by difference. Since the excess dye concentration influences the amount of dye bound by the protein, readings were only accepted when the residual dye concentration was in the range of 1.0 - 1.9 m-mol/liter. Any run giving a value outside this range was repeated with a different weight of test milk sample.

Dye-binding lysine (available lysine) was calculated by subtracting the dye-binding capacity (DBC) of the propionylated sample (B) from that of the untreated sample (A).

Effect of Heat and Added Salts on the Availability of Lysine

Skim evaporated milk samples were heated at 118.3 C for 10, 15, and 20 minutes, respectively. Available lysine was determined as describe above.

To determine the effect of adding salts on the availability of lysine, 0.2 ml of 10% solutions of sodium citrate, calcium chloride, sodium hexametaphosphate, and sodium bisulfite were added to 15 ml of skim evaporated milk. The milk samples were sterilized at 118.3°C (245°F) for 15 minutes and available lysine was determined as described above.

Electron Microscopy Study

A JEOL-JSM-35C scanning electron microscope (SEM) was used to observe the shape and size of casein micelles. The effects of heating at 118.3°C (245°F) for 10, 15, and 20 minutes and addition of salts on casein particles was studied.

Two-tenths of a milliliter of 10% solutions of calcium chloride, sodium-citrate, disodium phosphate, sodium hexametaphosphate, sodium sulfite, and sodium bisulfite were added to 15 ml portions of skim

evaporated milk. The milk samples were sterilized at 118.3°C (245°F) for 15 minutes.

Carroll et al.'s (1968) procedure, with modifications in procedure and technique, was used to prepare milk samples for SEM study.

Skim milk was fixed in a 1% glutaraldehyde solution for 15 minutes: 0.2 ml of skim evaporated milk + 2 ml of glutaraldehyde. The fixed milk was diluted with water (1:50) and dispersed on coverslips by dipping the coverslips in the diluted fixed milk. The coverslips with dispersed milk were mounted on stubs using a double-stick tape. The coverslips were mounted just slightly off-center on the stubs so that a good conductive pathway could be maintained from the top of the coverslips to the metal stubs once they had been sputter coated. The mounted coverslips were air-dried, then transferred to desiccator under vacuum for 24 hours. The dried mounted coverslips were coated with gold by using Emscope Sputter Coater.

Sensory Evaluation of Recombined Evaporated Milk

Sensory evaluations of the final products were conducted in individual booths equipped with daylight fluorescent lighting. Three types of milk were prepared: Ca-caseinate evaporated milk, Na-caseinate evaporated milk, and micellar-casein evaporated milk. Nine samples were prepared by mixing the micellar-casein

evaporated milk with the other two types of milk in different combinations. Twenty participants were asked to judge the samples on a scale of 1 to 5. Eight of the 20 participants were considered dairy product experts. Figure 13 represents a copy of the evaluation form used.

Check the samples and choose one of the following numbers:

1. Very poor 2. Poor 3. Fair 4. Good 5. Excellent

Sample	Color	Visual texture (body)	Flavor	Mouth feel (smooth, coarse)	Odor

Figure 13. Sensory Evaluation Form

Statistical Analysis

Results were analyzed using one-way analysis of variance (ANOVA) for a completely random design (Gill, 1978). The data were analyzed by the following statistical model:

$$Y_{ij} = u + T_i + E_{(i)j}$$

where Y_{ij} = random sampling variables

u = true mean of the distribution of Y

T_i = effect of the ith treatment

$E_{(i)j}$ = random experiment error.

To analyze the results of sensory evaluations of the final products, a randomized complete block design (Gill, 1978) with the following statistical model was used:

$$Y_{ijk} = u + T_i + \delta_j + J_j + (T\delta)_{ij} + E_{(ij)}$$
$$(i = 1, 2, \ldots, t;\ j = 1, 2, \ldots, r)$$

where T_i = fixed effect of the ith treatment

δ = fixed effect of the jth block

J_i = random effect of the randomization

$(T\delta)_{ij}$ = the interation of the effects of treatments and blocks

$E_{(ij)}$ = random experiment error.

Differences between means were analyzed for significance by Tukey's test (Gill, 1978).

RESULTS AND DISCUSSION

Following the production of recombined skim evaporated milk, selected properties were determined (Table 3). Na-caseinate milk had a higher viscosity and exhibited a darker color and lower calcium (ionic and total) content than the other two types of milk.

Forewarming Effect on the Heat Stability of Recombined Skim Evaporated Milk

Forewarming is one of the most important steps in the manufacture of evaporated of evaporated milk from the heat stability point of view. For successful production of conventional evaporated milk, a preliminary heat treatment (forewarming) of milk before concentration confers a significant increase in the initial heat stability of milk after concentration.

Data in Table 4 show the influence of forewarming on the heat stability of recombined skim evaporated milk. Forewarming increased the heat coagulation time, significantly ($p \leq 0.05$). Comparison of M-CN milk with Ca-caseinate milk indicated there was similarity between the response of the two kinds of milk. Forewarming at 93.3°C (200°F) for more than ten minutes did not increase the HCT significantly ($p \leq 0.05$). However,

Table 3—Selected Properties of Recombined Skim Evaporated Milks.[a]

Property	NFDM	Na-Caseinate	Ca-Caseinate
pH	6.53	6.64	6.71
viscosity (cp)[b]	4.3	4.9	3.8
color	17.8	21.0	15.5
protein (%)	6.80	6.97	6.84
lactose (%)	9.82	9.70	9.75
total calcium (mg%)	252	237	243
ionic calcium (mg%)	29	19	23

[a]milk forewarmed at 93.3°C (200°F) for 10 minutes and sterilized at 118.3°(245°F) for 15 minutes.

[b]viscosity in centipoises and 25°C (77°F).

Table 4--Influence of forewarming on the heat stability of recombined skim evaporated milk

Forewarming		Heat Coagulation (HCT) at 140°C		
		Type of Milk		
Temperature (°C)	Time (min)	Micellar-casein	Na-caseinate	Ca-caseinate
without forewarming		4.13 ± 0.086a	5.67 ± 0.067a	3.28 ± 0.044a
93.3	5	5.04 ± 0.010b	9.12 ± 0.052b	4.82 ± 0.146b
93.3	10	7.94 ± 0.087c	11.38 ± 0.086c	6.63 ± 0.086c
93.3	15	8.02 ± 0.123c	12.21 ± 0.161d	6.75 ± 0.163c

Within each type of milk, means not followed by the same letter are significantly different ($p \leq 0.05$).

forewarming of Na-caseinate milk for 15 minutes at 93.3°C (200°F) increased the HCT significantly ($p \leq 0.05$).

According to Muir (1984), the effectiveness of the forewarming treatment is related to the extent of whey protein denaturation. Forewarming at 90°C (194°F) for 10 minutes causes major denaturation of whey proteins and results in a marked improvement of the initial heat stability of concentrates. Data in Table 4 agree with the findings of Muir (1984); forewarming M-CN milk at 93.3°C (200°F) for 10 minutes increased the HCT from 4.13 minutes to 7.94 minutes. Heat coagulation time was increased from 5.67 to 11.32 minutes and from 3.28 to 6.75 minutes for Na-caseinate and Ca-caseinate milks, respectively. Precipitation of whey proteins on casein micelle surfaces during forewarming prevents later coagulation by diminishing the number of K-casein sites available for clotting (Payens, 1978).

The higher protein and ionic calcium concentration in the concentrates lowers the heat stability (Morr, 1975). Therefore, it is necessary to forewarm the milk prior to its concentration to complex the denatured B-lactoglobulin with K-casein sites on the casein micelles and to lower soluble and ionic concentrations of calcium, thus providing adequate heat stability during the sterilization process.

There is strong evidence that whey proteins are implicated since their removal from milk renders the evaporated milk made from it heat stable without forewarming, as demonstrated by Newstead et al. (1977).

Forewarming improves the heat stability and viscosity and can be a factor in color development and flavor of evaporated milk. In general, temperatures from 93.3°C (200°F) to the boiling point for 5 to 10 minutes are employed in the manufacture of evaporated milk. Time and temperature depend on the seasonal variation of milk in composition, the concentration of evaporated milk, and the method of forewarming. Since there was no significant increase in HCT as a result of increasing the time of forewarming at 93.3°C (200°F) (except for Na-caseinate milk), the three types of milks were forewarmed at 93.3°C (200°F) for ten minutes to eliminate differences in the manufacturing processes.

Effects of Salts on Heat Coagulation Time (HCT) of Skim Evaporated Milk

Of primary importance in the manufacture of evaporated milk is the ability of concentrated product to withstand heat sterilization. Heat stability of milk can be defined either in terms of the time required to induce coagulation at a given temperature or the temperature required to induce coagulation in a given time. For convenience, a fixed temperature 140°C (284°F) was chosen.

Casein exists as complex proteins or micelles containing inorganic phosphate, calcium, and citrate. Many problems associated with heat-treated dairy products depend on the behavior of this system.

Sodium-caseinate skim evaporated milk was more stable than micellar-casein and Ca-caseinate skim evaporated milks. Heat coagulation times (HCT) were 11.25, 7.97, and 6.71 minutes for Na-caseinate, M-CN, and Ca-caseinate milks, respectively (see Tables 5, 6, and 7). Calcium chloride markedly decreased the HCT at all concentrations, whereas Na-citrate, Na_2HPO_4 and sodium hexametaphosphate (SHMP) increased the HCT.

Data in Table 5 show that addition of 10% solution of $CaCl_2$ to M-CN milk reduced the HCT significantly ($p \leq 0.05$) at all concentrations except that by increasing the amount of addition from 0.10 ml to 0.15 ml the reduction in HCT was not significant.

Na-citrate increased the heat coagulation time significantly at all concentrations; adding 0.2 ml of 10% Na-citrate (the highest concentration) to 15 ml of M-CN milk increased the coagulation time significantly from 7.97 minutes to 12.98 minutes. A comparison of Na-citrate with SHMP and Na_2HPO_4 shows that all increased the HCT significantly. Na-citrate was more effective in increasing the HCT of M-CN than SHMP and Na_2HPO_4 at all concentrations, but at low rates of

Table 5--Effect of salts on heat stabilty of micellar-casein skim evaporated milk

Rate of Addition		Heat Coagulation (HCT) at 140°C (min)			
		Salt Type			
10% Sol. (ml)	H_2O (ml)	$CaCl_2$	Na-citrate	Na_2HPO_4	SHMP*
0.0	0.20	7.97 ± 0.107d	7.97 ± 0.107d	7.97 ± 0.107d	7.97 ± 0.107d
0.05	0.15	6.81 ± 0.124e	8.25 ± 0.163d	8.92 ± 0.134c	8.04 ± 0.153d
0.10	0.10	3.69 ± 0.104f	9.38 ± 0.131c	9.08 ± 0.122c	8.31 ± 0.124d
0.15	0.05	3.25 ± 0.139f	11.27 ± 0.179b	12.11 ± 0.084b	10.42 ± 0.141c
0.20	0.0	2.44 ± 0.138g	12.98 ± 0.123a	12.69 ± 0.141a	10.22 ± 0.122c

*sodium hexametaphosphate

Different concentrations of salts were added to 15 ml of skim evaporated milk.

Means within each column not followed by the same letter are significantly different ($p \leq 0.05$).

Table 6—Effect of salts on heat stabilty of Na-caseinate skim evaporated milk

Rate of Addition		Heat Coagulation (HCT) at 140°C (min) Salt Type			
10% Sol. (ml)	H_2O (ml)	$CaCl_2$	Na-citrate	Na_2HPO_4	SHMP*
0.0	0.20	11.25 ± 0.122e	11.25 ± 0.122e	11.25 ± 0.122e	11.25 ± 0.122e
0.05	0.15	10.65 ± 0.198e	11.89 ± 0.140d	12.19 ± 0.176d	11.42 ± 0.188e
0.10	0.10	7.83 ± 0.124f	12.92 ± 0.108c	13.48 ± 0.213c	12.23 ± 0.151d
0.15	0.05	6.81 ± 0.141g	15.62 ± 0.185b	15.33 ± 0.181b	14.31 ± 0.219c
0.20	0.0	4.23 ± 0.145h	17.19 ± 0.137a	15.02 ± 0.123b	14.63 ± 0.122c

*sodium hexametaphosphate

Different concentrations of salts were added to 15 ml of skim evaporated milk.

Means within each column not followed by the same letter are significantly different ($p \leq 0.05$).

Table 7--Effect of salts on heat stabilty of Ca-caseinate skim evaporated milk

Rate of Addition		Heat Coagulation (HCT) at 140°C (min) Salt Type			
10% Sol. (ml)	H_2O (ml)	$CaCl_2$	Na-citrate	Na_2HPO_4	SHMP*
0.0	0.20	6.71 ± 0.141d	6.71 ± 0.141d	6.71 ± 0.141d	6.71 ± 0.141d
0.05	0.15	4.46 ± 0.120e	7.11 ± 0.208d	6.83 ± 0.122d	6.97 ± 0.078d
0.10	0.10	3.81 ± 0.104f	10.34 ± 0.194b	7.47 ± 0.123c	8.06 ± 0.228c
0.15	0.05	3.31 ± 0.091f	11.23 ± 0.181a	9.65 ± 0.126b	8.92 ± 0.108b
0.20	0.0	1.92 ± 0.179g	11.89 ± 0.173a	10.94 ± 0.115a	9.48 ± 0.099b

*sodium hexametaphosphate

Different concentrations of salts were added to 15 ml of skim evaporated milk.

Means within each column not followed by the same letter are significantly different ($p \leq 0.05$).

addition (0.05 ml) Na_2HPO_4 was more effective than the other two types of salts (Table 5).

After the addition of salt solutions to the Na-caseinate milk and Ca-caseinate milk there was no difference in the coagulation behavior except that the HCT of Na-caseinate milk was longer than that of M-CN milk, and Ca-caseinate milk gave the lowest HCT among the three types of milk (Tables 6 and 7).

It is known that the proteins contain negatively charged groups. They bind different cations such as calcium. A large part of the calcium is united with the casein to form the inorganic constituents of the casein micelles, and mostly are referred to as colloidal calcium phosphate (CCP). Heating of milk caused a gradual reduction of soluble calcium phosphate with a corresponding increase in CCP. Sterilization of the concentrate resulted in a further reduction of soluble calcium phosphate and transfer of calcium and phosphate from the serum to the colloidal phase (Hardy et al., 1984). Tables 5, 6 and 7 show that addition of calcium chloride caused destabilizing of the milk protein system. This result agrees with the finding of Sweetsur and Muir (1982a) that the heat stability of concentrated skim milk was significantly correlated with soluble calcium in raw milk.

Skim evaporated milk which was not supplemented with salts of citrate and phosphate exhibited low heat

stability. This could be ascribed to the deposition of calcium in micelles or on the casein-B-lactoglobulin complex.

Citrate did not vary greatly with heating, whereas the amounts of soluble calcium and phosphorus decreased progressively with heating (Kudo, 1980; Dalgleish, 1987). Because heating had little effect on citrate, the heat coagulation time increased markedly by the addition of citrate solution, especially at high concentration (0.2 ml) as compared to Na_2HPO_4 and SHMP (Tables 5, 6 and 7).

The stabilizing effect against heat coagulation during sterilization of skim evaporated milk containing phosphate and citrate salts might be attributed to the chelating of calcium. According to Vujicic et al. (1962), disodium phosphate reduced the level of soluble and ionic calcium, probably by precipitation of insoluble calcium phosphates, because the amount of insoluble phosphate increased. Sodium hexametaphosphate removed the calcium from the soluble and ionic state and is bound to the protein. In contrast to SHMP, addition of citrate resulted in increased levels of soluble calcium. The calcium-citrate complex does not have as great an affinity for protein as does the calcium-SHMP complex. Citrate, Na_2PHO_4, and SHMP are powerful complexing agents, but SHMP reduced soluble calcium to a lesser extent.

According to Herreid and Wilson (1963), added polyphosphate compounds combined with milk proteins and calcium, penetrating into the colloidal caseinate particles to bind calcium, thus forming a more stable linkage of the caseinate-calcium phosphate complex.

From these findings, one can observe that the salts of milk have an important role in maintaining or disrupting the heat stability of evaporated milk. The addition of stabilizing salts to evaporated milk, to the extent of 0.10 percent by weight of finished product, has been legalized in the United States.

From data in Tables 5, 6 and 7, one can conclude that the lower amount of added ionic calcium required to affect heat coagulation time (HCT) significantly ($p \leq 0.05$) was 0.05 ml (equivalent to 12 mg%) for M-CN and Ca-caseinate milks and 0.10 ml (24 mg%) for Na-caseinate milk. The lowest concentration of Na-citrate which increased the HCT significantly ($p \leq 0.05$) was found to be 0.10 ml (58.5 mg%) for both M-CN and Ca-caseinate milks, and 0.05 ml (29.25 mg%) for Na-caseinate milk. The lower amount of added Na_2HPO_4 which caused significant increase in the HCT was found to be 0.05 ml (33.3 mg%) for M-CN and Na-caseinate milks and 0.10 ml (66.6 mg%) for Ca-caseinate milk. The lower amounts of sodium hexametaphosphate (SHMP) which increased the HCT significantly ($p \leq 0.05$) were found to be 0.15 ml (100

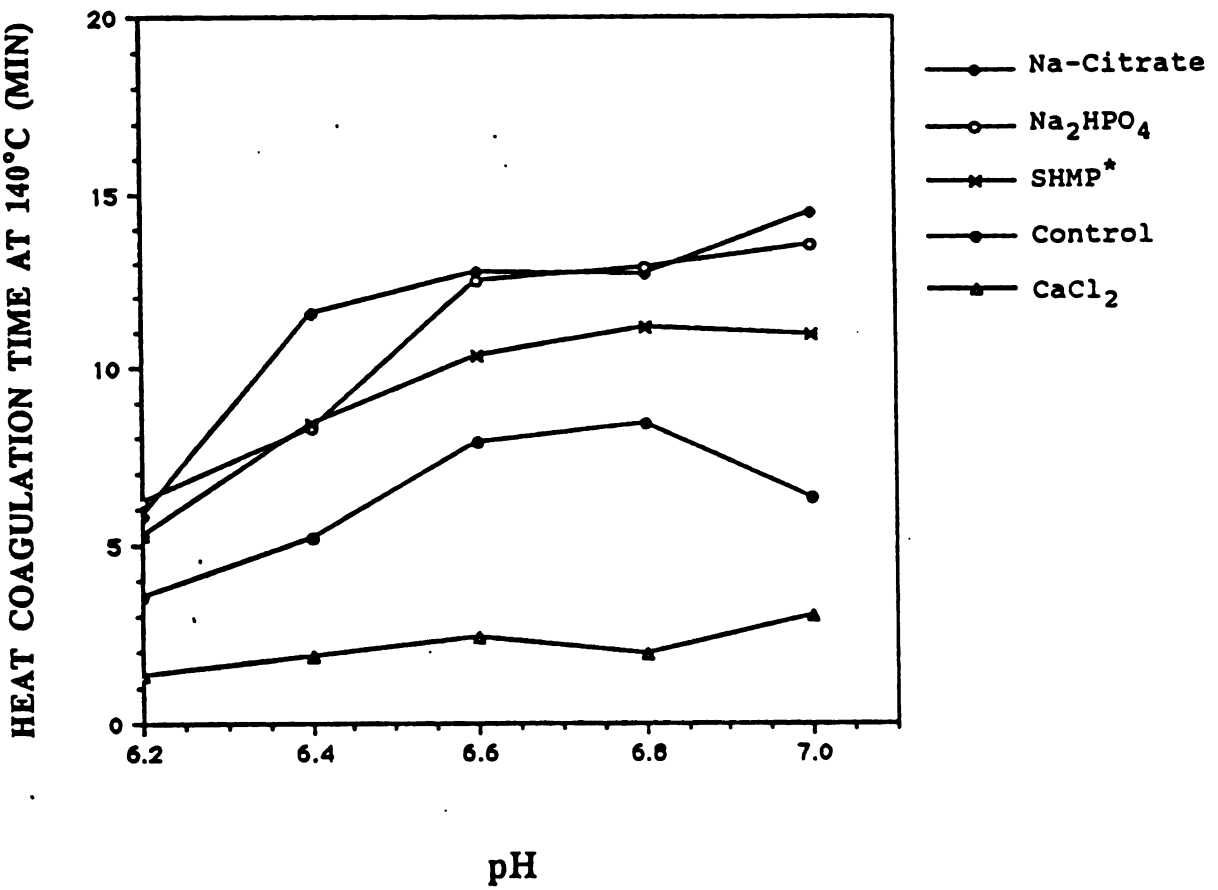
mg%) for M-CN milk and 0.10 ml (66.6 mg%) for Na-caseinate and Ca-caseinate milks.

Therefore, the caseinate particles are very sensitive to ionic calcium which causes coagulation. Citrate and phosphate exert an opposite action to that of calcium because they decrease the effect of calcium concentration. Furthermore, increasing the heat stability of the caseinate system is not achieved solely by the addition of phosphates or citrate salt. According to Hunziker (1949a), some evaporated milk is stabilized by the addition of calcium and destabilized by the addition of citrate or phosphates, but in most cases low heat stability is found to be due to an excess of calcium. This atypical behavior is attributed to a distortion in the salt balance of milk.

If heat instability is due to an excess of phosphate or citrate, it can be prevented by adding the proper amount of calcium chloride. On the other hand, if this phenomenon is due to an excess of calcium, appropriate amounts of phosphate or citrate will assist in preventing heat coagulation. Thus, a salt test is needed to find the type and amount of salt to be used.

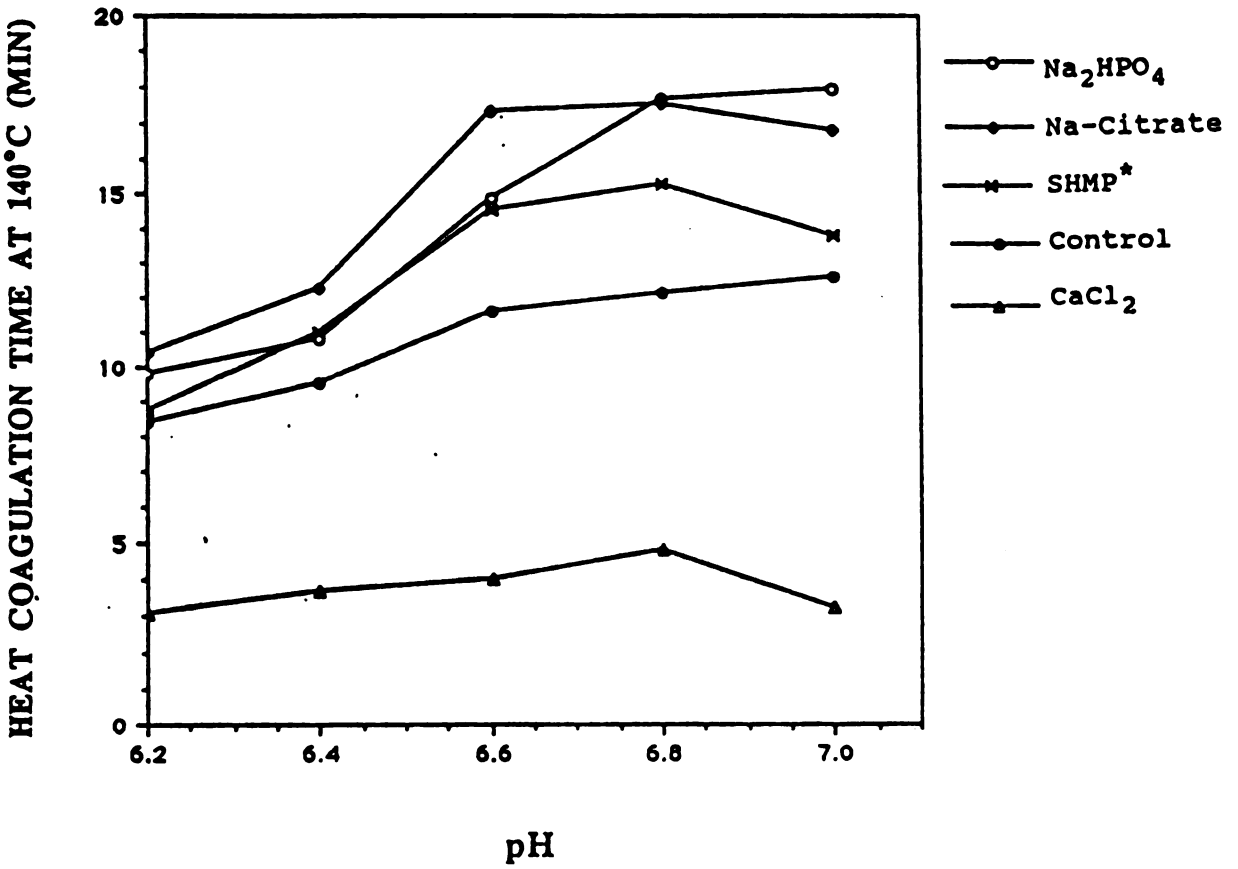
Influence of Salts on the HCT/pH Profile of Skim Evaporated Milk

The heat coagulation time versus pH curves for M-CN, Na-caseinate, and Ca-caseinate skim evaporated milks are shown in Figures 14, 15, and 16, respectively.



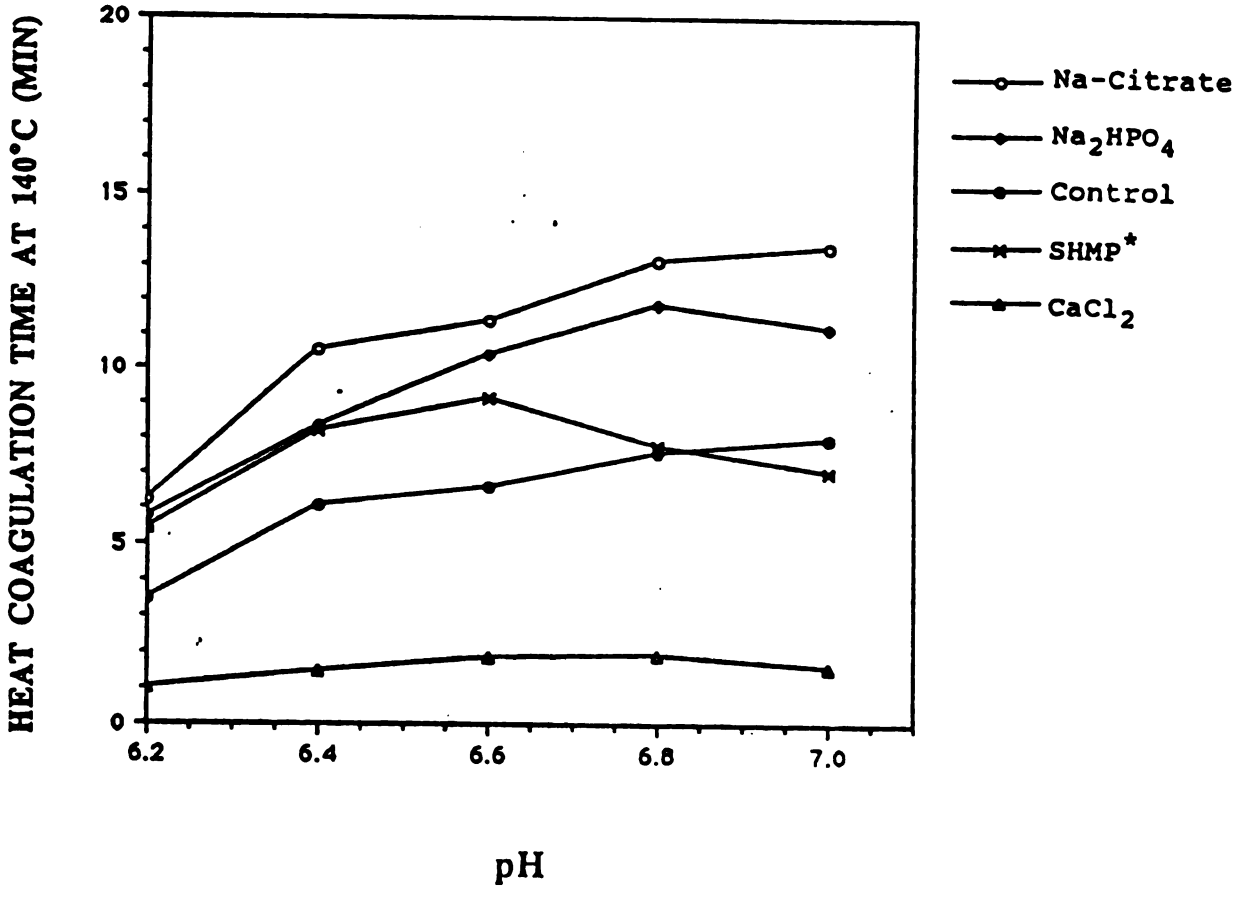
*sodium hexametaphosphate

Figure 14. Influence of salts on the heat coagulation/pH profile of micellar-casein skim evaporated milk.



*sodium hexametaphosphate

Figure 15. Influence of salts on the heat coagulation/pH profile of Na-caseinate skim evaporated milk.



*sodium hexametaphosphate

Figure 16. Influence of salts on the heat coagulation/pH profile of Ca-caseinate skim evaporated milk.

Before addition of salts, increasing the pH level caused an increase in HCT for Na-caseinate and Ca-caseinate milks with a maximum and minimum in HCT at pH 7.0 and 6.2, respectively (see Figures 15 and 16). For M-CN milk, maximum heat stability occurred at pH 6.8 and above that the HCT decreased (Figure 14).

Among the three types of skim evaporated milk, without addition of salts, Ca-caseinate milk showed the lowest HCT at all pH values, but at pH 7.0 the increase in HCT for Ca-caseinate milk was more than that of (M-CN) milk.

Figure 14 shows the effect of adding salts on the HCT/pH profile of M-CN milk. Addition of $CaCl_2$ to M-CN milk caused a dramatic destabilization at all pH values studied. The maximum and minimum in HCT occurred at pH 7.0 (~3.0 minutes) and pH 6.2 (1.3 minutes), respectively. At pH 6.2 and 6.8, Na_2HPO_4 was more effective than Na-citrate and SHMP with HCT of 6.18 and 12.88 minutes, respectively. At pH 6.4 and 7.0, Na-citrate was more effective with HCT of 11.56 and 14.48 minutes, respectively. SHMP showed a maximum and minimun in HCT at pH 6.8 (11.15 minutes) and pH 6.2 (5.27 minutes), respectively.

Figure 15 shows the effect of adding salts on the HCT/pH profile of Na-caseinate milk. Adding $CaCl_2$ to Na-caseinate milk decreased the HCT at all pH values, with a maximum and minimum in HCT at pH 6.8 (~5.0

minutes) and pH 6.2 (~3.0 minutes), respectively. Na-citrate, Na_2HPO_4, and SHMP increased the HCT with increased pH values. Na_2HPO_4 was more effective at the highest pH values; pH 6.8 and 7.0, with HCT of 17.69 and 17.94 minutes, respectively. When Na-citrate and SHMP were added, the optimum pH which exhibited the highest heat stability shifted from pH 7.0 (for samples which did not have added salts) to pH 6.8. Addition of Na-citrate and SHMP caused an increase in HCT to 17.51 and 15.26 minutes, respectively, which occurred at pH 6.8.

Figure 16 shows the effect of adding salts on the HCT/pH profile of Ca-caseinate milk. When $CaCl_2$ was added to Ca-caseinate milk, the effect on the HCT/pH profile was nearly the same as for Na-caseinate milk. It is clear that Ca-caseinate milk was less heat stable than M-CN and Na-caseinate milks over the pH range studied. The only noticeable difference was that the optimum pH after the addition of Na_2HPO_4 to Ca-caseinate milk was pH 6.8, with HCT of 13.56 minutes, whereas pH 7.0 was the optimum for M-CN and Na-caseinate milks.

According to Singh and Fox (1985, 1987), heating milk at temperatures $\geq$ 90 C in the pH range 6.5-6.7 caused whey protein to complex with the casein micelles, while heating at pH $\geq$ 6.9 resulted in the dissociation of whey protein-K-casein complexes from the micelles.

The complexing of B-lg with K-casein on the micelles at pH < 6.9 increased the surface charge and hydration and prevented the dissociation of K-casein, thus stabilizing casein micelles in the pH range 6.5-6.7. Our results, which show that HCT of M-CN milk decreased by increasing the pH values from pH 6.8 to 7.0, agreed with the finding of Singh and Fox (1985, 1987). For Na-caseinate milk and Ca-caseinate milk, increasing the pH froml pH 6.8 to 7.0 caused an increase in HCT. Thus, the decrease in HCT is unlikely to be due only to the failure of the interaction between B-lg and K-casein at pH 7.0, but milk salts, calcium and phosphate could be another factor influencing the heat stability of milk at high pH values. Accordin to Morrissey (1969a), the maximum in the HCT/pH curve is a consequence of low stability at pH $\simeq$ 6.9, which is attributed to heat-induced precipitation of Ca-phosphate on casein micelles sensitized by complexation with heat-denatured whey proteins.

Since reduction of the level of Ca^{2+} and colloidal calcium phosphate increases the heat stability of evaporated milk, it was not surprising that citrate, Na_2HPO_4, and sodium hexametaphosphate (SHMP), which are strong calcium chelators, increased heat stability. Figures 14, 15, and 16 show that addition of citrate, Na_2HPO_4, and SHMP to skim evaporated milk changed the HCT/pH curves. As one can observe, some additives

(such as SHMP when added to micellar-casein milk) show a very pronounced stability, maximum and minimum, while others (such as Na-citrate when added to micellar-casein milk) increased heat stability progressively with increasing pH and showed no maximum or minimum.

Effect of Intermittent Neutralization on Heat Coagulation Time (HCT) of Skim Evaporated Milk

Data in Tables 8, 9, and 10 show that heating of skim evaporated milk at 140°C (284°F) reduced the pH. Heating micellar-casein skim evaporated milk for 2.5 minutes caused a 0.08 unit reduction in pH, whereas for Na-caseinate milk and Ca-caseinate milk the declines in pH were 0.09 and 0.05 units, respectively. For micellar-casein milk, readjusting the pH increased the heat coagulation time (HCT) markedly from 7.69 minutes to 12.17 minutes (Table 8). HCT increased from 11.50 to 19.89 minutes and from 6.76 to 11.81 minutes for Na-caseinate and Ca-caseinate milks, respectively (Tables 9 and 10).

Data in Tables 8, 9, and 10 demonstrate that a stabilizing change occurs on heating not only by forewarming and salt balance but also by readjusting the pH occasionally to its original pH value. Also, the decrease in pH on heating is one of the most important factors leading to coagulation of milk during heating, which apparently can be delayed by periodic neutralization.

Table 8--Effect of intermittent neutralization on the heat stability of micellar-casein skim evaporated milk

No. of 2.5 min heating invervals at 140°C	Total preheating time (min)	pH after each preheating	Heat Coagulation time at 140°C (min)	Total heating time at 140°C (min)
0	0	6.60	7.69	7.69
1	2.50	6.52	7.15	9.65
2	5.00	6.55	5.74	10.74
3	7.50	6.53	4.53	12.03
4	10.00	6.56	2.17	12.17

Milk samples were readjusted to pH 6.60 with 1N NaOH solution.

Table 9--Effect of intermittent neutralization on the heat stability of Na-caseinate skim evaporated milk

No. of 2.5 min heating invervals at 140°C	Total preheating time (min)	pH after each preheating	Heat Coagulation time at 140°C (min)	Total heating time at 140°C (min)
0	0	6.60	11.50	11.50
1	2.50	6.51	10.60	13.10
2	5.00	6.54	8.90	13.90
3	7.50	6.56	7.81	15.31
4	10.00	6.57	6.70	16.70
5	12.50	6.54	5.61	18.11
6	15.00	6.55	3.63	18.63
7	17.50	6.57	2.39	19.89

Milk samples were readjusted to pH 6.60 with 1N NaOH solution.

Table 10--Effect of intermittent neutralization on the heat stability of Ca-caseinate skim evaporated milk

No. of 2.5 min heating invervals at 140°C	Total preheating time (min)	pH after each preheating	Heat Coagulation time at 140°C (min)	Total heating time at 140°C (min)
0	0	6.60	6.76	6.76
1	2.50	6.55	5.54	8.04
2	5.00	6.52	5.13	10.13
3	7.50	6.54	4.31	11.81
4	10.00	6.57	1.98	11.98

Milk samples were readjusted to pH 6.60 with 1N NaOH solution.

According to Pyne (1958), if the pH of milk is adjusted occasionally to its original pH value, its heat stability is more or less infinite. According to Fox et al. (1980), the stabilizing action of urea in milk is due to its pH buffering capacity following thermal decomposition.

Since the decline in pH is one of the principal factors to heat coagulation, finding ways of buffering pH might lead to significant improvement in the heat stability of milk.

Effect of Organic Acids on the Browning of Skim Evaporated Milk

Evaporated milk usually exhibits darker color than the whole unconcentrated fresh milk. This so-called "browning" or "discoloration" of evaporated milk is a result of the high temperatures required for the sterilization process. To many consumers, evaporated milk should possess the basic properties of fresh whole milk except for its more highly concentrated nature.

Data in Tables 11, 12, and 13 show the effect of adding organic acids on the browning of M-CN, Na-caseinate, and Ca-caseinate skim evaporated milks, respectively. Hydroxymethylfurfural (HMF) content was used as a chemical parameter in judging the browning reaction.

All three types of milk showed an increase in HMF values after sterilization. Na-caseinate milk exhibited

Table 11—Effect of adding organic acids on the browning of micellar-casein skim evaporated milk

Type of Acid	10% sol.: H_2O	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Acetic		35.33 ± 0.28a	35.11 ± 0.27a	35.66 ± 0.28a	35.88 ± 0.18a	36.31 ± 0.60a
Lactic		35.33 ± 0.28a	36.09 ± 0.28ab	36.64 ± 0.37abc	37.30 ± 0.41bc	37.73 ± 0.37c
Propionic		35.33 ± 0.28a	36.31 ± 0.47a	37.95 ± 0.49c	38.83 ± 0.21c	39.20 ± 0.25c
Tartaric		35.33 ± 0.28a	36.20 ± 0.21a	37.08 ± 0.49ab	38.28 ± 0.55bc	38.94 ± 0.40c
Gluconic		35.33 ± 0.28a	36.86 ± 0.28ab	38.28 ± 0.28bc	39.81 ± 0.64c	39.59 ± 0.58c
Citric		35.33 ± 0.28a	36.53 ± 0.13ab	37.89 ± 0.46b	39.92 ± 0.37c	40.25 ± 0.47c

*hydroxymethylfurfural, micromoles/liter

Different concentrations of organic acids were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

Table 12--Effect of adding organic acids on the browning of Na-caseinate skim evaporated milk

Type of Acid	10% sol.: H_2O :	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Acetic		48.23 ± 0.41a	48.45 ± 0.68a	48.56 ± 0.36a	49.44 ± 0.36a	49.11 ± 0.74a
Lactic		48.23 ± 0.41a	48.67 ± 0.58a	49.22 ± 0.38ab	49.70 ± 0.42ab	51.30 ± 0.58b
Propionic		48.23 ± 0.41a	49.33 ± 0.37a	51.56 ± 0.52b	53.05 ± 0.37b	52.94 ± 0.36b
Tartaric		48.23 ± 0.41a	49.66 ± 0.55ab	50.86 ± 0.60bc	52.39 ± 0.21c	52.72 ± 0.75c
Gluconic		48.23 ± 0.41a	49.55 ± 0.28a	54.14 ± 0.21c	54.36 ± 0.33c	54.58 ± 0.58c
Citric		48.23 ± 0.41a	49.88 ± 0.47a	53.48 ± 0.33b	55.67 ± 0.49c	55.56 ± 0.36c

*hydroxymethylfurfural, micromoles/liter

Different concentrations of organic acids were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

Table 13—Effect of adding organic acids on the browning of Ca-caseinate skim evaporated milk

Type of Acid	10% sol.: H_2O	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Acetic		32.48 ± 0.47a	31.94 ± 0.40a	32.16 ± 0.38a	32.59 ± 0.55a	32.81 ± 0.47a
Lactic		32.48 ± 0.47a	32.27 ± 0.28a	32.70 ± 0.28a	33.80 ± 0.55a	33.36 ± 0.49a
Propionic		32.48 ± 0.47a	32.92 ± 0.49ab	33.27 ± 0.46ab	34.02 ± 0.58ab	34.67 ± 0.37b
Tartaric		32.48 ± 0.47a	32.77 ± 0.29ab	33.03 ± 0.28ab	34.34 ± 0.52bc	34.98 ± 0.20c
Gluconic		32.48 ± 0.47a	33.23 ± 0.09ab	34.17 ± 0.18bc	34.93 ± 0.38c	36.90 ± 0.31d
Citric		32.48 ± 0.47a	33.18 ± 0.43a	35.77 ± 0.21d	36.68 ± 0.19d	36.20 ± 0.28d

*hydroxymethylfurfural, micromoles/liter

Different concentrations of organic acids were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

the highest browning (48.23 uMol/l), whereas Ca-caseinate milk showed the lowest browning (32.48 uMol/l).

Data in Table 11 show that addition of organic acids to M-CN milk increased browning. Even though acetic acid increased browning, the increase was not significant ($p \leq 0.05$). The highest browning was developed by the addition of gluconic and citric acids. At highest concentrations of addition (0.2 ml), the HMF values were 39.59 and 42.25 uMol/l for gluconic and citric acids, respectively. Propionic acid was more effective in producing browning than lactic or tartaric acids; the HMF values were 39.20, 37.73, and 38.94 uMol/l, respectively.

Although all acids, except acetic acid, increased browning significantly, there were differences among the rates of acids which increased browning. For instance, the lowest amount of addition (0.05 ml) of propionic and tartaric acids did not cause a significant increase in browning but increasing the rate of addition more than that promoted browning significantly.

Table 12 shows the effect of adding organic acids on the browning of Na-caseinate milk. All acids, except acetic acid, increased browning reaction ($p \leq 0.05$). At the lowest concentration (0.05 ml), none of the acids promoted browning significantly, but when the acids were gradually increased, browning also increased

significantly. The highest browning values were found with samples containing citric, gluconic, and propionic acids; the HMF values were 55.56, 54.58, and 52.94 uMol/l, respectively.

Table 13 shows the effect of adding organic acids on the browning of Ca-caseinate milk. Comparison of Ca-caseinate milk with micellar-casein and Na-caseinate milks shows that there is a broad similarity, i.e, addition of organic acids promote browning. Ca-caseinate, as mentioned previously, exhibited the lowest amount of browning (32.48 uMol/l). The only noticeable differences were that lactic acid did not increase browning significantly at all concentrations, and tartaric acid was more effective in producing brown discoloration than propionic acid.

According to Reynolds (1965), the degradation of reducing sugars to furfurals occurred in the presence of organic acids and their presence in foods would be a good catalyst for the degradation of sugars. Hass and Stadtmann (1949) and Livingston (1953) presumed that the organic acids reacted with reducing sugars. Lewis et al. (1949) also suggested that organic acids reacted with sugars to produce brown pigments. They found that solutions of glucose and citrate browned more rapidly at 90°-110°C than equivalent solutions of glucose and glycine. Felix (1983) found that the Maillard reaction

of sucrose-glycine model systems was directly proportional to the concentration of citric acid.

According to Klostermeyer et al. (1981), galactose and glucose, the breakdown products of lactose, are much more reactive with protein than with lactose itself.

Thus, the role of organic acids is essentially catalytic. The results indicated that all organic acids studied increased browning. Acetic acid developed less browning than the other acids studied. The three-carbon chain, propionic acid gave a more pronounced effect on browning than either lactic or tartaric acid, except for Ca-caseinate milk, in which tartaric acid was more effective than propionic acid. The six-carbon chain acids, gluconic anc citric, which have polyhydroxyl and polycarboxyl groups, respectively, exhibited the highest browning for all three types of milks.

Effect of Alkali Chemicals on the Browning of Skim Evaporated Milk

Data in Tables 14, 15, and 16 show the effect of adding ammonium hydroxide and urea on the browning of micellar-casein M-CN, Na-caseinate, and Ca-caseinate skim evaporated milks, respectively. Addition of NH_4OH and urea to M-CN milk caused an increase in browning. The gradual increase in addition of both alkali caused an increase in browning significantly ($p \leq 0.05$). When NH_4OH and urea were added at the highest

Table 14—Effect of alkali chemicals on the browning of micellar-casein skim evaporated milk

		HMF uMol/l* Rate of addition (ml)				
Type of Alkali	10% sol.: H_2O :	0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Ammonium hydroxide		35.33 ± 0.28a	36.53 ± 0.46ab	37.09 ± 0.56ab	37.82 ± 0.61bc	39.27 ± 0.28c
urea		35.33 ± 0.28a	36.64 ± 0.49ab	37.41 ± 0.58b	38.47 ± 0.15b	40.36 ± 0.49c

*hydroxymethylfurfural, micromoles/liter

Different concentrations of alkali chemicals were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

Table 15--Effect of alkali chemicals on the browning of Na-caseinate skim evaporated milk

Type of Alkali	10% sol. : H_2O :	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Ammonium hydroxide		48.23 ± 0.41a	49.00 ± 0.47a	49.77 ± 0.21ab	50.64 ± 0.37bc	52.17 ± 0.28c
urea		48.23 ± 0.41a	49.44 ± 0.47a	51.52 ± 0.41b	51.84 ± 0.38b	52.28 ± 0.52b

*hydroxymethylfurfural, micromoles/liter

Different concentrations of alkali chemicals were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

Table 16--Effect of alkali chemicals on the browning of Ca-caseinate skim evaporated milk

Type of Alkali	10% sol.: H_2O :	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Ammonium hydroxide		32.48 ± 0.47a	32.64 ± 0.25a	32.81 ± 0.56a	33.38 ± 0.33a	33.73 ± 0.47a
urea		32.48 ± 0.47a	32.53 ± 0.51a	33.45 ± 0.46ab	34.78 ± 0.58b	34.61 ± 0.36b

*hydroxymethylfurfural, micromoles/liter

Different concentrations of alkali chemicals were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

concentration (0.2 ml), the HMF values were 39.27 and 40.36 uMol/l, respectively (Table 14).

By increasing the amount of NH_4OH and urea added to Na-caseinate milk, the browning reaction was increased significantly ($p \leq 0.05$)(Table 15). As one can observe, at the highest concentration of addition (0.2 ml) of NH_4OH and urea, the HMF values were 52.17 and 52.28 uMol/l, respectively. Even though the browning was increased by adding urea, the increase in HMF values was not significant ($p \leq 0.05$) when the concentration of addition was more then 0.10 ml.

The results presented in Table 16 reveal that the browning reaction of Ca-caseinate milk was not increased significantly ($p \leq 0.05$) with the addition of NH_4OH, whereas Ca-caseinate milk samples containing urea promoted brown discoloration significantly ($p \leq 0.05$).

According to Jenness and Patton (1959), variation of urea concentration in milk affects browning reaction. Urea was reported to favor browning. Fox et al. (1980) reported that addition of urea to milk promotes browning via interaction between lactose and urea or its degradation products. Urea is capable of involvement in Maillard browning with lactose and reduce the rate of pH decline during heating. Whistler and BeMiller (1959) noted that the formation of 1,2 enols of reducing sugars occurred more easily in the presence of alkalies than in acids.

Effect of Adding Salts on the Browning of Skim Evaporated Milk

Data in Table 17, 18, and 19 show the effect of adding different salts on the browning of micellar-casein, Na-caseinate, and Ca-caseinate skim evaporated milks, respectively. The amount of browning reaction for milk samples which contained Na_2HPO_4 and Na-citrate increased significantly ($p \leq 0.05$), whereas addition of $CaCl_2$, sodium hexametaphosphate (SHMP), Na-sulfite, and Na-bisulfite decreased the browning reaction significantly ($p \leq 0.05$).

By adding these salts to micellar-casein milk at highest concentration (0.2 ml), the HMF values were 44.41, 47.03, 25.92, 24.65, 19.69, and 18.81 uMol/l for Na_2HPO_4, Na-citrate, $CaCl_2$, SHMP, Na-sulfite, and Na-bisulfite, respectively (Table 17). Na-citrate gave the highest browning, whereas Na-bisulfite was most effective in reducing the amount of browning. Adding 0.05 ml of SHMP did not decrease browning significantly ($p \leq 0.05$), but when SHMP was beyond 0.05 ml up to 0.2 ml, the HMF values were decreased significantly ($p \leq 0.05$).

For Na-caseinate milk, the browning reaction was increased significantly ($p \leq 0.05$) when Na_2HPO_4 and Na-citrate were added; the HMF values were 59.06 and 59.83 uMol/l, respectively (Table 18). When $CaCl_2$, SHMP, Na-sulfite, and Na-bisulfite were added, the

Table 17—Effect of adding salts on the browning of micellar-casein skim evaporated milk

Type of Salt	10% sol.: H_2O	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Na_2HPO_4		35.33 ± 0.28e	37.01 ± 0.25d	39.70 ± 0.37c	44.84 ± 0.13b	44.41 ± 0.38b
Na-citrate		35.33 ± 0.28e	37.23 ± 0.36d	39.26 ± 0.28c	45.17 ± 0.41b	47.03 ± 0.28a
$CaCl_2$		35.33 ± 0.28e	34.71 ± 0.27ef	33.91 ± 0.38f	28.11 ± 0.37g	25.92 ± 0.28h
SHMP**		35.33 ± 0.28e	34.45 ± 0.37e	30.84 ± 0.58f	26.91 ± 0.42g	24.65 ± 0.24h
Na-sulfite		35.33 ± 0.28e	32.05 ± 0.33f	28.33 ± 0.60g	22.42 ± 0.28h	19.59 ± 0.18i
Na-bisulfite		35.33 ± 0.28e	31.98 ± 0.18f	28.55 ± 0.54g	23.51 ± 0.28h	18.81 ± 0.47i

*hydroxymethylfurfural, micromoles/liter

**sodium hexametaphosphate

Different concentrations of salts were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

Table 18--Effect of adding salts on the browning of Na-caseinate skim evaporated milk

Type of Salt	10% sol. : H_2O :	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 : 0.15	0.10 : 0.10	0.15 : 0.05	0.20 : 0.0
Na_2HPO_4		48.23 ± 0.41d	49.81 ± 0.28d	53.27 ± 0.65c	55.61 ± 0.60b	59.06 ± 0.36a
Na-citrate		48.23 ± 0.41d	50.09 ± 0.52d	53.59 ± 0.58c	57.31 ± 0.36b	59.83 ± 0.37a
$CaCl_2$		48.23 ± 0.41d	47.36 ± 0.49d	41.78 ± 0.38e	39.59 ± 0.28f	37.99 ± 0.24f
SHMP**		48.23 ± 0.41d	47.58 ± 0.32d	43.20 ± 0.37e	41.02 ± 0.37f	33.14 ± 0.21g
Na-sulfite		48.23 ± 0.41d	46.05 ± 0.28e	40.36 ± 0.28f	36.64 ± 0.38g	31.39 ± 0.36h
Na-bisulfite		48.23 ± 0.41d	45.61 ± 0.37e	39.48 ± 0.21f	36.86 ± 0.37g	27.56 ± 0.31h

*hydroxymethylfurfural, micromoles/liter

**sodium hexametaphosphate

Different concentrations of salts were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

Table 19--Effect of adding salts on the browning of Ca-caseinate skim evaporated milk

Type of Salt	10% sol.: H_2O	HMF uMol/l* Rate of addition (ml) 0.0 : 0.2	0.05 0.15	0.10 0.10	0.15 0.05	0.20 0.0
Na_2HPO_4		32.48 ± 0.47c	33.40 ± 0.21c	36.97 ± 0.42b	37.99 ± 0.19ab	38.43 ± 0.24a
Na-citrate		32.48 ± 0.47c	33.29 ± 0.37c	35.92 ± 0.32b	39.86 ± 0.18a	39.97 ± 0.31a
$CaCl_2$		32.48 ± 0.47c	30.06 ± 0.35d	26.03 ± 0.28e	24.98 ± 0.18ef	23.95 ± 0.21f
SHMP**		32.48 ± 0.47c	30.95 ± 0.37c	27.06 ± 0.35d	25.94 ± 0.35d	22.14 ± 0.25e
Na-sulfite		32.48 ± 0.47c	30.12 ± 0.41d	26.18 ± 0.36e	19.73 ± 0.18f	19.95 ± 0.25f
Na-bisulfite		32.48 ± 0.47c	30.23 ± 0.18d	25.64 ± 0.30e	19.36 ± 0.28f	17.83 ± 0.37g

*hydroxymethylfurfural, micromoles/liter

**sodium hexametaphosphate

Different concentrations of salts were added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means within each row not followed by the same letter are significantly different ($p \leq 0.05$).

browning reaction was reduced significantly ($p \leq 0.05$), with HMF values of 37.99, 33.14, 31.39, and 27.56 uMol/l, respectively. At the lowest amount of addition (0.05 ml), Na_2HPO_4, Na-citrate, $CaCl_2$, and SHMP did not change the HMF values significantly, whereas Na-sulfite and Na-bisulfite reduced the HMF values significantly ($p \leq 0.05$).

Data in Table 19 show that by adding Na_2HPO_4, Na-citrate, $CaCl_2$, SHMP, Na-sulfite, and Na-bisulfite to Ca-caseinate milk at the highest concentration (0.2 ml), the HMF values were 38.43, 39.97, 23.95, 22.14, 19.95, and 17.83 uMol/l, respectively. Increasing the amount of Na-citrate and Na-sulfite beyond 0.15 ml did not change the HMF values significantly ($p \leq 0.05$).

According to Burton et al. (1963), in the presence of phosphate (Na_2HPO_4) the rate of color development in the sugar/amino system increased. They suggested that the increase in browning is due mainly to direct action on sugars; i.e., phosphates increase browning by an accelerated degradation of sugar.

The data in Tables 17, 18, and 19, indicate that sodium hexametaphosphate eliminated the browning significantly for all three types of milk. This indicates that the mechanism involved is different from that of Na_2HPO_4. Leviton (1964) found that approximately 60% of the phosphorus in added hexametaphosphate was converted to pyrophosphate

phosphorus in 15 seconds at 137.8°C (280°F). He further speculated that the pyrophosphate formed by this hydrolysis formed cross-linkages within the casein micelle, thereby stabilizing the micelle. Leviton further proposed that when pyrophosphate was added directly to the milk, it interacted with the calcium ions to form insoluble colloidal complexes. Herreid and Wilson (1963) reported that the polyphosphates penetrate the casein micelle to combine with the protein and calcium. According to Vujicic et al. (1968), polyphosphates complex calcium and simultaneously combine with protein.

It is known that casein and lactose are the two principal reacting materials for browning in milk. Thus, when sodium hexametaphosphate reacts with milk casein, the amino-carbonyl reaction is reduced. This may be the mechanism by which SHMP eliminates browning in the milk system.

Stalberg and Radaeva (1966) found that the addition of 0.15 percent of sodium hexametaphosphate to the sweetened condensed milk inhibited the formation of brown color. They also found that the amino-sugar reaction proceeded at a slower rate when compared with control samples. Comparing the results in Tables 17, 18, and 19 with the results of Stalberg and Radaeva (1966) demonstrates that the amount of sodium

hexametaphosphate needed to inhibit browning was less (0.13 percent) than that of Stalberg and Radaeva.

Citrate is a powerful complexing agent and it was obvious from the results of the heat coagulation time (Tables 5, 6, and 7) that citrate had the highest heat stability compared to other salts (i.e., Na_2HPO_4 and SHMP). Since heating had little effect on citrate (Kudo, 1980; Dalgleish, 1987), and the calcium-citrate complex did not have as great affinity for protein as did the calcium-hexametaphosphate complex (Vujicic et al., 1983), citrate gave the highest browning reaction compared to all other salts studied (see Tables 17, 18, and 19).

Calcium chloride reduced the browning reaction significantly for all three types of micelles (Tables 17, 18, and 19). Thus calcium has an opposite effect to that of citrate and Na_2HPO_4. According to Vujicic et al. (1968), Na_2HPO_4 reduced the level of soluble and ionic calcium and increased the amount of insoluble phosphate, whereas citrate increased the levels of soluble calcium.

A definite explanation of the mechanism by which calcium chloride serves to retard browning in the milk system cannot be offered at this time. The calcium may be acting in some manner to block the amino group, whereby the latter is restrained from entering into the browning reaction. Another possibility is that calcium

binds phosphate, therefore there should be less browning because phosphate increases browning.

According to Nagayama (1961), calcium inhibited the browning completely of glucose-lysine solution. In their study of non-enzymatic browning in the glucose-glycine and sucrose-glycine systems, Burton et al. (1963) suggested that calcium tends to slow sugar-amino browning.

The action of both Na_2HPO_4 and citrate salts upon browning of skim evaporated milk was similar in trend. However, sodium citrate produced a greater effect in terms of relative value of browning. Na_2HPO_4 and Na-citrate increased the browning reaction but at the same time both salts gave the highest heat stability for all three types of milks. In contrast, calcium chloride reduced the discoloration but gave the lowest heat stabilty.

Among the salts studied, Na-sulfite and Na-bisulfite were the most effective in minimizing the browning reaction (Tables 17, 18, and 19). The possible mechanism of sulfite or bisulfite in prevention of browning reactions probably involves sulfite interactions with active carbonyl groups; sulfite salts combine with reducing sugars. Whistler and Daniel (1985) showed the possible mechanism of bisulfite prevention of browning:

$$R - CHO + HSO_3^- \longrightarrow R - \underset{\underset{OH}{|}}{\overset{\overset{H}{|}}{C}} - SO_3^-$$

McWeeney et al. (1969) and Nagayama (1961) found that the Maillard reaction was inhibited by sulfite. According to Song et al. (1967), the inhibition by sodium bisulfite is due to the free radicals derived from sodium bisulfite.

Na-bisulfite was more effective in reducing the browning than Na-sulfite. This may be attributed to the difference in free radicals. A ten percent solution of sodium bisulfite has more sulfite ions than a ten percent solution of sodium sulfite, and thus should exhibit a greater effect upon the color.

Effect of pH on Browning of Skim Evaporated Milk

Data in Table 20 show the effect of different pH values on the browning of skim evaporated milks. In general, increasing the pH within the pH ranges studied (6.2-7.0) promoted browning for all three types of milk. At the same pH value, Ca-caseinate milk showed the lowest browning, whereas Na-caseinate milk exhibited the highest browning.

For M-CN milk, increasing the pH value from 6.2 to 6.8 increased the browning reaction significantly ($p \leq 0.05$); the HMF values were 32.09 and 39.53 uMol/l,

Table 20--Effect of different pH values on the browning reaction of skim evaporated milk

pH	HMF uMol/l*		
	Milk Type		
	Micellar-casein	Na-caseinate	Ca-caseinate
6.2	32.09 ± 0.103d	45.67 ± 0.274a	29.36 ± 0.184a
6.4	33.54 ± 0.427b	47.69 ± 0.399b	29.90 ± 0.103a
6.6	35.26 ± 0.107c	48.28 ± 0.371b	32.57 ± 0.200b
6.8	39.53 ± 0.336d	50.36 ± 0.184c	36.86 ± 0.109c
7.0	40.12 ± 0.238d	54.95 ± 0.107d	36.64 ± 0.109c

*hydroxymethylfurfural, micromoles/liter

pH was adjusted by addition of IN HCl and IN HaOH to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means for each type of milk within each column not followed by the same letter are significantly different ($p \leq 0.05$).

respectively. Beyond pH 6.8 the increase in browning was not significant ($p \leq 0.05$).

Similarly, Na-caseinate milk developed browning when the pH values were elevated from 6.2 to 7.0; the HMF values were 45.76 and 54.36 uMol/l, respectively. The degree of browning with pH 6.4 and pH 6.6 was not significant ($p \leq 0.05$).

Even though Ca-caseinate milk promoted browning by increasing the pH values, changing the pH from 6.2 to 6.4 or from 6.8 to 7.0 did not produce significant increases in HMF values. Therefore pH changes had less effect on Ca-caseinate milk compared to micellar-casein or Na-caseinate milks. The lowest and highest browning was found to be at pH 6.2 and 6.8, with HMF values of 29.36 and 36.86 uMol/l, respectively. Presumably, the increase in pH was a contributing factor in color formation for all three types of milk.

According to Patton (1955), elevated hydrogen ion concentrations depressed discoloration, whereas elevated hydroxyl ion concentrations increased discoloration. Ellis (1959) mentioned that browning occurred both in alkaline and acidic media, but the amino-carbonyl reaction was very weak in acid.

Influence of Adding Selected Substances on the Color of Skim Evaporated Milk

The color of evaporated milk is of considerable commercial importance since it is one of the fundamental

characteristics by which the consumer judges the product.

Data in Table 21 show the influence of adding selected substances on the color of skim evaporated milk as measured in terms of "+b" value, which is a measurement of the yellowness. Before the addition of chemical solutions to micellar-casein, Na-caseinate, and Ca-caseinate milks, the "b" values were 17.83, 21.03, and 15.53, respectively. It is obvious that addition of citric acid, urea, Na_2HPO_4, and Na-citrate increased the browning significantly ($p \leq 0.05$), whereas addition of $CaCl_2$, sodium hexametaphosphate (SHMP), Na-sulfite, and Na-bisulfite reduced the "b" values significantly ($p \leq 0.05$).

For micellar-casein milk, there was no significant difference in color between samples which contained SHMP or Na-sulfite. Similarly, the difference in color for Ca-caseinate milk samples which contained urea or citric acid was not significant. Adding Na-sulfite or Na-bisulfite to Na-caseinate milk gave the same color.

The relative degree of browning (color) for all three types of milk were as follows: Na-citrate > Na_2HPO_4 > citric acid > urea > control (no chemical added) > $CaCl_2$ > SHMP > Na-sulfite > Na-bisulfite, which was the most effective in reducing the color.

In general, the "b" values were more in accord with the hydroxymethylfurfural (HMF) results, but HMF

Table 21—Influence of adding selected substances on the color of skim evaporated milk.

Chemical Type (0.2 ml of 10% sol.)	"+b" value		
	Milk Type		
	Micellar-casein	Na-caseinate	Ca-caseinate
Control	17.83 ± 0.085e	21.03 ± 0.138d	15.53 ± 0.193c
Citric acid	20.03 ± 0.138c	24.85 ± 0.104c	18.38 ± 0.111b
Urea	19.33 ± 0.149d	24.23 ± 0.155c	17.98 ± 0.085b
Na_2HPO_4	23.95 ± 0.185b	26.73 ± 0.256b	20.43 ± 0.149a
Na-citrate	25.48 ± 0.239a	28.63 ± 0.095a	20.18 ± 0.111a
$CaCl_2$	13.68 ± 0.639f	18.93 ± 0.085e	12.78 ± 0.125d
SHMP*	11.98 ± 0.085g	16.83 ± 0.111f	12.05 ± 0.065e
Na-sulfite	11.88 ± 0.125g	15.33 ± 0.111g	10.33 ± 0.103f
Na-bisulfite	10.73 ± 0.125h	15.35 ± 0.096g	9.68 ± 0.170g

*sodium hexametaphosphate

0.2 ml of 10% solution of selected chemicals was added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

Means for each type of milk within each column not followed by the same letter are significantly different ($p < 0.05$).

values were a more sensitive indicator of the browning reaction.

On heating skim evaporated milk, the apparent whiteness at first intensifies. This may be attributed to the changes (i.e., increase) in size of the casein particles or it may be due to the increase in scattering as the whey proteins denature. On further heating, the Maillard reaction leads to the milk acquiring a brown appearance.

The white or "milky" appearance of cow's milk arises from light scattering by the colloidal calcium caseinate and colloidal calcium phosphate and fat globules. Dispersions of each of these ingredients are milky when prepared separately in concentrations similar to those occurring in milk (Johnson, 1974). The reflectance at different wavelengths in the visible range, however, is not uniform because of the presence of various colored components in milk. The most important of these are the greenish-yellow of riboflavin in the aqueous phase and the yellow of carotene in the fat phase (Priestley, 1979).

Effect of Heating and Adding Salts on the Availability of Lysine of Skim Evaporated Milk

When conventional sterilization is applied in the manufacture of evaporated milk, the heat effect on the milk protein is more pronounced. Hurrell and Carpenter

(1981) indicated that the most easily damaged of the essential amino acids is lysine. Therefore, the availability of lysine can be reduced as a result of severe heating.

Data in Tables 22, 23, and 24 show the effect of heating and adding different salts on the availability of lysine for micellar-casein milk, Na-caseinate milk, and Ca-caseinate milk, respectively. For non-sterilized milk, the available lysine for micellar-casein, Na-caseinate, and Ca-caseinate milks were 7.29, 7.71, and 7.62 g/16gN, respectively.

Heating micellar-casein milk reduced the available lysine markedly. After 10, 15, and 20 minutes at 118.3° C (245° F) heating, the availability of lysine was reduced to 91.63%, 85.87%, and 82.44%, respectively, compared to the non-sterilized milk (100%). Furthermore, addition of Na-citrate reduced the availability lysine to 84.44% (Table 22). Similarly, Na-caseinate milk and Ca-caseinate milk was affected by the addition of Na-citrate and heating which caused a reduction in available lysine (Tables 23 and 24).

Addition of calcium chloride, sodium hexametaphosphate (SHMP), and Na-bisulfite to all three types of milk increased the availability of lysine, as compared with milk samples which contained no salts, but the loss in available lysine was not prevented. Sodium hexametaphosphate was more effective in increasing the

Table 22—Effect of heating and adding salts on the availability of lysine in micellar-casein skim evaporated milk[a]

Milk Sample	Dye-binding capacity (mmoles/16gN) A (untreated)	Dye-binding capacity (mmoles/16gN) B (after propionylation)	Dye-binding lysine (mmoles/16gN) A - B	Available Lysine (g/16gN)	Available Lysine (%)
Non-sterilized milk	88.34	38.49	49.85	7.29	100
Heat-treated milk					
118.3°C/10 min	80.46	34.77	45.69	6.68	91.63
118.3°C/15 min	76.05	33.37	42.68	6.24	85.60
118.3°C/20 min	73.87	32.75	41.12	6.01	82.44
Na-citrate	75.64	33.55	42.09	6.15	84.36
$CaCl_2$	77.98	33.70	44.28	6.47	88.75
Na-bisulfite	77.74	33.83	43.91	6.42	88.07
SHMP[b]	80.13	35.41	44.72	6.54	89.71

[a]0.2 ml of 10% solution of salts was added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

[b]sodium hexametaphosphate

Table 23--Effect of heating and adding salts on the availability of lysine in Na-caseinate skim evaporated milk[a]

Milk Sample	Dye-binding capacity (mmoles/16gN) A (untreated)	Dye-binding capacity (mmoles/16gN) B (after propionylation)	Dye-binding lysine (mmoles/16gN) A - B	Available Lysine (g/16gN)	Available Lysine (%)
Non-sterilized milk	92.03	39.27	52.76	7.71	100
Heat-treated milk					
118.3°C/10 min	82.57	35.90	46.67	6.82	88.46
118.3°C/15 min	74.87	32.06	42.81	6.26	81.19
118.3°C/20 min	72.16	30.83	41.33	6.04	78.34
Na-citrate	74.31	32.07	42.24	6.18	80.16
$CaCl_2$	77.20	33.51	43.69	6.39	82.88
Na-bisulfite	77.15	33.62	43.53	6.36	82.49
SHMP[b]	78.72	34.67	44.05	6.44	83.53

[a]0.2 ml of 10% solution of salts was added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

[b]sodium hexametaphosphate

Table 24—Effect of heating and adding salts on the availability of lysine in Ca-caseinate skim evaporated milk[a]

Milk Sample	Dye-binding capacity (mmoles/16gN) A (untreated)	Dye-binding capacity (mmoles/16gN) B (after propionylation)	Dye-binding lysine (mmoles/16gN) A - B	Available Lysine (g/16gN)	Available Lysine (%)
Non-sterilized milk	91.15	39.03	52.12	7.62	100
Heat-treated milk					
118.3°C/10 min	84.52	35.69	48.83	7.14	93.70
118.3°C/15 min	80.05	34.20	45.85	6.70	87.93
118.3°C/20 min	76.96	32.82	44.14	6.45	84.65
Na-citrate	77.32	32.93	44.39	6.49	85.17
$CaCl_2$	81.25	34.83	46.42	6.79	89.11
Na-bisulfite	80.55	34.58	45.97	6.72	88.19
SHMP[b]	82.38	35.63	46.75	6.83	89.63

[a]0.2 ml of 10% solution of salts was added to 15 ml of skim evaporated milk and sterilized at 118.3°C/15 min.

[b]sodium hexametaphosphate

availability of lysine followed by $CaCl_2$ and Na-sulfite.

Previous results (Tables 17, 18, and 19) indicated that Na-bisulfite was more effective in reducing the browning reaction than sodium hexametaphosphate, whereas the availability of lysine was less for milk samples containing Na-bisulfite. This behavior is attributed to the difference in the mechanism by which the two salts reduce the browning reaction.

Na-bisulfite interacts with the reducing sugars as reported by Whistler and Daniel (1985):

$$R - CHO + HSO_3^- \longrightarrow R - \underset{\displaystyle OH}{\overset{\displaystyle H}{\overset{|}{\underset{|}{C}}}} - SO_3^-$$

Data in Tables 22, 23, and 24 indicated that Na-bisulfite has little effect on the nutritive value of milk proteins compared to SHMP. This finding agrees with the suggestion of Whistler and Daniel (1985) that although sulfites inhibit browning, it is not likely that they prevent loss of nutritive value of the amino acid involved in the Maillard reaction. This is because the utilization and subsequent degradation of amino acids (i.e., lysine) occurs before the point of action of sulfite in browning inhibition.

On the other hand, sodium hexametaphosphate (SHMP) interacts with milk proteins. According to Herreid and Wilson (1963), polyphosphates penetrate the casein

micelle to combine with proteins and calcium. Leviton and Pallansch (1962) reported that the interaction of the negatively charged polyphosphate with the positively charged groups on the protein at the normal pH of concentrated milk results in a building up of the net negative charge on the micelles, an effect which conduces to an expansion of the micelles.

Because sodium hexametaphosphate forms ionic linkages with the basic amino groups of the protein molecules, as reported by Ellinger (1972), lysine is prevented from entering into the browning reaction. Therefore, the availability of lysine was increased and the browning reaction was reduced by the addition of sodium hexametaphosphate.

Calcium chloride increased the available lysine content markedly for all three types of milk. For instance, the available lysine content of micellar-casein milk increased from 6.26 to 6.47 g/16gN. From previous results (Tables 17, 18, and 19), calcium chloride reduced the browning reaction. According to Burton et al. (1963) and Nagayama (1961), calcium tends to slow the sugar-amino reaction. Calcium may interact with milk proteins and thus restrain lysine from entering into the browning reaction.

On the other hand, Na-citrate reduced available lysine markedly. According to Vujicic et al. (1968), the calcium-citrate complex did not have a great

affinity for proteins. Also, Na-citrate increased the amount of soluble calcium. When Na-citrate binds calcium there should be more browning and less available lysine because calcium, as mentioned previously, interacts with proteins. Therefore Na-citrate has an opposite effect to that of calcium chloride.

Certainly the time and temperature of sterilization are important factors which contribute significantly to the formation of brown color and availability of lysine. Thus, evaporated milk should be cooled immediately after the sterilization process. Otherwise milk will be exposed to excessive heat which will increase the formation of brown color and reduce the available lysine.

Sensory Evaluation of Recombined Evaporated Milk

Sensory evaluation is an important part of the processing and development of new milk products. The method employed to evaluate the recombined milks developed in the study consisted of a performance survey given to 20 participants. This number was sufficient to provide a "working" opinion about the products which were produced.

Data in Table 25 show the results of sensory evaluation of recombined evaporated milk. Panelists easily pointed out the differences in color in recombined milk. The color of Ca-caseinate milk was

Table 25—Sensory evaluation of recombined evaporated milk.*

Sample	Color	Visual texture (body)	Flavor	Mouth Feel (smooth, coarse)	odor
Na-caseinate evaporated milk	2.20d	3.05c	2.70b	3.25c	3.55b
Ca-caseinate evaporated milk	4.15a	3.55bc	2.85b	3.90ab	3.95ab
Micellar-casein evaporated milk (control)	3.90ab	3.80ab	4.15a	4.05ab	4.00ab
90% (V/V) Na-caseinate milk plus 10% (V/V) micellar-casein milk	3.05c	3.70ab	3.55a	3.70bc	4.05ab
80% (V/V) Na-caseinate milk plus 20% (V/V) micellar-casein milk	3.40bc	3.60bc	3.90a	3.90ab	4.20a
70% (V/V) Na-caseinate milk plus 30% (V/V) micellar-casein milk	3.75ab	4.35a	4.05a	4.25ab	4.40a
90% (V/V) Ca-caseinate milk plus 10% (V/V) micellar-casein milk	4.05ab	3.60bc	3.75a	4.15ab	4.35a
80% (V/V) Ca-caseinate milk plus 20% (V/V) micellar-casein milk	3.90ab	3.90ab	3.95a	4.20ab	4.15a
70% (V/V) Ca-caseinate milk plus 30% (V/V) micellar-casein milk	3.90ab	3.95ab	4.00a	4.45a	4.35a

*a scale of 1 to 5 (5 being the most desirable) (n = 20)

Means within each column not followed by the same letter are significantly different ($p \leq 0.05$).

considered the best among the recombined milks, whereas Na-caseinate milk color was rated the worst among all samples. Mixing micellar-casein milk (control) with Na-caseinate milk increased the acceptance of the color significantly ($p \leq 0.05$).

Similarly, Na-caseinate milk and Ca-caseinate milk had the same flavor range scores, which were lower than that of micellar-casein milk. When 10%, 20%, and 30% of micellar-casein milk were mixed with Na-caseinate milk or Ca-caseinate milk, the panelists were unable to detect any significant differences in flavor. However, the acceptability of caseinate milks was increased by increasing the amount of micellar-casein milk.

After tasting the samples, the panelists described the products as smooth and with no graininess in the mouth or throat. They showed no significant differences ($p \leq 0.05$), except for Na-caseinate milk, which was significantly different from all other samples.

In general, the following conclusions may be drawn. Na-caseinate milk had the highest heat stability and browning, and the lowest acceptance in flavor among the three types of milk. However, Ca-caseinate milk had a lighter color, and micellar-casein milk showed the highest acceptance in terms of flavor, mouth feel, texture, and odor. Therefore, mixing the three types of milk (one-third each) should probably produce a product

possessing high heat stability, light color, and good flavor, texture, and odor.

Effect of Heat and Addition of Salts on the Size and Shape of Casein Micelles

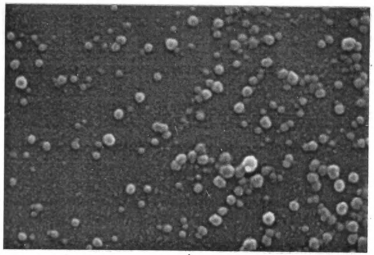
Casein occurs in milk as micelles and the properties of these micelles largely determine the behavior of milk during technological processes such as sterilization and concentration. The casein micelles are largely composed of protein but they also contain a small ($\simeq$7%) but essential amount of inorganic constituents of which calcium and phosphate are the most important.

Micellar-casein skim evaporated milk was heated at 118°C (245°F) for 10, 15, and 20 minutes (Figure 17). When milk was heated, a marked change occurred in size and appearance of casein micelles. It can be seen that the casein micelles are shaped as more or less regular globules, however, with different diameters. For non-sterilized milk (Figure 17a), the casein micelles were spherical and had diameters in the range of 70-300 nm; most of the micelles had a diameter of about 200 nm.

Heating evaporated milk caused an increase in particle size. When milk was heated at 118.3°C (245°F) for 10 minutes, the diameter of most of the casein micelles was 370 nm. Increasing the time of the heat treatment at 118.3°C (245°F) had an even greater effect on the micelles. A comparison of effects of exposure

Figure 17. Micrographs of micellar-casein skim evaporated milk showing the effect of heating (magnification 10,000X).
(a) non-heat treated milk
(b) 118.3°C (245°F) / 10 minutes
(c) 118.3°C (245°F) / 15 minutes
(d) 118.3°C (245°F) / 20 minutes

0.2 ml of 10% solutions were added to 15 ml of skim evaporated milk and sterilized at 118.3°C (245°F) for 15 minutes (magnification 10,000X)



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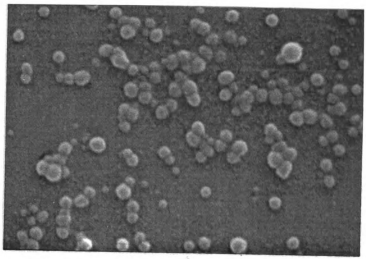

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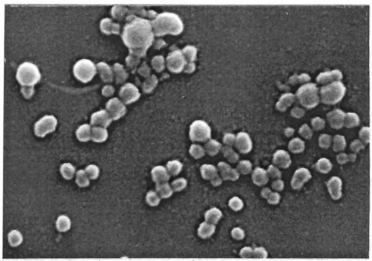
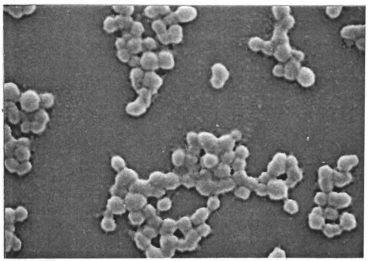


C



D

times for a given temperature revealed that a treatment of 20 minutes yielded micelles with the largest diameter (490 nm), whereas heating treatments of 15 minutes resulted in micelles with a diameter of 440 nm. Additionally, increasing the heat treatment caused an increase in aggregation and the casein micelles' shape remained unchanged (nearly spherical). Thus, the increase in micelle size seems mainly to be the result of heat treatment.

According to Schmidt (1968), the increase in particle size in conventionally sterilized milk (118°C for 13 minutes) was 40% greater than that in UHTST-sterilized milk (135°C for 15 seconds). However, Morr (1965) reported some increases in particle size due to concentration. Our micrographs show that the increase in micelle size was due to heating because all samples at the same concentration.

According to Carroll and Thompson (1971), the increase in micelle size is attributed, first, to the interaction of denatured whey protein (predominately B-lactoglobulin-K-casein complex function) and, second, to the effect of heat on the state of ionic calcium in milk. The amount of serum calcium is decreased by heating and precipitates on the surface of the casein micelle.

Addition of Na-citrate, disodium phosphate, and calcium chloride changed the shape and size of casein

micelles (Figure 18). When Na-citrate or disodium phosphate were added to micellar-casein milk, the micelles became spherical and small with diameters of 310 and 400 nm, respectively. This was attributed to chelation properties of both Na-citrate and Na_2HPO_4 because they bind Ca^{2+} and thus reduce the colloidal phosphate level.

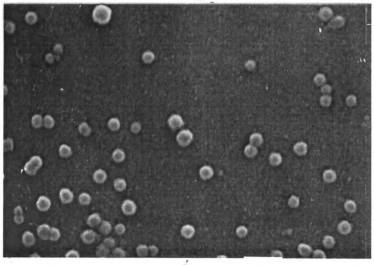
From previous tests (Tables 5 and 17), both Na-citrate and Na_2HPO_4 exhibited the highest heat stability and browning reaction. This may be attributed to the reduction in size and disaggregration of the casein micelles.

Addition of calcium chloride to micellar-casein milk caused an aggregation of casein micelles. The smaller micelles in particular (450 nm) were of regular spherical shape, whereas the larger ones (600 nm) which emerged from the aggregation of smaller micelles resulted in micelles with less well-defined edges. According to Thompson et al. (1969), an increase in calcium content would certainly lead to calcium bridging among micelles with a concomitant increase in micelle size.

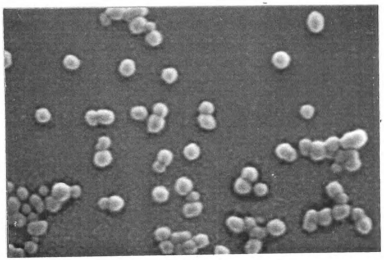
Figure 19 shows the effect of addition of sodium hexametaphosphate (SHMP), Na-sulfite, and Na-bisulfite to micellar-casein skim evaporated milk. Addition of sodium hexametaphosphate resulted in disaggregation of the micelle and a decrease in its diameter. The casein

Figure 18. Micrographs of micellar-casein skim evaporated milk showing the effect of adding
(a) sodium-citrate
(b) disodium phosphate
(c) calcium chloride

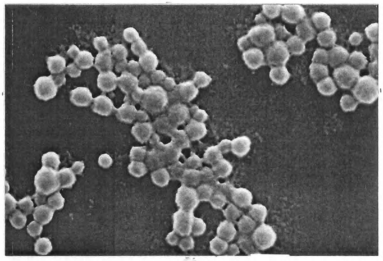
0.2 ml of 10% solutions were added to 15 ml of skim evaporated milk and sterilized at 118.3°C (245°F) for 15 minutes (magnification 10,000X)



A



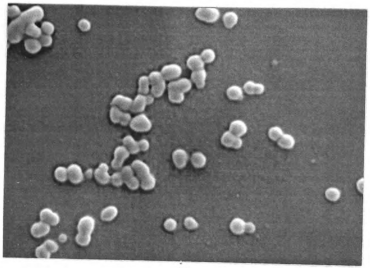
B



C

Figure 19. Micrographs of micellar-casein skim evaporated milk showing the effect of adding
(a) sodium hexametaphosphate
(b) sodium sulfite
(c) sodium bisulfite

0.2 ml of 10% solutions were added to 15 ml of skim evaporated milk and sterilized at 118.3°C (245°C) for 15 minutes (magnification 10,000X)



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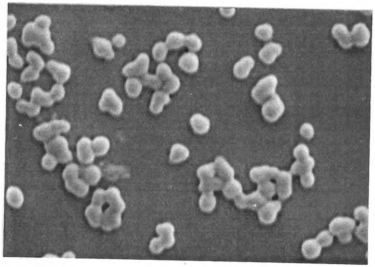
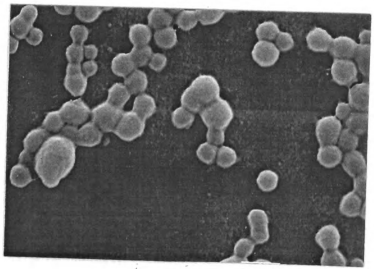


B



C

micelles are irregularly shaped with a diameter of 390 nm. It is known that sodium hexametaphosphate reduced the soluble and ionic calcium. According to Herreid and Wilson (1963), added polyphosphate compounds combined with milk protein and calcium, penetrating into the caseinate particles to bind calcium, thus forming a more stable complex.

Heat stability of milk increased significantly with the addition of sodium hexametaphosphate (Table 5). is may be attributed to the disaggregation of the casein micelles.

Addition of sodium sulfite and sodium bisulfite to milk caused partial aggregation of casein micelles of different size. The shape of the micelles was chain-like aggregates with irregular and less well-defined edges. The micelles had diameters of 510 nm and 650 nm for milk containing sodium sulfite and sodium bisulfite, respectively.

SUMMARY AND CONCLUSIONS

Recombined evaporated milk can be prepared utilizing sodium-caseinate, calcium-caseinate, whey protein concentrates, lactose, unsalted butter, artificial evaporated milk flavor, and nonfat dry milk. Three types of recombined evaporated milk were prepared: sodium-caseinate, calcium-caseinate, and micellar-caseinate.

Results obtained indicated that the casein type is an important factor in determining the heat stability and browning of recombined evaporated milk. Sodium-caseinate milk exhibited the highest heat stability and browning reaction, whereas calcium-caseinate milk showed the lowest heat stability and browning.

Forewarming milk at 93.3°C (200°F) for 10 minutes, readjusting the pH to its original value, and addition of Na-citrate and disodium phosphate showed a significant increase in the heat stability of all three types of milk.

Among the salts studied, sodium-citrate and disodium phosphate increased the browning significantly, whereas calcium chloride, sodium hexametaphosphate, sodium sulfite, and sodium bisulfite reduced the

browning significantly. Heating evaporated milk and addition of Na-citrate showed a reduction in available lysine. However, evaporated milk samples containing calcium chloride, sodium hexametaphosphate, or sodium bisulfite showed an increase in available lysine as compared with the control (milk containing no salt).

Heating and addition of calcium chloride, sodium sulfite, and sodium bisulfite caused an increase in the size of casein micelles, whereas sodium citrate, disodium phosphate, and sodium hexametaphosphate showed a reduction in the size of casein micelles.

Results from the sensory evaluation by a consumer panel indicate that there were no significant differences in acceptability when 10%, 20%, and 30% of micellar-casein milk were mixed with Na-caseinate milk or Ca-caseinate milk. The color and flavor of sodium-caseinate milk and the flavor of calcium-caseinate are the two major problems. By adding the appropriate salt, such as sodium hexametaphosphate (0.13%), the color of sodium-caseinate milk improved significantly. Addition of artificial evaporated milk flavor (0.10%) improved the flavor of caseinate milks. Therefore, mixing the three types of milk (one-third each) should probably produce a product possessing improved quality.

More work is needed to study the storage life of recombined evaporated milk. It is believed, however, based on the present study, that recombined evaporated milk can be successfully produced.

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