

# A Rapid Method for Laboratory Preparation of High Solids Casein and Caseinate Dispersions

E. D. STRANGE and R. P. KONSTANCE  
US Department of Agriculture  
Eastern Regional Research Center  
600 East Mermaid Lane  
Philadelphia, PA 19118

## ABSTRACT

A laboratory technique for the dispersion of casein or caseinate into liquid using a food processor as the mixing tool is described. The liquid, as crushed ice, was added to the processor before or after the casein or caseinate. The progress and completeness of mixing was monitored by variances of the moisture content of product sampled from different sections of the processing bowl. Total time for complete dispersion was 8 min or less, much faster than other dispersion methods.

(Key words: high solids casein, casein dispersions)

## INTRODUCTION

The preparation of dispersions or solutions of caseinates and caseins poses a problem. The method used for dispersion has a profound effect on the functional properties of milk protein particularly viscosity (4, 6). Kinsella (4) and Mulvihill and Fox (6) emphasized the need for standardized methods of dispersion.

Casein and caseinates are difficult to solubilize or disperse. Towler (11) added spray-dried caseinate at rates of .15 to .25 g per s and sampled after an additional 5, 15, and 30 min of stirring and found it impossible to measure the dispersion of 10% solutions because of floating agglomerates. The dry protein, particularly if it has a low bulk density, may float on top of the solution (11).

Techniques have been developed to handle such dispersion and solubility problems. The most common approach to the problem is to add dry casein or caseinate powder slowly by mechanical shaking into the vortex of the rapidly stirring solvent at 60°C (1, 11). The dispersing or dissolving solvents frequently contain sodium tetraborate (5, 7) or sodium triphosphate (1), which alters the pH of the protein dispersion. Other procedures for preparing solutions or dispersions are based on the method of Dolby (2). In his procedure, the casein is soaked in water for 5 to 10 min before heating at 60°C for 30 min.

Hooker and Roeper (3, 8) prepared high solids caseinate solutions for viscosity measurements with fresh caseinate made by disintegrating "dry" acid casein in a colloid mill and adding the appropriate mineral base. This approach avoided the problem of preparing uniform dispersions of the dried proteins.

The objective of this study is to describe a simple and rapid method for the preparation of solutions or dispersions of dried commercial casein and caseinates that subsequently may be used for rheological studies or in the preparation of solid or semisolid food products from milk proteins.

## MATERIALS AND METHODS

### Equipment

A Cuisinart Model CFP 5A (Robot-Coupe sa, France)<sup>1</sup> food processor equipped with a stainless steel blade was used to make all dispersions. The blade rotated at 1790 rpm, and the processing bowl held 750 ml of water.

Temperature of casein or caseinate slurries was measured using a glass temperature probe from a Corning model 135 pH/Ion Meter (Corning Science Products, Medfield, MA).

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<sup>1</sup>Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

### Materials

Commercial (New Zealand Milk Products, Inc., Petaluma, CA) calcium caseinate (Alanate 310, 89.8% protein, 4.1% ash, 4.5% water, 1.1% fat, and .1% lactose), sodium caseinate (Alanate 130, 82.4% protein, 4.3% ash, 9.0% water, 1.1% fat, and .1% lactose), and rennet casein (Alanate 771, 80.6% protein, 7.8% ash, 11% water, .5% fat, and .1% lactose) were used to prepare dispersions. Proximate analyses were obtained from the manufacturer.

### Ice to Casein

The appropriate amount of calcium caseinate (47 g for 15% solids, 93 g for 30% solids, and 140 g for 45% solids), sodium caseinate (100 g for 30% solids), or rennet casein (100 g for 30% solids) was added to the dry food processor bowl, and the lid was attached. The processor was run for 1 s to distribute the casein evenly. The lid was removed, and crushed ice was added to a total of 300 g (e.g., 253 g of ice for 15% calcium caseinate dispersion). The lid was replaced, and processing was begun. After 15 s, the processing was stopped, the ice and casein slurry was hand mixed, and the processing continued for an additional 15 s. Total mixing time was 30 s. Four 2.5- to 3.0-g samples were removed for moisture determination, one from each quadrant of the processing bowl. Processing was continued with additional hand mixing at 45 s, 1 min 30 s, 3 min, and 6 min after the start of processing. Subsequent samples were removed after 1, 2, 4, and 8 min so that variances of the moisture content could be determined as a function of mixing time.

Moisture content of the samples was determined by the following procedure. The ice and casein slurry sample was put into tared aluminum drying dishes 5 cm in diameter, and the exact weight of the sample was determined by difference on an analytical balance. The samples were predried at 110°C for 2 h in an oven to remove most of the moisture before final drying at 60°C under vacuum for 18 h. A predrying step can be used with caseinates because they have a low carbohydrate content. The residue weight was measured, and the moisture content of each sample was calculated.

Each calcium caseinate dispersion and sampling was carried out in duplicate. Sodium ca-

seinate (30%) and rennet casein (30%) were not duplicated.

### Caseinate to Ice

In this series of experiments, the weighed crushed ice was added to the processing bowl, and the processor was run for 1 s to distribute the ice more evenly in the bowl. The processor was stopped, and the caseinate powder was sprinkled over the crushed ice. Processing, hand mixing, and sampling were carried out as described.

### Statistical Analysis

The variances were calculated as described by Steel and Torrie (10), and the 95% confidence limits for the variances were calculated as described by Snedecor and Cochran (9).

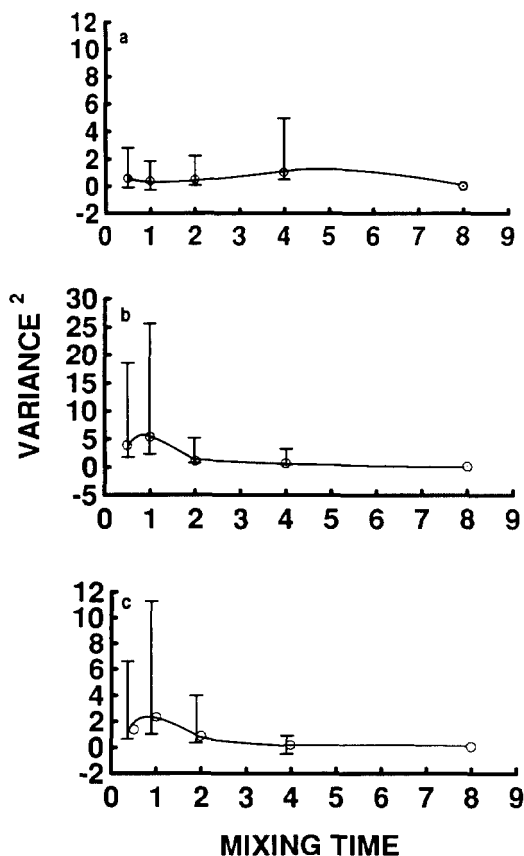


Figure 1. Ice added to caseinate A) 15% calcium caseinate, B) 30% calcium caseinate, and C) 45% calcium caseinate. All experiments were run in duplicate.

Photographs

Samples of a 30% ice to calcium caseinate dispersion were removed at the same times as samples for moisture determination. These samples were spread on plastic weighing dishes and photographed through a stereo microscope using 35 mm Kodak TMAX 100 film (Eastman Kodak Co., Rochester, NY). Total original magnification was 17 $\times$ .

RESULTS AND DISCUSSION

The variances and their 95% confidence limits versus mixing time for moisture determinations on 15, 30, and 45% calcium caseinate (ice added to caseinate) are shown in Figure 1. As mixing time increases, the 95% confidence

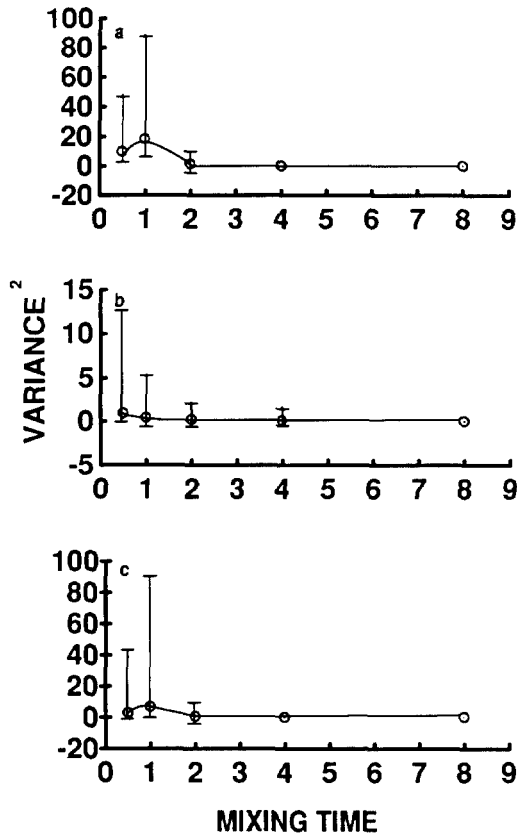


Figure 2. A) Caseinate added to ice, 30% calcium caseinate (run in duplicate); B) ice added to caseinate, 30% sodium caseinate (single experiment run); and C) ice added to casein, 30% rennet casein (single experiment run).

limits become much smaller, indicating better dispersion. For 15 and 30% calcium caseinate, mixing was not completed until 8 min, when the confidence limits were  $.001 < s^2 < .01$  for 15% and  $.02 < s^2 < .28$  for 30% calcium caseinate. For 45% calcium caseinate, mixing was complete after 4 min (95% confidence limits  $.07 < s^2 < .8$ ). However, the confidence limits decreased with continued mixing to  $.02 < s^2 < .25$  at 8 min.

When calcium caseinate was added to ice (Figure 2A), the initial variances and confidence limits were much higher than those found for ice added to caseinate, but by 4 min the mixing was completed (95% confidence limits  $.02 < s^2 < .25$ ), and by 8 min the 95% confidence limits had decreased to  $.006 < s^2 < .07$ .

These results illustrated that the order of addition, casein to ice or ice to casein, was of little import provided sufficient mixing time was allowed. In all cases, 8 min was sufficient time for complete mixing to occur.

When sodium caseinate was used (Figure 2B), the mixing pattern was identical to calcium caseinate, i.e., mixing was complete after 8 min (95% confidence limits  $.0004 < s^2 < .018$ ). However, when rennet casein (an insoluble material) was processed, another pattern was observed (Figure 2C). The smallest vari-

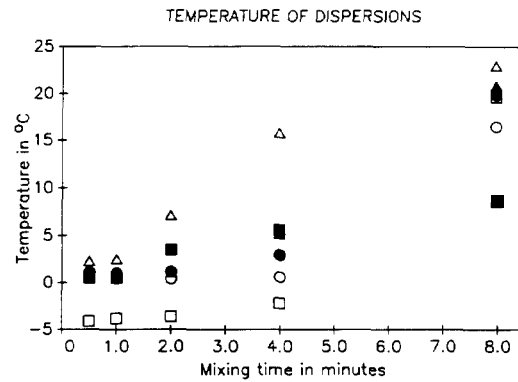


Figure 3. Ice added to caseinate: Open circle, 15% calcium caseinate; filled circle, 30% calcium caseinate; open triangle, 45% calcium caseinate. The experiments were run in duplicate. Open square, 30% sodium caseinate; filled square, 30% rennet casein. Single experiments were run. Caseinate added to ice: filled triangle, 30% calcium caseinate. The experiment was run in duplicate.

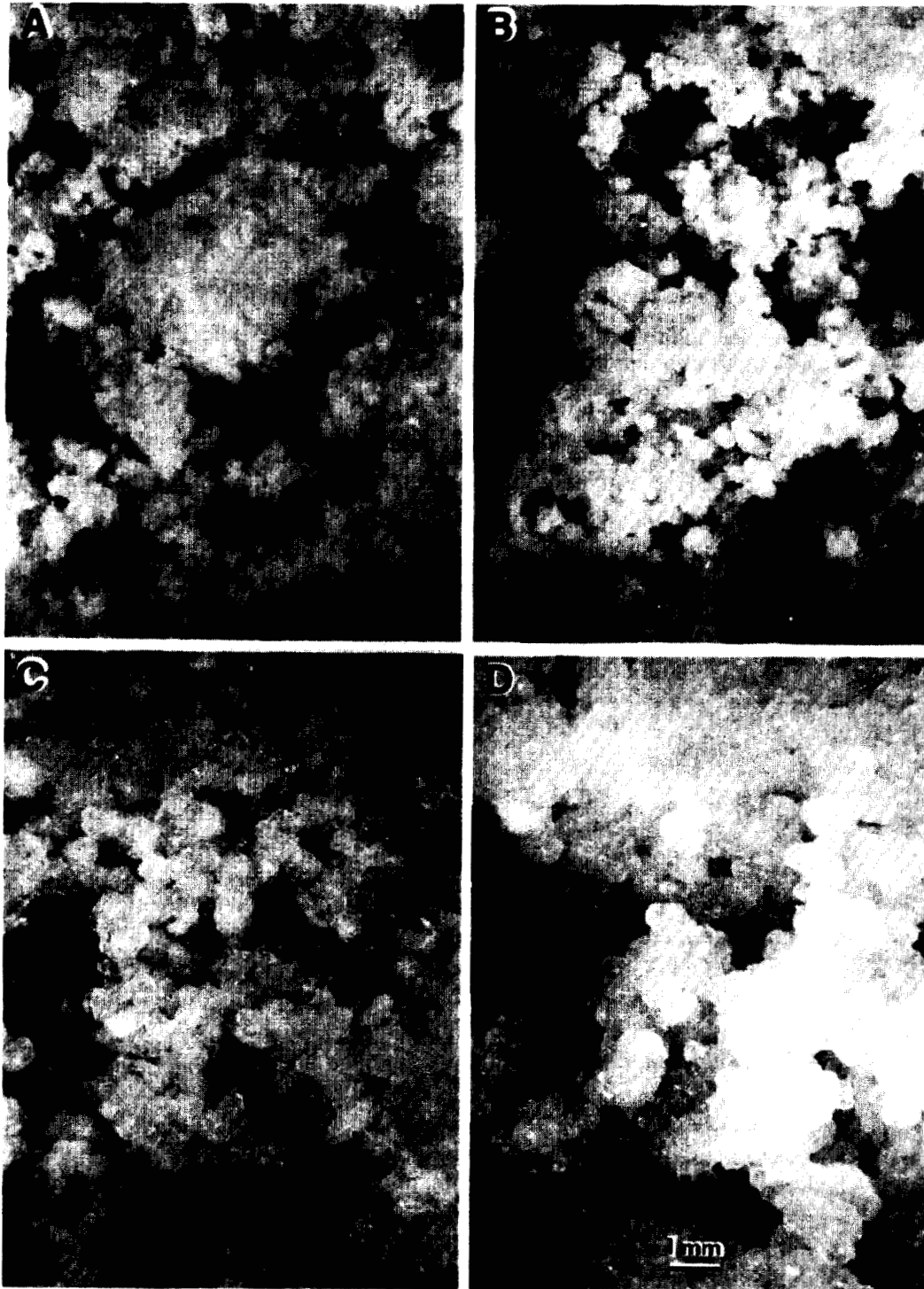


Figure 4. Photomicrographs of 30% calcium caseinate solutions: A, after 30 s mixing; B, after 2 min mixing; C, after 4 min mixing; D, after 8 min mixing.

ance (.03) and 95% confidence limits ( $.01 < s^2 < .43$ ) were observed at 4 min. When mixing was continued, the variance increased to .26, and the 95% confidence limits increased to  $.08 < s^2 < 3.68$ . This was because the ice in the mixture melted between 4 min (temperature of the slurry = 5.6°C) and 8 min (temperature of the slurry = 8.6°C) and the rennet casein settled out from the melted ice. This effect increased the variance.

Temperatures of the slurries increased during mixing due to the input of mechanical energy from the shearing action of the rotating blade and the resistance to such action by the protein solutions. Figure 3 shows how the temperature varied with mixing time. Sodium caseinate slurries showed supercooling, which commonly occurs when the freezing point of a solution (in this case sodium caseinate in water) is less than the freezing point of the solvent (water). Another factor that affected the temperature of the slurries was the amount of ice contained in the initial mixture. Ice has a large heat of fusion, and mechanical energy equal to the heat of fusion must be added before an increase in temperature occurs. The temperature versus mixing times behavior of the three different concentrations of the calcium caseinates illustrated this effect. Rennet casein, which did not form a solution, showed the least resistance to mechanical shear, and as a result the final temperature of the rennet casein-water dispersion was the lowest of any mixture tested.

A trial run using ice added to high heat treated nonfat dry milk at a 30% solids concentration had mixing behavior similar to that of calcium and sodium caseinate, although the physical appearance of the ice and milk slurry was considerably more liquid like than any of the proteins.

When cold water was used rather than ice for preparing 30% solids sodium and calcium caseinate dispersions, viscous gel-like agglomerates formed within 15 s from the start of mixing. Sodium caseinate formed a solid ball of dough that resisted any further mixing. Calcium caseinate continued to mix after the first 15 s but became extremely warm with 1 min mixing time. No detailed moisture analyses of the mixing behavior were carried out on these trials.

Figure 4 illustrates the structural changes in the caseinate powders that occur during mixing.

The temperatures of the slurries pictured in Figure 4A and 4B were 2.0°C or less; in Figure 4C, 3.3°C; and in Figure 4D, 20.7°C.

In Figure 4A (after 30 s of mixing), the calcium caseinate powder was clustered into aggregates with highly irregular contours surrounding ice particles. In Figure 4B (after 2 min of mixing), some water droplets were visible, and the aggregates consisted of coarser particles than in A. In Figure 4C (after 4 min of mixing), the droplets of water were incorporated, and the particles of powder formed irregular round structures, which finally, in Figure 4D (after 8 min of mixing), had swollen to .5 mm in diameter and formed grape-like clusters.

This procedure can be used to prepare casein, caseinate, and nonfat dry milk dispersions or solutions in less than 10 min in almost any high solids concentration desired. Some air is incorporated into the slurry, but foaming is not a problem unless the processing is continued after complete dispersion is accomplished. The ice used can be prepared from buffer or sugar solutions according to individual needs. Crushing of ice can be accomplished in the processor. The preparation of dispersion or solutions can be shortened by additional hand mixing, because all of the mixtures tested tend to form bridges of slurry above and below the blades when processing. Hand mixing breaks these bridges. To scale up in quantity, a silent cutter of the type used to make meat emulsions could be used, and to scale down, mini food processors could be used.

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