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# ENZYME ACTIVITY OF ICE-CREAM IMPROVERS

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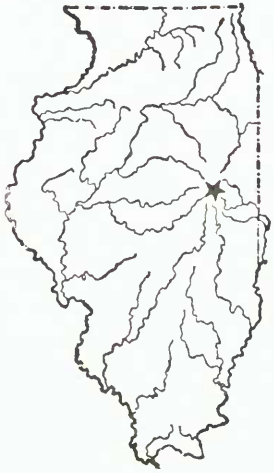

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# ENZYME ACTIVITY OF ICE-CREAM IMPROVERS

P. H. Tracy and H. A. Ruehe[1]

During recent years several different brands of so-called ice-cream improvers have been placed on the market. The manufacturers claim certain merits for the use of these products such as shorter aging period, better body for the ice cream, and improved flavor. These advantages have been made the subject of a study by other investigators and will therefore not be considered here.[2] The exact composition of the improvers, however, and their action in ice cream is not generally understood. If the ice cream manufacturer is to use these products intelligently he should be familiar with the properties of the improver he is adding to his mix and also with the underlying principles of its action both on the mix and on the ice cream. It was for the purpose of obtaining such information that this study was made.

## COMPOSITION OF IMPROVERS

Samples of twelve different improvers and of rennet and pepsin were secured for this study. Qualitative tests for enzymes, sugar, starch, and gum were made on each sample. In addition, the amount of titratable acidity, as measured with N/10 NaOH, and the number of bacteria per gram were determined for each improver. The results are recorded in Table 1.

*Determination of Enzymes.*—According to Cole,[3] "pepsin is almost completely destroyed by heating for ten minutes at 38° C. at a pH = 7.25. Rennin loses only a small fraction of its activity . . . Heating for two minutes at 70° C. at a pH = 5, completely destroys rennin, but has no effect on pepsin." Using this as a basis, an attempt was made to determine whether either of the two enzymes was present in the improvers studied.

The colorimetric method was used in standardizing the pH concentration of the improver solutions. The indicator solutions used were methyl red for the pH = 5, and cresol red for the pH = 7.25 concentrations. The indicator solutions were prepared according to Clark[4] as follows:

[1] P. H. Tracy, Assistant Chief Dairy Manufactures and H. A. Ruehe, Chief Dairy Manufactures.

[2] Isenberg, G. H. and Ross, A. C., Okla. Agr. Exp. Sta. Bul. 158, Jan., 1926. Sommer, H. H., Wis. Agr. Exp. Sta. Bul. 396, 1927.

[3] Cole, Sydney William. Practical physiological chemistry. 2-8. 1923.

[4] Clark, W. M. Cole, plant molecule, prepared acids. "The Determination of Hydrogen Ions." 1920.

*Methyl Red.*—Two one-hundredths of a gram of the indicator was added to a mixture of 60 cc. of 95-percent alcohol and 40 cc. of distilled water.

*Cresol Red.*—One-tenth gram of the indicator was added to 2.65 cc. of N/10 NaOH diluted to 500 cc. with distilled water.

One-percent water solutions of each improver were prepared. In standardizing the pH concentrations, .3 cc. of the methyl red indicator solution and .5 cc. of the cresol red indicator solution were added to 10 cc. of the filtrate from the improver solutions. One-tenth normal

TABLE 1.—COMPOSITION OF ICE-CREAM IMPROVERS

| Improver No. | N/10 alkali to neutralize 1 gram | Bacteria per gram | Starch | Sugar | Gum[1] | Enzyme[2] |
|---|---|---|---|---|---|---|
| | *cc.* | | | | | |
| 1 | alkaline | 0 | 0 | + | + | P+R |
| 2 | 2.82 | 0 | 0 | + | + | P |
| 3 | 2.85 | 750 | 0 | + | + | P |
| 4 | alkaline | 8 000 | + | + | 0 | P |
| 5 | alkaline | 210 | + | + | 0 | R |
| 6 | alkaline | 1 850 | 0 | + | 0 | 0 |
| 7 | alkaline | 76 000 | + | + | 0 | R |
| 8 | 2.28 | 1 400 | 0 | + | + | R |
| 9 | 3.42 | 2 700 | 0 | + | + | R |
| 10 | 4.57 | 1 000 | 0 | + | + | P |
| 11 | 1.14 | 6 800 | + | + | + | P |
| 12 | alkaline | 250 | 0 | + | 0 | P+R |
| Rennet | alkaline | 800 | 0 | 0 | 0 | R+P[3] |
| Pepsin | 11.1 | 30 000 | 0 | 0 | 0 | P+R[4] |

[1]Improver 1 gave a test for gelatin. [2]P = pepsin, R = rennet. [3]Very slight reaction for pepsin. [4]Very slight reaction for rennet.

NaOH and one-tenth normal HCL were used to adjust the pH concentrations. The solutions standardized to pH = 5 were heated for two minutes at 70° C., while those standardized to pH = 7.25 were heated for 10 minutes at 38° C. The heated solutions were then added to 30 cc. of sterilized milk to which 1 cc. of 10 percent $CaCl_2$ had been added. The samples were all held in a 45° C. incubator. The activity of the enzyme was checked by the control sample (10 cc. of the improver solution, 1 cc. of $CaCl_2$ and 30 cc. of sterilized milk). In every case the coagulation of a sample occurred practically at the same time as the coagulation of the control. Eleven of the 12 improvers were found to contain an enzyme. Five contained pepsin, 4 contained rennet, and 2 contained both pepsin and rennet.

*Determination of Presence of Sugar.*—Using Fehling's solution, the different improvers were tested for the presence of sugar. With the exception of one improver in which it was necessary to hydrolize the

sugar, the tests were easily performed. The hydrolization was accomplished by adding a small amount of concentrated $H_2SO_4$ (one drop to 5 cubic centimeters of the solution) and boiling the mixture.

All of the commercial improvers contained sugar (Table 1). No attempt was made to determine the kind of sugar present.

*Determination of Gums and Starch.*—The Congdon and Patrick[1] methods were used to detect the presence of gums, while starches were detected by the starch-iodin test.

Gums formed a part of 7 of the 12 improvers, while 4 contained starch.

*Determination of Acidity and Bacterial Count.*—Using phenolphthalein as an indicator the amount of N 10 NaOH required to neutralize the acidity of each improver was determined. It is apparent that gum increases the acidity of the improver since all of those containing gum had a rather high titratable acidity while those without gum were alkaline. The number of bacteria per gram was determined by the plate method, using agar media containing 1 percent peptone, 1 percent lactose, and a small amount of beef extract. The plates were incubated at 37° C. for 48 hours. None of the improvers contained an appreciable number of bacteria, the count ranging from none to 76,000 per gram.

It seems from the data recorded in Table 1 that the main constituent of an improver is the enzyme that it contains, which, together with the gum, is the "ripening agent" that improvers are commonly credited with containing. The sugar and starch present in the various improvers are used primarily as fillers or carriers for the gum and enzyme.

## FACTORS AFFECTING THE ENZYME ACTIVITY OF IMPROVERS

Inasmuch as the active agent of most of the improvers investigated was an enzyme, a study was made to determine what factors affect enzyme activity of improvers in ice cream.[2]

### Variations in Activity of Enzyme Contained in Different Improvers

One gram of each of the improvers and of pepsin and rennet was added to a 100-cc. portion of pasteurized milk (142° F. for 30 minutes)

[1] Congdon, L. A., Jour. Indus. and Engin. Chem. 7, 606, 1915.

[2] When considered practical, skimmed or whole milk was used in place of ice-cream mix.

and the samples were held at room temperature (75° F.). The time required for each lot to become coagulated is indicated below.

| Sample | Time to coagulate | | Sample | Time to coagulate | |
|---|---|---|---|---|---|
| | *hrs.* | *min.* | | *hrs.* | *min.* |
| Control..... (no change after 7 hrs.) | | | 8..... | 2 | 30 |
| 1..... | 3 | 30 | 9..... | 1 | 15 |
| 2..... | 2 | 30 | 10..... | 1 | 10 |
| 3..... | 2 | 30 | 11..... | 2 | 30 |
| 4..... | 2 | 30 | 12..... | 3 | 30 |
| 5..... | 5 | 30 | Pepsin..... | 0 | 50 |
| 6..(no enzyme: no change after 7 hrs.) | | | Rennet..... | 0 | 50 |
| 7..... | 4 | 0 | | | |

It will be seen that there is considerable variation in the enzyme activity of the different improvers. This no doubt accounts for the wide variation in recommendations made by the different improver manufacturers for the use of their products.

## Enzyme Activity as Affected by Certain Salts

In order to obtain further information regarding the active agents in ice-cream improvers, various studies were made to determine the effect that the addition of $NaHCO_3$ and $CaCl_2$ would have upon the activity of the enzyme contained in the improver.

According to Effront and Prescott[1] the activities of rennet are lessened by the addition of an alkali. These authors also state that heating milk to high temperatures will precipitate the calcium ions which are necessary for enzymic coagulation of casein, but that the addition of a soluble calcium salt seems to restore the necessary equilibrium.

*Effect of Adding $NaHCO_3$.*—One cc. of 2-percent rennet solution was added to 10 cc. of milk with an acidity of .18 percent, and a similar amount of the same rennet solution was added to 10 cc. of milk with its acidity reduced to .11 percent. The tubes were held at room temperature (70° F.). In the first case coagulation took place in $8\frac{1}{2}$ minutes, whereas in the second case about 3 hours were required. That the $NaHCO_3$ had an inhibiting effect upon enzyme activity is evidenced by results obtained when the acidity of the milk was reduced from .18 to .11 percent.

*Effect of Adding Soluble Calcium Salts.*—One gram of each of the improvers, as well as one gram each of rennet and pepsin, was added to 100-cc. portions of milk that had been sterilized by heating in the autoclave under 12 pounds pressure for 45 minutes. Ten cubic centimeters of each lot was put into test tubes and eight drops of 10-percent

[1] Effront, Jean and Prescott, Samuel C. Biochemical catalysts in life and industry. 100-101, 173-176. 1917.

calcium chlorid solution added to each tube. Immediately all but the control and Sample 6 (which contained no rennet or pepsin) coagulated. Both the control and Sample 6 were still unchanged after 4 hours at 45° C. and after 16 hours at 20° C.

The effect of adding calcium lactate upon enzyme activity was also studied.

| Sample | 5-percent calcium lactate | Rennet added | Time to coagulate |
|---|---|---|---|
| | cc. | cc. | |
| 10 cc. raw skim milk | 1 | .1 | Instant |
| 10 cc. raw skim milk | 0 | .1 | 4 min. |
| 10 cc. skim milk previously heated to 180° F. | 1 | .1 | 5 min. |
| 10 cc. skim milk previously heated to 180° F. | 0 | .1 | 50 min. |

These results are in accordance with the accepted belief that soluble calcium salts are necessary for the action of rennet on milk. Several theories as to rennet action have been advanced. Palmer and Richardson[1] in discussing these theories conclude that, " . . . there are at least two stages in rennet clotting. The first stage is the change of casein to paracasein by the rennin. The second stage is confined to the precipitations of the paracasein by the soluble calcium salts of the milk."

## Heating Milk Decreases Enzymic Activity of Improvers

The importance of the presence of soluble calcium salts is further brought out by a study of the effect of heating milk upon the enzymic activity of improvers. The procedure follows.

Three lots of milk were used together with 9 different improvers and rennet and pepsin.

*Lot 1.*—The milk was pasteurized by heating to 142° F. for 30 minutes. One gram of each of the improvers and rennet and pepsin was added to 100-cc. portions of milk after pasteurization.

*Lot 2.*—The milk was heated in the autoclave under 15 pounds steam pressure for 45 minutes. One gram of each of the improvers and of rennet and pepsin was added to 100-cc. portions of milk after heating.

*Lot 3.*—This lot was treated the same as Lot 2 except that 5 drops of 10-percent calcium chlorid were added to each 20 cc. of milk.

All three lots were placed in an incubator at 15° C. Lot 2 showed no change at the end of 48 hours. The changes in Lots 1 and 3 are shown in Table 2.

The improvers (with the exception of No. 6) and rennet and pepsin caused coagulation in the milk which had been heated to 142° F. for 30 minutes. The time required was from 2 to 19 hours, varying with the different kinds of improvers.

[1] Palmer, L. S. and Richardson, G. A. Colloid symposium monograph. 3. 112-134. 1925.

TABLE 2.—EFFECT ON ENZYME ACTIVITY OF ICE-CREAM IMPROVERS OF HEATING THE MILK BEFORE ADDING THE ENZYME

| Sample | Lot 1[1] | | | | Lot 3[2] |
|---|---|---|---|---|---|
| | After 2 hours | After 3 hours | After 4 hours | After 19 hours | After 46½ hours[3] |
| Control | No change | No change | No change | No change | No change |
| Improver 1 | No change | No change | Slight coagulation | Slight wheying off | Slight coagulation |
| Improver 2 | Slight coagulation | Coagulated | Slight wheying off | Wheyed off | Coagulated |
| Improver 3 | Slight coagulation | Coagulated | Slight wheying off | Wheyed off | Coagulated |
| Improver 4 | No change | Slight coagulation | Coagulated | Wheyed off | Coagulated |
| Improver 5 | No change | No change | No change | Coagulated | Slight coagulation |
| Improver 6 (No enzyme) | No change | No change | No change | No change | No change |
| Improver 7 | No change | No change | No change | Slight whey | Slight coagulation |
| Improver 8 | Slight coagulation | Coagulated | Slight whey | Whey | Coagulated |
| Improver 9 | Slight coagulation | Coagulated | Slight whey | Whey | Coagulated |
| Pepsin | Wheying off | Extreme wheying | Extreme wheying | 2/3 whey | Slight coagulation |
| Rennet | Wheying off | Extreme wheying | Extreme wheying | 1/2 whey | Coagulated |

[1]Milk heated to 142° F. for 30 minutes plus 1 gram of each improver, of rennet, and of pepsin for each 100 grams of milk (Lot 4). [2]Milk heated in autoclave under 15 pounds steam pressure for 45 minutes. One gram of each of the improvers was added to 100 cc. of milk plus 5 drops of 10% $CaCl_2$ to each 20 cc. of milk (Lot 3). [3]There were no changes at the end of 30 hours.

Milk heated to a high temperature did not coagulate upon the addition of rennet, pepsin, or the improvers. However, coagulation did take place between 30 to 46 hours, when a small amount of calcium chlorid was added to this milk. This occurred in all instances with the exception of Improver 6, which contained no enzyme.

It seems, therefore, that the amount of heat the mix has been subjected to previous to addition of the enzyme is very important from the standpoint of enzyme activity. Mixes pasteurized at high temperatures, or made from products heated to high temperatures in the process of manufacture, such as superheated condensed milk or evaporated milk, will not react in the same way toward improver enzymes as will mixes that have been subjected to lower temperatures.

## Dry Heat Slightly Reduces Improver Activity

According to Wells,[1] "Enzymes stand dry heat over 100° C." Such being the case, the activity of improvers containing enzymes should not be destroyed by dry heat at 100° C. The following results are in accordance with Wells' observations.

About 25 grams of each of several improvers and pepsin (powdered) were placed in open containers and held in a 100° C. constant-temperature oven for 45 hours. One gram of each of the heated and unheated improvers was added to a flask containing 50 cc. of sterilized milk. The sterilized milk was used in order to reduce the tendency for bacterial action to take place. One cc. of 10-percent $CaCl_2$ solution was then added to each flask. The samples were all held at room temperature (70° to 75° F.).

The time for coagulating reaction to take place follows:

| Improver[1] | Unheated | Heated |
|---|---|---|
| Control | Unchanged after 21 hrs. | Unchanged after 21 hrs. |
| 1 | Immediately | Immediately |
| 2 | Immediately | 13 minutes |
| 4 | Immediately | Immediately |
| 5 | 30 minutes | 3 hours 15 minutes |
| 7 | 13 minutes | 3 hours 15 minutes |
| 8 | 19 minutes | 3 hours 15 minutes |
| 9 | 13 minutes | 3 hours 15 minutes |
| 11 | 19 minutes | 3 hours 15 minutes |
| 12 | 13 minutes | 21 hours |
| Pepsin | Immediately | Immediately |

[1]Improver 6, which contained no enzyme and the liquid rennet were not used.

Improver 12 was badly charred while being heated. No doubt this was partially due to its high sugar content. This charring apparently destroyed some of its enzymic activity. The continued heating at 100° C. for 45 hours did not totally destroy the enzymes altho it did tend to reduce their activity.

[1]Wells, H. G. The chemical aspects of immunity. 41. 1925.

It may be concluded that improvers containing enzymes can be subjected to high temperatures with safety providing they are kept in a dry state.

### Enzymes Not Destroyed by Storage in Hardening Room

It is the opinion of some ice-cream manufacturers that the active principle of the ice-cream improver is destroyed after the ice cream is frozen and stored at hardening-room temperatures. The following data, however, do not support this contention.

Eleven weeks after being frozen, three samples of ice cream, one containing Improver 2, one containing rennet, and one containing pepsin, were removed from the hardening room. One-half pint of each of the ice creams was allowed to melt at room temperature. Five hundred cc. of distilled water was then added and the mixture filtered thru filter paper. One cc. of each of the filtrates was added to 5 cc. of milk (that had been sterilized by heating in the autoclave at 15 pounds pressure for 30 minutes) in each of two sets of test tubes. Four drops of 10-percent $CaCl_2$ solution were then added to one set of the tubes. The samples were placed in a 45° C. incubator. The following results were obtained:

| Sample | Time to coagulate |
|---|---|
| Improver 2 filtrate | No change after 120 min. |
| Improver 2 filtrate plus $CaCl_2$ | 60 minutes |
| Rennet filtrate | No change after 120 min. |
| Rennet filtrate plus $CaCl_2$ | 60 minutes |
| Pepsin filtrate | No change after 120 min. |
| Pepsin filtrate plus $CaCl_2$ | 10 minutes |
| Control | No change after 120 min. |
| Control plus $CaCl_2$ | No change after 120 min. |

The uncoagulated samples were then held at 45° F. overnight (14 hours) at the end of which time no change had taken place.

Thus we observe that the enzymes in the rennet, pepsin, and Improver 2 samples were active after they were in ice cream which had been stored in the hardening room for 11 weeks.

A similar experiment was performed using ice creams containing Improvers 2 and 5, and rennet and pepsin. The experiment was performed 5 months and 9 days after the ice cream had been made and placed in the hardening room. One-fourth pint sample of each ice cream was melted in 100 cc. of sterile distilled water and filtered. One cc. of the filtrate was put into a sterile test tube. Five cc. of sterilized milk and 4 drops of 10-percent $CaCl_2$ were added. Another set of samples was then prepared similar to the first set except that no $CaCl_2$ was added. A third set was prepared similar to the first except that the filtrate from the ice creams was not added. All the samples were placed in a 45° C. incubator. The samples coagulating were as follows:

| Sample | Time of coagulation |
|---|---|
| Improver 5 filtrate, plus milk, plus $CaCl_2$ | 5 hrs. 40 min. |
| Improver 2 filtrate, plus milk, plus $CaCl_2$ | 1 hr. 40 min. |
| Rennet filtrate, plus milk, plus $CaCl_2$ | 40 min. |
| Pepsin filtrate, plus milk, plus $CaCl_2$ | 20 min. |

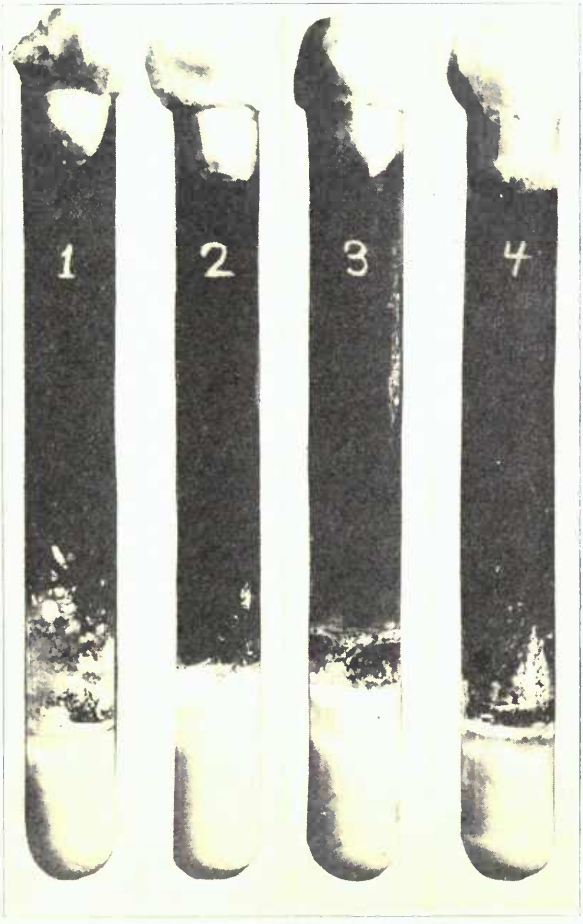


FIG. 1.—ACTIVITY OF ENZYMES AFTER STORAGE IN ICE CREAM FOR OVER FIVE MONTHS

The uncoagulated samples were held at 40° F. overnight, yet on the following morning none of them had coagulated. Figure 1 shows the condition of the coagulated samples after being held at 40° F. overnight.

Thus it is seen that the enzyme in Improvers 2 and 5 and in rennet and pepsin proved to be active even after they were contained in frozen ice cream for over 5 months. This agrees with the statement of Wells[1] to the effect that enzymes can withstand extremely low temperatures.

## ADDITION OF IMPROVERS TO ICE-CREAM MIX BEFORE PASTEURIZATION

Some attempt has been made commercially to develop an enzymic improver that can be added to an ice-cream mix previous to pasteurization. The plan is to pasteurize the mix after the desired improver action has taken place in order to destroy the enzymes and thus prevent the difficulties arising from continued enzymic action.

In order to obtain information regarding this procedure, a study was undertaken to determine some of the factors affecting the results obtained when mix[2] containing an enzyme is pasteurized.

### The Pasteurization of Milk Containing an Enzyme

In order to determine approximately the amount of rennet and pepsin which milk can contain and yet be successfully pasteurized, varying amounts of these two enzymes[3] were added to whole milk having a fat content of 4 percent and an acidity of .15 percent and the mixture heated to 145° F. in a flask submerged in hot water. The following results show that it is possible to heat milk containing an enzyme to pasteurizing temperature without coagulation taking place.

| Amount of enzyme per pound of milk | Initial temperature | Temperature at time of coagulation | Time to coagulate | Time to reach 145° F. without coagulating |
|---|---|---|---|---|
| | | | *min.* | *min.* |
| .075 gram pepsin.. . | 43° F. | ..... | ... | 8½ |
| .100 gram pepsin.. . | 44° F. | 113° F. | 5½ | ... |
| .30 cc. rennet..... | 45° F. | ..... | ... | 9½ |
| .40 cc. rennet..... | 46° F. | 118° F. | 5 | ... |

The Marchall rennet test was used to determine the relative coagulating values of the rennet and pepsin as used.

| *Enzyme* | *Reading on Marchall cup* |
|---|---|
| .5 gram pepsin..... | 2.5+ |
| 2.5 cc. rennet..... | 2.5− |

[1]Wells, H. G. The chemical aspects of immunity. 44. 1925.

[2]When practical, milk was used instead of mix.

[3]In each case a 5-percent solution was used tho the amounts recorded in the tables refer to the undiluted product.

The results show that the coagulating action of one gram of pepsin used was equivalent to that of about 5 cc. of the rennet extract.

## Acidity Favors Coagulation in Pasteurization of Milk Containing an Enzyme

Raw skim milk with an acidity of .165 percent and raw skim milk with an acidity of .285 percent were used in this experiment.

| Amount of rennet per pound of milk | Initial temperature | Temperature at time of coagulation | Time to coagulate | Time to reach 145° F. without coagulation |
|---|---|---|---|---|
| | *Skim milk, acidity .165 percent* | | | |
| *cc.* | | | *min.* | *min.* |
| .30 | 61° F. | ..... | ... | 6½ |
| .32 | 64° F. | 124° F. | 5 | ... |
| | *Skim milk, acidity .285 percent* | | | |
| .02 | 41° F. | ..... | ... | 8 |
| .04 | 39° F. | 138° F. | 8 | ... |

These data show that lactic acid favors heat coagulation for it was necessary to use a smaller amount of the rennet in order to bring the temperature of the milk to 145° F. without coagulating.

## Milk-Solids-Not-Fat Favor Coagulation in Pasteurization of Milk Product Containing an Enzyme

Two mixtures were prepared using skim-milk powder, one containing approximately 20 percent serum solids and the other approximately 15 percent. The acidity of the 20-percent mixture was .38 percent and that of the 15-percent mixture was .30 percent. The procedure was the same as that previously described. In this experiment pepsin was used as the enzyme. The results follow:

| Amount of pepsin per pound of milk | Initial temperature | Temperature at time of coagulation | Time to coagulate | Time to reach 145° F. without coagulation |
|---|---|---|---|---|
| | *15 percent m.s.n.f. solution, acidity 30 percent* | | | |
| *grams* | | | *min.* | *min.* |
| .04 | 80° F. | ..... | ... | 5 |
| .05 | 80° F. | 124° F. | 4 | ... |
| | *20 percent m.s.n.f. solution, acidity 38 percent* | | | |
| .02 | 80° F. | ..... | ... | 4½ |
| .03 | 80° F. | 125° F. | 3 | ... |

As these data indicate, the greater the percentage of milk-solids-not-fat the more danger there is of curdling. It seems therefore that less enzyme should be used in mixes containing a high percentage of serum solids.

## Relation of Sugar and Vegetable Gum to Coagulation in Pasteurization of Milk Containing an Enzyme

This experiment was conducted with both raw skim milk and with skim milk that had been heated to 180° F. Three lots of each kind of milk were used as follows: (1) milk alone, (2) milk plus sugar (1 pound of sugar to 5 pounds of milk), and (3) milk plus sugar (1 pound to 5 pounds of milk) plus gum (.5 percent). The gum was added by first mixing it with the sugar and then dissolving both in the milk. Rennet was used as the enzyme in this experiment. The following results were obtained:

| Amount of rennet per pound of milk | Initial temperature | Temperature at time of coagulation | Time to coagulate | Time to reach 145° F. without coagulation |
|---|---|---|---|---|
| | *Raw milk, acidity .195 percent* | | | |
| *cc.* | | | *min.* | *min.* |
| .20 | 68° F. | ..... | ... | 8 |
| .25 | 65° F. | 125° F. | 6 | ... |
| | *Raw milk and sugar, acidity .16 percent* | | | |
| .25 | 47° F. | ..... | ... | 8 |
| .30 | 44° F. | 130° F. | 6½ | ... |
| | *Raw milk, sugar, and gum, acidity .21 percent* | | | |
| .08 | 44° F. | ..... | ... | 8½ |
| .10 | 52° F. | 118° F. | 6 | ... |
| | *Pasteurized milk, acidity .195 percent* | | | |
| .30 | 57° F. | ..... | ... | 10 |
| .40 | 57° F. | 136° F. (fine curd) | 8 | ... |
| | *Pasteurized milk and sugar, acidity .165 percent* | | | |
| .40 | 44° F. | ..... | ... | 8 |
| .50 | 49° F. | 134° F. (very fine curd) | 6½ | ... |
| | *Pasteurized milk, sugar, and gum, acidity .21 percent* | | | |
| .04 | 47° F. | ..... | ... | 6 |
| .08 | 57° F. | 132° F. (fine curd) | 5½ | ... |

It is to be seen that sugar reduces enzyme reaction possibly thru its effect upon the concentration of the serum solids. Vegetable gum on the other hand increases the coagulating activity of an enzyme. It was thought that the acid contained in the gum was a factor in causing increased enzyme activity.

## Relation of Gelatin to Heat Coagulation During Pasteurization of Milk Containing Enzymes

Gelatin was added to pasteurized whole milk at the rate of .5 percent and 1 percent respectively. The gelatin was added dry and the temperature of the milk was then raised to 130° F. After thoroly agitating the milk it was cooled before adding the enzyme (pepsin).

The procedure was similar to that previously explained. That gelatin increases the coagulating activity of the enzymes is shown by the following:

| Amount of pepsin per pound of milk | Initial temperature | Temperature at time of milk coagulation | Time to coagulate | Time to reach 115° F. without coagulation |
|---|---|---|---|---|
| *grams* | | | *min.* | *min.* |
| .100 | 47° F. | ... | ... | 10 |
| .125 | 19° F. | 105° F. | 5½ | ... |
| | *Milk and .5-percent gelatin* | | | |
| .075 | 52° F. | ... | ... | 10 |
| .100 | 56° F. | 105° F. | 5 | ... |
| | *Milk and 1-percent gelatin* | | | |
| .075 | 62° F. | 98° F. | 3 | ... |
| .100 | 54° F. | 95° F. | 4 | ... |

These data are in agreement with the statement of Palmer and Richardson[1] that "gelatin exerts no retarding effect on rennet coagulation. On the contrary the rate of clotting is accelerated."

### Pasteurization of Mix Containing an Enzyme

Ice-cream mix containing 10.95 percent fat, 33.68 percent total solids (14 percent sugar), and having an acidity of .19 percent was used in this experiment. When a 5-percent rennet solution was added at the rate of .05 cc., .10 cc., .15 cc., and .20 cc. of rennet per pound of mix, the mix could be heated to 145° F. without curdling. Five minutes were required to raise the temperature from 70° F. to 145° F. When the mix containing the rennet added at the rate of .20 cc. was heated to 150° F., curdling occurred. When the amount of rennet was increased to .25 cc. per pound of mix, curdling occurred at 140° F.

Assuming mix viscosity to be a suitable measurement of the desired action of an enzyme added to a mix before pasteurization, it is to be expected that the desired results will depend to a certain extent on the time required to reach the pasteurizing temperature. That such is the case is indicated by the following:

| Rennet per pound of mix | Time to reach indicated temperature | Viscosity of mix at 40° F. 24 hours after heating |
|---|---|---|
| *cc.* | *min.* | *sec.* |
| Control | 145° F.— 6 5 | 57.0 |
| .20 | 145° F.— 1 5 | 64.0 |
| .20 | 145° F.— 8 | 139.0 |

## RELATION OF IMPROVERS TO SHRINKAGE OF ICE CREAM

One of the physical problems that is troublesome to some ice-cream manufacturers is the occasional shrinkage in volume that occurs while

[1]Palmer, L. S. and Richardson, G. A. Colliod symposium monograph. 3. 112-134. 1925.

ice cream is in storage. Usually this defect does not occur until after the ice cream has been placed in the dealer's cabinet, altho it sometimes develops while the ice cream is still in the hardening room. A study made at a plant experiencing the shrinkage difficulty, revealed a situation in which half a dozen or more 5-gallon cans of ice cream were being returned daily to the manufacturer because the ice cream had pulled away from the sides of the can and had dropped, in some cases, as much as 8 inches. The product was leaving the hardening room in good condition, but for some reason the contents of an occasional can would shrink after reaching the dealer.

The formula used at this plant in preparing the mix was as follows:

70 gallons of 8-percent plain bulk condensed milk
70 gallons 30-percent cream
150 pounds 84-percent butter
112 pounds skim-milk powder
247 gallons 3.6-percent milk
602 pounds cane sugar
19.5 pounds gelatin
4 pounds ice-cream improver

The results of fat and total-solids determinations made on five different mixes, using a Mojonnier milk tester, were as follows:

| Mix | Fat | Total solids |
|---|---|---|
| | *percent* | *percent*[1] |
| 1 | 10.11 | 36.61 |
| 2 | 10.02 | 36.77 |
| 3 | 9.34 | 36.10 |
| 4 | 10.32 | 35.71 |
| 5 | 10.93 | 36.53 |

The entire mix, with the exception of the improver, was pasteurized at 145° F. for 30 minutes and then viscolized at 2,000 pounds pressure. After the mix was cooled, the improver was added and the mix aged for 24 to 48 hours. The ice cream was drawn from the freezer when the amount of overrun,[2] as measured by the overrun tester, was 90 percent.

The data obtained in this survey served as a basis for a systematic study of the problem in the laboratories of this Station the results of which are reported in this publication. While the figures presented do not include all the data taken, they are representative.

## Improvers Cause Shrinkage

Since it was found that adding the above improver to milk caused the casein to be precipitated, and since the casein in the ice cream that

[1]The calculated sugar content of the mix was 14.3 percent.

[2]The overrun refers to the increase in volume of the ice cream in excess of the volume of mix used.

shrunk appeared to be in a precipitated form, experiments were made to determine whether or not there was any relation between the use of an improver and the shrinkage of ice cream. All the mixes used in these experiments were prepared from 40-percent cream, plain bulk condensed whole milk, skim milk, sugar, and gelatin, and were homogenized at 2,000 pounds pressure at a temperature of 145° F. Before freezing, vanilla flavoring was added.

The mixes were aged for 24 to 48 hours in 10-gallon milk cans at a temperature ranging from 35° F. to 40° F. The freezing was done in a 50-quart brine freezer. The percentage of overrun was measured with a Mojonnier overrun tester. Samples were taken in Sealright pint containers (except in Experiment A, when 3-gallon cans were used) and were stored in an ice-cream hardening room.

*Experiment A.*—A batch of mix was prepared having approximately the following composition: 12 percent fat, 11 percent milk-solids-not-fat, 13 percent sugar, and .5 percent gelatin.

The homogenized mix was divided into two lots of 50 pounds each. To one lot, one ounce of ice-cream improver was added. At the end of the aging period, the mix containing the improver was much more viscous than the control. Samples of ice cream were taken from the freezer when each batch had a 95 percent overrun.

Twenty-three days after being frozen, a 3-gallon can of each of the ice creams was removed from the hardening room and placed in a room having a temperature of 35° F. At the end of 3-hours the ice cream containing the improver had pulled away from the sides of the can and sunk about three centimeters, whereas the control can was still normal. The ice creams were returned to the hardening room for future study.

Five days later the 3-gallon cans were again removed from the hardening room to a room having a temperature of 35° F. At the end of 30 hours the volume of the control sample was unchanged, whereas the sample containing the improver had sunk a total of 8.5 centimeters.

*Experiment B.*—Batches of ice cream similar to the ones described in the previous experiment were prepared. The overrun in this case was 96 percent on both lots, when samples were taken in pint containers.

Three days after being frozen, a sample of each lot was removed from the hardening room and placed in a 60° F. incubator. At the end of 4½ hours, both samples had softened and that containing the improver had a slight indication of shrinkage.

Nine days after being frozen, these same samples were removed from the hardening room and placed in a 60° F. incubator. At the end of one hour the sample containing the ice-cream improver had

dropped in the container .7 centimeter and at the end of two hours the amount of shrinkage was 1.5 centimeters. The control sample, altho soft, had not shrunk, even at the end of two hours.

Sixteen days after being frozen, a sample of each lot that had never been out of the hardening room was placed in the 60° F. incubator for 1½ hours. At the end of this time the sample containing the ice-cream improver had pulled away from the sides of the container and had begun to settle. These same samples were later removed from the hardening room and placed in the 60° F. incubator at six different times during a period of 19 days. The total time held at the higher temperature was 12¾ hours. At the end of this period the sample containing the improver had settled 2.1 centimeters in the container, and had shrunk from the sides to the extent of .35 centimeter. The control sample, however, showed no signs of shrinkage.

To determine the effect of a more gradual rise in temperature upon the shrinkage of the ice cream, pint samples of both lots were placed in an iceless packer which was held at room temperature. The refrigerant for the packer had been stored in the hardening room for several days. The temperature of the hardening room was 8° F. at the time the packer was loaded. Four hours and fifteen minutes after being placed in the packer, the sample containing the improver had begun to shrink and pull away from the sides of the container. The control was unchanged. The temperature in the packer was 18° F.

Seven and one-half hours after being placed in the packer, the sample containing the improver had completely pulled away from the sides of the carton and had settled further. The control was still unchanged. The temperature in the packer at this time was 20° F.

Twenty-three hours after being placed in the packer, the sample containing the improver had shrunk about half-way down the carton and was very much smaller in diameter. The control sample was still standing as it was in the beginning of the experiment. The temperature in the packer was 28° F.

Four and one-half months after being frozen, pint samples of both lots which had been kept continuously in the hardening room were examined, and it was found that the control sample had not changed in volume, whereas that containing the improver had pulled away from the sides of the container and sunk .55 centimeter.

Two and one-half months later these same samples (the control still unchanged) were removed from the hardening room and a small portion of each was added to about ten times its volume of cold water. The control sample mixed with the water as would milk, whereas the sample containing the improver only partly dissolved, small curd particles settling to the bottom upon standing. Figure 2 shows this comparison.

The remainder of the ice cream was placed in milk bottles, which were in turn placed in water having a temperature of 140° F. to 120° F. for 15 minutes. Then they were removed and held at room temperature for 4 hours. At the end of this time the sample containing the improver had about 2½ centimeters of whey at the bottom,


FIG. 2.—MELTED ICE CREAM DILUTED WITH WATER

(A) control sample and (B) sample with Improver 2 added. Sample B was taken from a typical "shrinker." Both bottles were shaken before the picture was taken.

whereas the control sample appeared to be a homogenous mixture. (Fig. 3).

**Experiment C.** It has been shown that ice-cream improvers are a mixture of several substances. It was thought that an enzyme present in the improver was responsible for the shrinkage. To prove this, some mix was divided into 45-pound lots and treated as follows:

| Lot | Mix |
|---|---|
| A | Control |
| B | 1 ounce of Improver 2 added |
| C | 5 cubic centimeters of rennet[1] added |
| D | 4.1 grams of pepsin[1] added |
| E | 11 grams of Improver 5 added |

[1] A number of improvers gave qualitative tests for either rennet or pepsin or both.

The mix contained approximately 12 percent fat, 12 percent milk-solids-not-fat, 12 percent sugar, and .5 percent gelatin.

Four days after being frozen, two samples of each lot drawn at 100 percent and 125 percent overrun were held for 2 hours at 38° F., rehardened, and measured the following day. The results are given in Table 3.

On the fifth and sixth days after freezing, the same samples were held at 38° F. for 1 and 2 hours respectively, and then returned to the

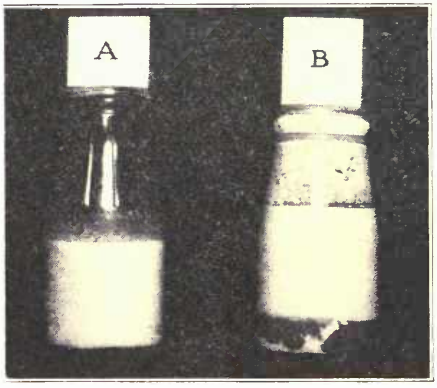


FIG. 3.—MELTED ICE CREAM AFTER BEING HEATED

(A) control sample and (B) sample with Improver 2 added. These are the same ice creams used in the experiment shown in Fig. 2. Bottles containing the frozen ice creams were placed in hot water (140° F. to 120° F.) for 15 minutes and then held at room temperature for 4 hours.

hardening room. On the seventh day the measurements were taken as recorded in Table 3.

Samples that had been kept at hardening room temperature for 23 days are shown in Fig. 4. The condition of the high overrun samples after being kept in the hardening room for 4 months is shown in Fig. 5.

It is evident from Experiments A, B, and C that under certain conditions some types of ice-cream improvers will cause the ice cream to shrink. It was interesting to note that the ice cream manufacturer who first reported the shrinkage trouble had no recurrence of the defect after he discontinued using improver. That the shrinkage is the result of an enzyme action on the milk proteins is evidenced by the results obtained when rennet and pepsin were used in the mix.

Some improvers when used according to the directions of the manu-

facturers caused greater shrinkage than others (Fig. 4 and Table 4). This difference was probably due to the difference in the amount or strength of enzyme in the improver as well as differences in the amounts recommended for use.

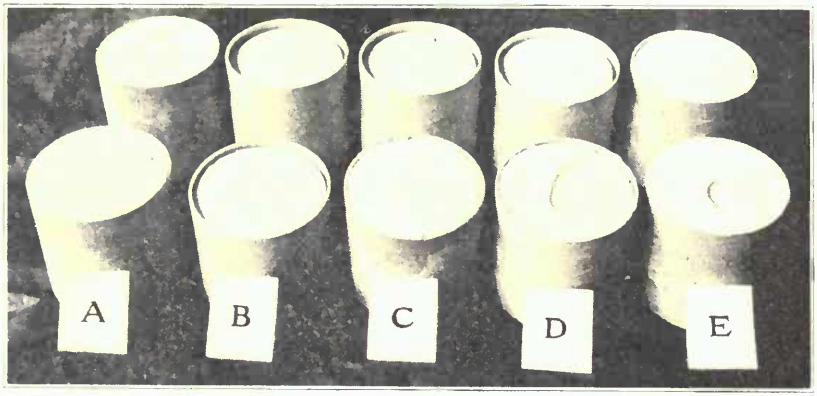


FIG. 4.—SHRINKAGE IN ICE CREAM STORED IN HARDENING ROOM 23 DAYS

(A) control sample, (B) sample with Improver 2 added, (C) pepsin added, (D) rennet added, (E) Improver 3 added. Lower row samples were drawn at 120 percent overrun. Upper row samples were drawn at 125 percent overrun.

TABLE 3.—SHRINKAGE OF ICE CREAM DUE TO THE PRESENCE OF CERTAIN ENZYMES[1]

| Sample and treatment | Shrinkage | | | |
|---|---|---|---|---|
| | Diameter | | Depth | |
| | 100-peret. overrun | 125-peret. overrun | 100-peret. overrun | 125-peret. overrun |
| After being frozen 4 days: | *cm.* | *cm.* | *cm.* | *cm.* |
| A. Control | 0 | 0 | 0 | 0 |
| B. Mix plus 1 ounce of Improver 2 | 0 | 0 | 0 | .30 |
| C. Mix plus 5 cc. of rennet | 0 | .15 | .25 | .30 |
| D. Mix plus 4.1 grams of pepsin | 0 | .10 | .20 | .30 |
| E. Mix plus 11 grams of Improver 5 | 0 | 0 | 0 | 0 |
| After being frozen 7 days: | | | | |
| A. Control | 0 | 0 | 0 | 0 |
| B. Mix plus 1 ounce of Improver 2 | .10 | .30 | .50 | .70 |
| C. Mix plus 5 cc. of rennet | .20 | .50 | .40 | .70 |
| D. Mix plus 4.1 grams of pepsin | .35 | .50 | .40 | .70 |
| E. Mix plus 11 grams of Improver 5 | 0 | 0 | 0 | .40 |

[1]The mix used contained approximately 12 percent fat, 12 percent milk-solids-not-fat, 12 percent sugar, and 5 percent gelatin. Samples were kept in Sealright pint containers.

## Factors That Encourage Shrinkage

The cause of the shrinkage of ice cream having been shown to be due to the use of certain ice-cream improvers containing enzymes it remained for the investigators to determine the factors or conditions that favor the development of this defect.

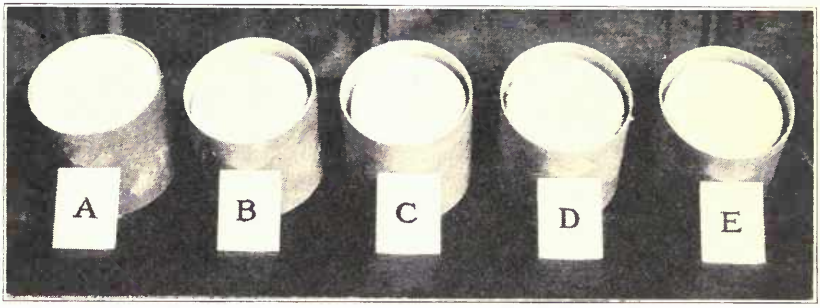


FIG. 5.—SHRINKAGE IN ICE CREAM STORED IN HARDENING ROOM 4 MONTHS

These samples are of the same series as those shown in the upper row of Fig. 4 after a prolonged period in the hardening room.

*Effect of Overrun on Shrinkage.*—The purpose of this experiment was to determine whether or not the amount of overrun was a factor contributory to the shrinkage of ice cream. In this study ice-cream rennet and three different brands of improvers were used according to the directions of the manufacturers. The mix used contained approximately 12 percent fat, 11 percent milk-solids-not-fat, 13 percent sugar and .5 percent gelatin.

When frozen, samples were taken from each batch at three different overruns as indicated below.

| Sample | Low overrun | Medium overrun | High overrun |
|---|---|---|---|
| | *percent* | *percent* | *percent* |
| Control | 83 | 97 | 125 |
| Improver 2 | 80 | 100 | 125 |
| Rennet | 76 | 100 | 130 |
| Improver 1 | 77 | 99 | 125 |
| Improver 3 | 77 | 100 | 125 |

At different intervals of time the 15 samples were removed from the hardening room and placed in the butter-storage room for a short time in an endeavor to bring about a shrinkage of the ice cream. The results are recorded in Table 4. In each case the measurements were taken after the ice cream had been rehardened.

Thirty-nine days after freezing the ice cream, it was noted that some of the samples that had never been out of the hardening room

had begun to shrink. The condition of the samples containing Improver 3 at that time can be seen in Fig. 6.

A high percentage of overrun is a condition that favors shrinkage. This probably helps to explain why the manufacturer mentioned had difficulty with only a few of the cans of ice cream sent to his dealers.

TABLE 4.—RELATION OF OVERRUN IN ICE CREAM TO THE AMOUNT OF SHRINKAGE CAUSED BY ENZYME

| Sample | Overrun | Shrinkage | |
|---|---|---|---|
| | | Diameter | Depth |
| 4 days after being frozen: held at 37° F. for 2¼ hours | | | |
| | *per cent.* | *cm.* | *cm.* |
| Improver 2 | 125 | .70 | .90 |
| Rennet | 130 | .40 | .30 |
| Improver 3[1] | 125 | .60 | 1.10 |
| 7 days after being frozen: held at 30° F. for 2 hours | | | |
| Improver 2 | 125 | .70 | 1.20 |
| Improver 2 | 100 | .25 | 1.00 |
| Rennet | 130 | .50 | .55 |
| Rennet | 100 | .40 | .20 |
| Improver 3 | 125 | .70 | 1.15 |
| Improver 3[1] | 100 | .20 | .50 |
| 10 days after being frozen: held at 31° F. for 2 hours | | | |
| Improver 2 | 125 | .90 | 1.30 |
| Improver 2 | 100 | .25 | 1.10 |
| Rennet | 130 | .50 | .65 |
| Rennet | 100 | .40 | .35 |
| Rennet | 76 | 0 | .10 |
| Improver 3 | 125 | .70 | 1.20 |
| Improver 3 | 100 | .25 | .70 |
| Improver 3[1] | 77 | 0 | .20 |
| 12 days after being frozen: held at 37° F. for 2 hours | | | |
| Improver 2 | 125 | .90 | 1.35 |
| Improver 2 | 100 | .40 | 1.15 |
| Rennet | 130 | .70 | .80 |
| Rennet | 100 | .60 | .60 |
| Rennet | 76 | 0 | .15 |
| Improver 3 | 125 | .90 | 1.30 |
| Improver 3 | 100 | .40 | .90 |
| Improver 3[1] | 77 | 0 | .30 |

[1] All other samples unchanged.

While studying the conditions in his plant, he determined the net weights on 22 five-gallon cans of ice cream filled in consecutive order at the freezer. The calculated overrun was found to vary from 71.4 percent to 128 percent. The average was 95.6 percent. Four of the 22 cans had an overrun above 100 percent.

*Relation of Acidity to Shrinkage.*—In order to determine whether or not increased acidity was a factor favoring shrinkage of the ice cream containing an improver, a mix containing approximately 12 per-

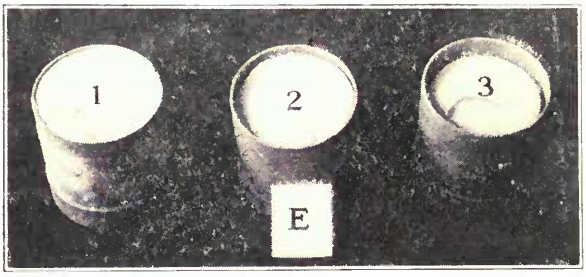


FIG. 6.—RELATION OF OVERRUN TO SHRINKAGE

These samples containing Improver 3 were taken from the same batch of ice cream but represent different percentages of overrun. Sample 1 contains 77 percent overrun, sample 2 contains 100 percent overrun, and sample 3 contains 125 percent overrun. The picture was taken after the samples had been stored in the hardening room for 39 days.

cent fat, 11 percent milk-solids-not-fat, 13 percent sugar and .4 percent gelatin, was divided into four 45-pound lots and treated as follows:

Lot 1—Control, 4 ounces water added
Lot 2—3 cc. rennet added in 4 ounces of water
Lot 3—20 grams of $NaHCO_3$ added, then 3 cc. of rennet in 4 ounces of water.
Lot 4—20 grams of lactic acid added, then 3 cc. of rennet in 4 ounces of water.

In each case the rennet was added to the cold mix.

Eighteen hours after being prepared, the acidity and relative viscosity of each batch of mix were determined. The acidity determinations were made by titrating 18 grams (by weight) of the sample with tenth-normal alkali. The viscosimeter used was a 17.6-cc. milk pipette with the tip removed to within 1 inch of the enlarged portion of the pipette. The results follow:

| Sample | Acidity *percent* | Viscosity[1] |
|---|---|---|
| Control | .210 | 1 min. 21.0 sec. |
| Rennet added | .215 | 8 min. 23.5 sec. |
| Rennet and $NaHCO_3$ added | .190 | 2 min. 3.5 sec. |
| Rennet and $C_3H_6O_3$ added | .300 | 30 min. for 5 cc. to discharge |

The mixes were frozen after being aged for 24 hours and samples were taken from each lot at 100 percent overrun.

[1]The viscosity determinations were made at 40° F.

Four days after being frozen samples of each lot were removed from the hardening room and held at 45° F. for two hours. After being rehardened none of the samples showed any signs of shrinking. This was repeated on the fifth, sixth, and seventh days. On the seventh day the ice cream containing the added acid shrunk slightly. The

TABLE 5.—RELATION OF TITRATABLE ACIDITY OF ICE-CREAM MIX TO THE AMOUNT OF SHRINKAGE CAUSED BY ENZYMES

| Sample and treatment | Shrinkage | |
|---|---|---|
| | Diameter | Depth |
| After being frozen 8 days; held at 45° F. for 2 hours | *cm.* | *cm.* |
| Control | 0 | 0 |
| Rennet added | .10 | .40 |
| Rennet and $NaHCO_3$ added | slight | 0 |
| Rennet and $C_3H_5O_3$ added | .10 | .50 |
| After being frozen 10 days; held at 48° F. for $2\frac{1}{4}$ hours | | |
| Control | 0 | 0 |
| Rennet added | .10 | .30 |
| Rennet and $NaHCO_3$ added | 0 | .30 |
| Rennet and $C_3H_5O_3$ added | .20 | .50 |
| After being frozen 11 days; held at 50° F. for 2 hours | | |
| Control | 0 | 0 |
| Rennet added | .25 | .55 |
| Rennet and $NaHCO_3$ added | .20 | .40 |
| Rennet and $C_3H_5O_3$ added | .30 | .65 |

samples were again subjected to a higher temperature and rehardened, after which measurements were taken. The results are given in Table 5.

According to Effront and Prescott[1] rennet and pepsin action in milk is increased by an increased acidity and retarded by a reduced acidity. The above data indicate that the same thing holds true for the action of rennet in ice cream. Considering the mixes to which rennet was added, increasing the acidity of the mix resulted in an increased viscosity, whereas the addition of sodium bicarbonate resulted in a reduced viscosity. The shrinkage tended to occur a little sooner and was a little greater in the case of the ice cream containing the increased acidity. Partial neutralization of the mix acidity reduced the tendency of the ice cream to shrink.

*Effect of Milk Solids on Shrinkage.*—In order to determine the relation between the milk solids in the ice cream and the amount of

[1] Effront, Jean and Prescott, Samuel C. Biochemical catalysts in life and industry. 100-101, 173-176. 1917.

shrinkage, three 50-pound batches of mix were prepared, having approximately the following composition:

| Lot | Fat | M.S.N.F. | Sugar | Gelatin |
|---|---|---|---|---|
| | *perct.* | *perct.* | *perct.* | *perct.* |
| 1 | 10 | 10 | 12 | .5 |
| 2 | 14 | 10 | 12 | .5 |
| 3 | 10 | 14 | 12 | .5 |

To each batch was added 5 cc. of rennet diluted with 100 cc. of water. The mixes were aged for 48 hours and then frozen, samples being taken at 100 percent and 110 percent overrun.

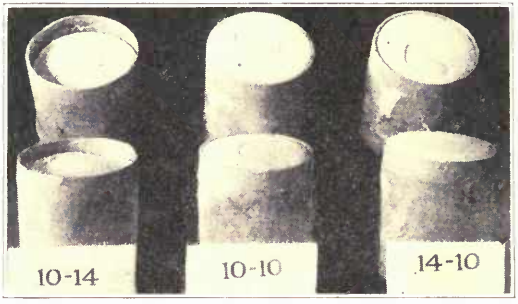


FIG. 7.—RELATION OF MILK SOLIDS TO SHRINKAGE

Rennet (10 cc. per 100 pounds of mix) was added to each of the three batches represented. The ice creams varied in milk solids as indicated, the first group containing approximately 10 percent fat and 14 percent m.s.n.f., and the third group, 14 percent fat and 10 percent m.s.n.f. The lower row samples were drawn at 100 percent overrun and the upper row samples were drawn at 110 percent overrun.

Three days after being frozen, a sample from each lot was held at 39° F. for two hours. After being rehardened no changes were noted. On the seventh and tenth days these samples were held at 44° F. for two hours. The measurements taken after the ice cream was rehardened are given in Table 6. The data show that an increased amount of milk-solids-not-fat or an increase in the concentration of these solids in the water of the mix increases the tendency for shrinkage to occur in ice cream containing an enzyme.

*Conditions Favorable for Enzyme Activity.*—That there are certain conditions which are favorable to the shrinkage of ice cream is evi-

denced by the results shown in Tables 3, 4, 5, and 6. This fact probably explains why not all manufacturers using the same improvers have trouble with shrinkage, and also why the manufacturer who experiences the difficulty does not have all of his ice cream shrink in the containers. Raising the temperature at which the ice cream is held will hasten the shrinkage, and the longer the ice cream is held in storage the greater the danger of shrinkage. It is for these two reasons that the trouble often does not occur until after the ice cream has been

TABLE 6.—RELATION OF MILK SOLIDS IN ICE-CREAM MIX TO AMOUNT OF SHRINKAGE CAUSED BY ENZYME

| Sample and treatment | Overrun | Shrinkage | |
|---|---|---|---|
| | | Diameter | Depth |
| After being frozen 7 days | *perct.* | *cm.* | *cm.* |
| 10 percent fat, 14 percent m.s.n.f. | 110 | 0 | .35 |
| 10 percent fat, 14 percent m.s.n.f. | 100 | 0 | .30 |
| 14 percent fat, 10 percent m.s.n.f. | 110 | 0 | .10 |
| 14 percent fat, 10 percent m.s.n.f. | 100 | 0 | 0 |
| 10 percent fat, 10 percent m.s.n.f. | 110 | 0 | 0 |
| 10 percent fat, 10 percent m.s.n.f. | 100 | 0 | 0 |
| After being frozen 10 days[1] | | | |
| 10 percent fat, 14 percent m.s.n.f. | 110 | .40 | 1.30 |
| 10 percent fat, 14 percent m.s.n.f. | 100 | .10 | 1.00 |
| 14 percent fat, 10 percent m.s.n.f. | 110 | 0 | .20 |
| 14 percent fat, 10 percent m.s.n.f. | 100 | 0 | 0 |
| 10 percent fat, 10 percent m.s.n.f. | 110 | 0 | 0 |
| 10 percent fat, 10 percent m.s.n.f. | 100 | 0 | 0 |

[1]Figure 7 shows the condition of the samples after the tenth day.

delivered and placed in the dealer's cabinet. However, the use of mechanically refrigerated cabinets may tend to lessen the importance of the temperature factor, since they generally maintain a uniformly low temperature.

It is apparent from these experiments that the composition of the mix is an important factor in the texture and quality of ice cream to which an improver has been added. A small increase in the amount of lactic acid present may so increase the enzyme activity of the improver that shrinkage results. More shrinkage is liable to occur in ice cream with a high serum solids content than in one with less of these solids. High overrun also favors shrinkage. It would seem therefore that a lack of careful control over manufacturing processes may produce conditions that are most favorable for the shrinkage of frozen ice cream.

## SUMMARY AND CONCLUSIONS

1. Of the 12 commercial ice-cream improvers studied, 11 contained either pepsin or rennet or both; 4 contained starch, 11 contained sugar, and 7 contained gum. It is evident that with most improvers the "ripening" activity is due primarily to rennet or pepsin.

2. There is a wide variation in the enzymic strength of the various improvers as shown by the time required for them to coagulate milk.

3. The bacterial content of the improvers studied was relatively low.

4. The addition of sodium bicarbonate inhibited the enzymic action of rennet in milk while the addition of calcium salts hastened this reaction.

5. Milk pasteurized at 145° F. for 30 minutes coagulated when either rennet or pepsin, or an improver containing these substances was added. Milk heated to extremely high temperatures (under 15 pounds of steam pressure for 45 minutes) did not show a coagulating reaction. However, when a small amount of calcium chlorid was added, this reaction did take place.

6. Improvers containing enzymes in powder form, are able to withstand a temperature of 100° C. for at least 45 hours. Altho their activity is somewhat reduced, it is not totally destroyed.

7. The enzymes in rennet, pepsin, and an improver remained active even tho the ice cream to which they were added was stored in a hardening room for 11 weeks. A further study proved the enzymes to be active after 5 months.

8. It is possible to add improvers containing enzymes to an ice-cream mix before pasteurizing without causing the mix to coagulate, providing an excessive amount of the enzyme is not present. The action of the improver added in this way is accelerated by the following factors:

(a) increased acidity
(b) increased serum solids
(c) presence of gums or gelatin
(d) slow heating of mix in pasteurizing process

9. The presence of certain types of improvers may cause ice cream to shrink. Some improvers are more troublesome in this respect than are others.

10. The factors which tend to hasten the shrinkage of ice cream to which certain improvers have been added are: (a) subjecting the ice cream to alternate high and low temperature; (b) long storage periods; (c) high percentage overrun; (d) increased acidity; (e) high percentage of milk-solids-not-fat or increased concentration of these solids in the water of the mix.