ENZYMIC INHIBITION OF GELATION IN FROZEN EGG YOLK

ANTHONY LOPEZ, CARL R. FELLERS AND WILLIAM D. POWRIE
Department of Food Technology, University of Massachusetts
Amherst, Massachusetts

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INTRODUCTION

Commercially egg yolk is generally mixed with sodium chloride, a sugar, or glycerol in order to inhibit changes that take place in the yolk during freezing, frozen storage, and thawing. Frozen, untreated yolk takes on a pasty, viscous consistency upon thawing. This "gelation" makes it unsuitable for many commercial and household uses. However, the yolk is still edible.

This report is concerned with the use of enzymes for inhibiting gelation in frozen yolks.

REVIEW OF LITERATURE

A patent on the use of pancreatin and other enzymes for inhibiting the gelation of yolk upon freezing was granted to Tressler4 in 1932. It has been found by Colmer1 that Bacillus cereus and related species produce a hardening of the yolk when the yolk of fresh shell eggs is inoculated with one of these species and incubated at 37° C. (98.6° F.). The explanation given is that the lecithoprotein of yolk is broken down by the action of the lecithinase produced by these bacteria on the lecithin. With the loss of the binder action of the lecithin, the fat and protein change from their dispersed state to that found after the bacteria have grown in the egg.

Romanoff and Romanoff5 state that a number of enzymes may be found in the active state in fresh eggs. Additional enzymes appear upon incubation. The fresh yolk contains a diastase, a lecithinase, and a monobutyrase. It is believed that yolk also contains erepsin, a salicylase and histidine (hippuricase). Phosphatase activity is said to occur in the yolk of infertile eggs. No true proteinase has been found in the yolk.

Lopez, Fellers, and Powrie6 reported that colloid milling of yolk previous to freezing inhibited gelation, and that very quick freezing combined with rapid thawing partially inhibited gelation in frozen and thawed yolk.

EXPERIMENTAL PROCEDURES AND RESULTS

The eggs used in these experiments were fresh hens' eggs from mixed breeds of the University flocks.

A 10 per cent aqueous suspension of the enzyme was added to 100 grams of fresh mixed yolk to make up the desired concentration of the enzyme in yolk. The mixture was blended for two minutes in a Waring blender. All samples were run in duplicate. Controls were prepared by mixing 100 grams of fresh yolk in a Waring blender with a volume of water equivalent to the volume of enzyme solution or suspension used for the samples.

Samples and controls were placed either in polyethylene bags or in glass jars, and incubated for various lengths of time. At the end of the incubation period they were stored in a freezer at -18° C. (0° F.) for various periods of time. They were thawed either by immersion in water at 50° C. (122° F.) or by standing at room temperature. The flavor, odor, color, and degree of gelation then were observed.

GELATION-INHIBITING ENZYMES - PRELIMINARY STUDY

The following enzymes were investigated in the concentrations indicated: papain, 2.00 and 0.33 per cent; pepsin, 0.2 and 0.02 per cent; trypsin, 0.5 and 0.05 per cent; lipase, 1.00 and 0.10 per cent; erepsin, 0.5, 0.2, 0.1 and 0.066 per cent; pancreatin, 0.5, 0.2, 0.1 and 0.066 per cent; and Rhozyme® 1.0, 0.5, 0.1 and 0.05 per cent. The incubation periods previous to freezing ranged for all samples from 15 seconds to 15 hours. It was observed that only papain, pepsin, trypsin, and Rhozyme® were able to inhibit gelation of frozen and thawed yolk. Under the conditions of this experiment, however, all samples had an off-flavor and sometimes a change in color. It was obvious that the enzyme concentrations were too high.
TABLE 1—Effect of Enzymatic Digestion of Yolk with Papain Previous to Freezing on the Gelation of Yolk

<table>
<thead>
<tr>
<th>Concentration of papain in yolk (percent)</th>
<th>Time (minutes) of incubation at 24° C. (75° F.)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Gelled</td>
<td>Low gelation</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No off flavors but lack of fresh flavor</td>
<td>Off flavor</td>
<td>Off flavor</td>
<td>Off flavor</td>
<td></td>
</tr>
<tr>
<td>0.066</td>
<td>Gelled</td>
<td>Gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lack of fresh flavor</td>
<td>Off flavor</td>
<td>Off flavor</td>
<td>Off flavor</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>Gelled</td>
<td>Gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No off flavor</td>
<td>No off flavor</td>
<td>No gelled</td>
<td>Slightly off flavor</td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>Gelled</td>
<td>Gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td>Not gelled</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Medium gelation</td>
<td>Off flavor</td>
<td>Off flavor</td>
<td>Off flavor</td>
<td></td>
</tr>
</tbody>
</table>

1 \text{pH of yolk was} 6.1
2 \text{Frozen in air at} -18° C. (0° F.). Kept frozen for 4 days. Thawed by immersion in water at 50° C. (122° F.)

**Effect of Freezing on Enzyme Activity**

An experiment was designed to find out whether there was a combination of enzyme concentration with time and temperature of incubation which would yield after freezing, frozen storage, and thawing, a yolk of normal appearance, flavor, odor, and color. The concentrations of papain, pepsin, trypsin and Rhozyme were lower than those used in the first experiment. At low concentrations papain was the only enzyme which could prevent gelation and still not cause any change in flavor, odor, and color.

Fifteen-gram portions of fresh yolk, containing various amounts of papain, were filled into polyethylene bags, incubated for various periods of time at room temperature, frozen and stored at -18° C. (0° F.). The yolk was thawed by immersion in water at 50° C. (122° F.) after various storage times.

Table 1 shows the thawed yolk with 0.05% papain and incubation periods of 15 and 20 minutes was not gelled and possessed normal flavor, odor and color after 4 days of storage. The thawed yolk was mixed with fresh egg white in the proportion of 1 part of yolk to 2 parts of white and was cooked as scrambled eggs. At the same time, fresh eggs were scrambled. In a taste panel test, no significant difference in flavor, odor or texture was found between the scrambled egg containing papain-treated yolk, and the scrambled fresh eggs.

Table 1 shows results obtained only when concentrations of papain ranging from 0.1 to 0.04 percent were used. Also, similar experiments were performed using several other concentrations of papain ranging from 0.1 to 0.4 percent, and incubating times ranging from 15 seconds to 15 hours. Results other than those presented in Table 1 are not reported inasmuch as they were all negative.

Long-time storage studies on yolk containing 0.05% papain with an incubation time of 15 minutes indicated that the thawed yolk in the third month of freezing storage was slightly gelled and was completely gelled in the sixth month. After three months the yolk viscosity had increased somewhat but not to the point where yolks did not reconstitute well. However, after six months in frozen storage the yolks were completely gelled. Additional work is in progress in an effort to explain the gelation of the papain-treated yolks during freezing storage.

**Effect of Container Size on Enzyme Activity**

This experiment was performed to study the effect of size of freezing containers upon gelation of papain-treated yolk. Yolk packaged in one quart glass jars (diameter 3.5 inches) showed gelation at all concentrations and incubation times tested. Of course, heat transfer takes place entirely by conduction. However, no off flavors nor gelation was produced in papain treated yolk packaged in polyethylene bags (yolk depth one inch) under the same conditions. The different results obtained with glass jars and with polyethylene bags were due undoubtedly to the difference in depth of the yolk in the jars and in the bags. That is, papain activity is dependent upon time, temperature and concentration. In large containers the papain continues to act until the center of the yolk mass is solidly frozen. If frozen commercially, the yolk mass should be frozen as rapidly as possible.

**Effect of Freezing and Thawing on Papain-Treated Yolk**

When freezing papain-treated yolk at -18° C. (0° F.), the enzyme is not entirely inactivated. Papain activity continues slowly at this temperature but does not prevent gelation after a few months. Providing some papain is still present in the frozen yolk activity is resumed upon defrosting. For this reason, off-flavors caused by products of protein hydrolysis develop rapidly in papain-treated frozen and thawed yolk within about 30 to 60 minutes after thawing. The
time depends upon yolk temperature.

Fifty-gram portions of mixed yolk, containing 0.05 percent papain, were placed in polyethylene bags. The sealed bags were held for 15 minutes at 24°C (75°F); then five bags were frozen by dipping in solid carbon dioxide-acetone mixture at approximately −68°C (−90°F), and held in the freezing mixture for 15 minutes; still another five bags were frozen in solid carbon dioxide-acetone mixture and held in it for five hours. After being frozen, all the bags were stored in the same freezer at −18°C (0°F.) for 4 days. Samples were simultaneously thawed by immersing in water at 50°C (122°F.). None of the samples gelled, and the flavor, color, and odor in all were normal. Samples were then placed under observation at 24°C (75°F.) to detect any change in organoleptic qualities. The results of the observations are shown in Table 2. Simultaneously, all samples started developing an off-flavor and off-odor. This means that papain was not affected by the different freezing conditions that were used.

Mechanism of Enzyme Action on Yolk

The gelation-inhibiting action of some enzymes may be caused by:

(a) the breaking down of the substances which in yolk is responsible for gelation and the formation of derivatives which do not have the property of producing gelation;

(b) the formation, upon the action of the enzyme on yolk components, of an inhibiting substance which prevents gelation;

(c) the enzyme itself, for it might have gelation-inhibiting properties. In order to ascertain whether the products of either acidic or enzymic hydrolysis when mixed with fresh yolk, have an inhibitory effect upon gelation, the following procedures were used to obtain the hydrolysates:

(a) Acid hydrolysis of yolk.

1) 3 ml. hydrochloric acid, density 1.19, were slowly added, while mixing, to 200 grams of fresh yolk to make an approximately 0.3 N solution of hydrochloric acid in yolk, considering the yolk as having 50 per cent water.

2) The acidified yolk was placed in a boiling water bath under reflux for 1 hour.

3) 100 grams of hydrolysate were removed.

4) The remaining hydrolysate was held in the water bath for a total of 5 hours. Temperature of yolk mixture during the period of hydrolysis was 95°C (203°F.).

5) The 1 hour and the 5 hour hydrolysates were neutralized with NaOH to pH 6.2.

6) Both hydrolysates were dialyzed for 24 hours using non-moisture-proof cellophane film and tap water. This dialysis was carried out for the purpose of eliminating the sodium chloride from the hydrolysates. During the dialysis, the hydrolysates increased in weight from 90 grams to approximately 200 grams.

(b) Enzymatic hydrolysis of yolk.

1) 2 grams of papain suspended in 5 ml. of water were added to 200 grams of fresh yolk, and mixed in a Waring blender.

2) The mixture was incubated at 40° - 42°C (104° - 108°F.).

3) After 4 hours of incubation, 100 grams of hydrolysate were extracted.

4) After 24 hours of total incubation time, the rest of the hydrolysate was taken out.

(c) Freezing tests with yolk hydrolysates.

(d) Freezing tests with meat, yeast, and vegetable hydrolysates.

Fresh yolk samples were well mixed with water suspensions of one of the following at the concentrations indicated: Bactopeptone, Difco, 2 per cent; Bacto-Tryptone, Difco, 4 per cent; Bacto-Autoysed Yeast, Difco, 2 per cent; Polypeptide 3 per cent and 10 per cent; Phytone of a vegetable hydrolysate, 3 per cent. The mixtures were placed in polyethylene bags, 50 grams in each, frozen and stored.
at $-18^\circ$ C. ($0^\circ$ F.). Prior to examination, they were thawed by immersion in water at $50^\circ$ C. ($122^\circ$ F.). After four days of storage all samples were highly gelled. Apparently neither acid nor enzymic hydrolysates had any appreciable effect on the degree of yolk gelation.

**Discussion of Results and Conclusions**

Of the enzymes tested, only papain, trypsin, papain and Rhizyme appreciably inhibited gelation in frozen and defrosted egg yolk. Erespin, pancreatin and lipase did not inhibit gelation.

Among the enzymes which were effective in inhibiting gelation, only papain did not seriously affect the organoleptic qualities of yolk. This result was obtained only at a concentration of approximately 0.05 per cent of papain in yolk, and only with incubation times of 15 or 20 minutes at $24^\circ$ C. ($75^\circ$ F.) previous to freezing. Other papain concentrations and other incubation times yielded thawed yolk with off-flavor and off-odor. It was difficult to control the enzyme activity so as to obtain non-gelled frozen yolk's of good culinary properties. While excellent frozen yolks and whole eggs were obtained in our laboratory, it is felt that it would be difficult to obtain acceptable products under field conditions such as on shipboard or in an Army mess.

The activity of papain added to yolk was not significantly affected by freezing at $-18^\circ$ C. ($0^\circ$ F.) and storage at same temperature for four months or longer, neither was the papain activity affected by freezing at $-68^\circ$ C. ($-90^\circ$ F.) for 5 hours and subsequent storage at $-18^\circ$ C. ($0^\circ$ F.). No effort was made to inactivate added papain in yolk by other means. For this reason, the hydrolytic action of papain upon yolk becomes very active upon thawing the yolk, producing flavor and odor changes in yolk. In order to be able to use papain commercially as a gelation inhibitor in frozen yolk, a method for inactivating the enzyme either before freezing the yolk, or while it is frozen, has to be developed. The flavor, odor, texture and color of yolk should not be affected by the enzyme inactivation procedure, neither should the nutritive value nor palatability of the yolk be affected. Effects of longer frozen storage periods upon gelation and organoleptic qualities of yolk should be studied further. Small diameter containers that allow fast thawing should be used because enzyme action continues in the unfrozen portion. Very rapid freezing and defrosting are distinctly advantageous.

In the experiments on the mechanism of action of enzymes in inhibiting gelation of yolk, the products of acid and of enzymatic hydrolysis of yolk, and the meat, yeast, and vegetable hydrolysates were not able to prevent the onset of gelation in fresh yolk. Furthermore, if the enzymes "per se" had gelation-inhibiting properties, the yolk samples with enzyme hydrolysate should not have gelled. These experiments tend to show that enzymes break down the component or components responsible for gelation. Since only proteolytic enzymes were found effective, it appears logical to consider a protein complex as being responsible in total or in part for the gelation of yolk.

**Summary**

Papain mixed with fresh yolk in a concentration of 0.05 percent and incubated for 15 or 20 minutes was found effective for inhibiting gelation of frozen and thawed egg yolk. The flavor of the raw or cooked yolk was not affected by this treatment. Papain, trypsin, and Rhizyme also inhibited gelation of yolk. However, these enzymes developed off-flavors and off-odors in yolk. Pancreatin, erespis and lipase did not inhibit gelation.

Papain-treated yolk intended to be frozen should be packaged in a container that makes fast freezing and thawing possible.

Upon thawing, the activity of papain in papain-treated yolk was found unaffected by freezing at $-68^\circ$ C. ($-90^\circ$ F.) for 5 hours, and subsequent storage at $-18^\circ$ C. ($0^\circ$ F.) for up to 4 months when the experiment was discontinued.

Gelation in frozen and thawed yolk was not inhibited by the addition to fresh yolk of either acid or enzymatic hydrolysates of yolk, a yeast hydrolysate, meat hydrolysates, and a vegetable hydrolysate. A protein or protein complex is believed responsible for gelation in frozen and thawed yolk.

**Acknowledgment**

The authors wish to thank Mr. Arthur C. Avery, Technical Director, Commissary Research Division, U. S. Navy Supply Research and Development Facility, Naval Supply Base, Bayonne, N. J., for suggesting this problem and for his many helpful suggestions during the course of the investigation. Thanks are also due Mr. Manohar Sathe who assisted in carrying on some of the laboratory experiments.

**References**


**Market Milk and Ice Cream Meetings to Be Held at Purdue**

Two one-day dairy meetings will be held in April, 1955 at Purdue University according to an announcement by Professor H. W. Gregory, Head, Department of Dairy Conference, April 6, and Ice Cream Institute, April 7.

The conferences are a continuation of the series held annually in cooperation with the Indiana Dairy Products Association. Specialists from the dairy industry and universities will be on the programs. Current problems relating to the processing and distribution of bottled milk and cottage cheese will be discussed at the market milk conference. Also, a clinic on commercial samples of market milk and cottage cheese will be held in connection with the conference. Ice cream samples submitted by plants to Purdue for analysis and scoring will be examined and discussed as a part of the ice cream meeting.

For further information write to: Professor V. C. Manhart, Smith Hall, Purdue University, Lafayette, Indiana.