



Hungarian University of Life Sciences

**EFFECTS OF FREEZING ON THE QUALITY ATTRIBUTES OF  
LIQUID EGG PRODUCTS**

DOI: 10.54598/003230

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Budapest

2022

**Doctoral school:** Doctoral School of Food Sciences

**Discipline:** Food sciences

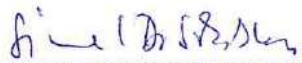
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## 1. INTRODUCTION AND OBJECTIVES

Eggs are widely food ingredients in the food industry, and are a popular ingredient in pasta, pastry, bakery and catering. For ease of handling and to reduce microbiological risks, manufacturers in the food industry mostly choose processed egg products, such as liquid egg products, egg powders and cooked egg products, instead of shelled eggs, including whole egg, egg yolk and egg white varieties (NÉMETH et al. 2011).

The most common preservation technology of liquid egg products in industrial practice is heat treatment. Due to the heat sensitivity of the egg proteins in, the heat treatment does not result in a commercially sterile product and the shelf life of liquid egg products is a 3 to 4 weeks (DAWSON and MARTINEZ-DAWSON 1998; DELVES-BROUGHTON et al. 1992).

Freezing is one of the most widely used, effective and affordable preservation technologies, which results in a long shelf-life product. However, food freezing also has drawbacks and limitations (FANG et al. 2021). For example, protein-rich foods may be subject to protein denaturation and aggregation. Ice formation often leads to a change in the conformation of the hydrophobic protein part due to contacting with ice crystals (CHANG et al. 1996). In addition, an increase in solute concentration due to freezing can also damage proteins (HATLEY and MANT 1993). This process is associated with a change in pH and denaturation, possibly aggregation, of proteins (FANG et al. 2021).

While only minor changes occur in egg whites during freezing, such as dilution of the dense protein (COTTERILL 1995), irreversible changes in yolk fluidity occur when the yolk is cooled to  $-6^{\circ}\text{C}$  or below. As a consequence, the yolk becomes a paste-like structure, making it difficult to transport and mix (MORAN 1925) and its usability is reduced (POWRIE et al. 1963). The most common explanation for the gelation of egg yolks during freezing is that the ice crystals formed concentrate the components of the yolk, resulting in the accumulation of low-density lipoproteins in the plasma fraction of the yolk.

To prevent or reduce the extent of gelation, researchers have used a variety of mechanical and chemical methods in recent decades. These have included various cryoprotective agents, of which sucrose and table salt have been found to be the most effective. In addition, the optimization of freezing and thawing processes was also studied and the effect of adding 0.05 wt.% papain was studied (LOPEZ et al. 1955).

However, very little information is available on the extent of changes in liquid whole egg and there is no solution available to prevent them and to increase the usability of frozen-thawed samples.

The objectives of the experiments were:

- To investigate whether freezing using in nitrogen, which has a significant operating cost, can be a solution to gelation of egg yolk. Therefore, I will investigate the effects of freezing in liquid nitrogen and subsequent frozen storage on the pH, colour, rheological, thermophysical and technofunctional properties of liquid egg yolk.
- To investigate the effects of slow freezing and subsequent frozen storage on the pH, colour, microbiological state, rheological, thermo-physical and technofunctional properties of liquid whole egg, liquid egg white and liquid egg yolk.
- To examine the cryoprotective effect of table salt and sucrose in the gelation of liquid egg yolk. To carry out systematic experiments to investigate the optimal concentrations at which they have a beneficial effect on the gelation of liquid egg yolk during freezing. To investigate the changes in pH, colour, rheological, thermophysical and technofunctional properties of liquid egg yolk due to the addition of common salt and sucrose before and after freezing and thawing.
- To investigate the effect of different commercially available enzyme preparations on the undesirable gelation processes of liquid egg yolk and liquid whole egg during freeze-thawing. To investigate the pH, colour, microbiological status, rheological, thermophysical and technofunctional properties of liquid egg yolk and liquid whole egg treated with different enzyme concentrations.

## 2. MATERIALS AND METHODS

### 2.1. Freezing and thawing procedures

In this study, pasteurised liquid egg products were used. In the first experiment, cryogenic freezing was performed on liquid egg drops formed by a steel strainer ( $d = 1.5$  mm) for 60 s in liquid nitrogen. Liquid nitrogen began to boil at a temperature of  $-196^{\circ}\text{C}$  due to the temperature difference between the liquid nitrogen and the liquid egg yolk. Frozen pellets were separated by a strainer from liquid nitrogen. After freezing, the samples were stored in foil bags at  $-24 \pm 1^{\circ}\text{C}$ . In further experiments, slow freezing was used to model room freezing, whereby different liquid egg samples filled into polypropylene bags and placed in a freezer at  $-24 \pm 1^{\circ}\text{C}$ . In each case, slow thawing was performed transferring the samples from the freezer to a laboratory refrigerator at  $4 \pm 1^{\circ}\text{C}$  24 hours before performing the tests. The control sample in each experiment was the freshly received non-frozen sample (day 0).

### 2.2. Design of experiments

In the first part, the effect of freezing with liquid nitrogen was investigated on the different properties of egg yolk samples. The samples frozen using liquid nitrogen were subjected to pH, colour, rheological and calorimetric tests after 1, 7, 14, 30, 60, 90, 120 and 150 days.

In the second part, the effect of slow freezing on different properties of liquid whole egg, liquid egg white and liquid egg yolk was investigated. Samples frozen by slow freezing were thawed after 1, 7, 14, 30, 60, 90, 120, and 150 days and then subjected to pH, colour, rheological and calorimetric analyses. In addition, total plate count of the samples was examined at a few selected time points and different methods were used to study the usability of each sample. For the liquid egg white samples, foaming properties were examined, for the liquid egg yolk samples the optical density (turbidity) at 660 nm was measured. In addition, they were used to prepare mayonnaise samples and to monitor the changes in their rheological properties. For the liquid whole egg samples, sponge cake was baked from fresh and frozen samples and the change in the sponge cake texture was analysed.

In the third part, cryoprotective procedures were applied. To reduce or prevent the extent of gelation in liquid egg yolk during freezing, various concentrations of common salt, sucrose and enzyme treatments were used. For liquid whole egg, the effectiveness of enzyme treatment was studied on the texture changes during freezing. For table salt, concentrations of 1, 2, 4, 5, 6, 7 and 10 wt.% were used. The concentrations of 1, 2, 5, 7, 9 and 10 wt.% were investigated in case of sucrose. The enzyme treatment was carried out without pH adjustment in water bath at  $40^{\circ}\text{C}$  for 120 min at concentrations of 0.05; 0.3 and 0.5wt.%. Among the 7 different commercially available enzymes with lipase and protease activity, the one that was found to be effective based on the visual

evaluation after freeze-thawing was selected. For the samples containing common salt, sucrose and enzyme-treated samples, pH, colour, optical density at 660 nm, rheological and calorimetric properties were assessed before and 60 days after freezing.

### *2.3. Tests carried out*

In this study, a digital pH meter was used to measure the pH of the samples, as pH can change during freezing, which affects the structure of the proteins. The objective colour of the samples using a Konica Minolta CR400 (Konica Minolta Inc., Japan) tristimulus colourimeter in the a CIELab system was also measured. In addition to comparing the colourimetric factors  $L^*$ ,  $a^*$  and  $b^*$ , the colour difference ( $\Delta E^*_{ab}$ ) was calculated to characterize the colour change due to freeze-thaw and cryoprotective treatments.

Protein state studies were carried out by a SETARAM MicroDSC III (SETARAM Instrumentation Caluire, France) thermoanalytical apparatus in the range of 25-90°C at a heating rate of 1.5°C/min. DSC was used to determine the amount of denaturable proteins, from which the extent of protein denaturation and aggregation during freeze-thawing was determined. Protein denaturation affects technofunctional properties. I performed different tests for different liquid egg products to investigate their technofunctional properties. In the case of liquid egg whites, the foam stability was investigated by measuring the amount of liquid after beating to a hard foam. For liquid egg yolk, the optical density was measured at 660 nm using a U-2900 spectrophotometer (Hitachi, Tokyo, Japan). The degree of turbidity can be used to interpret the emulsifying capacity of the egg yolk. The liquid egg yolk was used to prepare a finished product, mayonnaise, and its strength, consistency, cohesion and viscosity index were measured by back extrusion method using a TA.XT Plus Texture analyser (Stable Micro Systems Ltd., Surrey, UK). For liquid whole egg, the technofunctional properties were also investigated through the preparation of a product. Fresh and frozen-thawed samples were used to make a sponge cake and then the consistency was tested by TPA (method. I compared the hardness, cohesion, elasticity and gumminess values.

Investigation of the rheological properties was carried out by an MCR 92 rheometer (Anton Paar, Graz, Austria). The apparatus was used in rotary mode with a measuring system in concentric cylinder geometry. The measurements were carried out first at increasing and then decreasing shear rates between 1 and 1000 1/s. In the first experiment, the rheological properties were investigated at 5°C, in the following experiments at 20°C. Using the measured data flow curves viscosity curves were evaluated. The Herschel-Bulkley model [Equation 1] was used to evaluate the flow curves.

$$\tau = \tau_0 + K \left( \frac{d\gamma}{dt} \right)^n \quad (1)$$

Where  $\tau$  is the shear stress (Pa),  $\tau_0$ : yield stress (Pa),  $K$ : consistency coefficient (Pa·s<sup>n</sup>),  $d\gamma/dt$  is the shear rate (1/s) and  $n$  is the flow behaviour index.

The model fitting was performed using Microsoft Excel 365 Solver plug-in, using the least squares sum of squares fitting method, where  $\tau_0$ ,  $K$  and  $n$  are the variable values. The software was used to minimize the sum of squares of the difference between the measured and calculated data points. In addition, total plate count of the samples was determined by colony counting at 30°C to assess the overall microbiological status.

The use of cryoprotectants changes the water binding forms and therefore Setaram DSC 131 evo (Setaram, Caluire, France) differential scanning calorimeter was used to measure the enthalpy required for the phase change during freezing, to determine the initial temperature of intensive thawing and to calculate the unfrozen water content.

Statistical evaluation of the measured data was performed using one-way ANOVA (SPSS Statistics 24, IBM Corp., USA) at 5% significance level ( $p < 0.05$ ). Normality of error terms was checked by Shapiro-Wilk test and homogeneity of variance by Levene's test. If the ANOVA was significant, Tukey's HSD test was used to separate the different groups if the homogeneity of variance condition was met, Games-Howell's test, if the homogeneity of variance condition was not fulfilled.

## RESULTS

In the first part, cryogenic freezing of liquid egg yolk was carried out, and it was observed that significant changes in rheological properties occurred on day 1 after freezing. The change in the rheological properties is mainly detectable by the appearance of a yield stress value. Yield stress is the minimum shear stress value required for the sample to start flowing. However, the changes in the values of the consistency coefficient and the flow index are not negligible, as the consistency coefficient has increased by almost 37 times and the flow index has decreased by half. The results of the studies contradict the results of early publications which found that cryogenic freezing can greatly reduce the gelation of liquid egg yolk. Cryogenic freezing reduced the amount of denaturable protein and the colour of liquid egg yolk was greatly lightened. During frozen storage, these changes increased, the yolk samples became lighter, the yield stress increased and the denaturation enthalpy decreased. However, it was observed that most of the properties changed most during the first part of the storage period (days 1-14).

In the following experiment, the effect of slow freezing was investigated on different liquid egg products. In the case of liquid egg white, a lightening of the sample occurs during freezing and frozen storage. The rheological properties did not change, the amount of denaturable proteins decreased. During usability testing, it was found that more liquid is released from the hard beaten egg white foam from day 30 after freezing, thus its foam stability is slightly reduced.

For liquid egg yolk, similar trends were observed for slow freezing as for freezing using liquid nitrogen. The samples became lighter and the rheological properties changed to a large extent, the yolk undergoing gelation. Mayonnaise made from frozen-thawed egg yolks was found to be firmer and more viscous, and the consistency and cohesion values were also increased. However, the extent of aggregation during freezing was more clearly demonstrated by the optical density measured by spectrophotometer, which increased significantly in the case of frozen liquid egg yolk.

The liquid whole egg faded and lightened in colour on freezing and became a watery, transparent pale yellow liquid which, after thorough mixing, became lumpy. As a result, its rheological properties have also changed considerably. Sponge cake made from frozen-thawed liquid whole egg became harder and more rubbery, but its elasticity and cohesion decreased.

In the third part of the thesis, different cryoprotective techniques were used to reduce the extent of gelation of liquid egg yolk during freezing. The two technological adjuvants used, table salt and sucrose, reduced the degree of gelation. They had different mechanisms of action and thus had different effects on the parameters studied. The colour of the liquid egg yolk was significantly altered by both substances both before and after freezing. After the addition of table salt, the



samples became darker and their red and yellow colour decreased, while after freezing they became slightly lighter and their yellow colour coordinate increased slightly. The addition of table salt also changed the rheological properties. However, after freezing, a decrease in the degree of gelation was seen, as frozen-thawed egg yolks were found to be liquid when applied at concentrations of 4-10 wt.%. The optimum concentration based on the rheological measurements is 6 wt.%. In addition, it was observed that table salt also has a strong influence on denaturation and melting properties. The effect of table salt decreases the denaturation enthalpy, increases the denaturation temperature and thus the thermal stability. The microbial count decreases slightly at a salt concentration of 6-10 wt.% after heat treatment, which can be explained by a decrease in water activity. In addition, eutectic formation, a strong increase in the unfrozen water content and a decrease in the melting temperature were also observed.

The addition of sucrose caused a smaller change in the colour of the egg yolk samples than table salt. It was observed that sucrose did not significantly affect the rheological properties of liquid egg yolk. After freezing, it had a cryoprotective effect. As the sucrose concentration increased, the gelation rate decreased, and a minimum sucrose concentration of 9 wt.% was required to achieve better rheological properties. The thermophysical factors show the same trend, but much smaller changes than with table salt.

Finally, the effects of 7 different commercially available protein and lipid-degrading enzyme preparations on the changes in texture and usability during freezing of liquid whole egg and liquid egg yolk were investigated. Treatment with Biocatalysts Flavorpro™ 750MDP resulted in a darker colour of both egg products and a loss of red and yellow colouration. The change in colour factors is particularly significant for the two higher concentrations (0.3 and 0.5 wt.%). However, the effect of freeze-thawing was to increase the lightness factor and the yellow-blue colour factor values, which brought them back closer to the colour of fresh egg products. In the rheological properties study, it was found that the enzyme treatment reduced the shear stress value during shearing of both liquid egg, and therefore the consistency coefficient value obtained by fitting the Herschel-Bulkley model was also reduced. In addition, a minimum yield stress value appeared. Enzyme-treated egg yolk samples behaved similarly to Newtonian fluids, while liquid whole egg samples exhibited dilatant behaviour. After freeze-thawing, the rheological properties of the enzyme-treated samples were similar to those of the fresh liquid egg samples for both liquid egg types. The samples treated with the enzyme preparation at a concentration of 0.3 wt.% had the lowest shear stress values. The amount of denaturable proteins significantly changed with both enzyme treatment and freezing for both liquid egg products. When the melting properties were examined, it was observed that the initial temperature of intensive melting decreased with enzyme treatment.

The decrease in the liquid egg yolk was equivalent to the decrease with 5 wt% sucrose and was less than the change with the lowest concentration of table salt tested.

The optical density values after enzyme treatment showed just a small increase as the enzyme treatment prevented large aggregation.

## CONCLUSIONS AND RECOMMENDATIONS

Based on the results, it can be concluded that both liquid nitrogen and slow freezing led to the gelation of liquid egg yolk. This is evidenced by the change in colour of the yolk, the decrease in the amount of denaturable proteins, the increase in apparent viscosity and shear stress, and the appearance of a yield stress calculated by fitting the Herschel-Bulkley model. The changes that occur are due to the concentration of egg yolk components, resulting in the accumulation of low-density lipoproteins in the plasma fraction. Under these conditions, lipoproteins aggregate and denature.

It was found that more liquid is released from the hard beaten egg white foam from day 30 after freezing, thus its foam stability is slightly reduced. To improve foam stability, investigation of different additives, cryoprotective substances is proposed.

The liquid whole egg has turned into a transparent, pale yellow liquid structure on thawing, which, after thorough mixing, has become lumpy. It is concluded that the changes in the texture and protein state of liquid whole egg and liquid egg yolk also caused a large deterioration in their usability, and that some cryoprotective treatment during freezing was necessary.

The change in colour, increase in apparent viscosity, shear stress, decrease in denaturable proteins, increase in denaturation temperature and change in melting properties due to the addition of table salt are due to protein coagulation by salt. Salting leads to the formation of a low-density lipoprotein-water-salt complex. The dissociated ions in the solution have a repellent effect, but the formation of the complex on freezing inhibits the freezing of the water and thus the gelation. This explains why samples containing 4-10 wt% salt were found to be liquid after freeze-thawing.

The addition of sucrose to the yolks resulted in only minor changes in colour, rheological and calorimetric properties. Sugars are generally used as stabilisers to protect proteins from degradation during lyophilisation and frozen storage. After freezing, a cryoprotective effect was observed, as the samples containing sugars showed lower shear stress and apparent viscosity results than those without cryoprotective agents during the applied shear.

It was found that the aminopeptidase enzyme preparation Biocatalysts Flavorpro™ 750MDP was effective for both liquid egg products at a concentration of 0.3 wt.%. The enzyme treatment condition (40°C, 2 h) was found to be appropriate both in terms of rheological properties, optical density values and microbial counts. The rheological properties of the frozen-thawed samples were most similar to those of fresh liquid egg products for the enzyme-treated samples. The enzyme treatment was the most suitable for reducing the degree of gelation of egg yolks during freeze-thawing. In addition, the texture of the enzyme-treated freeze-thawed liquid whole egg is very similar to that of the fresh sample.

To prevent gelation of liquid egg yolk during freezing, it is recommended to use 6 wt.% common salt, 9 wt.% sucrose or 0.3 wt.% enzyme treatment. Biocatalysts Flavorpro™ 750MDP. The selection of the appropriate one of these options should consider the field of application, the technological possibilities and the economic aspects. Whole egg texture changes can also be prevented by using Biocatalysts Flavorpro™ 750MDP at 0,3 wt.%.

## NEW SCIENTIFIC RESULTS

1. It was found that freezing of liquid egg yolk using liquid nitrogen (by dripping through a 1.5 mm hole diameter stainless steel filter into a refrigerated medium and allowing 60 s residence time) and subsequent frozen storage at  $-24^{\circ}\text{C}$  caused gelation. The findings are based on the apparent viscosity, shear stress measured with a rotational rheometer (between 10 and 1000 1/s shear rate) and the stepwise increase in  $\tau_0$  and K values (calculated by fitting Herschel-Bulkley models to the flow curves).
2. It was found that the effect of slow freezing followed by 150 days of frozen storage at  $-24^{\circ}\text{C}$  does not significantly change the  $\tau_0$ , K and n values of pasteurized egg whites measured by rotational rheometer (using the Herschel-Bulkley model fitted to the flow curve at shear rates between 10 and 1000 1/s). In contrast, the rheological properties of the pasteurised liquid whole egg are altered. After freezing, the shear stress values increase, and the consistency coefficient obtained by fitting the Herschel-Bulkley model increases. It is found that the colour of pasteurised liquid egg yolk undergoes a large change during slow freezing, mainly due to an increase in the lightness.
3. It was demonstrated by systematic experiment that the gelation of pasteurized liquid egg yolk during freeze-thawing was reduced by 4-10 wt.% salt concentration, the most similar rheological properties (apparent viscosity, shear stress measured by a rotational rheometer between 10 and 1000 1/s shear rate, and  $\tau_0$ , K and n values calculated by fitting Herschel-Bulkley models to the flow curves) to the pasteurised unfrozen sample were obtained for the sample containing 6 wt.% of common salt.
4. It was demonstrated by a systematic experiment that the applied sucrose concentration (1-10 wt.%) reduces the gelation of egg yolk during freeze-thawing. As the concentration increased, the gelation rate decreased, in order to achieve the rheological properties (apparent viscosity, shear stress measured by rotational rheometer between 10 and 1000 1/s shear rate, shear stress and  $\tau_0$ , K and n values calculated by fitting Herschel-Bulkley model to the flow curves) that best resemble fresh unfrozen samples, at a minimum 9 wt.% sucrose concentration.
5. It was firstly demonstrated that enzyme treatment with the aminopeptidase enzyme preparation Flavorpro<sup>TM</sup> 750MDP at a concentration of at least 0.3 wt.% (180 U/kg egg products) for 120 min at  $40^{\circ}\text{C}$  without adjusting the pH of the liquid egg products prevented gelation of liquid egg yolk during freeze-thawing and texture changes in liquid whole egg. After freeze-thawing, the viscosity curves and flow curves of the samples treated were similar to those of fresh liquid egg products for both liquid egg products.

## LIST OF JOURNAL PUBLICATIONS IN THE FIELD OF STUDIES

**Hidas, K. I.**, Visy, A., Csonka, J., Nyulas-Zeke, I. Cs., Friedrich, L., Pásztor-Huszár, K., Alpár, B., Hitka, G., Felföldi, J., Fehér, O., Gere, A. (2020): Development of a Novel Gluten-Free Egg Pie Product: Effects of Sensory Attributes and Storage. SUSTAINABILITY. 12 (24) Paper: 10389 <https://doi.org/10.3390/su122410389> **Q1**

**Hidas, K. I.**, Nyulas-Zeke, I. Cs., Visy, A., Baranyai, L., Nguyen, L. P. L., Tóth, A., Friedrich, L., Nagy, A., Németh, Cs. (2021): Effect of Combination of Salt and pH on Functional Properties of Frozen-Thawed Egg Yolk. AGRICULTURE-BASEL. 11 (3) 257. pp. 1-18. <https://doi.org/10.3390/agriculture11030257> **Q2**

**Hidas, K. I.**, Németh, Cs. Nguyen, L. P. L., Visy, A., Tóth, A., Barkó, A., Friedrich, L., Nagy, A., Nyulas-Zeke, I. Cs. (2021): Effect of cryogenic freezing on the rheological and calorimetric properties of pasteurized liquid egg yolk. CZECH JOURNAL OF FOOD SCIENCES. 39 (3) pp. 181-188. <https://doi.org/10.17221/37/2021-CJFS> **Q3**

**Hidas, K. I.**, Németh, Cs., Visy, A., Tóth, A., Friedrich, L., Nyulas-Zeke, I. (2020): Comparison of different thawing methods effect on the calorimetric and rheological properties of frozen liquid egg yolk. PROGRESS IN AGRICULTURAL ENGINEERING SCIENCES 16 (S2) pp. 37-44. 8 p. (2020) <https://doi.org/10.1556/446.2020.20005> **Q3**

**Hidas, K. I.**, Németh, Cs., Csonka, J., Visy, A., Friedrich, L., Zeke, I. Cs. (2020): How does freezing in liquid nitrogen influence the reological properties of liquid egg products? JOURNAL OF HYGIENIC ENGINEERING AND DESIGN 31 pp. 24-30. **Q4**

**Hidas, K. I.**, Németh, Cs., Csonka, J., Visy, A., Friedrich, L., Zeke, I. Cs. (2019): Effect of natural preservatives on the shelf life and calorimetric properties of salted, liquid whole egg. JOURNAL OF HYGIENIC ENGINEERING AND DESIGN 26 pp. 36-41. **Q4**

**Hidas, K. I.**, Németh, Cs., Nguyen, L. P. L., Visy, A.; Tóth, A., Friedrich, L., Nyulas-Zeke, I. Cs. (2021): Effect of different salt concentration on the physical properties of frozen thawed egg yolk. PROGRESS IN AGRICULTURAL ENGINEERING SCIENCES 17: S1 pp. 29-36. <https://doi.org/10.1556/446.2020.10007> **Q4**

**Hidas, K. I.**, Németh, Cs., Visy, A., Barkó, A., Horváth-Mezőfi, Zs., Tóth, A., Nguyen, L. P. L., Nyulasné Zeke I. (2021): Krioprotektív anyagok alkalmazásának hatása fagyasztott-felengedett tojássárgájából készült majonézek technofunkciós tulajdonságaira. ACTA AGRONOMICA ÓVÁRIENSIS. 62.:III. pp. 46-61.

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