# EFFECT OF DIFFERENT SALT CONCENTRATION ON THE PHYSICAL PROPERTIES OF FROZEN THAWED EGG YOLK

# Short, running title: Effect of salt on egg yolk freezing

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# Abstract

Freezing can enhance the storage time of liquid egg products, but egg yolk undergoes an irreversible textural and structural change when it is cooled to -6 °C. In this study, the effects of different salt concentrations on the physical properties of frozen-thawed egg yolk were investigated.

The pasteurised liquid egg yolk (LEY) was treated with 4, 5, and 6% of NaCl before freezing and it was stored at -18 °C for 4 weeks. The colour, pH, and rheological characteristics (firmness, consistency, cohesiveness, and index of viscosity) of yolk samples were evaluated before and after freezing.

Salt treatment resulted in preventing gelation, with decreasing firmness consistency and viscosity compared to control samples. The pH of all yolk samples increased during frozen storage. The more salt the egg yolk contained, the smaller the pH change was. The lightness values increased when salt was added before freezing. It decreased in treated samples and increased in the control sample after freezing.

The results indicated that the applied salt concentrations could inhibit protein aggregation of LEY induced by freezing during storage the period. The salt concentration of 6% could reduce the changes in pH, colour, and rheological properties.

# Keywords

Liquid egg yolk, Freezing, Gelation, Back-extrusion rheology, Salt concentration

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### Introduction

Eggs are very versatile in the food industry because of their excellent nutrient content and various functional properties. Processed egg products, such as separated, homogenized, and pasteurized liquid egg products are generally used for industrial purposes. Liquid egg yolk (LEY) is a popular ingredient in the food industry because of its excellent gelling, emulsifying, colouring, and coagulating properties, high nutritional value, and unique sensory characteristics. It is often used as a raw material of mayonnaise and confectionery products such as lemon curd, ice cream, créme brulée, and pudding (COOK & BRIGGS, 1986; UYSAL ET AL., 2019).

Since egg yolk undergoes a phenomenon called gelation, when it is cooled to -6 °C or lower temperatures, the freezing of egg yolk has been a popular topic in numerous researches (AU ET AL., 2015; PRIMACELLA ET AL., 2018; WANG ET AL., 2020). The most common explanation of gelation process is that large ice crystals form during the freezing process and it results in the concentration of components, that leads to the accumulation of low-density lipoproteins in plasma (ZHAO ET AL., 2021). Au and his co-authors examined the effect of freezing at -20 °C and frozen storage for 168 days on the kinetics and gelation process (AU ET AL., 2015). They found that plasma and granules participate in the irreversible texture change. Wang and his co-authors examined the gelation time of egg yolk at -18 °C. They found that egg yolk lost its fluidity after 7 hours (WANG ET AL., 2020).

To prevent the gelation process, antigelation agents can be used. The first study about preventing gelation established, that the gelation of yolk can be decreased by the addition of sucrose (MORAN, 1925). Other cryoprotectant agents were also found effective, sucrose and NaCl are widely used in 10% concentration (LAI, 2016). Researchers found that the addition of 5% salt resulted in a less hard texture than the addition of 10% (MA ET AL., 2021; PRIMACELLA ET AL., 2018).

In our study, 4, 5, and 6% salt was added to LEY before freezing to determine which concentration is the most effective to reduce the gelation. In our experiment, the pH, colour, and rheological properties of the samples were examined to determine which sample had the most similar properties to those of the fresh egg yolk.

# Materials and Methods

### Materials

Three kilograms of homogenised, pasteurized LEY (pH =  $6.38 \pm 0.02$ ) was obtained from a liquid egg plant (Capriovus Ltd., Szigetcsép, Hungary). The separated and homogenised LEY was pasteurized for 600 s at 65 °C with a flow rate of 600 kg/h. The product was then filled into

'Elopak' carton boxes with a weight of 1.0 kg. The LEY containing no additives should be stored between 0  $^{\circ}$ C and 4  $^{\circ}$ C with a shelf life of 3 days. Before treating, the samples were stored at a temperature of 4  $^{\circ}$ C.

### Sample preparation, freezing and thawing

Three kilograms of LEY was divided into four equal sections. Salt was added to the sample sections to achieve 4% w/w, 5% w/w and 6% w/w concentration. One section was examined without the addition of salt as control sample. All samples were stored at  $-18.0 \pm 1.0$  °C for four weeks. The measurements were performed before freezing (0 day) and on 14<sup>th</sup> and 28<sup>th</sup> days of frozen storage. The frozen yolks were thawed at 4 °C for 24 h.

### Determination of pH

The pH was measured at 4 °C using a digital pH meter (206-pH2, Testo SE & Co. KGaA, Titisee-Neustadt, Germany) in triplicate.

### Colour measurement

The colour of the samples was measured with a Konica-Minolta CR-410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan) at 4 °C. Measurements were performed five times.

# Examination of the rheological properties

The rheological properties of the LEY samples before freezing and after freezing-thawing were examined by back extrusion rheology. Measurements were performed by a TA.XT plus texture analyser (Stable Micro Systems, Surrey, UK) according to Wang et al. (2020). LEY (100 mL) was filled into the cylindrical measuring container of the equipment and a probe (d = 35 mm) was used to test the rheological characteristics. Measurements were performed at a constant velocity of 1 mm/s. The applied trigger force was 5.0 g, the trigger type was automatic and the test distance was 15 mm. The measurement was repeated 10 times.

### Statistical evaluation

Statistical analysis was performed by IBM Statistics 24 software. The significance level was 5% (p<0.05) The normality of the error terms was tested by Shapiro-Wilk test (pH (k(36)=0.962; p=0.252), L\* (k(60)=0.976; p=0.280), a\* (k(60)=0.994; p=0.995), b\* (k(60)=0.990; p=0.894), Firmness (k(60)=0.989; p=0.867), Cohesiveness (k(60)=0.968; p=0.115)). In case of Consistency and Index of viscosity, normality of the error terms was not proved, so statistical analysis was performed without the values of the control sample (Consistency (k(45)=0.975; p=0.423), Viscosity index (k(45)=0.987; p=0.876)). Levene's test was used for the determination of the equality of variances. Variances were assumed equal in each cases: pH (F(11;24)=0.606; p=0.805), L\* (F(11;48)=1.265; p=0.273, a\* (F(11;48)=2.028; p=0.050), b\* (F(11;48)=1.901; p=0.063), Firmness (F(11;48)=0.853; p=0.590), Consistency

(F(8;36)=1.945; p=0.083), Cohesiveness (F(11;48)=1.511; p=0.159), Viscosity index (F(8;36)=2.175; p=0.053). ANOVA was used for the statistical analysis of variance. The result of ANOVA test was significant in each case (p < 0.05), so Tukey HSD post hoc test was used to decide which groups differ significantly.

# **Results and discussion**

### Changes in pH

Figure 1. showes the effect of NaCl on pH of LEY before freezing and after freezing-thawing.



Figure 1. Effect of different salt concentration on pH of liquid egg yolk

NaCl did not affect the pH of sample before freezing. However, freezing caused significant changes in pH of samples. It was observed that pH of frozen-thawed yolk increased during storage at -18°C. The control samples had higher values in pH than others after 14 and 28 days of storage. The pH of salted yolk also increased over storage time but at different rates. Addition of 4 % NaCl resulted in a large increase in pH of frozen-thawed yolk after freezing compared to the treatment with 5 or 6 % of NaCl. The pH of all samples increased with the increasing of storage time. This finding was similar to other reports (HIDAS ET AL., 2021; HUANG ET AL., 1997).

### Changes in colour parameters

Changes of colour parameters of LEY were shown in Figure 2. As can be observed, the lightness of LEY changed dramatically after salt treatment compared to the fresh sample (Figure 2A). L\* of salted yolks had higher values than control before freezing. However, L\* of treated yolk decreased significantly and obtained lower values at 14<sup>th</sup> day and 28<sup>th</sup> day of frozen storage compared to control samples. No significant difference in L\* values among treated yolk before and over storage time. There was a great increase in L\* value of control samples at the first 14<sup>th</sup>

day of frozen storage, but after that only minor changes were observed. Huang and his coauthors also reported a rising trend of  $L^*$  of egg yolk for 1 day and a significant decrease at 60 days of frozen storage (HUANG ET AL., 1997).

Figure 2B showed that there was no significant difference in a\* values between control and treated yolk before freezing but after freezing, a\* values of those samples changed at different rates. The a\* values of control samples declined gradually during storage, whereas the frozen-thawed salted yolk had a notable decrease in a\* values over storage period. No significant difference in a\* values between treated yolk was observed.

The b\* values of LEY were greatly influenced by NaCl (Figure 2C). Salted yolks had lower values in b\* compared to fresh sample before freezing. At the 14<sup>th</sup> days of storage, b\* values of all samples increased significantly after freezing but at different rates. Control samples had higher values in b\* compared to treated yolks during storage. At the first 14<sup>th</sup> day, there was a notable increase in b\* for all samples, however, after that a slight decline was observed for those samples. However, no significant difference in b\* among treated samples was found before and after freezing.



Figure 2. Effect of different salt concentration on colour of liquid egg yolk. A - CIE lightness coordinate (L\*), B - (CIE red(+)/green(-) colour attribute (a\*), C - (CIE yellow(+)/blue(-) colour attribute (b\*).

### Changes in the rheological behaviour

The results of the Back-extrusion rheological measurement are shown in Figure 3. The firmness values of the egg yolk sample did not change significantly when salt was added to it before freezing (Figure 3A). After freezing and thawing, the firmness of the control sample without salt increased dramatically. Although, the firmness of the treated samples was much lower compared to the frozen and thawed control sample. Increasing the salt concentration resulted in lower firmness. In case of samples containing 4 and 5% salt, a significant decrease can be seen on the 28<sup>th</sup> day of storage. The firmness of the samples containing 6% salt have not changed on day 28.

Figure 3B shows the consistency values of the LEY samples before freezing and after freezing and thawing. The trend is similar to the trend of firmness data. No significant difference was observed before freezing when salt was added to the LEY. After freezing and thawing, the consistency of the control sample showed a large increase. On the 28<sup>th</sup> day of frozen storage, a decrease can be seen. Treated samples showed much lower consistency values after freezing and thawing, as well. Samples with different salt concentration had significantly different consistency values. Samples did not show significant difference on the 28<sup>th</sup> day compared to the 14<sup>th</sup> day.

Cohesiveness values also did not change significantly before freezing when different salt concentrations were used (Figure 3C). There is a huge difference between the control and the treated samples after freezing and thawing. In case of the control sample, the cohesiveness value of the fresh egg yolk is  $-8.27 \pm 1.11$  g, on day 14 it is  $-218.43 \pm 1.91$  g, on day 28 it is  $-260.84 \pm 2.44$  g. However, the cohesiveness values of treated samples were between  $-17.57 \pm 0.41$  g and  $-40,69 \pm 1.43$  g. The more salt the sample contained, the smaller values were obtained. Samples did not change significant difference on different measurement days after freezing and thawing.

There was no significant difference in case of the index of viscosity of the samples before freezing (Figure 3D). However, the viscosity index of the control sample changed a lot after freezing. Significant difference was observed on each measurement day in case of the control sample. Samples containing salt had similar values. The sample containing 6% salt had the lowest viscosity.

Physical properties, such as firmness, consistency, cohesiveness and viscosity index of LEY are important factors affecting the processing line. Previous studies (PRIMACELLA ET AL., 2018) also reported, that the rheological properties of LEY were more similar to the fresh egg yolk when salt is added.



Figure 3. Effect of salt concentration and frozen storage on the rheological characteristics of frozen-thawed liquid egg yolk. A – Firmness, B – Consistency, C – Cohesiveness, D – Index of viscosity.

# **Conclusions**

NaCl treatment caused several changes in physical properties of LEY. NaCl could help to prevent the gelation after freezing. The results presented in this study showed that salt treatment greatly influenced firmess, consistency, cohesiveness and viscosity index of frozen-thawed egg yolk. Addition of 5 or 6% of NaCl could decrease protein aggregation induced by freezing.

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### References

Au, C., Acevedo, N. C., Horner, H. T., & Wang, T. (2015) Determination of the Gelation Mechanism of Freeze–Thawed Hen Egg Yolk. *Journal of Agricultural and Food Chemistry*, 63(46): 10170–10180.

- Cook, F., & Briggs, G. M. (1986) The Nutritive Value of Egg. In W. J. Stadelman & O. J.Cotterill (Eds.), *Egg Science and Technology* (pp. 141-164.). Food Products Press.
- Hidas, K. I., Nyulas-Zeke, I. C., Visy, A., Baranyai, L., Nguyen, L. P. L., Tóth, A., Friedrich,
  L., Nagy, A., & Németh, C. (2021) Effect of Combination of Salt and pH on
  Functional Properties of Frozen-Thawed Egg Yolk. *Agriculture*, *11*(3): 257.
- Huang, S., Herald, T., & Mueller, D. (1997) Effect of electron beam irradiation on physical, physiochemical, and functional properties of liquid egg yolk during frozen storage. *Poultry Science*, 76(11): 1607–1615.
- Lai, L.-S. (2016) Quality and Safety of Frozen Eggs and Egg Products. In D.-W. Sun, Handbook of Frozen Food Processing and Packaging (pp. 529–548). CRC Press.
- Ma, Z., Ma, Y., Wang, R., & Chi, Y. (2021) Influence of antigelation agents on frozen egg yolk gelation. *Journal of Food Engineering*, 302: 110585.
- Moran, T. (1925). The effect of low temperature on hens' eggs. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, 98(691): 436–456.
- Primacella, M., Fei, T., Acevedo, N., & Wang, T. (2018) Effect of food additives on egg yolk gelation induced by freezing. *Food Chemistry*, 263: 142–150.
- Uysal, R. S., Sumnu, G., & Boyaci, I. H. (2019) Effects of heat-treated liquid whole egg on cake batter rheology and the quality of baked cake. *Journal of Food Process Engineering*, *42*(2): e12977.
- Wang, R., Ma, Y., Ma, Z., Du, Q., Zhao, Y., & Chi, Y. (2020) Changes in gelation, aggregation and intermolecular forces in frozen-thawed egg yolks during freezing. *Food Hydrocolloids*, 108: 105947.
- Zhao, Y., Feng, F., Yang, Y., Xiong, C., Xu, M., & Tu, Y. (2021) Gelation behavior of egg yolk under physical and chemical induction: A review. *Food Chemistry*, *355*: 129569.