




## Article

# Effect of Combination of Salt and pH on Functional Properties of Frozen-Thawed Egg Yolk

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**Abstract:** Egg yolk undergoes an irreversible gelation process at temperatures below  $-6^{\circ}\text{C}$ , which greatly impairs its application and increases its apparent viscosity. This work was aimed to investigate the effect of salt and pH in preventing the gelation of frozen-thawed egg yolk. Before freezing, 5% of salt was added into the pasteurized liquid egg yolk, then pH was adjusted to different levels (5.7, 6.0 and 6.3) with citric acid. After that, the yolk was stored at  $-18^{\circ}\text{C}$  for four weeks. Rheological and thermal properties of the fresh and frozen-thawed egg yolk were measured. In addition, the colour, turbidity and emulsifying properties were also determined. The results showed that pH of all samples increased during frozen storage, but at different rates. The combination of 5% of salt and pH at 6.0 and 6.3 could prevent the gelation, resulting in rheological properties more like the fresh liquid egg yolk. In addition, emulsifying properties also obtained better results for treated yolk. Moreover,  $L^*$  value of treated egg yolk was higher before freezing and became lower after storage compared to control. The results of this work found that the combination of 5% of salt and adjusted pH could prevent the gelation of frozen-thawed liquid yolk.

**Keywords:** egg yolk; gelation; additive; rheological properties; calorimetric properties



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## 1. Introduction

Eggs play a prominent role in the human diet due to their nutritional values including high-quality proteins, essential long-chain fatty acids, iron, phosphorus, trace minerals and vitamins A, D, E, K and B [1]. Chicken egg proteins, phospholipids, vitamin A, vitamin E, selenium and lutein also show biological activities, such as antioxidant activity [2,3]. The coagulating, emulsifying, colouring and flavouring properties also contribute to eggs' utilization in food industry [4,5].

The amount of shelled eggs consumed per capita varies greatly around the world, but the market for processed egg products is clearly on the rise [6]. Two groups of processed egg products are distinguished. Products from “first processing” are mainly for the food industry (such as liquid, frozen and powdered egg products), while speciality egg products (such as formulated and cooked eggs) are for consumers [7]. Processed egg products are used in the food industry for a wide variety of purposes, including: ingredients for pasta, bakery products, sea-food products, meat products, dairy products, mayonnaise, sauces and salad dressings [8,9].

Recently, liquid egg yolk freezing has been an interesting topic in numerous research. Most researchers have focused on understanding the mechanism of egg yolk gelation

and looked for different methods to prevent it [8,10–13]. Au et al. [10] experimented with freezing egg yolk at  $-20\text{ }^{\circ}\text{C}$  for 168 days and studied the gelation process and kinetics. Both plasma and granules were found to be involved in the gelation process [10]. Primacella et al. [8] concluded that all low-density lipoprotein, high-density lipoprotein, and livetins were also involved in the egg yolk gelation process. Wang et al. [13] found that at  $-18\text{ }^{\circ}\text{C}$ , egg yolk became gel-like in 7 h, and the thawed yolk contains hydrophobic interactions and disulfide bonds.

To prevent gelation, several studies have been conducted using cryoprotectants [11,12], protein-degrading enzymes [14] or mechanical treatments such as colloid milling [15,16]. The first cryoprotectant was sucrose [17], which is widely used in 10% concentration [4]. Other agents such as arabinose and dextrose were found to be effective against gelation in the concentration of 10% [16]. Primacella et al. [12] examined the effect of salt concentration on the hardness of frozen-thawed egg yolk and found that in the short term, the addition of 5% salt resulted in a less hard texture than 10% salt.

The drawback of using high concentration of sugar or salt is the negative effect on both nutritional and sensory properties, which limits the application of treated yolk [12]. Salt or sugar can both affect blood pressure and can cause hypertension and cardiovascular disease; however, salt has a much stronger effect [18]. Sugar intake contributes to body weight increasing. Overweight plays a huge role in the development of diabetes and many cancers [19]. In addition, the heat resistance of microorganisms is increased with the application of additives such as sugar or salt [20]. Therefore, novel gelation inhibitors such as hydrolysed carboxymethyl cellulose, proline, hydrolysed egg white and yolk were also tested. These alternatives were effective in inhibiting the gelation of frozen-thawed yolk [12]. However, from an economic point of view, these methods may not be widely applicable.

Citric acid is widely used in the commercial industry for liquid egg products due to its benefits. Previous reports found that citric acid could prevent colour loss during cooking, enhance the effect of pasteurisation treatment on liquid whole egg (LWE) [21] and increase the protein solubility of pasteurized LWE during storage period [22,23]. The pH is one of the main factors in quality retention and processing of liquid egg products [24]. However, the information about the effect of citric acid and salt on the attributes of frozen-thawed egg yolk is still limited.

In this context, the experiment was carried out to investigate the effect of the combination of salt and pH on physicochemical and functional properties of frozen-thawed liquid egg yolk. In addition, the correlation of these properties was also evaluated.

## 2. Materials and Methods

### 2.1. Materials

Eight kilograms of pasteurized liquid egg yolk ( $\text{pH} = 6.40 \pm 0.02$ ; dry matter content:  $43.31 \pm 0.18\text{ g}/100\text{ g}$ ; protein content:  $14.85 \pm 0.11\text{ g}/100\text{ g}$ ; fat content:  $25.42 \pm 0.11\text{ g}/100\text{ g}$ ) was obtained from a liquid egg plant (Capriovus Ltd., Szigetcsép, Hungary). Liquid egg yolk was produced from caged hen eggs that have been laid for up to 3 days prior. The pasteurization process lasted for 600 s at  $65\text{ }^{\circ}\text{C}$  with a flow rate of 600 kg/h and the eggs were then filled into ‘Elopak’ carton boxes with a weight of 1.0 kg. The shelf life of this product containing no additives is 3 days, and it should be stored between  $0\text{ }^{\circ}\text{C}$  and  $4\text{ }^{\circ}\text{C}$ . Samples were stored at a temperature of  $4\text{ }^{\circ}\text{C}$ , until treatment, for 3 h.

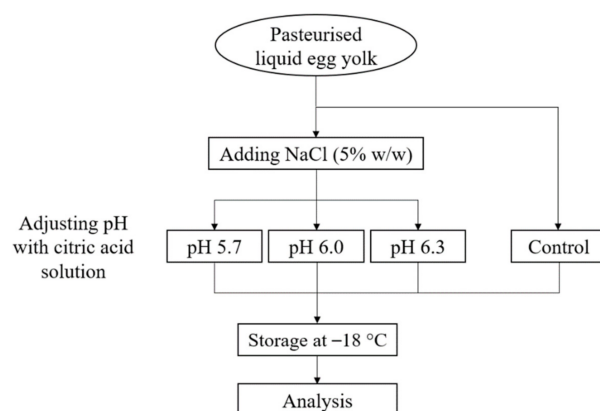
Citric acid monohydrate was provided by Foodchem International Corporation (Shanghai, China). A solution of citric acid (20% (*w/v*)) was used to adjust the pH.

### 2.2. Sample Preparation, Freezing and Thawing Procedure

Samples were prepared according to Figure 1. 300 g of salt was added into 6 kg of liquid egg yolk (5% *w/w*), then the yolk mixture was stirred for homogeneity. After that, the mixture was divided into 3 groups and pH was adjusted with citric acid to 5.70, 6.00 and 6.30 ( $\pm 0.02$ ). The amount of citric acid added into liquid egg yolk was less than 1%



(v/v) in order to minimize the dilution effect. Liquid egg yolk that did not receive any treatment served as the control. All samples were stored at  $-18.0 \pm 1.0$  °C for four weeks.



**Figure 1.** Flowchart of the experiment.

The frozen yolks were thawed at 4 °C for 24 h before analysis.

### 2.3. Measurements

The measurements were performed before freezing (0 day) and on 14 days and 28 days of storage.

#### 2.3.1. Determination of Composition and pH

Moisture and fat content data were measured according to the Association of Official Analytical Chemists [25] in triplicates. Moisture was determined by drying of around 2 g liquid egg yolk sample in an air-forced oven (Labor Műszeripari Művek, Budapest, Hungary) at 105 °C until constant weight. The Soxhlet method was used to evaluate the fat content with a Soxtec system (Tecator AB, Höganäs, Sweden). Protein was measured by the Kjeldahl method [26].

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

#### 2.3.2. Colour Measurement

Tristimulus colour measurements were performed with a Konica-Minolta CR-410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan) at 4 °C. Measurements were performed five times.

#### 2.3.3. Differential Scanning Calorimetry

Calorimetric properties of liquid egg yolk samples were examined by differential scanning calorimeter MicroDSC III (Setaram, Caluire-et-Cuire, France). The samples of  $210 \pm 5$  mg were put into 1 mL aluminium pans and sealed. Samples were heated from 20 °C to 95 °C with a heating rate of 1.5 °C/min, then cooled to 20 °C with a cooling rate of 3.0 °C/min. Distilled water was used as reference sample. All data were processed with Calisto Processing software (Setaram, Caluire-et-Cuire, France). Straight baselines were set to the cooling phase of the thermograms and enthalpy change ( $\Delta H$ , [J/g]) was determined from the peak area. Denaturation temperature ( $T_d$ , [°C]) was also recorded. Measurements were performed in triplicate.

#### 2.3.4. Examination of Rheological Properties

Examination of the rheological behaviour of liquid egg yolk was performed by MCR 92 rheometer (Anton Paar, Les Ulis, France) in rotational mode equipped with a concentric cylinder (cup diameter 28.920 mm, bob diameter 26.651 mm, bob length 40.003 mm, active length 120.2 mm, positioning length 72.5 mm). Anton Paar RheoCompass software was

used to control the equipment. The temperature of rheological measurements was kept constant at 20 °C. Shear stress was measured by logarithmically increasing and decreasing shear rate between 1 and 1000 1/s for 31 measurement points with a period of 3 s.

The Herschel—Bulkley model (Equation (1)) was used to analyse the flow curves (shear rate-shear stress diagrams), according to Atilgan and Unluturk [27]. This model was used to describe the rheological properties of liquid egg yolk at 4 °C [27]. In agreement with literature, the Herschel—Bulkley model fitted well to the experimental data in this study with  $R^2 > 0.99$ .

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (1)$$

where  $\tau$ —refers to shear stress (Pa);  $\tau_0$ —indicates the yield stress (Pa);  $\dot{\gamma}$ —is the shear rate (1/s),  $K$ —refers to the consistency coefficient (Pa·s<sup>*n*</sup>) and  $n$ —is the flow behaviour index (dimensionless).

### 2.3.5. Back Extrusion Rheology

The flow properties of the liquid egg yolk samples were also examined by back extrusion rheology with a TA.XT plus texture analyser (Stable Micro Systems, Surrey, UK) according to Wang et al. [13]. A cylindrical container was filled with 100 mL of liquid egg yolk and a probe with Ø35 mm was used. The back extrusion force was measured at constant velocity of 1 mm/s. The applied trigger was 5.0 g, the trigger type was automatic and the test distance was 15 mm. The measurement was repeated 10 times.

### 2.3.6. Emulsifying Properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined using the turbidimetric method of Pearce and Kinsella [28], with minor modifications made by Tang et al. [29]. Two *w/v* percent of fresh or frozen-thawed liquid egg yolk sample was dissolved in 0.5 M NaCl at pH 6.5 and the solutions were emulsified with sunflower oil in a 60:40 ratio [30,31]. Homogenization was performed by a T 25 digital Ultra-Turrax® homogenizer (IKA®-Werke GmbH & Co. KG, Inc., Staufen, Germany) at 11,000 RPM for 60 s. An aliquot of the bottom of the homogenized emulsion was taken by pipette immediately (0 min) and after 5 min and it was diluted with 0.1% (*w/v*) sodium dodecyl sulphate solution. Absorbance measurement was performed by a U-2900 spectrophotometer (Hitachi, Tokyo, Japan) at a wavelength of 500 nm against a blank (diluting solution).

*EAI* (m<sup>2</sup>/g) and *ESI* (min) values were calculated by Equations (2) and (3).

$$EAI = \frac{2 \cdot 2.303 \cdot A_0 \cdot DF}{c \cdot \varnothing \cdot (1 - \theta) \cdot 1000} \quad (2)$$

$$ESI = \frac{A_0}{A_0 - A_5} \cdot 5 \quad (3)$$

where *DF* is the dilution factor (150), *c* is the weight of protein per unit volume of protein aqueous (mg/mL) emulsion,  $\varnothing$  is the optical path (0.01 m),  $\theta$  is the fraction of oil used in the emulsion (0.4) and *A*<sub>0</sub> and *A*<sub>5</sub> are the absorbance of the diluted emulsions at 0 and at 5 min. Measurements were performed in 6 replications.

### 2.3.7. Turbidity

Turbidity measurements were carried out following the method by Wang et al. [13]. Fresh and frozen-thawed liquid egg yolk samples were dispersed in 10% NaCl solution (1:100 dilution). The absorbance of the diluted samples was measured at a wavelength of 660 nm with a U-2900 spectrophotometer (Hitachi, Tokyo, Japan).

### 2.3.8. Statistical Analysis

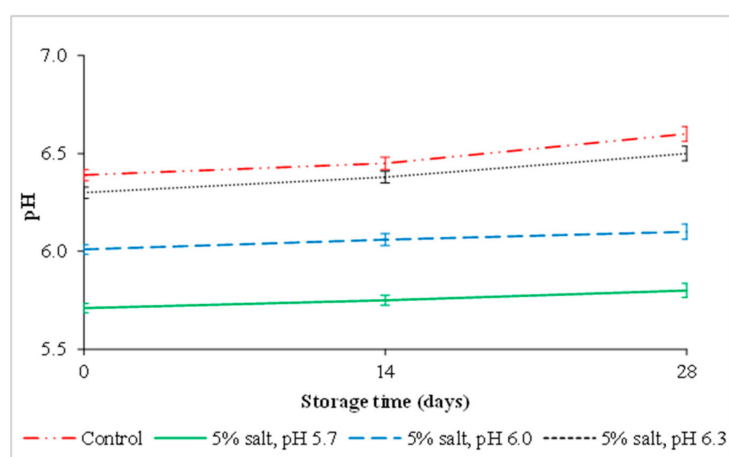
Data were collected, pre-processed and visualized on charts using Microsoft® Excel® (version 16.45). Statistical analysis was performed using R (version 4.0.3, R Foundation

for Statistical Computing, Vienna, Austria). Analysis of Variances (ANOVA) test was performed to discover significant effects. Besides the main effects of treatment and storage time, interaction effect was also included in the models. Homogeneity of variances was evaluated by Bartlett test. Following the ANOVA test, parameters of homogeneous variances were further analysed by TukeyHSD post-hoc test, while others of inhomogeneous variances were further analysed by non-parametric Kolmogorov-Smirnov test. Significant differences were determined using  $p < 0.05$ . Relationship between measured parameters was evaluated using linear correlation coefficient (Pearson, London, UK). Data are presented on figures with mean value  $\pm$  standard deviation.

### 3. Results

#### 3.1. Changes in pH

The pH of pasteurized liquid egg yolk (LEY) for this experiment was around 6.39 at the beginning. It was observed that frozen storage had a significant effect ( $p < 0.05$ ) on the pH of all samples. The pH values increased during storage for all samples, but at different rates. The pH of frozen-thawed samples with initial pH 5.7 and 6.0 increased slightly during storage at  $-18\text{ }^{\circ}\text{C}$ . Samples of pH 6.3 and the control were observed to increase significantly ( $p < 0.05$ ) within 28 days. Greater change in pH was found for samples with higher initial pH value. The results of the pH measurement are shown in Figure 2.



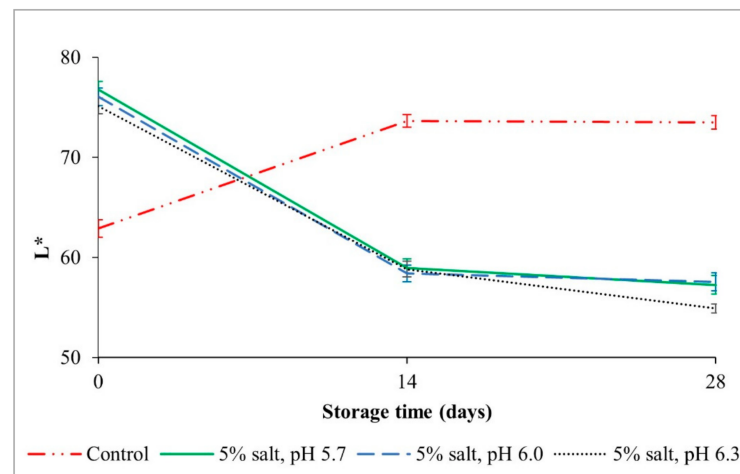
**Figure 2.** The effect of treatment on the pH of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

#### 3.2. Changes in Colour

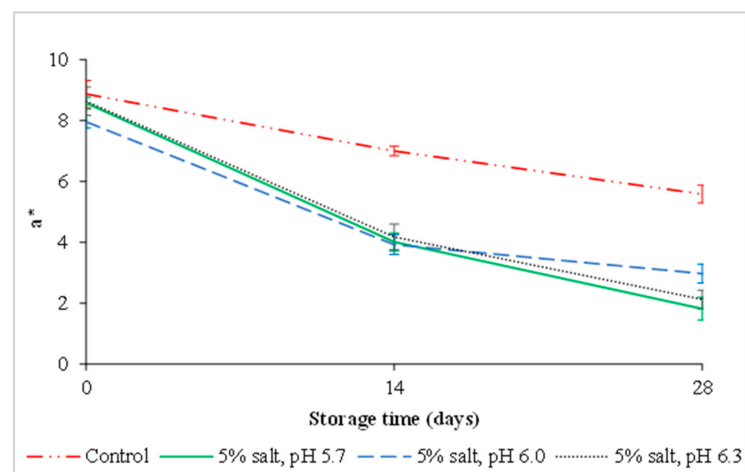
Colour properties of frozen-thawed liquid egg yolk samples are shown in Figures 3–5. According to the results, the colour of the liquid egg yolk changed significantly ( $p < 0.05$ ) with treatment and storage period.  $L^*$  values showed an increasing trend for treated samples before freezing. This work shows that, after freezing and thawing, the  $L^*$  value of the control sample increased during the first 14 days. The  $L^*$  value of the treated samples decreased significantly ( $p < 0.05$ ) after freezing and thawing at 14 days and 28 days, showing opposite trend to that of control samples. Measured lightness changed primarily during the first 14 days. The control samples had higher value in  $L^*$  after 14 days of freezing but did not show significant change afterwards during storage.

This study also revealed that all the samples became less red and more strongly yellow as a result of freezing and thawing. The  $a^*$  values (Figure 4) showed a decreasing trend after freezing and thawing. Significant difference ( $p < 0.05$ ) in  $a^*$  was found between the control sample and the treated samples; however, treated samples had similar values at 14 days of storage period. The  $b^*$  values (Figure 5) showed an increasing trend in the case of the control sample. The results show an increase in  $b^*$  after 14 days and a decrease at

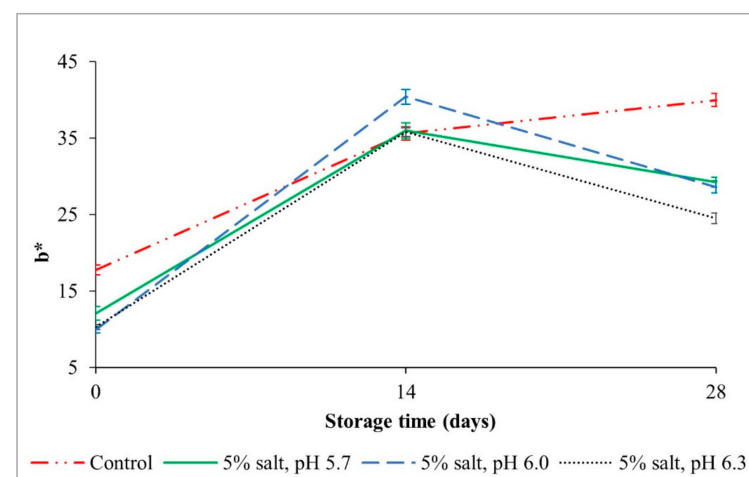
28 days for treated samples. Regarding the  $b^*$  value, the behaviour of the control sample was significantly different ( $p < 0.05$ ) from the treated samples after 14 days.



**Figure 3.** The effect of treatment on  $L^*$  (CIE lightness coordinate [32]) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.



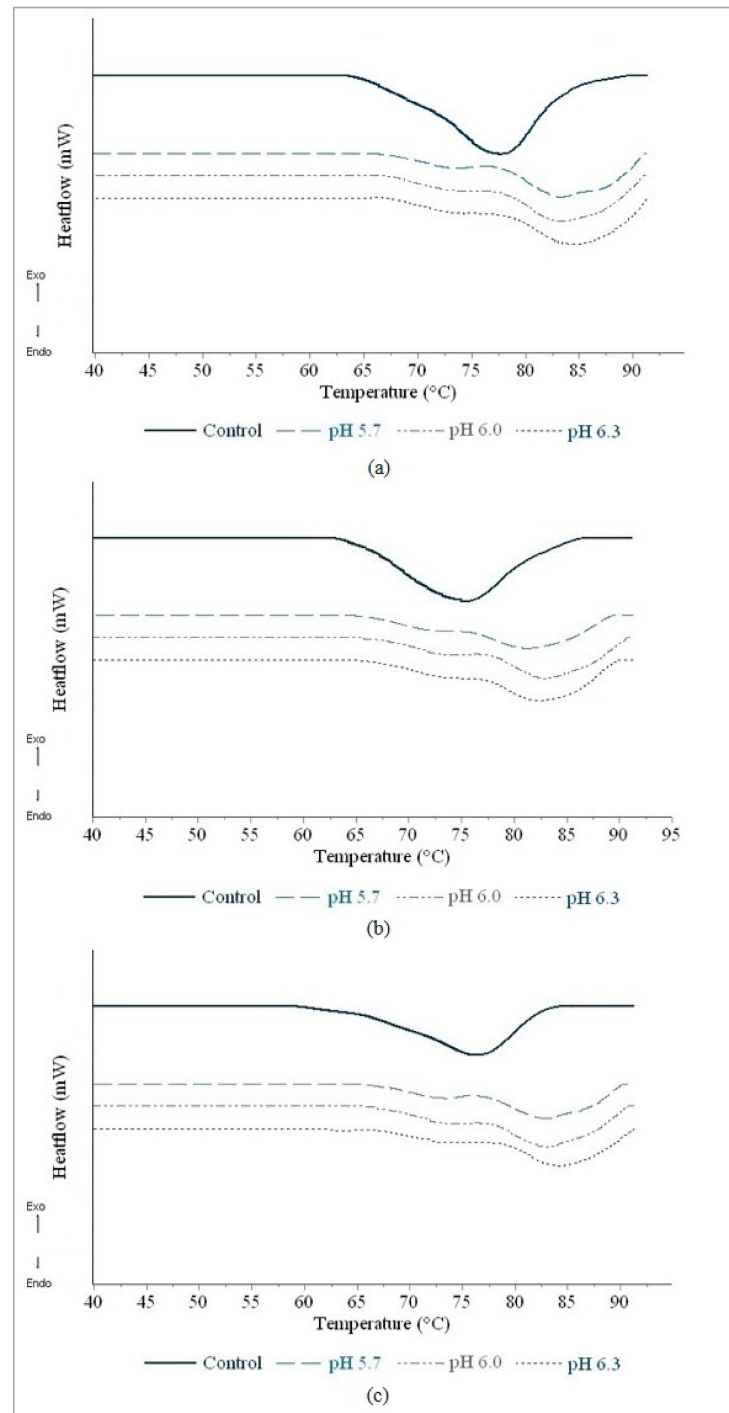
**Figure 4.** The effect of treatment on  $a^*$  (CIE red(+)/green(−) colour attribute [32]) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.



**Figure 5.** The effect of treatment on  $b^*$  (CIE yellow(+)/blue(−) colour attribute [32]) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

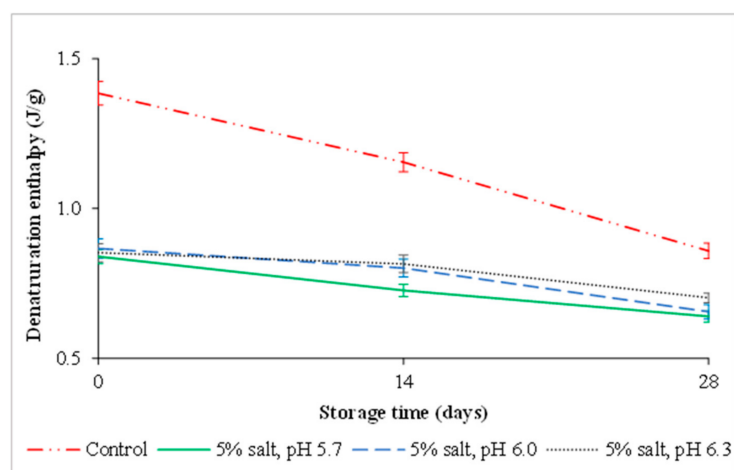
### 3.3. Thermodynamic Properties

Thermograms of liquid egg yolk samples obtained by differential scanning calorimetry (DSC) are represented in Figure 6. Enthalpy change and denaturation temperature values are shown in Figures 7 and 8.

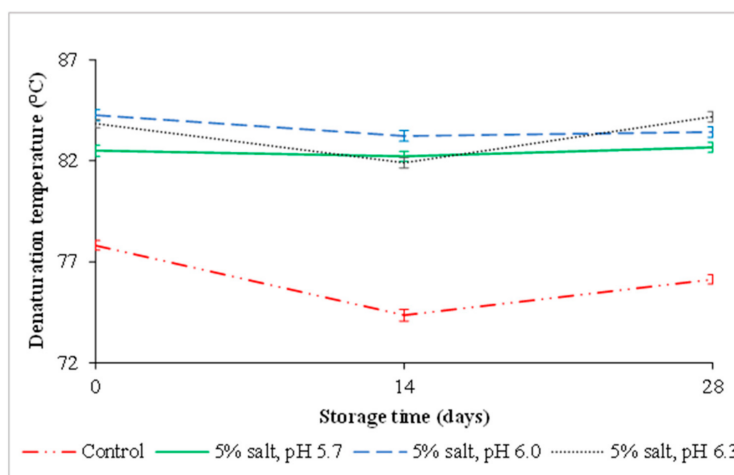


**Figure 6.** Differential scanning calorimetry thermograms of liquid egg yolk samples before freezing (a), and frozen-thawed after 14 days (b) and 28 days (c) of storage at  $-18^{\circ}\text{C}$ .





**Figure 7.** The effect of treatment on denaturation enthalpy of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.



**Figure 8.** The effect of treatment on the denaturation temperature of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

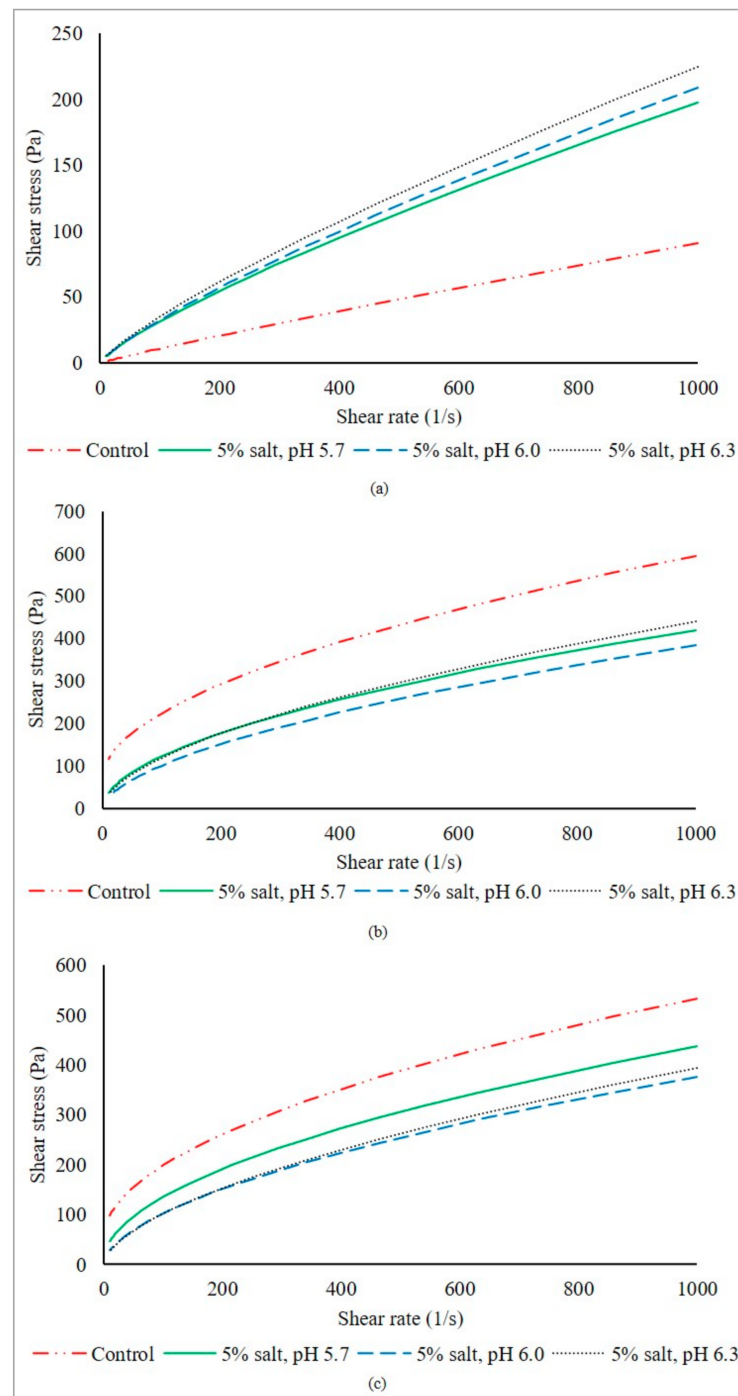
The denaturation enthalpy fell to lower values for treated samples before freezing, compared to control (Figure 7). However, the results of the treated groups show very similar values. After freezing, a decreasing trend of the denaturation enthalpy can be observed for all samples. The final measured denaturation enthalpy values are in the order of adjusted pH. The control sample obtained obviously higher denaturation enthalpy values compared to treated ones during the whole experiment.

In this study, a single peak was observed for control egg yolk samples for the whole experiment period. The denaturation temperatures of control samples were approximately 75–77 °C (Figure 8). The thermograms of treated samples show that the characteristic peak is elongated and divided into a smaller and a larger peak and the denaturation temperature shows a large increase compared to the control sample. Freezing and thawing had a minor effect on the shape of the thermograms. However, a significant change ( $p < 0.05$ ) in denaturation temperature can be observed in the case of the control sample and yolk at pH 6.3. A decrease in denaturation temperature can be found at 14 days and an increase at 28 days in the case of the control sample and yolk at pH 6.3.

### 3.4. Rheological Measurement as a Function of Shear Rate

The flow curves of fresh and frozen-thawed egg yolk samples are shown in Figure 9. An increase of shear stress was observed in the case of treated samples compared to the fresh sample before freezing (Figure 9a). Moreover, the effect of different pH levels was

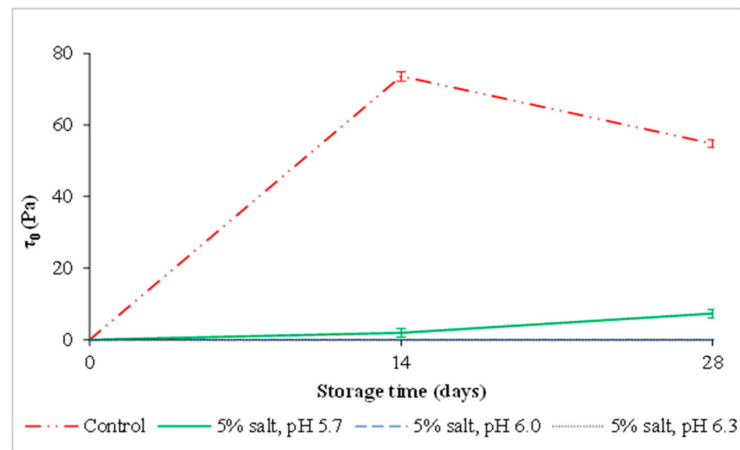
also detected, lower pH resulted lower shear stress values for all samples. Freezing also caused an increase in the shear stress values. This study demonstrates that the shear stress values of control sample were the highest after freezing. Whereas the shear stress values of treated samples were lower due to the inhibition of gelation process. Among the salted yolks, samples with pH 6.0 had the lowest shear stress values after freezing and thawing.



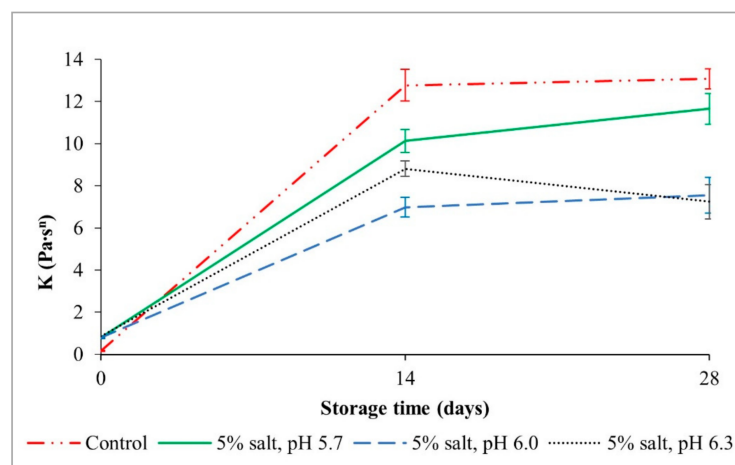
**Figure 9.** Flow curves of liquid egg yolk samples before freezing (a), and frozen-thawed liquid after 14 days (b) and 28 days (c) of storage at  $-18^{\circ}\text{C}$ .

The Herschel—Bulkley model parameters are represented in Figures 10–12. According to the results in this study, the yield stress value appeared in the control sample and in the sample of pH 5.7 after freezing. The minimum stress required to move the material (yield

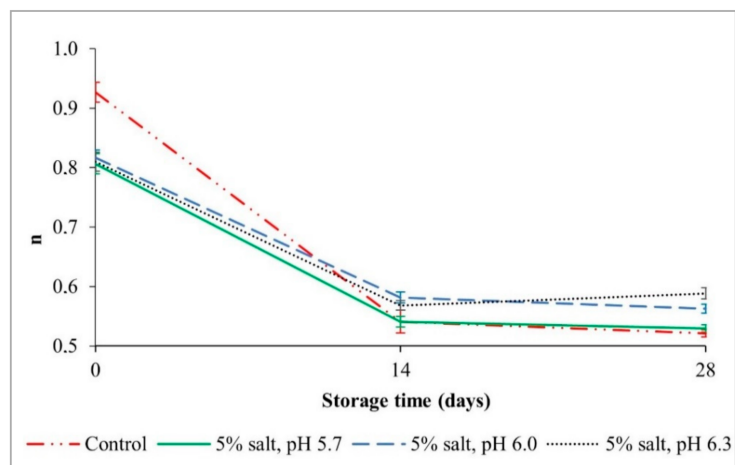
stress) was the highest for the control sample after freezing, which decreased after 14 days (Figure 10). In the case of the sample with pH of 5.7, its value increased continuously during storage.



**Figure 10.** The effect of treatment on the yield stress ( $\tau_0$ ) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.



**Figure 11.** The effect of treatment on the consistency coefficient (K) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.



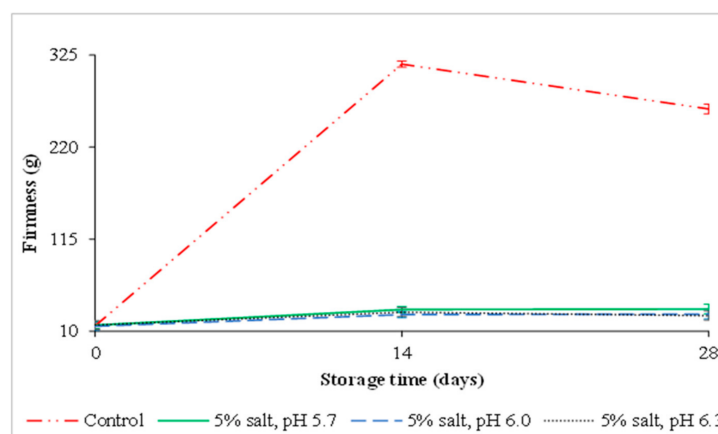
**Figure 12.** The effect of treatment on the flow behaviour index (n) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

The initial consistency coefficient already increased for treated samples. According to the results, the treatment changed the value of the consistency coefficient significantly ( $p < 0.05$ ) after freezing (Figure 11). The consistency coefficient increased with the increase of storage period in the case of salted yolk with pH 5.7, whereas an increase in consistency coefficient was observed until 14 days for all other samples (control and pH 6.0, pH 6.3).

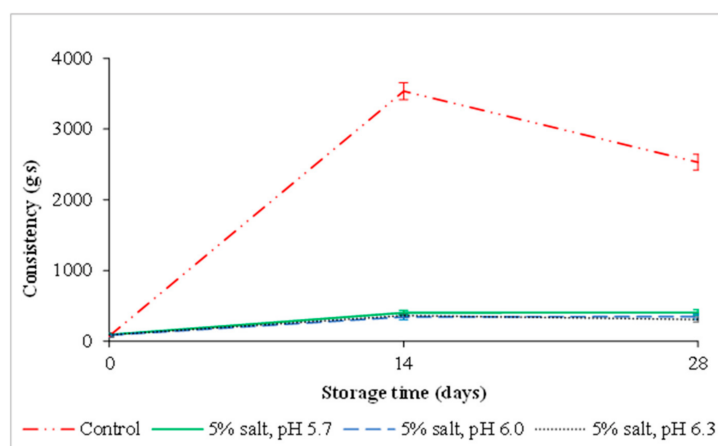
The flow behaviour index ( $n$ ) values of samples before freezing and frozen-thawed yolks were less than 1.0 (Figure 12). Decrease in flow behaviour index values was observed as an effect of treatment, before freezing. However, samples among the treated group had similar values. After freezing,  $n$  values showed a decreasing trend with a large reduction during the first 14 days.

### 3.5. Viscosity and Consistency Analysis

Figures 13 and 14 represent the data obtained by the back extrusion rheology method. The effects of the frozen storage and treatment were significant ( $p < 0.05$ ) for all parameters. Firmness increased at 14 days and then decreased at 28 days for the control sample. Similar behaviour was observed for treated samples of pH 6.0 and 6.3, but changes were significant until 14 days. There was very large difference between the firmness of the control sample and the treated groups. Among the treated samples, the yolk with pH 6.0 had the smallest values in firmness before and after freezing. The observed behaviour of consistency values was the same as that of firmness values.



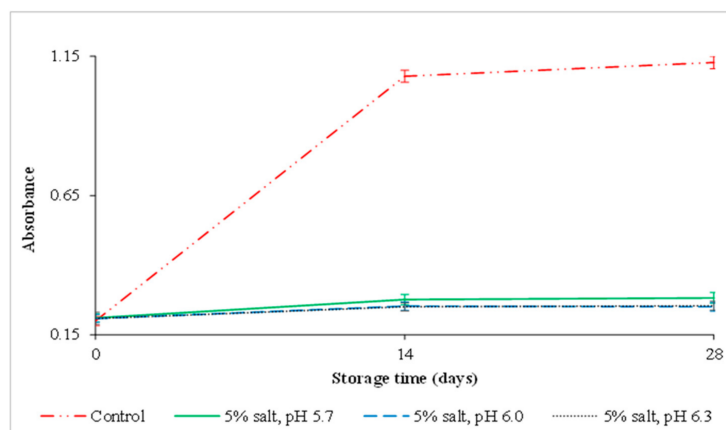
**Figure 13.** The effect of treatment on the firmness of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.



**Figure 14.** The effect of treatment on the consistency of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

### 3.6. Turbidity Analysis

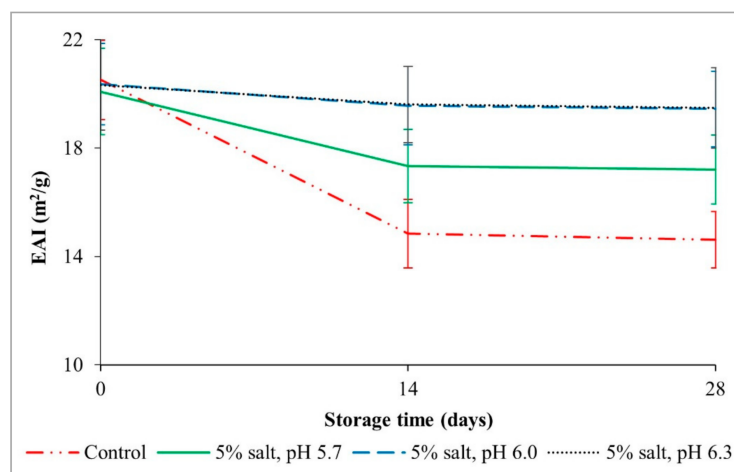
The effect of treatment on turbidity of the frozen-thawed yolk sample is presented in Figure 15. This study showed that the combination of NaCl and pH inhibited the gelation of frozen-thawed yolk. There was a significant difference ( $p < 0.05$ ) in turbidity among samples during storage (Figure 15). The control sample had the highest values in turbidity after 14 days and 28 days of storage, compared to treated groups. Only a minor change was detected for all samples between 14 days and 28 days. Salted yolk at pH 6.0 and 6.3 obtained better results in preventing the gelation than that of pH 5.7. This work found that salt and a mild change of pH could prevent protein aggregation.



**Figure 15.** The effect of treatment on the turbidity of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

### 3.7. Emulsifying Properties

Emulsifying properties were determined by absorbance of emulsion solutions prepared with yolk. As shown in Figure 16, frozen storage caused a decrease in EAI values compared to the control sample at 0 day (fresh yolk).

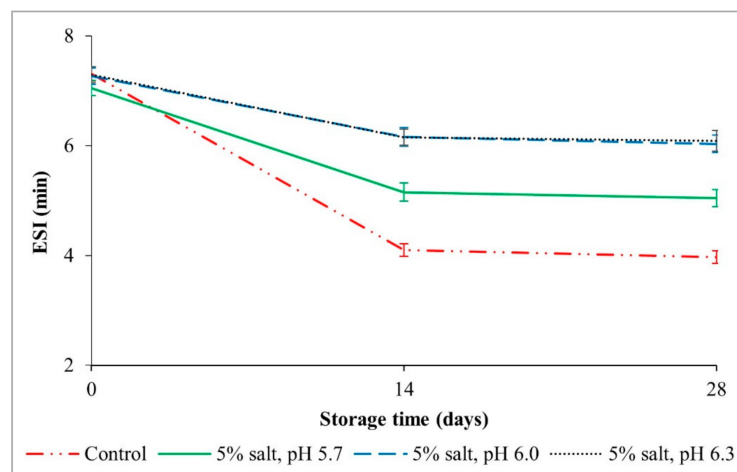


**Figure 16.** The effect of treatment on the emulsifying activity index (EAI) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

The combination of salt and pH resulted in a slight decrease in EAI for samples of pH 6.0 and 6.3. In the case of pH 5.7, it showed lower EAI values than that of other treated groups, but still higher than the control. No significant difference in EAI was observed between 14 days and 28 days for all samples. Control samples obtained the lowest EAI values after freezing.



Figure 17 shows the results of emulsion stability index measurements. ESI also decreased after freezing for all samples, however, only minor change was detected between 14 days and 28 days. There was a significant ( $p < 0.05$ ) decline in ESI for all samples after freezing. ESI of frozen-thawed treated yolk obtained better results than control over the storage period. Samples with initial pH 6.0 and 6.3 had higher values in ESI than that of pH 5.7.



**Figure 17.** The effect of treatment on the emulsifying stability index (ESI) of frozen-thawed liquid egg yolk. Data are presented with mean  $\pm$  standard deviation.

### 3.8. Correlation of Parameters

The correlation table (Table S1) compares all parameters to each other. Linear correlation coefficient has been calculated and significant values are highlighted by colour, while obvious and expected relationships are marked with grey background. Significance levels ( $p$  values) have been calculated and presented in a separate table. According to the observed correlation values for time, primarily yolk colour (CIE  $a^*$ ) and yolk rheology parameters ( $K$ ,  $n$ ) changed with storage time. On the other hand, rheology parameters  $K$  and  $n$  strongly correlated with CIE  $b^*$  as  $r = 0.859$  and  $r = -0.861$ , respectively. Among DSC parameters, denaturation temperature correlated well with rheology parameter  $\tau_0$ , followed by viscosity, consistency features and turbidity. The parameter turbidity obtained the highest correlation value of  $r = -0.996$  with cohesiveness. Turbidity achieved the highest number of significant correlation values with other parameters.

## 4. Discussion

The pH of yolk of freshly laid eggs is around 6.0 [32], however, the pH of liquid egg yolk is higher due to the presence of egg white [33]. This can be also observed in this study. The natural increasing trend of pH with increasing time of frozen storage was also observed by Huang et al. [34]. Those authors found that the pH of liquid egg yolk stored at  $-15^\circ\text{C}$  increased significantly at 60 days. In this study, minor changes in pH were detected for salted yolk at pH 5.7 and 6.0. Low pH is desirable because it helps to prevent microbiological spoilage [35]. According to Lai [4], normally less than 1% of the bacteria in raw egg products survive after pasteurization. However, during thawing, the growth of surviving microorganisms may occur again [36].

Compared to the control sample, the treated egg yolk samples had larger initial  $L^*$  values. Radovic et al. [22] also identified that the addition of citric acid causes an increase in the  $L^*$  value in the case of liquid whole egg. Those authors did not find a significant difference in  $a^*$  values, but they reported an increase in  $b^*$  values. It was found that protein denaturation can be responsible for colour change in liquid whole egg [22]. In this study,  $L^*$  value of control samples increased until 14 days. Huang et al. [34] also reported a rising trend of  $L^*$  of egg yolk for 1 day and a significant decrease at 60 days. However, those

treated samples did not show significant difference before 30 days. In agreement with literature, presented results show that egg yolk colour changes during frozen storage.

The enthalpy decrease measured by DSC is due to the unfolding of the examined protein molecules [37]. The change can be characterized by the simultaneous exothermic (e.g., protein aggregation) and endothermic (such as breakdown of hydrogen bond) processes. However, the endothermic nature of the curves indicates that hydrogen bonds have been disrupted in large quantities, causing protein denaturation [35]. In this study, the only peak on the thermogram of the control sample represents the denaturation enthalpy of high-density lipoproteins, phosphitin and low-density lipoproteins [36,38]. The measured denaturation temperatures for the control sample are in agreement with the results of Primacella et al. [11]. Freezing had no effect on the shape of the thermograms, similar to the measurements reported by de Souza and Fernández [38]. In this work, the shape of the thermogram was observed to change as a response to the treatment. This is caused by the dissociation of proteins and changes in protein conformation [12]. Present study revealed that the application of 5% salt and pH caused a large increase in the denaturation temperature. Wang et al. [39] reported that the denaturation temperature of salted egg yolk plasma is higher than that of native egg yolk. A previous study [11] found that the addition of 10% salt increased the denaturation temperature to 91.5 °C compared to 76.8 °C of the fresh yolk. The addition of salt increases surface hydrophobicity due to protein dissociation [12]. This indicates that salting can raise the thermal stability of egg yolk plasma proteins. According to the results, a steady decrease was found in denaturation enthalpy caused by the increase of frozen storage period in the case of the control sample. According to Au et al. [10], the reason is the aggregation of lipoprotein particles via hydrophobic interactions caused by water removal during slow freezing. Those authors also stated that the granules are also involved in the aggregation process.

Liquid egg yolk products have a shear-thinning (pseudoplastic) rheological behaviour [40]. This behaviour is characterized by a convex profile on the shear rate-shear stress diagram. As the shear rate increases in such materials, the shear stress increases due to the attenuation of molecular interactions [41]. According to the shape of the flow curves and flow behaviour index data ( $0 < n < 1$ ), liquid egg yolk samples examined in this study had pseudoplastic flow behaviour. The power-law model is often used to evaluate the flow behaviour of egg yolk products [11,38,42,43]. However, the Herschel—Bulkley model better describes samples that suffer from gelation after freezing. Therefore, this model was preferred. In the present study it was found that the shear stress values increased for treated samples. This also indicates an increase in apparent viscosity. Previous studies [12,43] also reported a remarkable increase in the viscosity of yolk mixture due to the addition of salt before freezing. In this study, freezing also had strong effect on the shape of the flow curves, especially in the case of control samples. Chang et al. [44] reported significant increase in shear stress and apparent viscosity even after 5 h of frozen storage at −14 °C. Defining the yield stress is important in many aspects of processing, handling, storage and performance properties [11]. The yield stress value obtained by model fitting is often referred as dynamic yield stress. It can be explained as the minimum stress required to maintain or stop flow [45]. In this study, the yield stress value appeared in the control sample and in the sample of pH 5.7 after freezing, which is an important feature of gelation. This work found that the combination of 5% salt and pH 5.7 did not completely prevent gelation, whereas salted yolk at pH 6.0 and 6.3 obtained better results in preventing gelation. The study of Primacella et al. [12] also revealed that 5% of salt can inhibit gelation. The consistency coefficient is often used as an indication of the viscosity of the liquid [46]. As expected, the consistency coefficient increased for treated samples before freezing, similar to the earlier report [11]. The value of flow behaviour index was below 1.0 for all samples in this study, like those of pseudoplastic fluids [40,41]. Ibarz [43] found that an increase in salt concentration resulted in a decreasing value of the flow behaviour index, in agreement with this study. In his research, flow behaviour index of the sample containing 7% salt examined at 20 °C was 0.77, which differs slightly from obtained values (around 0.8) of this study for treated groups.

This can be explained by the difference in dry matter content and salt concentration. The rheological characteristics of semisolid materials, like liquid egg yolk, can be also described by back extrusion [47]. Values of the control sample obtained in this study are similar to those reported by Wang et al. [13]. The firmness and consistency of the control sample were very high after freezing and thawing in agreement with Wang et al. [13]. According to a previous study, the egg yolk protein denaturation, disruption and aggregation are possibly responsible for this phenomenon [13]. The results in this study indicated remarkable changes in the rheological parameters of treated yolks compared to the control sample, in agreement with the findings of Primacella et al. [12]. This study demonstrated that treatment was able to prevent the gelation after freezing and thawing. Previous studies suggested that, with the addition of salt, the amount of unfreezable water is increased because of the formation of LDL-water-NaCl complex [12,48].

Nowadays, the market for processed egg products including frozen and powder egg products is increasing [6]. The use of products with a longer shelf life, such as frozen liquid egg products, as a raw material for various food products becomes more popular when the supply chain of eggs sometimes has a temporary interruption due to avian influenza or cases of poisonous eggs [49,50]. Frozen egg products can be kept for up to a year under appropriate conditions [10]. However, egg yolk undergoes a phenomenon called gelation when cooled to  $-6^{\circ}\text{C}$  or below [4]. Gelation results in an irreversible loss of fluidity, an increase in viscosity and a decrease in the functionality of the egg yolk [10]. Presently, this is an important issue in commercial practice, particularly for liquid egg producers who are facing the demand of the market for providing liquid egg yolk for food. The results of this work found that the combination of 5% salt and modified pH could prevent the gelation of frozen-thawed liquid yolk. Functional properties of treated yolk were also improved compared to the control samples. Salted egg yolk at pH 6.0 and 6.3 attained better results in rheology.

Freezing caused damage to the yolks resulting in protein aggregation [13]. This work showed that salted yolk had lower values in turbidity compared to control samples. It could be explained by the reduced particle size of frozen-thawed yolk due to addition of NaCl [12]. Moreover, NaCl also caused dissociation of protein [12]. The results of this study are coincident with previous report [22]. Among sample groups, the turbidity of control samples increased noticeably more than that of others after freezing. The increase in turbidity of measured samples was a results of yolk aggregation after freezing and thawing.

In this work, the control sample had a strong decline in EAI and ESI during storage due to gelation. Earlier study indicated that the emulsifying activity is related to the protein solubility [30]. Protein aggregation occurring during frozen storage decreased the solubility of protein, and therefore the EAI of frozen-thawed yolk declined dramatically. In agreement with literature [13], treatment was able to prevent the gelation of frozen-thawed egg yolk; however, freezing damage to yolk had negative effect on the attributes of protein. The molecular flexibility of protein is one of the influential factors in emulsion stability [30]. Earlier reports found that protein aggregation caused a decline in molecular flexibility resulting in lower values of ESI. In this work, control sample and salted yolk at pH 5.7 also showed higher values in viscosity compared to other groups, what may relate to a decrease in ESI.

Although a strong correlation ( $r > 0.9$ ) and low  $p$  values ( $p < 0.01$ ) were calculated, no mathematical models were made for deeper analysis of the relationship, due to the low number of observations. The significant correlation values among the parameters representing similar features confirm observed changes. On the other hand, standard CIE colour coordinate  $b^*$  correlated well with rheology parameters and turbidity measured as absorbance at 660 nm correlated well with texture parameters. These relationships indicate that the changing rheology of egg yolk might be visible and could be monitored by spectroscopy. The present study does not have enough data to conclude such a model or causation, but the results are promising towards starting new experiments in this direction.

## 5. Conclusions

This work provided basic information for liquid egg products industry, particularly for yolk freezing and storage. Frozen storage caused changes in structure and physicochemical properties of liquid egg yolk. The results of this study found that the combination of 5% salt and pH adjustment could inhibit the yolk gelation induced by freezing. However, the turbidity and calorimetry results indicated that different levels of pH induced difference in aggregation of frozen-thawed yolk. Addition of 5% NaCl and pH at 6.0 and 6.3 could prevent the gelation of frozen-thawed yolk. Moreover, emulsifying properties of salted yolk at pH 6.0 and 6.3 attained better results than that of others.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2077-0472/11/3/257/s1>, Table S1: Correlation table and corresponding *p* values.

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