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Effect of cryogenic freezing on the rheological and calorimetric properties of pasteurized liquid egg yolk

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Abstract: The egg yolk undergoes an irreversible gelation process when freezing to –6 °C or lower. In this experiment, liquid egg yolk (LEY) was frozen in liquid nitrogen and stored at –18 °C for 150 days. The measurement of pH and colour of LEY were performed. The examination of the rheological and calorimetric properties of samples was also carried out. The results indicated that the pH of LEY changed significantly during frozen storage, increasing from 6.37 ± 0.02 to 6.58 ± 0.03 over five months. The colour of the samples also showed a significant change compared to the fresh sample. The rheological properties of the LEY also changed significantly after 1 day of freezing and during frozen storage, with a clear increasing trend of the yield stress. The results of the calorimetric study showed that freezing and frozen storage did not affect the denaturation temperature, however, the denaturation enthalpy was reduced by about half after five months of frozen storage.

Keywords: differential scanning calorimetry; Herschel-Bulkley model; frozen storage; liquid nitrogen; shear test

Eggs are widely used as food ingredients in the food industry due to their nutritional value. Egg contains high-quality proteins, essential long-chain fatty acids, iron, phosphorus, trace minerals and vitamins A, D, E, K, and B (Chambers et al. 2016). Moreover, the coagulating, foaming, emulsifying, colouring, and flavouring properties are prominent (Lai 2016). Nowadays, the market of processed egg products is on the rise (Bertechini 2016). The products from the so-called "first processing" are liquid, frozen, and powdered egg products, which are marketed mainly in the food industry. These products are used for a wide variety of purposes, such as ingredients of pasta, dairy products, mayonnaise, sauces, and salad dressings (Primacella et al. 2018).

However, the supply chain of eggs sometimes had a temporary interruption or an increase in prices. Between January 2014 and November 2016, 13 strains of avian influenza were identified in 77 countries. As a result, hundreds of wild and domestic birds had to be killed (OIE 2020). It is also important to mention the 2017 European "poisonous eggs" case caused by fipronil identified in eggs, which affected at least 40 countries. Millions of eggs were recalled, costing the industry millions of dollars (Tu et al. 2019).

During the temporary shortage of eggs, the use of products with a longer shelf life, such as frozen liquid egg products, as a raw material for various food products becomes evident. Frozen egg products can be kept for up to a year under appropriate con-

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ditions (Au et al. 2015). While only minor changes in egg white occur during freezing, egg yolk occurs a phenomenon called gelation when cooled to –6 °C or below (Lai 2016). The results of the gelation process are an irreversible loss of fluidity, an increase in viscosity and a decrease in the functionality and dispersibility of the egg yolk (Au et al. 2015).

Au et al. (2015) experimented with freezing egg yolk at –20 °C for 168 days and studied the gelation process and kinetics. They found that the rheological properties were affected significantly by the length of frozen storage. Both plasma and granules were found to be involved in the gelation process. Fernández-Martín et al. (2018) also examined the effect of magnetic assisted freezing on the gelation of yolk. According to their result, this freezing technique had significant effect on the thermal denaturation, free sulfhydryl content, emulsifying activity, emulsifying stability, colour, and rheological characteristics.

Researchers concluded that freezing is a time-temperature dependent technique (Lopez et al. 1954). Fast freezing rates can be achieved at very low temperature [–78.5 °C with CO_2 and –195.8 °C with liquid nitrogen (LN)] (Mulot et al. 2019). Lopez et al. (1954) found that egg yolk frozen in LN and thawed at 54 °C suffers from a very low degree of gelation, while after thawing at room temperature, the degree of gelation was medium. Jaax and Travnicek (1968) stated that freezing in LN has a significant effect on the apparent viscosity of egg yolk if thawed at 35 °C.

In previous studies about cryogenic freezing, samples were usually thawed at warm temperature, which is not easy to apply in the industry. In addition, the effect of frozen storage after cryogenic freezing were not evaluated. Therefore, the aim of this study was to investigate the effect of cryogenic freezing and subsequent frozen storage on the LEY. The calorimetric and the rheological properties of LEY were investigated during the storage.

MATERIAL AND METHODS

Material

Pasteurised LEY [pH = 6.37 ± 0.02; dry matter content: 44.21 ± 0.86% (w/w)] was obtained from a liquid egg plant (Capriovus Ltd., Hungary). LEY was pasteurized for 600 s at 65 °C with a flow rate of 600 kg h^{-1} (Actitube; Actini, France) and filled into Elopak carton boxes with the filling mass of 1.0 kg. The shelf life of this product is 3 days, and it should be stored between 0 °C and 4 °C. Samples were stored at 4 °C until treatment.

LN (purity: 99.995%; boiling point at atmospheric pressure: –196 °C; stored in Dewar container at 0.3–0.5 MPa overpressure) was provided by Messer Hungarogáz Ltd. (Hungary).

Freezing of LEY

Figure 1 shows the cryogenic freezing procedure. Cryogenic freezing was performed on liquid egg drops formed by a steel strainer [Figure 1(3)] (d = 1.5 mm) for 60 s in LN [Figure 1(2)]. The volume of LN was kept constant at 10 L during the experiment. 3 L of LEY [Figure 1(1)] was poured into LN in 100 mL portions. LN began to boil at a temperature of –196 °C due to the temperature difference between the LN and the LEY and the room temperature of 10 °C. Frozen pellets were separated by a strainer [Figure 1(4)] from LN. Sample pellets were filled into polypropylene bags and stored at –18 ± 1 °C for 150 days. Figure 2 shows the flowchart of the experiment.

Measurements

Sampling was carried out on day 0 (before cryogenic freezing) and 1, 7, 14, 30, 60, 90, 120, and 150 days after frozen storage. The yolks were thawed at 4 °C for 24 h before analysis.

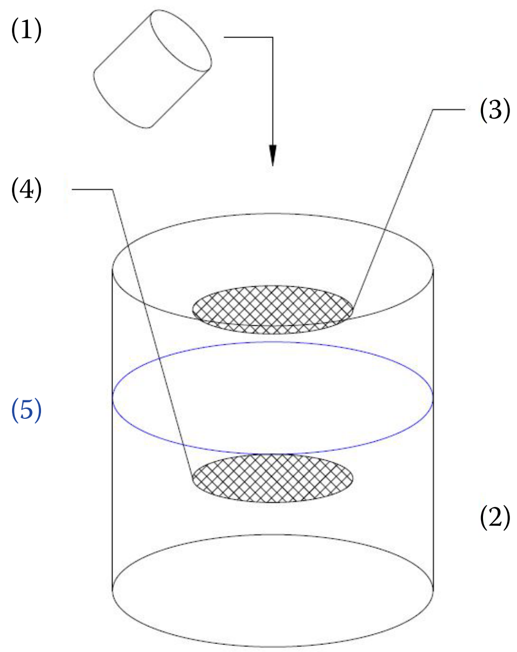

Figure 1. Cryogenic freezing procedure during the experiment; (1) sample inlet, (2) polystyrene container filled with liquid nitrogen, (3) steel strainer to form the drops of liquid egg yolk, (4) steel strainer to remove the formed frozen pellets, (5) the blue oval indicates the level of liquid nitrogen

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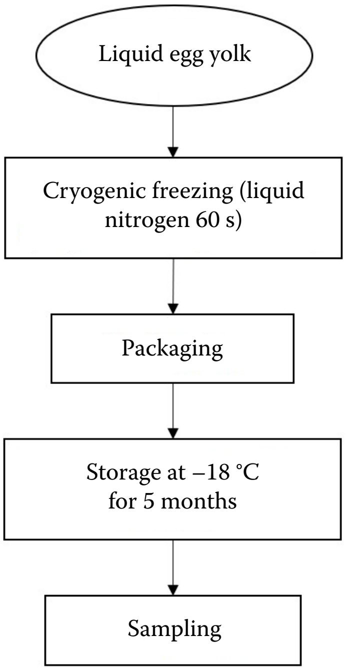

Figure 2. Flowchart of the experiment

Determination of pH and dry matter content. The pH of samples was measured at 4 °C using a portable digital pH meter (206-pH2; Testo SE & Co. KGaA, Germany).

Moisture was determined by drying of around 2 g LEY sample in an air-forced oven (Labor Műszeripari Művek, Hungary) at 105 °C until constant mass. The difference between the initial and final dry mass was considered as dry matter content and calculated as percentages on the initial mass. The measurements were performed in triplicate.

Colour measurement. Tristimulus colour measurement was performed with a Konica-Minolta CR-410 chromameter (Konica Minolta Sensing Inc., Japan) at 4 °C. L^* is the CIE lightness coordinate of the sample, a^* is the CIE red(+)/green(–) colour attribute and the b^* is the CIE yellow(+)/blue(–) colour attribute, respectively (Pathare et al. 2013). Measurements were performed in 5 replicates. Euclidean distance (ΔE^*) was calculated according to Kim et al. (2014) to compare the colour of frozen-thawed and fresh LEY. The ranges of ΔE^* were 0–0.5 (not noticeable), 0.5–1.5 (slightly noticeable), 1.5–3.0 (noticeable), 3.0–6.0 (well visible), and > 6.0 (great), based on a previous study (Cserhalmi et al. 2006). Chroma (C^*) was calculated according to Ahn and Lee (2008) and hue difference (ΔH^*) was calculated according to Perez et al. (2011) to compare the hue of frozen-thawed and fresh LEY samples.

Differential scanning calorimetry. Calorimetric properties of the LEY samples were examined by differential scanning calorimeter MicroDSC III (Setaram, France). The samples of 210 ± 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^{-1}, then cooled to 20 °C with a cooling rate of 3.0 °C min^{-1} (MicroDSC III; Setaram, France). Distilled water was used as reference sample. All data were processed with Calisto Processing software (Setaram, France). Straight baselines were set to the cooling phase of the thermograms and transition enthalpy [ΔH, (J g^{-1})] was determined from the peak area. Denaturation temperature [T, (°C)] was also recorded. Measurement was performed in triplicate.

Examination of rheological properties. Examination of the rheological behaviour of LEY was performed by MCR 92 rheometer (Anton Paar, 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 (version 1.21.852) was used to control the equipment. The temperature of rheological measurements was kept constant at 4 °C. Shear stress was measured by increasing and decreasing shear rate between 1 and 1 000 s^{-1} for 31 measurement points with a period of 3 s.

The Herschel-Bulkley model (Equation 1) was used to analyze the flow curves (shear rate-shear stress diagrams). This model was used to describe the rheological properties of LEY at 4 °C. All R^2 values of the fitted model were higher than 0.99.

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

where: τ – shear stress (Pa); τ_0 – yield stress (Pa); $\dot{\gamma}$ – shear rate (s^{-1}), K – consistency coefficient (Pa s^n); n – flow behaviour index (dimensionless).

Statistical analysis. Data were collected, pre-processed and visualized on charts using Microsoft Excel® (version 16.45). The Kolmogorov–Smirnov test was used to test the normality of the pH, dry matter content, colour, differential scanning calorimetric, and rheological variables. Levene's test was used to test the homogeneity of variances. Analysis of variance (ANOVA) along with post hoc tests was used to compare the variables of samples examined at different times. In case of homogeneity of variances (pH, a^*, b^*, C^*, denaturation enthalpy, τ_0, K and n) Tukey test was performed. Games-Howell test was used in case of not equal variances (L^*) to determine different inter-

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vals. Data analysis was carried out by SPSS Statistics software 22 (IBM, US).

RESULTS AND DISCCUSION

Changes in pH. The results of the pH measurement are shown in Table 1. The pH of the LEY can be higher than that of the yolk of the freshly laid egg around pH 6.0 (Mine 2008). Huang et al. (1997) observed that slow freezing at –15 °C caused a significant change after one day of frozen storage. According to the results, pH did not alter significantly on day 1, but a slight decrease can be seen. This study reports an increasing trend in pH from day 1 to day 150. Significant change was observed on day 14 and day 120. The increasing trend in pH during frozen storage was also observed by Huang et al. (1997) during a frozen period of 60 days.

Changes in colour. Colour has a very important role in the perception of food products, and it is also a key aspect in case of determination of food quality (Bovšková et al. 2014). The colour properties of LEY before freezing and during the storage period are shown in Figure 3. The results show that L^* values increased significantly during the 150 days of frozen storage (Figure 3A). The increase occurred in the initial phase (in the first 14 days), and no significant change was seen thereafter. The effect of cryogenic freezing on lightness is obvious. Huang et al. (1997) also stated that the L^* value of the egg yolk increased after 1 day of frozen storage at –15 °C, but longer frozen period did not cause significant change until day 60, when a decrease was observed.

Table 1. The effect of cryogenic freezing and storage period at –18 °C on the pH of liquid egg yolk

Time (day)	pH	
	mean	SD
0 (before freezing)	6.37^a	0.02
1	6.35^a	0.03
7	6.36^a	0.03
14	6.45^b	0.02
30	6.45^b	0.02
60	6.48^b	0.03
90	6.46^b	0.04
120	6.55^c	0.04
150	6.58^c	0.03

[a,b,c]different letters show significant differences ($P < 0.05$) in time; SD – standard deviation

The a^* values decreased (Figure 3B), which means that the LEY lost its red colour as a result of freezing. Minor fluctuations in red colour are seen during frozen storage. The b^* values increased with freezing (Figure 3C), which means a stronger yellow colour. During long term storage, by day 60, the b^* values decreased significantly. As a result of freezing, the total colour difference (ΔE^*) between the fresh LEY and the frozen-thawed samples was great on each measurement days. The C^* value changed significantly during frozen storage (Figure 3D), its trend was the same as the trend of b^*. However, the ΔH^* showed a small difference compared to the control sample (Figure 3E). This shows that the main change occurred in the L^* values.

Rheological properties. Rheological behaviour plays an important role in the industrial practice during material handling. Figure 4 shows the results of the rheological analysis obtained in this study, where samples were examined freshly and after freezing and thawing throughout storage period. LEY has non-Newtonian flow behaviour even in the fresh and frozen-thawed state. This means that they do not have a constant viscosity, but apparent viscosity and shear stress values depending on flow conditions (Figura and Teixeira 2007). Among the non-Newtonian fluids, LEY shows pseudoplastic flow behaviour characterized by a convex profile on the shear rate-shear stress diagram (Severa et al. 2010). As the shear rate increases in such materials, the shear stress increases due to the attenuation of molecular interactions (Figura and Teixeira 2007).

The flow behaviour index (n) obtained by fitting the Herschel-Bulkley model shows that fresh LEY also has pseudoplastic behaviour ($n < 1$). According to the results in this study, the rheological behaviour of LEY changed due to freezing and thawing. The shear stress values showed a steady increase during the frozen period (Figure 4A). Chang et al. (1977) also reported an increase in the shear stress and apparent viscosity values of LEY even after 5 h of frozen storage.

After freezing and thawing, yield stress has appeared in the rheological behaviour of LEY. In this case, initial minimum shear stress is needed for the material to start flowing (Larsson and Duffy 2013). Defining this parameter is important in many aspects of processing, handling, storage, and performance properties. This study shows an increasing trend of yield stress value due to the frozen period from day 0 to day 14 (Figure 4B). No significant difference was detected between day 14 and day 30. After a decrease on day 60, no significant change was observed until day 120. At the end of the measurement, there was a strong increase in yield stress.

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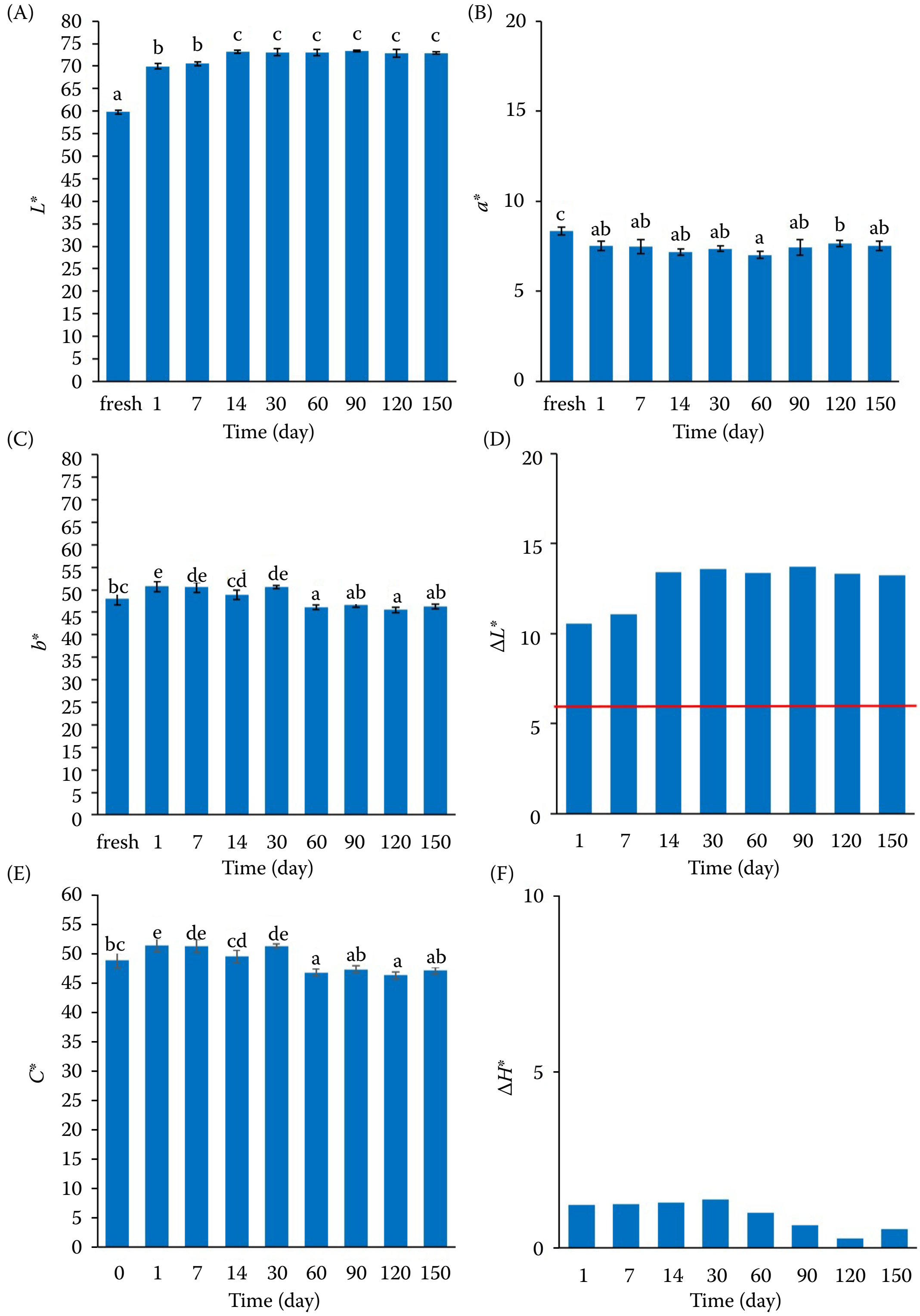

Figure 3. Results of colour measurements; (A) lightness (L^*), (B) redness (a^*), (C) yellowness (b^*), (D) Euclidean distance (ΔE^*) between the colour of fresh liquid egg yolk and the egg yolk on the days after freezing [red horizontal line – lower limit of the ΔE^* range "great"], (E) chroma (C^*), (F) hue difference (ΔH^*) between the fresh liquid egg yolk and frozen-stored samples

[a–e]Different letters show significant differences ($P < 0.05$) in time

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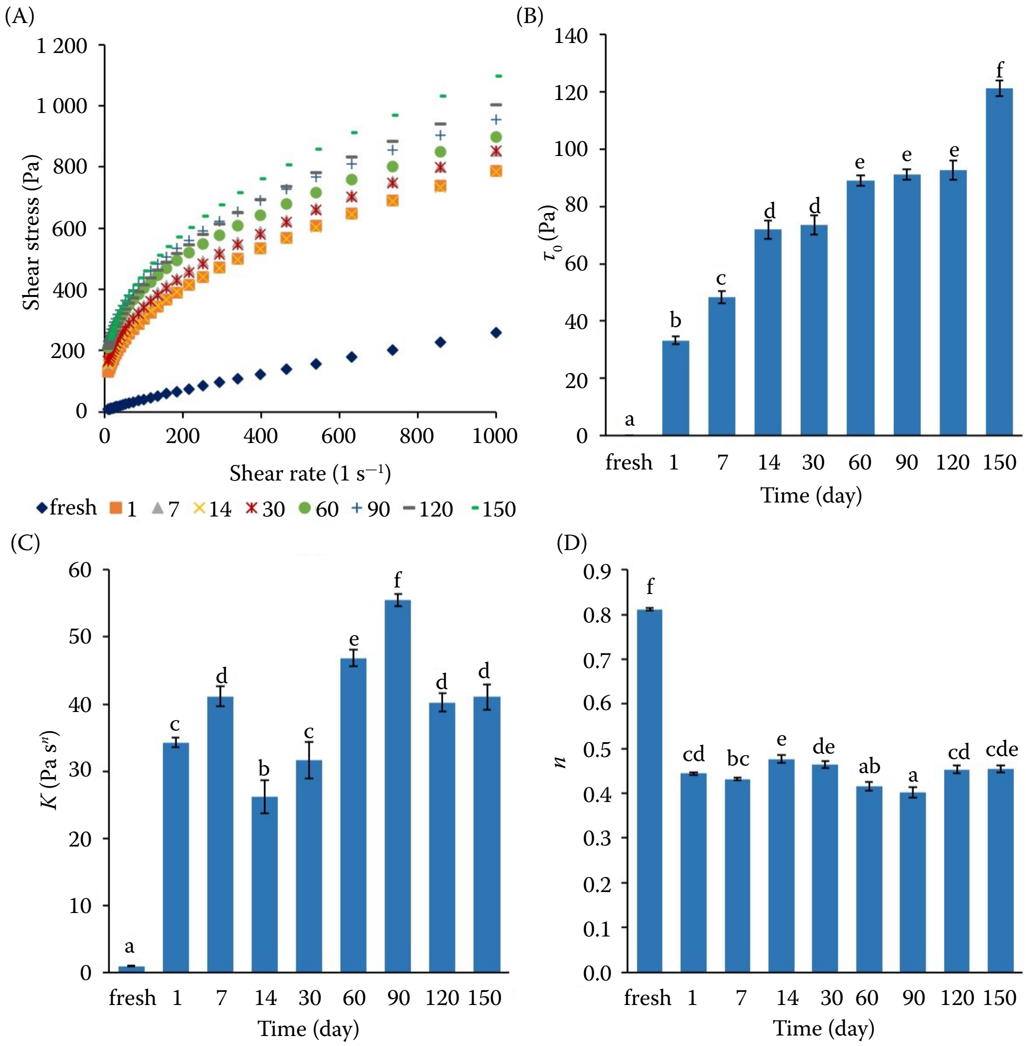

Figure 4. Results of rheological analysis; (A) the effect of time on the flow curves of liquid egg yolk, data series marked with different notations indicate samples tested on different days, numbers below the figure indicate storage period in days, (B, C, D) the effect of time on the parameters of the Herschel-Bulkley model fitted to the flow curve of liquid egg yolk

[a–f]Different letters show significant differences ($P < 0.05$) in time; τ_0 – yield stress (Pa); K – consistency coefficient (Pa s^n); n – flow behaviour index (dimensionless)

However, for the consistency coefficient and flow behaviour index values, no such clear trend can be seen. The value of the consistency coefficient shows the viscosity of the material (Primacella et al. 2019). In this report, the consistency coefficient of the fresh sample is clearly different from that of the frozen-thawed samples (Figure 4C). However, during frozen storage, an upward trend is observed until day 7, followed by a decline on day 14. From day 14 to day 90, its value rises again and then decreases on day 120. Au et al. (2015) also reported the fluctuating rheological behaviour of LEY frozen at –20 °C and thawed at 25 °C. That research proposed a two-stage gelation process during prolonged storage. The first proposed stage was observed from day 1 to day 28, the second stage between day 28 and day 84.

In this study, the flow behaviour index values were less than 1.0 (Figure 4D) like those of pseudoplastic fluids (Figura and Teixeira 2007). Cryogenic freezing and thawing at 4 °C resulted in a significant change of flow behaviour index value. During the frozen period, a fluctuating trend is observed. However, as the consistency coefficient increased, the flow behaviour index decreased and vice versa.

Calorimetric properties. Table 2 shows the results of the calorimetric measurements. The egg yolk contains a large amount of lipoprotein, which DSC cannot separate into fractions (Cordobés et al. 2003).

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Table 2. The effect of cryogenic freezing and storage at –18 °C on the denaturation enthalpy and denaturation temperature of liquid egg yolk

Time (day)	Denaturation enthalpy ($J g^{-1}$) mean ± SD	Denaturation temperature (°C) mean ± SD
0 (before freezing)	1.01^{e} ± 0.05	72.97 ± 0.20
1	0.85^{d} ± 0.01	72.83 ± 0.18
7	0.74^{c} ± 0.02	72.92 ± 0.30
14	0.69^{bc} ± 0.01	72.96 ± 0.31
30	0.65^{b} ± 0.03	73.12 ± 0.19
60	0.65^{b} ± 0.02	72.90 ± 0.19
90	0.64^{b} ± 0.02	73.08 ± 0.14
120	0.55^{a} ± 0.02	72.74 ± 0.17
150	0.52^{a} ± 0.01	72.76 ± 0.35

[a,b,c]different letters show significant differences ($P < 0.05$) in time; SD – standard deviation

In this study, one well pronounced endothermic peak can be seen on the heat flow curves in fresh and frozen--thawed samples. The enthalpy of the reaction, that is generally accepted as denaturation enthalpy (Arntfield and Murray 1981), was significantly reduced by storage time, but denaturation temperature had not changed significantly. The enthalpy of denaturation already shows a large decrease on day 1 after freezing and thawing, and the rate of decrease slows down during storage. After 5 months of storage, the denaturation enthalpy of frozen-thawed yolk decreased half compared to that of fresh sample. According to Au et al. (2015), freezing in the first stage (from day 1 to day 28) causes an aggregation of lipoprotein particles via hydrophobic interactions caused by water removal. In the second stage (from day 28 to day 84) of the gelation process the proteins are released. These protein interactions are responsible for the decreasing denaturation enthalpy.

CONCLUSION

In this study, it was found that the gelation process also occurs after cryogenic freezing and thawing at cold temperatures. LEY underwent a clear discolouration, and denaturation enthalpy decreased half due to protein interactions after freezing and frozen storage. The rheological properties also changed as a result of cryogenic freezing, and they also changed continuously during frozen storage.

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