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Comparison of different thawing methods effect on the calorimetric and rheological properties of frozen liquid egg yolk

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ABSTRACT

Eggs are commonly used in the food industry because of their excellent nutrient value and also for their coagulating, foaming, emulsifying, colouring and flavouring properties. Manufacturers substitute shell eggs with processed egg products, such as liquid whole egg, liquid egg yolk or albumin. They have a shelf life of a few weeks, but freezing can increase it to 1 year. However, freezing causes gelation in case of egg yolk. This process is highly dependent on the conditions of freezing and thawing.

In our study, raw liquid egg yolk was frozen and stored for 14 days at −18 °C. On days 1, 7 and 14 samples were thawed by two different methods. Denaturation temperature and enthalpy were investigated by differential scanning calorimetry. Besides, rheological properties were examined at 20 °C, Herschel–Bulkley model was fitted to flow curves of the examined samples. The dry matter content was also recorded during the experiment. Two-way ANOVA was used to analyse data.

The results of the study showed that method of thawing had no significant effect on calorimetric and rheological properties and dry matter content. In contrast, freezing and frozen storage had a significant effect on denaturation enthalpy and rheological properties.

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KEYWORDS

differential scanning calorimetry, rheology, Herschel–Bulkley, slow freezing, thawing method

INTRODUCTION

The egg yolk is a popular ingredient in the food industry because of its emulsifying, gelling and coagulating properties (Primacella et al., 2020). In addition, it provides desirable organoleptic and nutritional properties, high quality proteins, vitamins, minerals, essential fatty acids, phospholipids and other lipids (Anton, 2013).

Nowadays, industrially processed egg products are preferred for easier and faster handling and to reduce microbiological risk. However, liquid egg products may be used for short periods of time, because due to the sensitivity of the proteins only low pasteurisation temperature is allowed (Lechevalier et al., 2011). Freezing increases the shelf life for up to 1 year, but the egg yolk undergoes an irreversible phenomenon called gelation, when it is cooled under −6 °C. The fluidity loss affects the functionality. Gelation process is not fully understood, but most researchers agreed that ice crystal formation leads to the aggregation of proteins (Au et al., 2015; Primacella et al., 2018; Chang et al., 1977).

Atilgan and Unluturk (2008) examined the rheological behaviour of liquid egg products at different temperatures. The Herschel–Bulkley model was found to fit well with the shear rate–shear stress data. Herald et al. (1989) examined the rheological behaviour of heat-treated liquid whole egg at −24 °C for 80 days. Thixotropic behaviour was found in all samples examined, and their rheological properties were completely altered, with near Newtonian rheological properties becoming pseudoplastic during frozen storage. During their studies, they noticed an increase in the viscosity due to frozen storage.

Differential Scanning Calorimetry (DSC) is a thermo-analytical method, which is used to examine different thermal effects quickly. Physical or chemical properties of material can be measured by this method as a function of temperature. During the measurement, an inert material and a sample are subjected to the same temperature program (heating then cooling) and the required heat flux is measured. Heat flow changes in the sample are recorded as a peak.

Studies about denaturation properties of egg and egg parts by DSC are not new (but most of the authors examined egg white (Höhne et al., 2003; Biliaderis, 1983; Cordobés et al., 2004; Rossi and Schiraldi, 1992; Ferreira et al., 1997; Donovan et al., 1975; Duan et al., 2017; Igarashi et al., 1999). In their differential scanning calorimetric study, Wootton et al. (1981) showed that denaturation enthalpy can be reduced by decreasing the freezing rate, increasing the thawing rate and increasing the storage temperature and storage time in case of liquid egg white.

In our study, we examine the effect of slow freezing and different thawing methods on the rheological and calorimetric properties of raw liquid egg yolk. Dry matter content is also measured for the better understanding of the gelation process.

MATERIALS AND METHODS

Materials

In this study, raw egg yolk (Capriovus Ltd., Szigetcsép, Hungary) was used, which was produced by the separation of fresh hen eggs and homogenisation. Liquid egg yolk (LEY) was cooled to

3 °C and it was filled into Polyethylene terephthalate (PET) bottles under industrial circumstances. Freshly maintained sample was measured as control sample.

Freezing and storage conditions

After opening the PET bottles, 0.9 L of LEY was filled into 9 plastic containers (0.1 L). They were placed into the laboratory freezer, where they were frozen at −18 °C. Samples were removed from the freezer on days 1, 7 and 14. On the measurement days, one sample was thawed by tap water (35 °C) in 2 h (thawing rate: 0.44 °C/min), and one in a laboratory refrigerator at 5 °C in 24 h (thawing rate: 0.96 °C/h). The measurement of dry matter content, calorimetric and rheological properties were carried out.

Measuring of dry matter content

Dry matter content (d.m.c.) was determined by oven method. Two to 3 g of samples were measured and dried to constant weight at 105 °C in Petri dishes. After drying, they were cooled to room temperature in a desiccator. Dry matter content (d.m.c.) was calculated by Eq. (1):

$$d.m.c. = \frac{m_{P+D} - m_P}{m_S} \cdot 100, \tag{1}$$

where $d.m.c.$ is dry matter content in m/m%, m_{P+D} is the mass of the Petri dish and the dried sample (kg), m_P is the mass of the Petri dish (kg) and m_S is the mass of the sample (kg) before drying.

Determination of the calorimetric properties

The thermal denaturation of frozen LEY samples was tested by the MicroDSC III type (SETARAM, France) differential scanning calorimeter in dynamic measurement mode. Bidistilled water was used as reference material (203.7 mg) and 203.7 ± 5 mg of each sample was weighed into the sample holders. The measurement program started by thermostating at 20 °C for 2 min, then heating the samples to 95 °C at a heating rate of 1.5 °C/min. At 95 °C, the samples were re-cooled to 20 °C with a cooling rate of 3 °C/min. Heat flow curves were recorded. The measurement program was controlled by SetSoft 2000 software. Three parallel measurements were performed per sample. The evaluation was carried out on the heat flow curves of the heating phase as a function of temperature with Callisto Processing version 1.076. A linear baseline was set to the heat flow curves and the area under the curve was calculated, which gives the denaturation enthalpy (ΔH, [J/kg]). Peak temperatures were also recorded.

Examination of the rheological properties

The rheological behaviour of the frozen and thawed LEY was investigated by the MCR 92 rotational rheometer (Anton Paar, Les Ulis, France). Properties of the probe are the following: 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. The device was operated using Anton Paar RheoCompass™ software. The flow curves of the samples were recorded at an increasing shear rate of 10–1,000 1/s and 1,000–10, 1/s in the deceleration phase at 20 °C. Three parallel measurements were performed per sample. The rheological properties of the samples were investigated by fitting the

Table 1. Results of the homogeneity test (Levene's test) and the test of normality (Shapiro–Wilk test)

Tested data	Shapiro–Wilk test			Levene's test			
	df	*F* value	*P* value	*df*1	*df*2	*F* value	*P* value
d.m.c.	24	0.968	0.609	7	16	1.959	0.126
τ_0	24	0.940	0.165	7	16	2.458	0.065
K	24	0.954	0.334	7	16	2.203	0.090
n	24	0.976	0.809	7	16	1.370	0.283
ΔH	24	0.932	0.106	7	16	1.689	0.182
T	24	0.967	0.600	7	16	3.281	0.023*

* $P < 0.05$, the null hypothesis of equal variances is rejected.

Herschel–Bulkley model (Eq. 2) to the decelerating phase of the flow curves, using Excel Solver Least Squares Fitting, where τ_0, K and n are variable values. Correlation coefficient (R^2) was calculated to check model fitting.

$$\tau = \tau_0 + K\left(\frac{d\gamma}{dt}\right)^n, \tag{2}$$

where τ is the shear stress (Pa), τ_0 is the yield stress (Pa), γ is the shear rate (1/s), K is the consistency coefficient (Pa s^n) and n is the flow behaviour index (dimensionless).

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 (Table 1) and Levene's test was used for the determination of the equality of variances. Variances were assumed equal except for n. Two-way ANOVA was used for the statistical analysis of variance. If the result of ANOVA test was significant, post hoc test were used to decide which groups differ. In case of equal variances, Tukey-test was used, and in case of not equal variances, Games-Howell test was used to decide which groups differ (Tabachnick and Fidell, 2013).

RESULTS

Effect of frozen storage and thawing mode on the dry matter content of liquid egg yolk

The dry matter content of the samples was examined during storage. This is because the rheological behaviour is highly dependent on the dry matter content. The dry matter content of the control and frozen LEY samples are shown in Fig. 1. Each sample contained approximately 45 m/m% dry matter. There was no significant difference between the dry matter content of egg yolk samples on measurement days ($d.m.c._{T\ \mathrm{(Time)}}$ ($F(3;16) = 1.786$; $P = 0.190$) and the type of thawing did not influence the dry matter content either ($d.m.c._{TH\ \mathrm{(Thawing\ type)}}$ ($F(1;16) = 3.823$; $P = 0.068$). There was no cross impact ($F(3;16) = 2.697$; $P = 0.081$) of thawing mode and time. From this it can be concluded that the change in rheological properties is not related to the change in dry matter content.

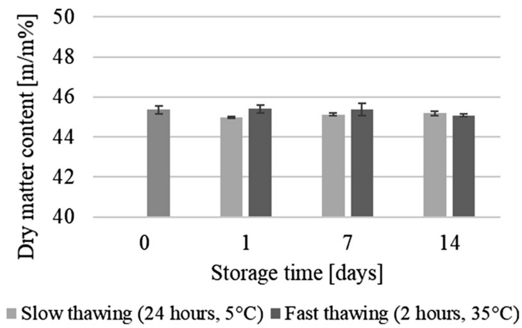

Fig. 1. Dry matter content of liquid egg yolk samples before freezing (day 0) and during the storage experiment

Effect of frozen storage and thawing mode on the rheological properties of LEY

The flow curves of raw and frozen LEY samples were recorded in the range of 10–1,000 l/s. The flow curves are shown in Fig. 2. LEY samples in all cases showed non-Newtonian behaviour. The rheological characteristics could be described by Herschel–Bulkley equation. Frozen samples have a yield point, in addition, the shear stress decreases with increasing shear rate less and less. Applying shear stress greater than the yield stress, the material begins to flow, that is to say, a continuous deformation occurs over time.

The Herschel–Bulkley model was fitted to the flow curves of raw and frozen and thawed LEY samples. In all cases the model fitted the measured data well, the correlation coefficient was above 0.999. Rheological properties of samples are shown in Table 2 and the results of the

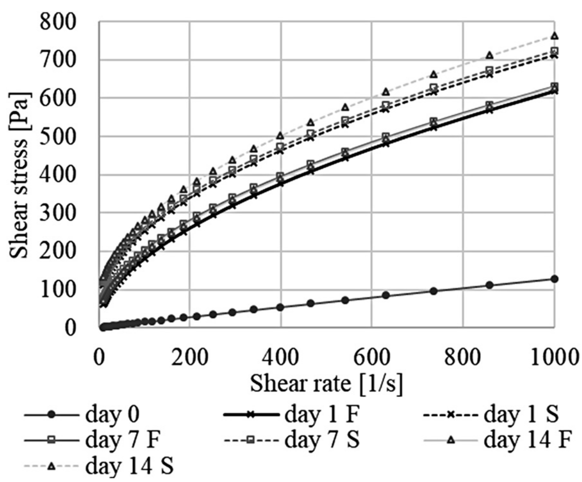

Fig. 2. Flow curves of liquid egg yolk samples before freezing (day 0) and during the storage experiment

Table 2. Rheological parameters (yield stress, τ_0; consistency coefficient, K and flow behaviour index, n) of raw and frozen-thawed LEY (applied model: Herschel–Bulkley)

Storage time (days)	Thawing method	τ_0 (Pa)		K (Pa s^n)		n		R^2
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean
0	–	0.00	0.00	0.21	0.01	0.929	0.001	1.0000
1	2 h, 35 °C	13.17	3.33	13.07	1.17	0.555	0.015	0.9999
	24 h, 5 °C	49.94	4.75	18.98	1.15	0.515	0.005	0.9999
7	2 h, 35 °C	24.23	0.91	14.73	0.97	0.538	0.010	0.9998
	24 h, 5 °C	60.92	3.24	19.31	1.61	0.513	0.016	0.9999
14	2 h, 35 °C	23.03	2.20	14.53	0.80	0.540	0.009	0.9999
	24 h, 5 °C	67.03	4.17	20.12	2.50	0.520	0.016	0.9999

Table 3. Results of the two-way ANOVA

Tested data	Effect of time			Effect of thawing			Interaction effect		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
τ_0	3	306.343	<0.001[a]	1	616.222	<0.001[a]	3	70.571	<0.001[a]
K	3	253.013	<0.001[a]	1	59.280	<0.001[a]	3	6.876	<0.05[a]
n	3	2005.112	<0.001[a]	1	23.258	<0.001[a]	3	3.583	<0.05[a]
ΔH	3	44.050	<0.01[a]	1	0.067	0.799	3	1.249	0.325
T	3	1.277	0.316	1	0.206	0.656	3	1.148	0.360

[a]Significant effect.

two-way ANOVA are shown in Table 3. Freezing time and thawing method had significant effect in the case of yield stress, consistency coefficient and flow behaviour index. Based on the results of the post hoc tests, yield stress was significantly different from the frozen samples, and the sample of day 1 differed significantly from day 7 and 14. The consistency coefficient and the flow behaviour index of the control sample differed significantly from the frozen samples.

Effect of frozen storage and thawing mode on the calorimetric properties of LEY

Results of the calorimetric study are shown in Table 4. As a result of freezing, denaturation enthalpy of the samples was increasingly reduced. The statistical results (Table 3) confirmed that the effect of freezing time had a significant effect on denaturation enthalpy values. However, there was no significant difference in the type of thawing and no cross impact could be seen. Based on the result of the Tukey test, the denaturation enthalpy differed significantly from the frozen samples and a significant change was also seen on day 14. However, there is no significant difference in peak temperatures, either between the two types of thawing or the freezing time. Denaturation temperatures were not affected by time or thawing. No cross impact could be seen.

DISCUSSION

Proteins and lipids make up the bulk of the egg yolk dry matter content. From the fact that the dry matter content does not change during frozen storage, it can be concluded that the water

Table 4. Calorimetric properties of liquid egg yolk samples before freezing (day 0) and during the storage experiment

Time (day)	Thawing method	Denaturation enthalpy (J/kg)		Denaturation temperature (°C)	
		Mean	S.D.	Mean	S.D.
0	–	1.20E−03	8.50E−05	78.62	0.16
1	2 h, 35 °C	1.02E−03	9.30E−05	78.41	0.54
	24 h, 5 °C	1.05E−03	2.50E−05	78.96	0.37
7	2 h, 35 °C	9.73E−04	6.60E−05	78.20	0.22
	24 h, 5 °C	8.79E−04	5.80E−05	78.39	0.71
14	2 h, 35 °C	7.57E−04	3.30E−05	78.49	0.65
	24 h, 5 °C	7.88E−04	4.70E−05	78.09	0.45

content of the egg yolk does not evaporate. This also indicates that water does not escape from the system during the gelling process.

The results of the rheological examination have shown that gelling by freezing does not produce a pattern with constant rheological properties, but that rheological properties also depend on the time and manner of thawing during freezing. Herald et al. (1989) have also identified this phenomenon. As a result of the faster thawing (2 h, 35 °C), the apparent shear viscosity of the samples in the examined range (10–1,000 1/s) was lower and the shear stress values were also lower.

Based on the results of the calorimetric study, protein denaturation and aggregation is due to freezing and frozen storage, but thawing mode has no effect on the denaturation enthalpy. Wootton et al. (1981) found that there was a significant difference in denaturation enthalpy between the differently thawed samples in case of egg white samples. The denaturation temperature of about 78 °C shows a big difference compared to a denaturation temperature of 81–86 °C measured by Cordobés et al. (2004). However, their work also showed that the denaturation temperature of egg yolk is very dependent on the heating rate used for the measurement.

CONCLUSIONS

According to our results, the rheological properties of slow-frozen LEY were influenced by both the duration of freezing and the mode of thawing, however, the calorimetric properties only changed depending on the duration of freezing during the 14-day storage period.

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