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HOW DOES CRYOGENIC FREEZING AFFECT THE CALORIMETRIC PROPERTIES OF LIQUID EGG PRODUCTS?

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Abstract
Eggs are widely utilized because of their high nutrient value, coagulating, foaming, emulsifying and sometimes even colouring or flavouring facilities in food manufacturing. Production of processed egg products shows an increasing trend. Frozen products belong to first processing, their shelf life can increase up to 1 year. By freezing, a large reduction in microbial loss can be achieved. But different undesirable processes can occur. The effect of freezing on animal cells is highly dependent on freezing parameters. It has a different effect on egg subtituents. Egg yolk undergoes a gelation process while proteins can denaturate.
In our study pasteurized liquid egg products (liquid egg white, liquid egg yolk and liquid whole egg) were frozen by dripping into liquid nitrogen. After that, a 14-day frozen storage experiment was carried out at -18°C. Before freezing and on the 1th, 7th and 14th days of storage experiment pH, dry matter content, colour and calorimetric properties (denaturation temperatures and enthalpy of denaturation) with differential scanning calorimetry were tested. For statistical analysis, one-way ANOVA ($\alpha = 0.05$) was employed. In our experiment, we found no significant change in calorimetric properties of liquid egg white after freezing, but significant decreasing of enthalpy and denaturation temperatures of liquid egg yolk and liquid whole egg was identified. In contrast, frozen storage had a decreasing effect in all these products. Freezing caused a clearly visible colour change in LEW, a visible change in colour of LWE and a very clearly visible change in colour of LEY. In case of LEW and LEY changes increased to clearly visible 14 days. In conclusion, our results show that frozen storage had a greater effect on liquid egg products properties than freezing in liquid nitrogen.

1. INTODUCTION

Eggs are a rich source of high-quality protein, unsaturated fatty acids, iron, phosphorus, trace minerals and vitamins A, D, E, K and the B vitamins (Watkins, 2017). However, eggs are used not only because of the high nutrient value as raw material in the food industry. They are widely utilized in cooking and manufacturing of different food products due to their coagulating, foaming, emulsifying and sometimes even colouring or flavouring facilities (Lai, 2015). The production of processed egg products shows an increasing trend. We distinguish two groups of processed egg products. There are products from "first processing", such as liquid, frozen and powdered egg products, and specialty egg products, such as formulated and cooked eggs (Lechevalier et al., 2011). Shelf life of frozen products can increase up to 1 year (Au, et al., 2015). By freezing, a large reduction in microbial loss can be

achieved and it has a slight effect on functional properties. But different undesirable processes can occur during freezing and frozen storage, such as gelation of egg yolk or denaturation of proteins and major textural changes (Lai, 2015; Cotteril, 1986). The effect of freezing on egg white is less intense (Cotteril, 1986). Wootton et al. (1981) revealed in their differential scanning calorimetric study, that enthalpy of denaturation can be decreased with decreasing of freezing rate, increasing of thawing rate, increasing of storage temperature and storage time. In addition, conalbumin was more sensitive to freezing than ovalbumin. They examined dependence of viscosity and foam stability from freezing parameters. Slow freezing rate, high thawing temperatures, longer storage time and decreasing of storage temperatures resulted in a more stable foam and reduced viscosity.

When freezing and storing raw egg yolk below -6°C, gelation occurs and viscosity becomes higher (Moran, 1925). This gelation has a negative effect because of difficult mixing and undesirable appearance. Different cryoprotectors can provide a solution for this phenomenon. 10 % of sodium chloride and sucrose are commonly used to control it. However, the range of foods in which they can be used will be limited. Besides that, syrups, glycerine, phosphates, and other sugars can also be used (Cotteril, 1986). But mechanical treatment, the use of proteolytic enzymes and novel solutions, such as hydrolysed egg white and egg yolk can decrease gelation process (Primacella, 2018). With increasing of freezing and thawing rates, smaller ice crystals are created, and proteins are less dehydrated (Powrie, et al., 1963). A less drastic gelation occurs in whole egg upon freezing and thawing. Miller and Winter (1950) found out that stability of mayonnaise from frozen whole egg is better than that from unfrozen. The aim of this study is to examine the effects of a fast-freezing method, dripping in liquid nitrogen, on the calorimetric properties, colour, pH and dry matter content of liquid whole egg (LWE), liquid egg white (LEW) and liquid egg yolk (LEY).

2. MATERIAL AND METHODS

2.1. Materials

Pasteurized (Tubular Pasteurizer) liquid egg white (LEW) (56 °C, 3 min holding time, 2000 kg/h), liquid whole egg (LWE) (70°C, 3 min holding time, 2000 kg/h) and liquid egg yolk (LEY) (65 °C, 10 min holding time, 600 kg/h) (Capriovus Ltd., Szigetcsép, Hungary) were used in our experiment. Products are made from "A" classified hen eggs with homogenization and pasteurization. LEW and LEY also undergo a separation process. The used liquid products contain 0.5% of citric acid and 0.3% of potassium sorbate. They are free from any strange smell and taste, the texture is fluid homogeneous, free from foam, knot, coat, shell or any contamination. 1 kg of LEW contains about 33 pcs egg white, 1 kg of LWE means about 22 pcs of egg and 1 kg of LEY is about 63 pcs egg yolk. pH of products is between 5.0 and 7.0. and they are packaged into 1.0 kg filling weight 'Elopak' carton box with polyethylene surface. They should be stored between 0- +4°C and shelf life is 21 days.

2.2. Freezing method

Products used in our experiment were produced the previous day. After opening carton boxes, 1-1 litre of liquid egg products were dripped into liquid nitrogen (-195,8 °C), (Messer Hungarogáz Ltd., Hungary) one after another. Drops of similar size were formed by pouring into a steel strainer (d=1,5 mm). Frozen sample balls were separated from liquid nitrogen after 60 seconds. They were put into polyethylene foil (10 µm), which was sealed with a foil welder. Samples were stored in freezer at a temperature of -18°C. 100 ml of each sample was taken out of the freezer on days 1, 7, and 14 and thawed with tap water at room temperature for 1 hour.

2.3. Determination of calorimetric properties

Calorimetric properties of control and frozen samples were examined by differential scanning calorimeter MicroDSC III, from Setaram (Caluire, France). Bidestilled water of 212.4 mg was used as reference and 212.4 5 mg of samples was measured to the tests. Samples were heated up 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. Thermograms of samples were recorded. Evaluation was performed by Calisto Processing software. We set straight baselines for the temperature-heatflow curves and the enthalpy of denaturation (ΔH, [J/g]) was calculated from peak area by the software. Besides that, denaturation temperatures (T1, T2, and T3), which were the peak maximum values, were determined.

2.4. Measurement of pH, dry matter content and colour

Dry matter content, pH values and colour parameters were measured on the above-mentioned measurement days. Dry matter content was determined by oven method. Two to three grams of samples were 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 with equation (1):

$$D.m.c. = \frac{m_{P+D} - m_P}{m_S} \cdot 100 \quad (1.)$$

where d.m.c. is dry matter content in g/100 g, mP+D is the combined mass of the Petri dish and the dried sample (g), mP is the mass of the Petri dish (g) and mS is the mass of the sample before drying. The pH values were measured by Testo 206 pH meter (Testo AG, Lenzkirch, Germany). Konica Minolta CR-410 colorimeter was used for colour measurement. We recorded a* (red-green hue), b* (yellow-blue hue) and L* (lightness) values. Colour difference values were calculated with equation (2):

$$\Delta E *_{ab} = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta c^{*2}} \quad (2.)$$

where ΔEab* is the colour difference of frozen samples to untreated samples, ΔL* is the difference in lightness of frozen to untreated samples, Δa* is the difference in red-green hue of frozen to untreated samples and Δb* is the difference in yellow-blue hue of frozen to untreated samples. With colour difference, we can express how big the difference between the colours of two different objects or two parts of an object is. It classifies the visibility of differences into 5 groups (table 1.).

Table 1. Classification of ΔE*ab

ΔE*ab	classification
0-0.5	not visible
0.5-1.5	barely visible
1.5-3	visible
3-6	clearly visible
6-	very clearly visible

2.5. Statistical analysis

Quadruplicates of measurements were carried out. IBM SPSS Statistics 24 was used to analyse the collected data. Analyses of variance were performed by the ANOVA procedure. Homogeneity tests were performed by Levene's tests, normality tests were performed by Kolmogorov-Smirnov and Shapiro-Wilk.

3. RESULTS AND DISCUSSION

3.1. Evaluation of pH, dry matter content and colour values

There is a significant increase in pH (table 2.) in all the examined samples. pH values of LEW and LWE rose already 1 day after the freezing and the change was significant. Feiser and Cotteril (1982) examined pH of scrambled eggs after cooking, freezing-thawing and microwave treatment.

Table 2. pH value, dry matter content, colour parameters and calculated colour different of control (day 0) and cryogenic frozen liquid egg white (LEW), liquid whole egg (LWE) and liquid egg yolk (LEY) stored at -18°C

Product	Time [day]	pH value		d.m.c. [g/100 g]		a*		b*		L*		ΔE*$_{ab}$
		mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.	
LEW	0	6.03^{a}	0.01	12.50^{a}	0.24	-0.32^{a}	0.41	10.39^{a}	1.75	46.23^{a}	4.04	-
	1	6.23^{b}	0.01	12.78^{a}	0.11	-0.88^{a}	0.06	12.21^{a}	1.64	42.41^{ab}	2.15	4.27
	7	6.26^{c}	0.02	11.91^{a}	0.28	-1.07^{b}	0.23	6.57^{b}	2.03	38.80^{b}	2.25	8.38
	14	6.66^{d}	0.02	14.11^{b}	0.55	-1.30^{b}	0.15	12.78^{a}	1.27	70.80^{c}	3.32	24.71
LWE	0	5.77^{a}	0.01	23.17^{a}	0.21	3.19^{a}	0.34	43.72^{ab}	0.78	78.37^{a}	0.77	-
	1	5.85^{b}	0.05	22.83^{ab}	0.12	3.13^{a}	0.04	45.22^{a}	0.36	79.59^{a}	0.32	1.93
	7	5.90^{b}	0.03	22.08^{b}	0.54	3.81^{b}	0.20	44.61^{ab}	1.64	78.75^{a}	0.93	1.14
	14	6.15^{c}	0.03	23.08^{a}	0.07	2.73^{ab}	0.67	42.59^{b}	2.12	78.44^{a}	0.68	1.22
LEY	0	5.57^{a}	0.01	45.05^{a}	0.38	8.33^{a}	0.22	47.99^{a}	1.32	59.81^{a}	0.47	-
	1	5.56^{a}	0.01	43.61^{a}	0.05	6.72^{b}	0.40	50.74^{b}	1.10	69.98^{b}	1.32	10.66
	7	5.56^{a}	0.03	42.79^{b}	0.11	7.49^{c}	0.38	50.58^{b}	1.10	70.53^{b}	0.46	11.06
	14	5.85^{b}	0.02	45.53^{ab}	1.30	7.10^{bc}	0.20	48.86^{ab}	1.03	73.14^{c}	0.32	13.41

$^{a, b, c, d}$ means with different letter in products are significantly different (P<0.05)

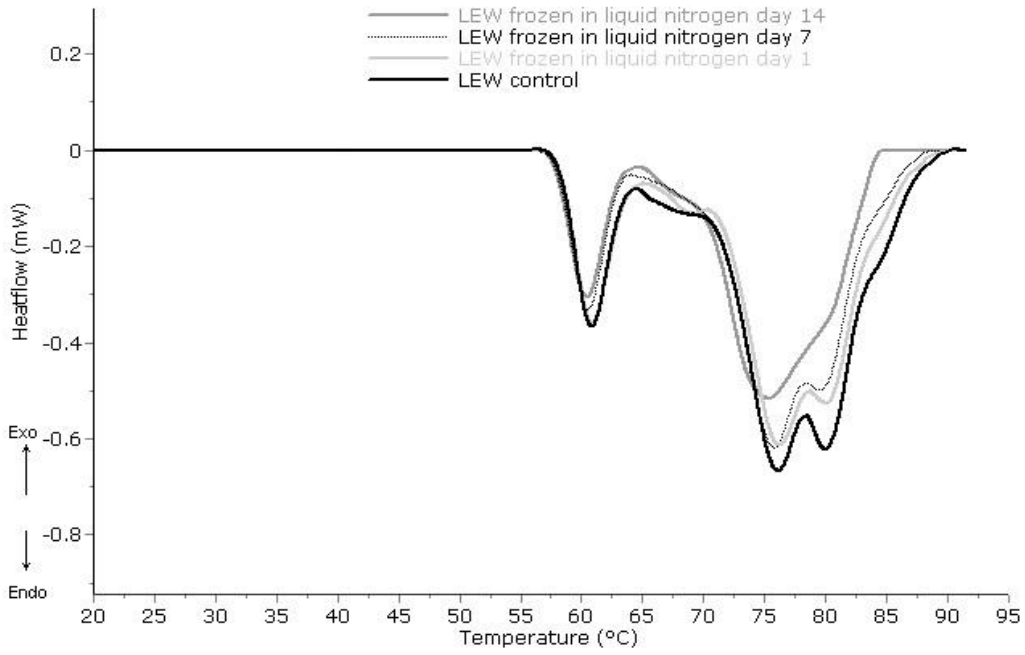

Figure 1. Thermograms of control and cryogenic frozen liquid egg white (LEW) stored at -18°C.

It has been observed that there is a continuous increase in pH during the process. In our experiment, freezing and thawing caused a slight pH rise. The pH of LEY was the most stationary after freezing and during frozen storage. But a significant change can be seen on day 14 also in this case. The tendency of d.m.c. (table 2.) is not as clear as it was in case of pH values. There is no significant difference in LEW, except on day 14. In contrast d.m.c. of LWE and LEY decreased in the intermediate days but increased again. No significant change can be seen between the d.m.c. of control sample and the sample after 14 days of frozen storage. Change of colour parameters (table 1.) was not significant after freezing in case of LEW and LWE. In contrast, freezing caused decreasing of a* values and increasing of b* and L* values in LEY. A reason of this can be the denaturation or agglomeration of proteins. Frozen storage of samples caused a significant change in almost every case. Lightness of LWE did not change during the storage period. Colour differences of frozen samples to control samples are shown also in table 2. Freezing caused a clearly visible colour change in LEW, a visible change in colour of LWE and a very clearly visible change in colour of LEY. In case of LEW and LEY changes increased during frozen storage. It is very clearly visible in these products on day 14.

3.2. Calorimetric properties of LEW

Figure 1. shows typical thermograms of LEW. The diagram shows one selected thermogram from the 4 measurements. All of the thermograms show characteristic endotherm reactions because of the protein denaturation. When examining proteins, the change in enthalpy is due to the unfolding of the protein molecules (Delben et al., 1969). Endothermic (such as breakdown of hydrogen bond) and exothermic reactions (e.g. protein aggregation) may occur simultaneously. However, the endothermic nature of the curves indicates that hydrogen bonds have been disrupted in large quantities, causing protein denaturation (Arntfield and Murray, 1981). The thermogram of LEW control shows 2 peaks. The first is due to the denaturation of conalbumin (ovotransferrin). The second is probably due to the denaturation of ovalbumin. According to Powrie and Nakai (1995) the denaturation temperatures of these proteins are 61 and 84 °C. There is a significant difference between the measured denaturation temperature of ovalbumin and the denaturation temperature in the study of Powrie and Nakai (1995). It may be caused by inhomogeneity of egg white, the difference in the technology of these products and the different measurement settings. Donovan et al. (1975) found that the selection of heating rate can influence the detected denaturation temperature of protein fractions. Lower heating rates caused lower denaturation temperatures. Table 3. shows results of thermogram evaluation. Cryogenic freezing caused

no significant change either in the enthalpy of denaturation or in denaturation temperatures, but a slight decrease in enthalpy and in the denaturation temperature of conalbumin is present. By meat freezing examined with DSC, authors revealed, that fast freezing has no significant impact on the calorimetric properties of samples (Leygonie et al., 2012). In contrast, a significant decreasing is shown in these values during frozen storage. Figures 2 and 3 show typical thermograms of LWE and LEY. In these cases, one peak can be seen because proteins of LWE and LEY cannot be separated by this calorimetric process. Table 4 shows results from LWE and LEY thermogram evaluation.

There is a clear decreasing tendency of enthalpy and denaturation temperature values. Enthalpy of denaturation decreased by cryogenic freezing, but denaturation temperature remained significantly unchanged. In contrast, frozen storage had significant effect on calorimetric properties in both cases. In these cases, the large amount of fat could influence the measurement. Furthermore, the gelation of egg yolk may affect measured tendencies. To eliminate this phenomenon, cryoprotive agents, such as salt and sugar, could be used (Primacella et al., 2018).

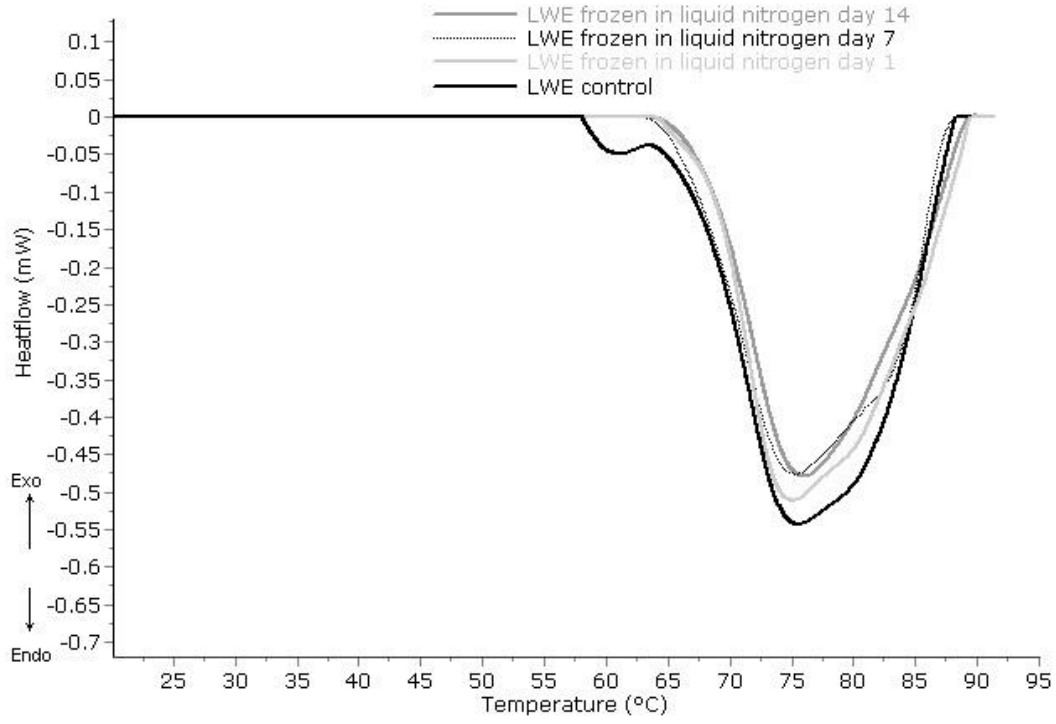

Figure 1. Thermograms of control and cryogenic frozen liquid whole egg (LWE) stored at -18°C.

Table 3. Enthalpy of denaturation and denaturation temperatures of control (day 0) and cryogenic frozen liquid egg white (LEW) stored at -18°C

Time [day]	ΔH [J/g]		T_1[°C]		T_2 [°C]		T_3 [°C]	
	mean	S.D.	mean	S.D.	mean	S.D.	mean	S.D.
0	1.60[a]	0.05	60.86[a]	0.1	76.16[a]	0.04	80.01 [a]	0.09
1	1.56[a]	0.05	60.81[a]	0.07	76.16[a]	0.07	80.07 [a]	0.1
7	1.42[b]	0.03	60.59[b]	0.04	75.79[b]	0.02	79.62[b]	0.04
14	1.25[c]	0.03	60.40[c]	0.06	75.27[c]	0.03	-	-

[a, b, c] means with different letter are significantly different (P<0.05)

Table 4. Enthalpy of denaturation and denaturation temperatures of control (day 0) and cryogenic frozen liquid whole egg (LWE) and liquid egg yolk (LEY) stored at -18°C

Time [day]	LWE				LEY			
	ΔH [J/g]		T_1[°C]		ΔH [J/g]		T_1[°C]	
	mean	S.D.	mean	mean	mean	S.D.	mean	S.D.
0	1.49[a]	0.02	75.43[a]	0.07	0.99[a]	0.06	72.98[a]	0.16
1	1.31[b]	0.02	75.25[ab]	0.05	0.85[b]	0.01	72.84[a]	0.15
7	1.28[b]	0.01	75.17[b]	0.12	0.74[c]	0.01	72.32[b]	0.03
14	1.18[c]	0.03	75.75[c]	0.18	0.65[d]	0.02	72.14[b]	0.08

a, b, c, d means with different letter are significantly different (P<0.05)

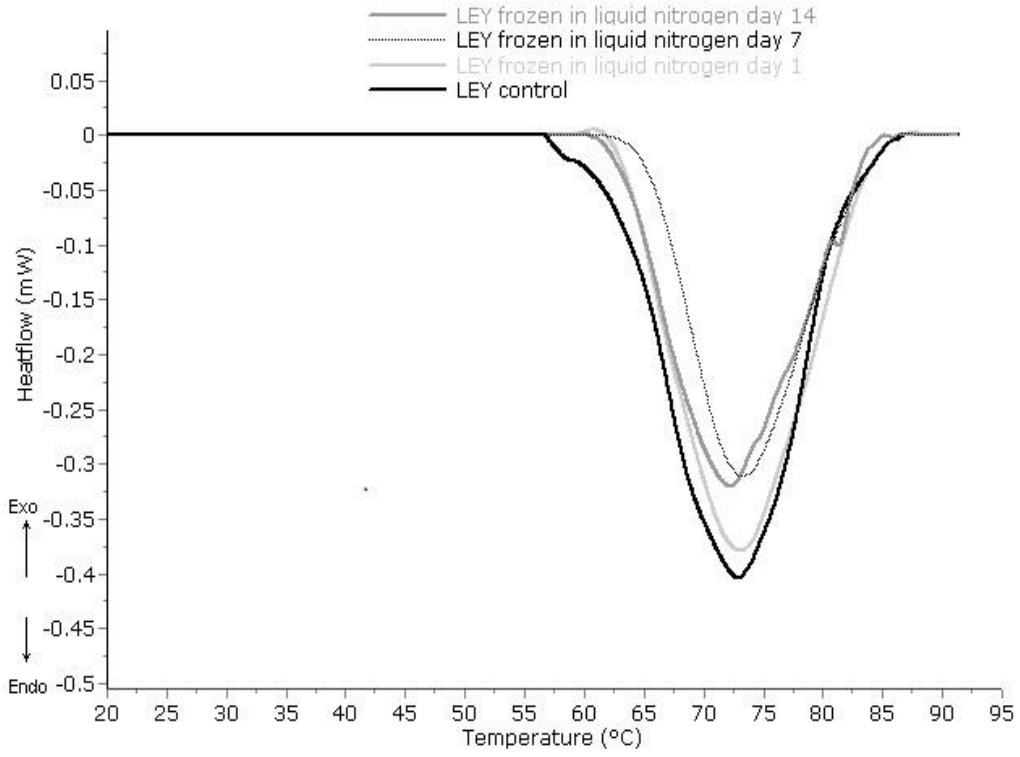

Figure 3. Thermograms of control and cryogenic frozen liquid whole egg (LEY) stored at -18°C.

4. CONCLUSIONS

This study shows that cryogen freezing does not affect calorimetric properties of LEW, but it does in the case of LWE and LEY. The reason for this phenomenon may be the composition of egg yolk. In contrast, frozen storage at -18°C decreased calorimetric properties of all three liquid egg products. Moreover, it had effect on the colour attributes and dry matter content of these products. We will carry out microbiological tests to find out the reason for the pH change.

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