Original scientific paper
UDC 637.43:[664.8.037.5:546.17-14

HOW DOES FREEZING IN LIQUID NITROGEN INFLUENCE THE REOLOGICAL PROPERTIES OF LIQUID EGG PRODUCTS?

Karina Ilona Hidas[1*], Csaba Németh[2], Judit Csonka[1], Anna Visy[1], László Friedrich[1], Ildikó Csilla Zeke[1]

[1]Department of Refrigeration and Livestock Products Technology, Faculty of Food Science, Szent István University, Ménesi Street 43-45., H-1118 Budapest, Hungary
[2]Capriovus Ltd., Dunasor 073/72., H-2317 Szigetcsép, Hungary

[*]e-mail: hidaskarina@gmail.com

Abstract

Liquid egg products are frequently used in the food industry. They serve as an excellent medium for microorganisms, therefore their shelf life is limited, even with the addition of preservatives it is only a few weeks. However, it can be increased by further preservative methods such as freezing. Liquid nitrogen is boiling when it contacts to a higher temperature material, the boiling temperature at atmospheric pressure is -195.6 ^{0}C. Due to the extreme large temperature difference, a frozen layer is formed on the food surface, which protects the product from drying out.

In our experiment, liquid egg products were frozen by dropping into liquid nitrogen. These included liquid whole egg (LWE), liquid egg white (LEW), and liquid egg yolk (LEY). After 60 seconds, samples were filtered and stored at -18 ^{0}C for 4 months. We examined the rheological properties of samples prior to freezing (control) and after freezing on days: 1, 7, 14, 60, and 120 after thawing in room temperature. Subsequently, shear stress data were recorded using a rotational viscometer at a shear rate of 1 - 1000 1/s. Herschel-Bulkley model was fitted to the shear rate-shear stress diagrams and evaluation was performed by ANOVA. In addition, dry matter content was reported during the experiment by drying to constant weight.

In our study, we found that the Herschel-Bulkley model fitted the shear rate-shear stress curves. Our results show that the rheological properties of LEW are not strongly influenced by cryogenic freezing. However, in the rheological properties of LWE and LEY, freezing and frozen storage caused significant changes.

Our results show that rheological characteristics of egg yolk change as a result of cryogenic freezing. This is probably due to the gelling process. Therefore, in our next experiment, various cryoprotectors will be used to eliminate gelling.

***Key words**: Liquid egg products, Rheological properties, Herschel-Bulkley, Freezing, Liquid nitrogen, Cryogenic.*

1. Introduction

Humans began to consume eggs in prehistoric time by discovering that eggs in bird's nest are easily available. Consumption of eggs is very variable among countries. Mexico, Malaysia and Japan are the leading countries, where people consume 329 or more eggs annually. On the other hand, in Africa eggs are not readily available.

New industries were developing from the egg industry that evolved greatly in further processing of eggs. Per capita consumption of shelled eggs decreased, but it was offset by an increase in consumption of further processed eggs [1]. An increasing trend is shown in the production of processed egg products. Two types of these are products from "first processing" (such as liquid, frozen and powdered egg products) and specialty egg products (such as formulated and cooked eggs) [2].

Processed egg products are widely utilized in manufacturing of different food products. For example, liquid whole egg (LWE) is a main ingredient in bakery products, in the catering and food industry. It is contained in pies, biscuits or pastas. Liquid egg white (LEW), which is produced by separation of whole egg, is appropriate for confectionery and bakery industry because of the whipping ability and foam stability. Liquid egg yolk (LEY) is mostly used in mayonnaise, sauces, pasta and other products [3, 4].

People in industrialized countries can easily obtain eggs on the market. But there may be a short-term deficit, such as an outbreak of the disease like avian influenza. For example, Influenza A virus in the United States caused approximately $3.3 billion economic losses because of chicken euthanasia [5]. Another incident was the European "poisonous eggs" incident, which involved at least 40 countries. Millions of hen eggs have been recalled across the continent. Experts diagnosed that eggs were contaminated with an insecticide called fipronil [6].

In such emergencies, some reserved egg products can be used to bridge the temporary egg deficit. In these times, products with long self-life, like egg powder or frozen liquid egg can be used instead of liquid egg products. Frozen products shelf life can increase up to 1 year [7]. Freezing can greatly reduce the number of microbes without significantly affecting functional properties. However, during freezing and frozen storage, different undesirable processes may occur, such as gelation of egg yolk or denaturation of proteins and significant textural changes [4, 8].

Stadelman and Cotterill, [4], found that egg whites texture is less affected by freezing. It has been known since 1925, that during freezing and storing raw egg yolk below -6 ^{0}C, gelation occurs and viscosity increases [9]. This phenomenon can be disadvantageous because of difficulties in mixing and it has an objectionable appearance [4]. One solution for this problem can be the increasing of freezing and thawing rates, because of the formation of smaller ice crystals, that dehydrate proteins less [10]. Small ice crystals cause higher quality with lower drip loss and good texture [11].

The flow behaviour of liquid foods can be investigated with the help of rheology [12]. Atilgan and Unluturk, [13], examined liquid egg products (LEW, LWE and LEY) rheological behaviour at different temperatures. They concluded, that Herschel-Bulkley model fitted well to the shear rate - shear stress data. Ahmed *et al.*, [14], studied the effect of high hydrostatic pressure (HHP) on different liquid egg products. They found that HHP caused the increasing of hysteresis loop on the flow curve of the samples and they also found that Herschel-Bulkley model properly approximated the flow curve. Herald *et al.*, [15], examined rheological behaviour of heat treated LWE stored at -24 ^{0}C for 80 days. They found thixotropic behaviour in all of the examined samples and flow properties of pasteurized liquid LWE changed from Newtonian to Bingham Plastic during frozen storage. They also diagnosed an increase of viscosity due to freezing.

The aim of this study is to investigate the effects of cryogenic freezing and frozen storage on the rheological properties of different liquid egg products.

2. Materials and Methods

2.1 Liquid egg products

Pasteurized liquid egg products heat treated by tubular pasteurizer were used in this experiment (Capriovus Ltd., Szigetcsép, Hungary). LEW was pasteurized for 180 s at 56 ^{0}C with a flow rate of 2000 kg/h, LWE was heat treated under the same circumstances except the temperature, that was 70 ^{0}C. LEY was pasteurized for 600 s holding time at 65 ^{0}C with a flow rate of 600 kg/h. "A" classified hen eggs are the raw materials of these products, which are homogenized and pasteurized during production. Besides that, LEW and LEY production contains a separation process. Products contain 0.5% of citric acid and 0.3% of potassium sorbate. About 33 eggs albumin makes 1 kg of LEW, while about 63 eggs yolk means 1 kg of LEY. 1 kg LWE contains about 22 pcs of eggs. Products are filled into 'Elopak' carton boxes with the filling weight of 1.0 kg. The shelf life is 21 days and products should be stored between 0 ^{0}C and +4 ^{0}C. We used liquid egg products produced on the previous day.

2.2 Freezing of liquid egg products

Freezing was performed by dripping into a liquid nitrogen (Messer Hungarogáz Ltd., Hungary) containing polystyrene container. 3 litres of each product were dripped into liquid nitrogen with the help of a steel strainer (d = 1.5 mm). Liquid nitrogen began to boil with a temperature of -195.8 ^{0}C due to the high temperature difference between the liquid nitrogen and the products. Frozen pellets were separated by a strainer from liquid nitrogen 60 s after dripping into liquid nitrogen. Sample pellets were filled into polyethylene foil, which was sealed with a foil welder and stored in laboratory freezer at -18 ^{0}C for 120 days. Sampling was carried out on following days: 1, 7, 14, 60, 90 and 120. An unfrozen (control) sample was also examined. Frozen samples were thawed with tap water at room temperature.

2.3 Examination of rheological properties

Examination of the rheological behaviour of liquid egg products 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. Temperature of rheological measurements was 5 ^{0}C. Shear stress was measured by increasing shear rate from 1 to 1000 1/s. Herschel-Bulkley model (1) was fitted to flow curves (shear rate-shear stress diagrams) by using the least square fit method of Excel Solver, where τ_0, K and n are the changeable values:

$$\tau = \tau_0 + K\left(\frac{d\gamma}{dt}\right)^n \qquad (1)$$

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

2.4. Measuring of dry matter content

Dry matter content (d.m.c.) was determined by drying to constant weight at 105 ^{0}C in Petri dishes by measuring the mass before and after drying.

2.5. Statistical evaluation

Rheological parameters and d.m.c. were analysed by IBM Statistics 24 software, in which ANOVA was used to decide which of the collected data differ. Levene's test was carried out for checking homogeneity of variances, while Kormogorov-Smirnov and Shapiro-Wilk tests were used for verifying the normality. Tukey tests were performed if variances were determined as equal and Games-Howell test was used in case of not equal variances to determine which groups differ.

3. Results and Discussion

Dry matter content was determined on all measurement days to make sure that changes in the rheological properties are not due to changes of water content. There was no significant change in d.m.c. of liquid products during frozen storage. LEW had the d.m.c. 12.53 ± 1.06 g/100 g, d.m.c. of LWE was 22.86 ± 1.43 g/100 g, while LEY had 44.04 ± 3.34 g dry matter in 100 g product.

Rheological properties of the liquid egg products were tested before freezing, the day after cryogenic freezing and during the frozen storage at 5 ^{0}C (in case of frozen samples a thawing process was performed) on the above mentioned days. Figures 1, 2 and 3, show flow curves of control (day 0) and on day after freezing (day 1).

Figure 1 shows that LEW did not show thixotropy at this temperature either before or after freezing, because approximately the same values were obtained for shear stress in the upward and downward stages, so no hysteresis loop was formed between the two legs of the curve.

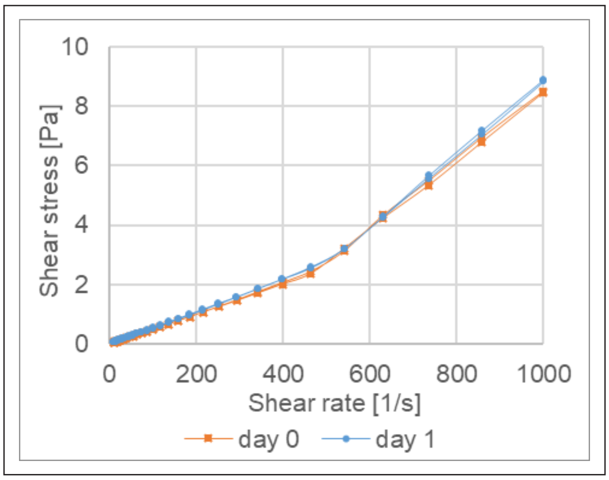

Figure 1. Thixotropy (at 5 ^{0}C) of control (day 0) LEW and LEW on day after cryogenic freezing and thawing in tap water (day 1)

Atilgan and Unluturk, [13], discovered thixotropy in egg white sample at 4 ^{0}C. This difference is probably due to the fact, that they used non-pasteurized samples. The LEW samples show a non-Newtonian shear-thickening property based on the shape of the curves, as shear rate increases, the shear stress curves slope increases, which means that the viscosity increases [16].

Figure 2 shows the thixotropy and rheological behaviour of LWE.

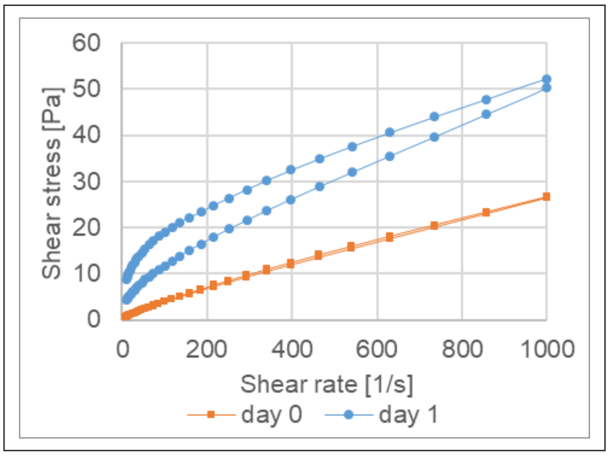

Figure 2. Thixotropy (at 5 ^{0}C) of control (day 0) LWE and LWE on day after cryogenic freezing and thawing in tap water (day 1)

Thixotropy can already be observed in the control sample for LWE samples. According to the accepted definition, a gradual decrease of the viscosity under shear stress is a thixotropy [16]. In addition, it can be stated that samples show no shear-thickening behaviour, as LEW samples, but shear-thinning, also called pseudoplastic behaviour. Control sample behaves almost as a Newtonian fluid, but after freezing and thawing, pseudoplasticity is obvious. Tóth *et al.*, [17], found also that examining LWE before and after HHP treatment. Herald *et al.*, [15], found frozen LWE Newtonian and Bingham plastic fluid based on the rheological behaviour. However, this is probably due to the use of shear rate values between 0 and 60, while a much higher maximal shear stress was used in this study. In addition, occurrence of yield stress can be seen after freezing and thawing, as Herald *et al.*, [15], also stated.

Figure 3 shows that LEY also has a hysteresis loop, so samples are considered to be thixotropic.

They have also a pseudoplastic behaviour. Atilgan and Unluturk, [13], also found pseudoplastic behaviour during their measurements. In addition, yield stress can be seen here after freezing and thawing, like seen in the case of LWE samples.

Figure 4, 5 and 6 show upward flow curves of examined samples before freezing, after freezing and during frozen storage on measurement days. The evaluation of viscosity attributes is summarized in Tables 1, 2, and 3. The model Herschel-Bulkley fitted well the shear-stress-shear rate data in every cases ($R^2 > 0.99$).

Changes of rheological properties caused by cryogenic freezing and frozen storage at -18 ^{0}C in LEW are summarized in Figure 4 and Table 1.

Flow behaviour index (n), as seen in table 1, is > 1 on each measurement day. That confirms the shear-thickening behaviour of these samples. This value increased slightly due to frozen storage. Slight changes occur

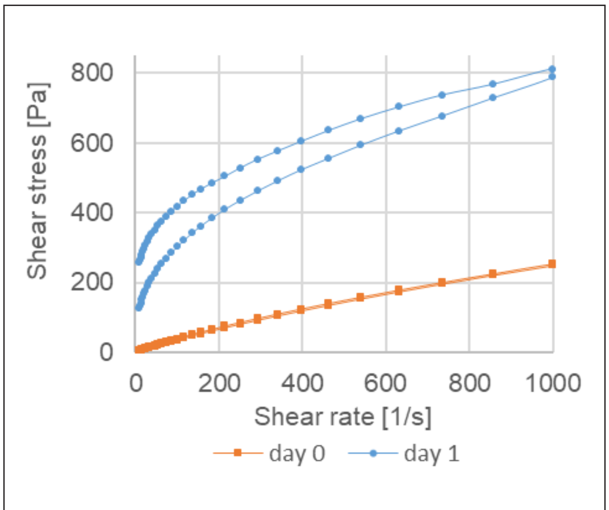

Figure 3. Thixotropy (at 5 ^{0}C) of control (day 0) LEY and LEY on day after cryogenic freezing and thawing in tap water (day 1)

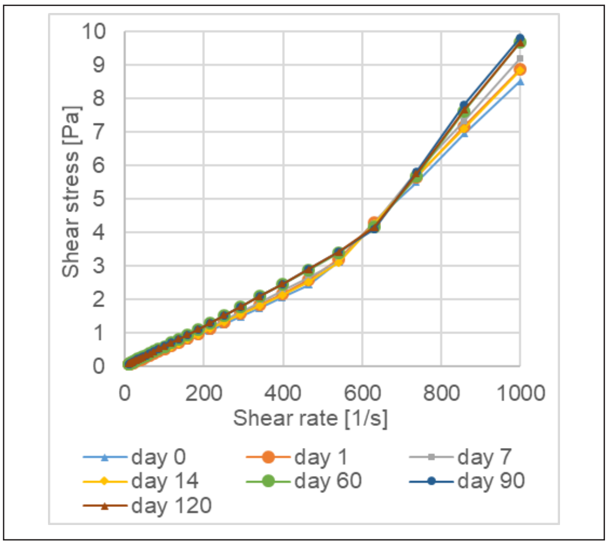

Figure 4. Effect of cryogenic freezing and frozen storage (at -18 0C) on the flow curve of LEW measured at 5 0C

Table 1. Rheological results of unfrozen and cryogenic frozen-thawed LEW after modelling flow curves with the model Herschel-Bulkley

Time [Day]	Yield stress, τ_o [Pa]		Consistency index, K [Pa x s^n]		Flow behaviour index, n		R^2
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	
0	0.1551^a	2.419E-03	1.926E-04^{ab}	1.997E-05	1.539^a	1.664E-02	0.9985
1	0.1872^{abc}	3.000E-02	1.971E-04^b	1.396E-05	1.549^a	1.533E-02	0.9983
7	0.2010^b	9.098E-03	1.661E-04^{ab}	1.015E-05	1.579^{ab}	5.692E-03	0.9979
14	0.1819^{ab}	1.621E-02	1.667E-04^{ab}	1.761E-05	1.569^{ab}	1.945E-02	0.9981
60	0.2386^c	8.926E-03	1.693E-04^{ab}	3.575E-05	1.580^{ab}	2.962E-02	0.9965
90	0.2478^c	8.262E-03	1.477E-04^a	8.416E-06	1.600^b	7.298E-03	0.9966
120	0.2523^c	1.573E-02	1.811E-04^{ab}	2.368E-05	1.568^{ab}	2.212E-02	0.9967

Legend: $^{a, b, c}$ means with different letter in a column are significantly different (P < 0.05).

due to freezing and frozen storage in the rheological characteristics of LEW, some of them are significant.

Figure 5 and table 2 show changes of rheological properties caused by cryogenic freezing and frozen storage at -18 ^{0}C in LWE.

Flow behaviour index (n) (Table 2) is < 1, which confirms the pseudoplasticity of samples. After freezing and frozen storage n decreases significantly, which means, that pseudoplastic behaviour becomes stronger. K stands for the slope of the curve, which increases significantly after freezing. The value of yield stress increases also significantly after cryogenic freezing and frozen storage. Herald *et al.*, [15], stated also this result in their study in the case of previously pasteurized samples.

Figure 6 and table 3 show changes of rheological properties caused by cryogenic freezing and frozen storage at -18 ^{0}C in LEY.

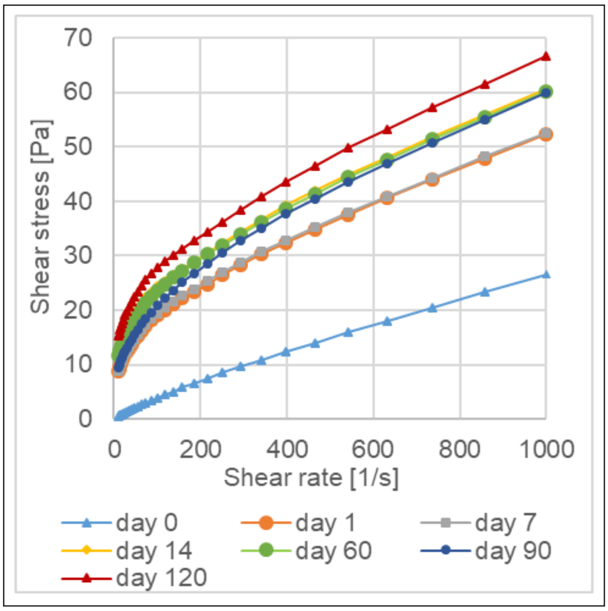

Figure 5. Effect of cryogenic freezing and frozen storage (at -18 ^{0}C) on the flow curve of LWE measured at 5 ^{0}C

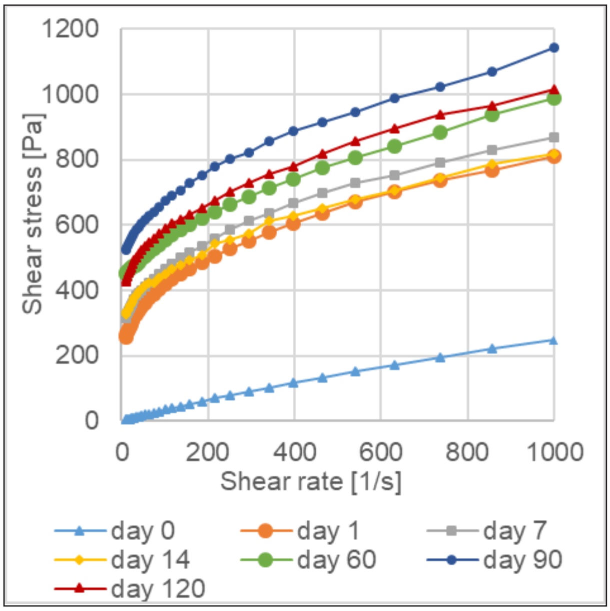

Figure 6. Effect of cryogenic freezing and frozen storage (at -18 ^{0}C) on the flow curve of LEY measured at 5 ^{0}C

Table 2. Rheological results of unfrozen and cryogenic frozen-thawed LWE after modelling flow curves with the model Herschel-Bulkley

Time [Day]	Yield stress, τ_o [Pa]		Consistency index, K [Pa x s^n]		Flow behaviour index, n		R^2
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	
0	3.039E-04^a	2.956E-04	0.086^a	0.004	0.836^a	0.008	0.9997
1	6.179^b	0.144	0.930^b	0.061	0.570^b	0.007	0.9994
7	5.805^b	0.163	1.048^{bc}	0.017	0.547^{cd}	0.002	0.9993
14	10.355^c	0.214	1.007^{bcd}	0.107	0.566^b	0.008	0.9994
60	8.831^{bc}	1.376	1.285^d	0.056	0.532^d	0.006	0.9994
90	5.916^b	0.856	1.151^{bcd}	0.111	0.555^{bc}	0.011	0.9996
120	11.983^c	0.818	1.165^{cd}	0.082	0.554^{bc}	0.010	0.9991

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

Table 3. Rheological results of unfrozen and cryogenic frozen-thawed LEY after modelling flow curves with the model Herschel-Bulkley

Time [Day]	Yield stress, τ_o [Pa]		Consistency index, K [Pa x s^n]		Flow behaviour index, n		R^2
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	
0	0.001[a]	0.001	0.727[a]	0.003	0.847[e]	3.714E-04	0.9998
1	161.305[b]	5.649	37.644[d]	2.081	0.411[a]	7.848E-03	0.9998
7	254.868[c]	2.656	19.256[b]	0.970	0.505[c]	7.058E-03	0.9997
14	257.179c	8.598	20.069[b]	1.108	0.486[bc]	9.952E-03	0.9994
60	418.128[e]	12.775	7.776[a]	2.889	0.623[d]	3.812E-02	0.9989
90	435.147[e]	18.927	29.300[c]	2.447	0.459[b]	1.119E-02	0.9995
120	350.887[d]	8.028	34.068[cd]	3.600	0.439[ab]	1.819E-02	0.9997

Legend: [a, b, c, d, e] means with different letter in a column are significantly different (P < 0.05).

Table 3 shows, that n is smaller than 1 before and after freezing, as well. A significant decrease appears in values after cryogenic freezing and during the storage experiment. Changes in yield stress and consistency index are significant.

4. Conclusions

- In our experiment, we found that the dry matter content of pasteurized liquid egg products frozen by dripping into liquid nitrogen and then stored at -18 ^{0}C for 120 days did not change significantly.

- In order to characterize the rheological behaviour of LEW, LWE and LEY, we used Herschel-Bulkley model. The model approximated the flow curves of each sample examined with adequate accuracy ($R^2 > 0.99$).

- LWE and LEY samples showed pseudoplastic flow behaviour and also tixotrophy at 5 ^{0}C. The flow curve of LEW samples had shear-thickening flow characteristics.

- We revealed that cryogenic freezing and frozen storage for 120 days had a very slight effect on the rheological parameters of LEW, but affected the flow behaviour of LWE and LEY significantly. This is due to the gelation of egg yolk, which appears under -6 ^{0}C [7].

Acknowledgement

Our research is sponsored by the project „Egg white based dairy product analogs with probiotic effects for allergic people and development of low-fat and carbohydrate products for people on a protein-dense diet VÁLLALATI KFI_16"". This work was supported by Doctoral School of Food Sciences of Szent István University.

5. References

[1] Bertechini A. G. (2016). *Economic and cultural aspects of the table eggs as an edible commodity.* In: Hester P. (Ed.), Egg innovations and strategies for improvements. Academic press, Cambridge, United Kingdom, pp. 223-233.

[2] Lechevalier V., Croguennec T., Anton M., and Nau F. (2011). *Processed egg products.* In: Nys Y., Bain M., van Immerseel F. (Eds.), Improving the Safety and Quality of Eggs and Egg Products: Egg Chemistry, Production and Consumption. Woodhead Publishing Limited, Cambridge, UK, pp. 538-581.

[3] Alvarez E., Cancela M. A., and Macerias R. (2006). *Comparison of rheological behaviour of salad sauces.* International Journal of Food Properties, 9, (4). pp. 907-915.

[4] Stadelman W. J., and Cotterill O. J. (Eds.). (1995). *Egg science and technology* (4th Ed.). The Haworth Press Inc, New York, USA.

[5] Looi F., Baker M., Townson T., Richard M., Novak B., Doran T., and Short K. (2018). *Creating Disease Resistant Chickens: A Viable Solution to Avian Influenza?* Viruses, 10, (10), pp. 1-9.

[6] Tu Q., Hickey M. E., Yang T., Gao S., Zhang Q., Qu Y., Du X., Wang J., and He L. (2019). *A simple and rapid method for detecting the pesticide fipronil on egg shells and in liquid eggs by Raman microscopy.* Food control, 96, pp. 16-21.

[7] Au C., Acevedo N. C., Horner H. T., and Wang T. (2015). *Determination of the gelation mechanism of freeze-thawed hen egg yolk.* Journal of agricultural and food chemistry, 63, (46), pp. 10170-10180.

[8] Lai L. S. (2005). *Quality and Safety of Frozen Eggs and Egg Products.* In: Sun D. W. (Ed.), Handbook of Frozen Food Processing and Packaging. CRC Press, Boca Raton, USA, pp. 503-518.

[9] Moran T. (1925). *The Effect of Low Temperature on Hens' Eggs*. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character, 98, (691), pp. 436-456.

[10] Powrie W. D., Little H., and Lopez A. (1963). *Gelation of egg yolk*. Journal of Food Science, 28 (1), pp. 38-46.

[11] Kolbe E., and Kramer D. E. (2007). *Planning for seafood freezing*. Alaska Sea Grant College Program, University of Alaska Fairbanks, USA.

[12] Rao M. A. (1977). *Rheology of liquid foods - A review.* Journal of Texture Studies, 8 (2), pp. 135-168.

[13] Atilgan M. R. and Unluturk S. (2008). *Rheological properties of liquid egg products (LEPS)*. International Journal of Food Properties, 11, (2), pp. 296-309.

[14] Ahmed J., Ramaswamy H. S., Alli I., and Ngadi M. (2003). *Effect of high pressure on rheological characteristics of liquid egg.* LWT-Food Science and Technology, 36, (5), pp. 517-524.

[15] Herald T. J., Osorio F. A., and Smith D. M. (1989). *Rheological properties of pasteurized liquid whole egg during frozen storage.* Journal of Food Science, 54, (1), pp. 35-38.

[16] Barnes H. A., Hutton J. F., and Walters K. (Eds.). (1993). *An introduction to rheology*. Elsevier, Amsterdam, The Netherlands.

[17] Tóth A., Németh C., Juhász R., Zeke I., Bertold S., Bényi D., and Friedrich, L. (2016). *Effect of high hydrostatic pressure at 400 MPa on quality attributes of liquid egg products.* Review on Agriculture and Rural Development, 5, (1-2), pp. 148-152.