Effects of HHP's Holding Time on Proteins of Liquid Egg Products

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***Abstract*—Today's consumers require food products with fresh, or fresh-like characteristics. Minimal processing technologies are indicated as technologies with a gentle impact on sensorial and techno-functional properties. High Hydrostatic Pressure (HHP) is a widely used minimal technology considered as a cold pasteurization method in food preservation. The effect of applied pressure of HHP is well understood, but the holding time of HHP treatment is not extensively investigated in topic of proteins. One of the most important attribute of liquid egg products are the great foaming ability, foaming stability as well as emulsifying properties. These techno-functional properties are highly influenced by the protein structures of egg products. In our study, liquid egg products were pressurized at 400 MPa, for 1, 3, 5 7 and 10 minutes. The protein structures of liquid egg white (LEW), liquid egg yolk (LEY) and liquid whole egg (LWE) were investigated using Differential Scanning Calorimetry (DSC).Our study pointed out that after HHP treatment at 400 MPa, for 10 minutes 12% decrease in denaturation enthalpy of LWE was observed. In LEY 30% decrease was detected after 10 minutes HHP treatment. The highest impact of HHP treatment (35% decrease in denaturation enthalpy) in LWE was noted. The changes in protein structures of liquid egg products were smaller, than the effects of higher pressure applied for preservation. In aspects of techno-functional properties applying a longer holding time is more favourable, than applying a higher pressure.[1]**

***Index Terms*—liquid egg products, protein, minimal processing technologies, DSC**

I. Introduction

Different food products require different pressure levels providing microbiological safe products. E. g. meat products are mainly pasteurized, which is generally done in the range of 300–600 MPa, inactivating vegetative cells [1], [2]. High Hydrostatic Pressure (HHP) treatment could induce the egg white proteins denaturation and aggregation, depending on pressure range, protein concentration, time, pH, and temperature [3]. HHP process has shown a great potential to modify the protein conformational structure (secondary, tertiary and quaternary), which is stabilized by electrostatic interactions, hydrogen bonds and hydrophilic interactions, provoking protein unfolding, while preserving the protein's primary structure stabilized by covalent bonds [4], [5]. Previous works, pointed out the extent of protein modification is strongly affected by the nature of protein as well as by the processing conditions applied, namely pressure level, treatment temperature and holding time [6]-[9].

Pressure processing of egg products has been used experimentally as an alternative to heat pasteurization and to eliminate *Salmonella* in several liquid egg products [10], [11]. The investigations pointed out that higher pressure ranges (above 450 – 500 MPa) minimalize microbiological spoilage of egg products [12], but it may cause a destruction of original structure [13] as well destroy techno-functional properties [14], [15].

In our study the effects of combinations of heat and HHP treatments are investigated on egg white's protein structures and microbiological safety.

II. Materials and Methods

A. Sample Preparing

Freshly laid, M size, traditional cage eggs were used for our measurements. Eggs were taken from a Hungarian layer farm, laid by farming Broilers. Homogenized, raw liquid egg products (liquid egg yolk LEY, liquid egg white LEW and liquid whole egg LWE) were taken from the production line of Capriovus Ltd (Szigetcsép, Hungary). Samples refrigerated at 4 °C were transported to Sznet Szent István University, Budapest. For differential calorimetrical measurements (DSC) three times 10-10 mL of samples were packaged in polyethylene bags, for every treatment. 3-3 packages were prepared. HHP processing was carried out in a RESATO FPU100 – 1200 HHP equipment at room temperature. Before measurements. Before and after HHP treatments samples were stored at 4 °C.

B. Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC**)** was used to assess the changes in proteins conformation induced by

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thermal denaturation [16]. Thermophysical calorimetric properties were examined on Micro DSC III (differential scanning calorimeter, SETARAM, Caluire, France). In each case approximately 778 mg of samples were sealed in a hermetic stainless-steel pan, for the measurements, and distilled water was used, in the reference cell. The heat-up ramp was from 20 to 95 ℃ 1.5 ℃/min, the speed of cooling was 1.5 ℃/min, controlled by SetSoft2000. The overall denaturation enthalpy (DH) was calculated from the peak area of the thermograms (between 45 and 90 ℃) using Callisto 7.6 software. For every treated and native samples 3-3 repetitions were measured, measurements were carried out in 24 hours after HHP treatment. Our method was similar to [15], [17], [18] and [20]. According to several literature [19], [20], up ramp and cooling is more depending on characteristics of applied DSC equipment, than on properties of samples. Normalized thermograms of liquid egg samples are standing in this work for illustrating the widely different shapes of HHP treated samples. Numerical results of DSC analysis are summarised in tables.

III. Results and Discussion

A. *Effects of Holding Time*

Fig. 1 shows the thermograms of LWE samples treated at 400 MPa for 1 – 10 minutes. The first peak decrased by longer holding times. It may a result of denaturation of ovotransferrin of egg white and LDL proteins of egg yolk.

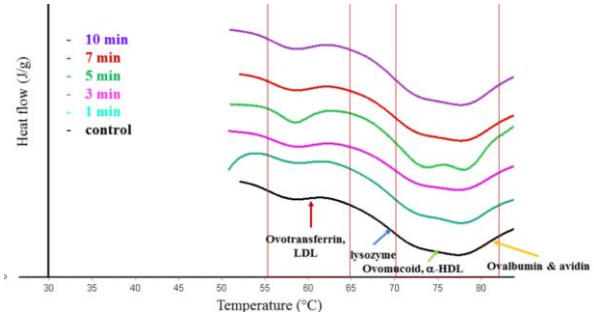

Figure 1. Thermograms of LWE treated between at 400 MPa for 1 – 10 min

TABLE I. DENATURATION ENTHALPY AND DENATURATION TEMPERATURES OF LWE AFTER HHP TREATMENTS AT 400 M?PA FOR 1 – 10 MINUTES

HHP	ΔH (J/g)	T_{d1}, ℃	T_{d2}, ℃
control	0,895 ±0,0039	58,11 ±0,78	73,69 ±1,52
1 min	0,839 ±0,0024 AB	57,78 ±1,01	72,99 ±2,02 AB
3 min	0,831 ±0,0023 AB	59,09 ±0,99	77,27 ±1,01 AB
5 min	0,828 ±0,0081 AB	57,39 ±0,84	76,48 ±0,45 AB
7 min	0,814 ±0,0018 AB	58,23 ±0,95	77,22 ±0,98 AB
10 min	0,785 ±0,0097 AB	58,07 ±0,77	77,83 ±1,11 AB

Table I represents the numerical data of DSC measurements of LWE after HHP treatments. Comparing to control, ΔH decreased 13% after 10 minutes of HHP at 400 MPa. First denaturation temperature T_{d1}, was not affected by the different holding times applied in the experiment, in contrast, second denaturation temperature, T_{d2}, showed an increasing tendency by increased holding time. It may a result of denaturation and aggregation of lysozyme and ovomuciod.

Using one-way ANOVA and post hoc tests, ΔH, T_{d1} and T_{d2}, were significantly influenced by HHP.

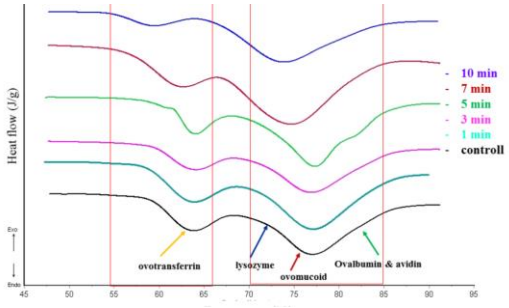

Figure 2. Thermograms of LEW treated between at 400 MPa for 1 – 10 min

Fig. 2 represents the thermograms of LEW samples treated at 400 MPa for 1 – 10 minutes. A similar tendendcy of peak maxima are viable to LWE. The shape of LEW treated for 10 minutes is highly influenced by HHP treatment: both peak maxima are smaller, as well having smaller temperatures compared to control. Denaturation enthalpy of LEW after 10 minutes decreased by 35% compared to control.

TABLE II. DENATURATION ENTHALPY AND DENATURATION TEMPERATURES OF LEW AFTER HHP TREATMENTS AT 400 M?PA FOR 1 – 10 MINUTES

HHP	ΔH (J/g)	T_{d1}, ℃	T_{d2}, ℃
control	2,091 ±0,239	64,21 ±0,66	77,02 ±0,59
1 min	1,986 ±0,313	58,75 ±1,67 AB	75,31 ±0,62
3 min	1,815 ±0,157	64,11 ±0,54	77,09 ±0,37
5 min	1,845 ±0,121	64,23 ±0,71	77,21 ±0,69
7 min	1,756 ±0,103	63,88 ±0,75	73,12 ±0,66
10 min	1,351 ±0,305 AB	59,61 ±1,54 AB	73,67 ±1,71

Table II represents the data of DSC measurements of LEW after HHP treatments at 400 MPa. A higher denaturation of proteins is viable in egg white compared to whole egg samples. It may a result of the lack of protective effects of egg yolk's proteins. In changes of temperature of denaturation there are no tendencys. But statistical evaluation pointed out, that HHP for 1 – 10 min at 400 MPa has no significant effects on values of TD_2.

Fig. 3 demonstrates the thermograms of LEY samples treated at 400 MPa for 1 – 10 minutes. Peak maxima are moved to a higher temperature after treatments, as long denaturation enthalpy decreased by longer holding time. Two peak maxima are demonstrated [12]. The first may a result of denaturation of LDL (low density lipoproteins)

and the second from denaturation of α-HDL (α-high density lipoprotein) fractions in egg yolk [20].

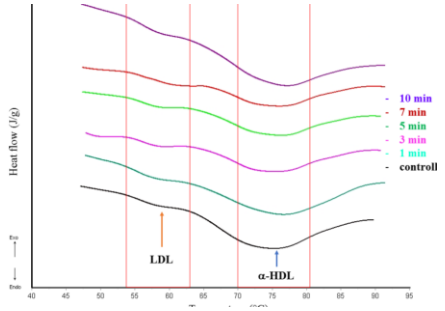

Figure 3. Thermograms of LEY treated between at 400 MPa for 1 – 10 min

TABLE III. DENATURATION ENTHALPY AND DENATURATION TEMPERATURES OF LEY AFTER HHP TREATMENTS AT 400 M?PA FOR 1 – 10 MINUTES

HHP	ΔH (J/g)	T_{d1}, ℃
control	0,714 ±0,0092	73,74 ±1,36
1 min	0,639 ±0,0078	77,49 ±1,17 ^{AB}
3 min	0,567 ±0,0039	74,09 ±0,86
5 min	0,541 ±0,0031	76,04 ±0,27 ^{AB}
7 min	0,506 ±0,0034	76,25 ±0,42 ^{AB}
10 min	0,499 ±0,0021	76,16 ±0,36 ^{AB}

Table III summarizes the numerical data of DSC analysis of HHP treated LWY at 400 MPa for 1–10 minutes.

The longest treatment affected a decrease of denaturation enthalpy about 13%, which means a statistical unsignificant change in ΔH. In contrast, temperatures of denaturation are highly influenced by the holding time of HHP. It showed an increase after HHP treatment. Every T_{d1} result is statistical significant compared to the control sample.

IV. CONCLUSION

Summarizing our data HHP's holding time may affect the protein structures of liquid egg products. The highest influence of HHP was established in liquid egg white. It means that techno-functional properties of egg white may highest changed by HHP holding time as well. In case of liquid whole egg and egg yolk HHP at 400 MPa for 1 – 10 minutes may a relevant solution for decontamination and fulfil the requirements of microbiological food safety.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Adrienn Tóth conducted the research; Csaba Németh analyzed the data; Karina Hidas and Attila Lőrincz wrote the paper; Annamária Barkó, Attila Nagy, Gergő Illés and László Friedrich corrected the conclusions and the paper and all authors had approved the final version.

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Dr. Adrienn Tóth finished her Phd in 2020 in the topic of shelf-life extension of liquid egg products. Her main topics and research projects are shelf-life extension of food products applying minimal processing technologies. During and after PhD she carried out research projects in field of product development of livestock origin food products.
After finishing Phd she became a research fallow and later a researcher of the Szent István University (now Hungarian University of Agriculture and Life Sciences, Institute of Food Science and Technology, Department of Livestock and Food Preservation Technology). Her newest research projects are focusing on development of egg based dairy replacement products for consumers following diets with high protein intake. Today she is an educational entrepreneur leading I living Labs in the EŦUDRES project of the European Union.

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