



EFFECTS OF HIGH HYDROSTATIC PRESSURE'S HOLDING TIME ON PROTEIN STRUCTURE OF LIQUID EGG PRODUCTS

Author(s):

A. Tóth¹, Cs. Németh², R. Pintér¹, E. Ayari¹, K. Noori¹ and L. Friedrich¹

Affiliation:

¹Department of Refrigeration and Livestock Products Technologies, Szent István University

Ménesi út 43-45., Budapest, H-1118, Hungary

²Capriovus Ltd., Dunasor 073/72 hrsz. Szigetcsép, H-2317, Hungary

Email address:

toth.drnn@gmail.com, nemeth.csaba@capriovus.hu

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.

Keywords: liquid egg products, protein, minimal processing technologies, DSC

1. Introduction

The Egg white is a key ingredient in many food products as it combines high nutritional quality (8) with excellent functional properties (1). The high content of essential amino acids in egg white proteins and the high bioavailability of these proteins are of great benefit to human nutrition (3),(5). However, the effects of industrial processing such as dry heating on the nutritional quality of egg white proteins have been poorly documented. Some studies considered the effect of dry heating on the in vitro digestibility of proteins as it is a prerequisite to nutritional quality (7), but the effects of minimal processing technologies are not investigated in case of egg white. Studies are viable about the digestibility assays confirmed previous findings that exposure of egg white to high temperatures increased digestibility markedly. However, it seems that the effects of pH and salt concentrations were found to be minimal (4).

In the egg product industry, microbiological safety of liquid products is mainly guaranteed by pasteurisation. The USDA requires that liquid whole egg is at least heated at 60°C for no less than 3.5 min, but in the United Kingdom the recommendations are to pasteurize at least at 64°C for 2.5 min [1], [2]. In France, there is no statutory heat treatment; only microbiological results are determined by regulations. To achieve this, the treatments classically used to pasteurize whole egg vary from 65 to 68°C for 2–5 min in order to ensure 5 to

6 decimal reductions of vegetative microorganisms and especially *Salmonella Enteritidis* and *Listeria monocytogenes* [3]. Pasteurisation temperatures used in egg industry are limited by the sensitivity of egg proteins to heat treatment. Thus, pasteurisation for 2–10 min from 60 to 68°C modifies whole egg electrophoretic pattern by especially decreasing ovotransferrin, livetin, ovalbumin, apovitellenin, lysozyme and/or ovomucin band intensity [4], [1], [5].

Liquid egg white (LEW) and egg white-based products are usually regarded as functional foods for their excellent source of high-quality proteins, trace minerals, and for the ability of their components to coagulate, and to form foams when whipped. HHP is one of the most promising minimal processing technologies in the food industry, but only a few scientific studies are existing about HHP treatment and its effects on egg products [6].

HHP has gained popularity as an alternative for conventional thermal treatment. It has advantages over thermal processing, including lower temperature and reduced extreme aggregation [7]. HHP is a powerful post-package treatment for controlling growth of microorganisms in different food products. Innovative processes have been reported by several researchers for improving the microbiological safety of eggs and egg products [8], [9], [10]. 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 [11], [12]. High hydrostatic pressure (HHP) treatment could induce the egg white proteins denaturation and aggregation, depending on pressure range, protein concentration, time, pH, and temperature [13]. 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 [14], [15]. 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 [16], [15], [17], [18].

Pressure processing of egg products has been used experimentally as an alternative to heat pasteurization and to eliminate *Salmonella* in several liquid egg products [19], [20]. The investigations pointed out that higher pressure ranges (above 450 – 500 MPa) minimize microbiological spoilage of egg products [21], but it may cause a destruction of original structure [20] as well destroy techno-functional properties [22], [23]. In our study the effects of combinations of heat and HHP treatments are investigated on egg white's protein structures and microbiological safety.

2. Materials and methods

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 Szent István University, Budapest. For differential calorimetric 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.

Differential Scanning Calorimetry (DSC) was used to assess the changes in proteins conformation induced by thermal denaturation [24]. 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°C 1.5°C/min, the speed of cooling was 1.5°C/min, controlled by SetSoft2000. The overall denaturation enthalpy (DH) was calculated from the peak area of the thermograms (between 45 and 90°C) 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 [25], [26], [27] and [28]. According to several literature [29], [30], 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.

3. Results

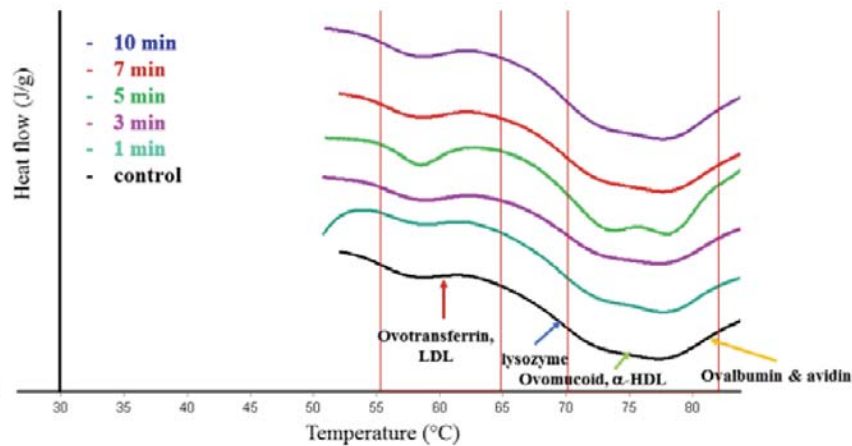


Figure 1. Thermograms of liquid whole egg treated at 400 MPa for 1 – 10 min

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

Table 1. Denaturation enthalpy and denaturation temperatures of LWE after HHP treatments at 400 M?Pa for 1 – 10 minutes

HHP	ΔH (J/g)	T_{d1} , °C	T_{d2} , °C
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}

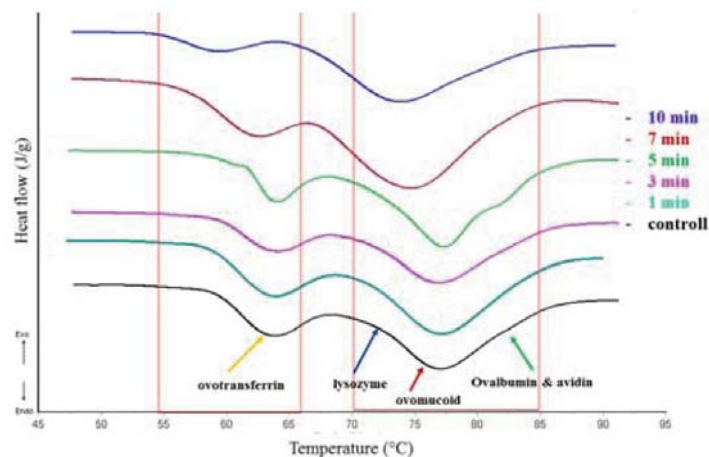


Figure 2 Thermograms of liquid egg white treated at 400 MPa for 1 – 10 min

Table 1 represents the numerical data of DSC measurements of LEW 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 represents the thermograms of LEW samples treated at 400 MPa for 1 – 10 minutes. A similar tendency of peak maxima are viable to LEW. The shape of LEW treated for 10 minutes is highly influenced by HHP treatment: both peak maxima are smaller, as well have smaller temperatures compared to control. Denaturation enthalpy of LEW after 10 minutes decreased by 35% compared to control.

Table 2. Denaturation enthalpy and denaturation temperatures of LEW after HHP treatments at 400 M?Pa for 1 – 10 minutes

HHP	ΔH (J/g)	T_{d1} , °C	T_{d2} , °C
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 2 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 tendencies. But statistical evaluation pointed out, that HHP for 1 – 10 min at 400 MPa has no significant effects on values of TD2.

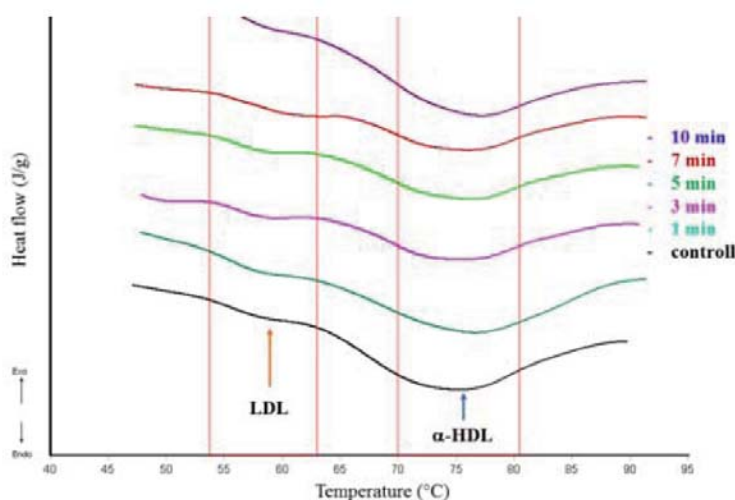


Figure 3. Thermograms of liquid egg yolk treated at 400 MPa for 1 – 10 min

Figure 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 [31]. 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 [29].

Table 3. Denaturation enthalpy and denaturation temperatures of LEY after HHP treatments at 400 MPa for 1 – 10 minutes

HHP	ΔH (J/g)	T_{d1} , °C
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 3 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 insignificant 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.

Conclusions

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.

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