HHP treatment of liquid egg products

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Abstract. High Hydrostatic Pressure (HHP) is one of the most promising minimal processing technologies in food preservation. HHP decreases microbiological spoilage of products and extend shelf life, while freshly-like properties are retained. For controlling microbiological safety of liquid whole egg (LWE), liquid egg white (LEW), liquid egg yolk (LEY) several preservation methods are viable in industry, but most of these apply heat or preservatives. On the one hand high temperatures are effective, but techno-functional properties could be declined, on the other hand the use of preservatives is rejected by consumers. In our study liquid egg samples are treated between 150 and 600 MPa, for 5 min. After treatments rheological properties and protein structures of samples were investigated. In evaluation of rheological results, Herschel-Bulkley model was fitted. Relevant changes in values of Herschel-Bulkley models were observed above 450 MPa. LWE after HHP treatment had a stronger pseudoplastic behaviour. Summarizing our data, using a higher pressure for preservation of LWE may have bad influence on techno-functional properties. But the border pressure for adequate techno-functional properties may differ depending on final use of LWE.

1. Introduction

HHP has gained popularity as an alternative for conventional thermal treatment. It has advantages over thermal processing, including lower temperature and reduced extreme aggregation [1]. 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 [2], [3], [4]. 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 [5], [6]. HHP treatment could induce the egg white proteins denaturation and aggregation, depending on pressure range, protein concentration, time, pH and temperature [7].
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 [8], [9]. 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 [10], [9], [11], [12].

Pressure processing of egg products has been used experimentally as an alternative to heat pasteurization and to eliminate *Salmonella* in several liquid egg products [13], [14]. The investigations pointed out that higher pressure ranges (above 450 – 500 MPa) minimalize microbiological spoilage of egg products [15], but it may cause a destruction of original structure [14] as well destroy techno-functional properties [16], [17].

In our experiment the rheological properties and protein structures were investigated for liquid egg samples after HHP treatments between 150 and 600 MPa.

2. Materials and Methods

2.1. Materials

2.1.1. 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 whole egg (LWE) was taken from the production of Capriovus Ltd (Szigetscép, Hungary).

Samples refrigerated at 4 °C were transported to Szent István University, Budapest. For protein structure and rheological measurements three times 100-100 mL of LWE, LEW and LEY were packaged in polyethylene bags, for every HHP treatment. 3-3 packages were prepared.

HHP processing was carried out in a RESATO FPU100 – 1200 HHP equipment at room temperature. Pressure levels were chosen between 150 and 600, holding time was 5 min. Pressure’s build up speed was 100 MPa/min, and the pressure decreasing was instantaneous. Before and after HHP treatments samples were stored at 3-5 °C.

2.2. Methods

2.2.1. Rheological measurements
Rheological properties were measured with an Anton Paar MCR 92 rheometer applying a concentric cylinder system (d= 27 mm). Shear stress was measured between 10 and 1000 1/s shear rate. For every sample the speed up and speed down tracks 31 – 31 points were taken for flow curve. For analysis of apparent viscosity, Herschel-Bulkly model was fitted on yield curve of every treated sample.

2.2.2. Investigation of protein structures

**Differential Scanning Calorimetry (DSC)** was used to assess the changes in proteins conformation induced by thermal denaturation [18]. 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 (ΔH) 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 [19], [20], [21] and [22].
According to previous studies, heating and cooling ramps depend more on the DSC equipment than on properties of samples [23,24]. Normalized thermograms of liquid egg samples are shown in this work for illustrating the widely different shapes for HHP treated samples. Numerical results of DSC analyses are summarised in tables.

3. Results and Discussion

3.1. Rheological properties

Figure 1 shows the liquid egg products after HHP treatments. LWE showed an increased viscosity on 550 and 600 MPa. LEY’s viscosity increased on 400 MPa and above. In case of LEW above 400 MPa protein aggregates are visible.

![Figure 1: Liquid egg samples after HHP treatments at different pressures](image)

![Figure 2: Shear curves of LWE after HHP treatments at different pressures](image)
Figure 2 presents the shear curves of LWE. As Figure 1 pointed out as well, the changes in apparent viscosity is visible on 550 and 600 MPa.

![Shear curves of LWE](image1.png)

Figure 3: Shear curves of LEW after HHP treatments at different pressures

Figure 3 represents the viscosity curves of LEW. Visible changes are observed above 400 MPa. Samples treated on 550 and 600 MPa were coagulated so the viscosity measurements were not possible in case of both samples.

![Viscosity curves of LEW](image2.png)

Figure 4: Shear curves of LEY after HHP treatments at different pressures

Figure 4 shows the viscosity curves of LEY. The shape of viscosity curves is the same in case of every HHP treated LEY sample. All the HHP treated liquid egg samples have pseudoplastic behaviour after treatments.
3.2. Changes in protein structures

Protein structures of liquid egg samples were highly influenced by the pressure of HHP. 350 MPa caused a statistically significant decrease in denaturation enthalpy of LWE ($\Delta H$) (Table 1). Denaturation temperatures are significantly changed even on 150 MPa. A previous study confirmed the same effect for 350 MPa, 5 min HHP treatment [25].

Table 1: Thermograms of LWE treated for 5 minutes at different pressures. Superscripts indicate significant difference from control sample ($A$: Turkey HSD, $B$: LSD post hoc test). $T_{d1}$ and $T_{d2}$ are the temperatures in denaturation maxima.

| HHP, MPa | $\Delta H$ (J/g) | $T_{d1}$, °C | $T_{d2}$, °C |
|----------|------------------|--------------|--------------|
| control  | 1.22±0.03        | 58.11±0.03   | 73.78±0.11   |
| 150      | 1.21±0.01        | 58.43±0.63   | 78.67±0.44   | $AB$ |
| 200      | 1.15±0.03        | 58.26±1.44   | 78.57±0.51   | $AB$ |
| 250      | 1.12±0.06        | 59.04±0.87   | 77.44±1.27   | $AB$ |
| 300      | 1.06±0.08        | 58.55±0.96   | 77.43±1.35   | $AB$ |
| 350      | 1.01±0.06        | 57.98±0.48   | 77.35±0.69   | $AB$ |
| 400      | 0.89±0.03        | 57.39±0.61   | 76.90±0.19   | $AB$ |
| 450      | 0.94±0.11        | 57.31±0.42   | 80.31±0.61   | $AB$ |
| 500      | 1.00±0.03        | 59.46±0.42   | 79.16±2.17   | $AB$ |
| 550      | 0.74±0.05        | $-            | 82.21±0.13   | $AB$ |
| 600      | 0.48±0.02        | $-            | 82.80±0.13   | $AB$ |

Table 2: Thermograms of LEW treated for 5 minutes at different pressures. Superscripts indicate significant difference from control sample ($A$: Turkey HSD, $B$: LSD post hoc test). $T_{d1}$ and $T_{d2}$ are the temperatures in denaturation maxima.

| HHP, MPa | $\Delta H$ (J/g) | $T_{d1}$, °C | $T_{d2}$, °C |
|----------|------------------|--------------|--------------|
| control  | 1.33±0.02        | 60.01±0.78   | 75.23±0.09   |
| 150      | 1.33±0.11        | 58.41±1.60   | 75.30±0.04   | $AB$ |
| 200      | 1.32±0.12        | 58.34±1.00   | 75.30±0.06   | $AB$ |
| 250      | 1.3±0.35         | 58.63±1.01   | 75.32±0.04   | $AB$ |
| 300      | 1.28±0.07        | 60.48±0.04   | 75.29±0.07   | $AB$ |
| 350      | 1.23±0.04        | 60.39±0.18   | 75.63±0.09   | $AB$ |
| 400      | 1.18±0.01        | 60.03±0.10   | 75.51±0.21   | $AB$ |
| 450      | 1.14±0.14        | 59.06±0.64   | 73.45±0.59   | $AB$ |
| 500      | 1.00±0.03        | 58.86±0.90   | 73.73±1.29   | $AB$ |
| 550      | 0.61±0.04        | 58.37±0.59   | 78.53±1.05   | $AB$ |
| 600      | 0.33±0.08        | 58.62±0.24   | 80.90±0.70   | $AB$ |

Data of DSC measurements are summarised for LEW in table 2. LEW is more resistant against HHP’s pressure: significant decrease is observed from 500 MPa in denaturation enthalpy. In case of beef lean meat similar results are reported in previous works [26], [27]. The higher resistance of LEW’s proteins may be clarified by the lower pressure resistance of LEY’s protein [23], however there are just few papers published in this topic.
Table 3 summarizes the DSC measurements in case of LEY. Denaturation enthalpy decreased by increasing pressures, being more significant from 200 MPa. The major protein denaturation is in LDL1 and LDL2 lipoproteins [28]. According to previous works [29,30] the most resistant proteins against pressure may be phosvitin.

4. Conclusion
In our study the protein structures and the rheological properties of liquid egg products are investigated. HHP highly influenced the protein structures. The highest influence on proteins was observed in LEY. Viscosity attributes are highly influenced by HHP as well. Changes in apparent viscosity are significant in all liquid egg products.

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