HHP treatment of liquid egg at 200-350 MPa

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Abstract. High hydrostatic pressure (HHP) treatment of egg proteins partially limits their sensitivity to pressure. According to the literature, at the 450 MPa level, denaturation of some proteins sets in to the extent that sensory and functional characteristics are impacted. This study involved treating liquid egg (egg white, yolk, and melange) at less than the above-mentioned value, after which the microbiological effect was examined. For the study, pressure pouches were filled with 100ml of raw liquid egg per pouch. Then the samples were treated at 200, 250, 300 and 350 MPa. In each case, the level was reached by increasing pressure at a rate of 100 MPa/min. Measurements were taken at the Corvinus University of Budapest, Faculty of Food Science, Dept. of Refrigeration and Livestock Products Technology RESATO FPU 100-2000 equipment. Denaturation was determined with calorimetric (DSC) tests. From our results, it appears that even at 250 MPa pressure treatment, the viable cell count decreases. Further, it can be said that microbe count went down in the egg white samples at 300-350 MPa, below the impact level. Significant denaturation was not detected during our examinations. In summary, we state that the most HHP-sensitive liquid egg type, egg white, can be pressure treated to reduce microbe count at a level less than that which causes denaturation. Microbe reduction was smaller in yolk and melange, so higher pressure values are appropriate for these products.

1. Introduction
Eggs have been classified as nature’s original functional food [1]. Eggs are very important in food for emulsification, leavening, smoothness and flavour, to name a few. Owing to these facts eggs are used in many food products. At present there is a tendency to use more and more eggshell processed products where term egg products refers to eggs that are removed from their shells, like liquid egg products, or boiled eggs [2, 3].

Non-thermal processes might be an excellent alternative to overcome the problems associated to the changes in egg functional properties [4]. Similar to thermal pasteurization, non-thermal pasteurization of eggs is challenging and despite substantial efforts, and none of the non-thermal technologies has been commercialized for liquid egg products, like pulsed electric fields [5], electron beam radiation [6] and gamma radiation, or high hydrostatic pressure [7, 8].

The recent development of alternative processing technologies for food preservation such as high hydrostatic pressure (HHP), offers potential to inactivate microorganisms, reduce loss of essential nutrients, and contribute to the development of novel egg products[8 - 11]. While the mechanism of high pressure-induced protein unfolding is not completely understood, but there is evidence of structural
changes in protein molecules that are distinct from those caused by thermal or chemical treatment. The underlying mechanism of pressure-induced protein denaturation involves water penetration into cavities within the molecule resulting in varying population of molecular conformations [12, 13].

The initial microflora of raw liquid egg consist of diverse Gram-negative and Gram-positive bacteria that originate from the shell, or occasional infected egg, or processing equipment (e.g.: egg breakers, pipes, shell filters), as well as from those who handle eggs. In order to produce ultrapasteurized egg products of good bacteriological quality, it is essential both to use eggs within a few hours of their being laid by dedicated flocks of hens and to pay critical attention to the cleanliness and hygiene of the processing equipment [14, 15].

The aim of the present paper is to study the effect of HHP treatment of liquid egg products (liquid whole egg LWE, liquid egg white LEW and liquid egg yolk LEY) between 200 and 350 MPa. Microbiological changes, color and protein denaturation are inspected.

2. Materials and methods
   2.1. Samples preparing
   Three different liquid egg products were used in our experiment: liquid egg white (LEW), liquid whole egg (LWE) and liquid egg yolk (LEY). All of the samples were taken from production line of Capriovus Ltd (Szigetscép, Hungary) directly after homogenization.

   Samples were stored at refrigerated temperature 2-5°C, a maximum of 24 hours. From every samples 100 mL were packed in polyethylene bags. For every pressure range and for control 3 packages were taken from prepared samples, so altogether 45 bags were prepared for measurements.

   2.2. Methods
   HHP treatments were carried out at 200, 250, 300 and 350 MPa for 5 min holding time, pressure increases were in every case 100 MPa/min, on a RESATO FPU 100-2000 HHP equipment. Treatment and measurements were at Corvinus University of Budapest (new name: Szent István University), Faculty of Food Science, Department of Refrigeration and Livestock Products Technology.

   For color measurement Minolta CR 200 colorimeter was used. From L* (brightness), a* (red-green hue) and b* (yellow-blue hue) values color differences (ΔEab*) were counted to untreated control samples.

   Thermophysical, calorimetric properties were examined on Micro DSC III (differential scanning calorimeter). In each case approximately 580 mg of samples were taken, reference was distilled water. Speed of heating was 1.5 °C/min, temperature of measuring was 95 °C and speed of cooling was 1.5 °C/min, controlled by SetSoft2000. Callisto 7.6 software was used to evaluate DSC thermograms.

   In case of microbiological load of samples mesophilic aerobe cell counts were inspected. As usual, TGA agar and plat clasing were used with an incubation of 30 °C and 48 hours.

   Statistical evaluation of results was carried out by paired t-tests.

3. Results and discussion
   3.1. Color
   Brightness of LWE increased after HHP, higher applied pressures caused higher L* values. By contrast a* and b* decreased effected by increasing pressure. Changes were statistically not significant.

   In case of LEW L* increased, therefore samples after processing became brighter, it can be caused of protein's denaturation, or agglomeration. Values of a* increased and b* decreased, but there were no statistically significant.

   LEY’s color showed a tendency like LWE. Color differences of samples to controls is shown in table 1. Color difference ΔEab* defines how visible differences are between color of objects for human eyes. Called our results there were mostly hardly visible, or visible changes in color of treated samples. Based on the results obtained slight color changes are very prospering for future industrial applications, because visible sensorial attributes may not be influenced by HHP treatment.
Table 1. The color differences ($\Delta E_{ab}^*$) of samples compared to untreated controls and explanation of classification.

| $\Delta E_{ab}^*$ | LWE  | LEW  | LEY  | classification |
|------------------|------|------|------|----------------|
| 200 MPa          | 0.38 | 1.40 | 1.41 | 0 - 0.5        |
| 250 MPa          | 1.04 | 1.73 | 0.38 | 0.5 - 1.5      |
| 300 MPa          | 2.44 | 0.58 | 0.78 | 1.5 - 3        |
| 350 MPa          | 3.51 | 2.09 | 0.52 | 3 - 6          |

3.2 Calorimetric parameters

In case of LEW and LWE two denaturation points were observed, that is shown on figure 1 and figure 2 respectively. Enthalpy of denaturation for LEW and temperature of denaturation were decreasing after HHP treatment and increasing of pressure range in both denaturation points. But there were not statistically significant differences, that means, that there is no, with DSC detectable, protein denaturation caused by HHP to 350 MPa.

Figure 1. Normalized thermogram of LWE after processings and control

Enthalpy of denaturation was decreasing with increasing pressure of processing’s, but the temperature of denaturation showed no interpretable tendency, 3-4°C differences between the samples may be caused by inhomogeneity of LEW. Statistically significant differences were not found.
Thermogram of LEY is shown in figure 3, DSC detected only one denaturation point of LEY samples. The enthalpy of denaturation was decreased by increasing pressure, but the main difference was between untreated control and treated samples. Because of decreased enthalpy can be assumed that during HHP treatment proteins are denatured. Temperature changed only 4-5 °C that is insignificant.

Overall we can be seen that our applied HHP treatments do protein denaturation only in the case of LEY, which is detectable by DSC.
3.3. Microbiological load
Mesophyll aerobe cell count of samples is shown in figure 4. Least decreasing of microbiological load was observed in LWE samples, this was only one log magnitude. In case of LEW and LEY the microbiological inactivation effect of HHP was 3 log magnitude.

Our microbiological results (excepted LEY, however microbiological load is still acceptable for commercialization) are promising than increasing pressure ranges effect increasing microbial inactivation, by LEW samples there was no detectable microbiological load at 300 MPa already.

![Figure 4. Mesophilic aerobic cell count of LWE, LEW and LEY caused by HHP treatment](image)

4. Conclusion
Our results show that HHP treatment of liquid egg products is a prosperous opportunity for preservation. HHP caused no detectable protein denaturation in LEW and LWE between 200 and 350 MPa, which may offer the same techno-functional properties like raw products (e.g. foaming ability). Color of every samples was not significant changed that is very important because of consumer’s choices.

In our study microbiological inactivation effect of HHP is shown in case of egg products. Examined low pressure ranges can effect already 3 log magnitude decreasing in microbiological load of LEW and LEY. Increasing of pressure we can enhance effectivity of HHP treatment and microbiological safe products can be produced.

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