THESIS OF THE DOCTORAL DISSERTATION

Adrienn Tóth

Budapest

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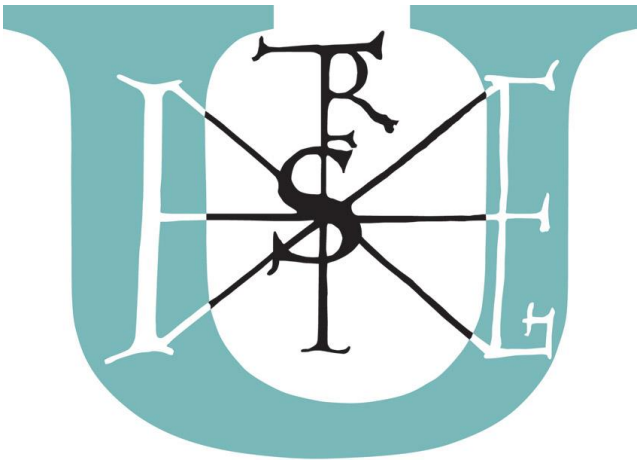

Szent István University

THESIS OF THE DOCTORAL DISSERTATION

INCREASING SHELF-LIFE OF LIQUID EGG PRoDUCTS BY THE APPLICATION OF COMBINED MINIMAL PROCESSING TECHNOLOGIES

Adrienn Tóth

Budapest

2019

Szent István University – Doctorial School of Food Sciences

Doctorial School

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Approval signature of Head of the doctoral school and supervisors:
The candidate has fulfilled all the conditions prescribed by the doctoral school of Szent István University, the comments and suggestion at the thesis workshop were taken into consideration when revising the thesis, so the dissertation can be submitted to a public debate.

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Head of Doctorial School Supervisors

1 INTRODUCTION AND OBJECTIVES

Because the birds preceded man in the evolutionary chain, both eggs and birds have existed longer than historians, but today's healthy eating consumers prefer egg consumption because of its high nutritional value. Other explanations are for the large amount of consumption the taste and techno functional properties of eggs. Moreover, almost all essential amino acids are found in eggs, while eggs are essentially free of carbohydrates.

In addition to its excellent nutritional composition and sensorial properties, its excellent techno-functional properties must be highlighted. In industrial food processing, and more and more often in households, the use of shell eggs is replaced by the use of liquid egg products. However, processing techniques such as heat treatment may negatively affect the techno-functional properties of liquid egg products. Innovative processing technologies are needed to produce foods that suit consumers' needs, as traditional methods often do not guarantee the adequate quality. It is also obvious that traditional methods are combined with new technologies. At the same time, in order to increase shelf life, the treatment parameters must be properly combined so that the treatments cause no, or minimal changes in the product properties and structure. In my dissertation I investigate the effect of minimal processing technologies on liquid egg products for reducing these negative effects. One of the most promising minimal processing technologies is Hgh Hydrostatic Pressure, HHP. In my thesis, the effects of HHP single and combined with heat treatments are investigated. Five attempts were made to find answers to the following questions:

- How does influence HHP between 150 and 600 MPa the sensorial attributes (colour, rheological properties), techno-functional properties (pH, denaturation of proteins) and mesophyll aerobe cell count of liquid egg products?
- How does influence HHP's holding time the sensorial attributes (colour, rheological properties), techno-functional properties (pH, denaturation of proteins) and mesophyll aerobe cell count of liquid egg products, how will change the sensorial characteristic cand texutre of products made from HHP treated liquid egg products?
- How does influence single and combined HHP treatment the sensorial attributes (colour, rheological properties), techno-functional properties (pH, denaturation of proteins) and mesophyll aerobe cell count of liquid egg white?
- How does influence the order of HHP and heat treatment the sensorial attributes (colour, rheological properties, sensorial attributes of omelettes made from liquid egg products), techno-functional

properties (pH, denaturation of proteins) and mesophyll aerobe cell count of liquid egg products? How does influence the order of treatments the shelf-life of liquid egg products?

- How do influence the parameters of HHP and heat treatment sensorial attributes (colour, rheological properties), techno-functional properties (pH, denaturation of proteins) and mesophyll aerobe cell count, foaming ability and stability, emulsifying ability and emulsion stability of liquid egg products?

2 RESULTS

In food industry and catering systems, the use of shell eggs is suppressed by the use of egg products, which have many advantages over shell eggs (e.g. lower labor requirements, simplifying technology, lower investment costs). Liquid egg products (LEP) have the largest market share among egg products. However, after breaking eggs, its content is an excellent medium for microbes. To ensure microbiological food safety, the food industry is currently using heat treatment (pasteurization). However, some valuable components (such as proteins) can be damaged by heat, which affects the further processing of LEP. But gentle (non-thermal) technologies are still not currently used in the industrial processing of LEP.

This problem raised the topic of my doctoral research, in which I investigated the effects of High Hydrostatic Pressure (HHP) technology single and in combination with gentle heat treatment on certain properties of LEP. The aim was to determine which technological parameters (pressure value, holding time, heat treatment temperature and time) are the most suitable for the gentle preservation of LEP based on examined properties.

In my experiments the changes were investigated in the physical, chemical and techno-functional properties of liquid whole egg (LWE), liquid egg white (LEW) and liquid LEY (LEY). My thesis is based on five experiments.

In the first experiment, the effects of the pressure value of HHP were investigated in the pressure range of 150 to 600 MPa with a holding time of 5 minutes. Based on my results, I concluded that the differences in the colour of the LEP were due to the pressure values above 350 MPa. A linear decrease in total mesophilic aerobic cell count was observed between 150 and 450 MPa for LWE, andLEW, and between 150 and 500 MPa for LEY. Higher pressures led to the formation of well visible protein agglomerates and gelling. The use of 550 and 600 MPa for LEW has already rendered the product unsuitable for further processing. Pressure values above 400 MPa in protein structures can already be considered as unfavorable based on DSC results.

Therefore, in the second experiment, the LEP were tested at 400 MPa with a holding time of 1-10 min. For each of the techno-functional properties (viscosity, protein structures), 5-7 minutes is the maximum holding time that does not yet affect the properties tested to become unsuitable for processing. From a microbiological point of view, holding time has less effect than increasing pressure level. An exponential correlation was found between the holding time and the decrease in mesophilic aerobic cell count. So, if microbial reduction is our goal with HHP treatment, I suggest using higher pressures instead of longer holding times. In the experiment I examined the texture and organoleptic properties of products prepared from HHP treated LEP. Based on my results, by

increasing the holding time, I was able to make harder egg products. So, longer HHP treatment is suitable for texture forming. Based on sensory judgments, these changes are favourable for sensory parameters. The changes in texture and organoleptic properties is likely to be due to changes in protein structures.

In the first two experiments, the use of HHP alone did not result a significant reduction in microbial numbers without significant protein denaturation and population change (5 orders of magnitude reduction). Therefore, in the third experiment, I applied heat treatment to LEW before HHP treatment. According to my results, the combination of prolonged heat storage (53 ° C, 6 hours) and HHP (350 MPa, 5 min) significantly influenced the rheological properties of egg white juice, but the combination of pasteurization (57 ° C, 7 min) and HHP was favourable from a microbiological and techno-functional point of view.

In the fourth experiment, I examined the combination of heat and HHP treatment for all LEP. This time, besides the treatment parameters, I also examined the effect of the order. Combined heat and pressure treatments resulted in greater changes in the colour of the egg juices than the treatments applied alone. The order of the treatments is more favourable regarding to the changes of the colour, first by heat and then pressure treatment of LEP. Combining heat and HHP treatment resulted in a reduction of 4 orders of magnitude in total egg juice, 3 orders of magnitude in protein broth, and more than 6 orders of magnitude in yeast reduction in total mesophilic aerobic cell count. In my DSC studies, I found that the combined treatment had a similar effect on the LWE as the 10-minute HHP at 400 MPa. The use of combination treatments over egg white juice is more beneficial than increasing holding time. The order of the treatments does not substantially affect the degree of protein denaturation in the protein broth, but the use of HHP after heat treatment is more advantageous than the yolk broth.

After the combined treatments, the organoleptic properties of the "omelettes" made from egg juices were judged by the panellists to be more favourable than after the simple heat or pressure treatments. According to the reviews, certain organoleptic properties are affected more favourably by HHP treatment after heat treatment than by the reverse treatment sequence.

In the fifth experiment, the effects of a combination of heat and HHP treatment parameters (temperature and pressure) were investigated using a centrally located, rotation experimental design at 53-67 ° C (12 minutes) and 330-470 MPa (5 minutes). After the heat treatment I applied HHP treatment based on the results of the n my n my first two experiments, the use of HHP alone did not

result in a significant reduction in microbial numbers without significant protein denaturation and population change (5 orders of magnitude reduction). Therefore, in the third experiment, I applied heat treatment to egg white juice before HHP treatment. According to my results, the combination of prolonged heat storage (53 ° C, 6 hours) and HHP (350 MPa, 5 min) significantly influenced the rheological properties of egg white juice, but the combination of pasteurization (57 ° C, 7 min) and HHP was favorable from a microbiological and techno-functional point of view.

In the fourth experiment, I examined the combination of heat and HHP treatment for all three egg juices. This time, besides the treatment parameters, I also examined the effect of the order. Combined heat and pressure treatments resulted in greater changes in the colour of the egg juices than the treatments applied alone. The order of the treatments is more favorable with regard to the change of the colour factors, first by heat and then pressure treatment of the egg juices. Combining heat and HHP treatment resulted in a reduction of 4 orders of magnitude in total egg juice, 3 orders of magnitude in protein broth, and more than 6 orders of magnitude in yeast reduction in total mesophilic aerobic cell count. In my DSC studies, I found that the combined treatment had a similar effect on the LWE as the 10-minute HHP at 400 MPa. The use of combination treatments over egg white juice is more beneficial than increasing holding time. The order of the treatments does not substantially affect the degree of protein denaturation in the protein broth, but the use of HHP after heat treatment is more advantageous than the yolk broth.

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Based on my results, different combinations are optimal for each trait and egg juices. For example, the colour and rheological properties of egg white juice were more strongly influenced by higher pressure values, while higher heat treatment temperatures tended to affect more than LWE.

Based on the results presented in my dissertation, the application of high hydrostatic pressure technology at pressures higher than 400 MPa in egg juices may be an appropriate microbiological risk reduction treatment, but at lower pressures it can be considered as a process for the formation of

stock and organoleptic properties. If the goal is technological preservation, microbial reduction, we need to combine HHP treatment with other technology (heat treatment).

Based on my results, the treatments and parameters applied affect the examined properties of egg juices in different ways. Depending on the properties tested, different pressure treatment parameters or treatment combinations used alone are most advantageous. I do not think we need to choose a "relatively favorable" parameter or combination of parameters, but to use the optimal parameter (s) for the property that best suits the research or industrial purpose.first two experiments, the use of HHP alone did not result in a significant reduction in microbial numbers without significant protein denaturation and population change (5 orders of magnitude reduction). Therefore, in the third experiment, I applied heat treatment to egg white juice before HHP treatment. According to my results, the combination of prolonged heat storage (53 ° C, 6 hours) and HHP (350 MPa, 5 min) significantly influenced the rheological properties of egg white juice, but the combination of pasteurization (57 ° C, 7 min) and HHP was favorable from a microbiological and techno-functional point of view.

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Based on my results, the treatments and parameters applied affect the examined properties of egg juices in different ways. Depending on the properties tested, different pressure treatment parameters or treatment combinations used alone are most advantageous. I do not think we need to choose a "relatively favorable" parameter or combination of parameters, but to use the optimal parameter (s) for the property that best suits the research or industrial purpose.previous experiment.

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Based on my results, the treatments and parameters applied affect the examined properties of egg juices in different ways. Depending on the properties tested, different pressure treatment parameters or treatment combinations used alone are most advantageous. I do not think we need to choose a "relatively favorable" parameter or combination of parameters, but to use the optimal parameter (s) for the property that best suits the research or industrial purpose.

2.1 NEW SCIENTIFIC RESULTS

1) *During the 5-minute HHP treatment of LWE and LEW the mesophilic aerobic cell count decreased linearly with increasing pressure in the range of 150-450 MPa, while in the case of LEY 150-500 MPa. I found that in the case of LWE, LEW and LEY, the reduction of the total aerobic cell count by 4 orders of magnitude can be achieved by applying a 450 MPa, 5-minute HHP treatment.*
2) *I found that in case of LWE (300 MPa, 5 min) and LEW (400 MPa, 5 min) decreased total mesophilic aerobic cell count by 2 and 4 orders of magnitude, respectively, without significant denaturation of protein denaturation and apparent viscosity. In the case of LEY, HHP treatment (350 MPa, 5 min) resulted a reduction in total cell count 3 orders of magnitude without a significant change in apparent viscosity.*
3) *In the case of LEP an exponentially decreasing correlation was found between the holding time and the mesophilic aerobic total cell count in the 0 - 10 minutes holding time at 400 MPa.*
4) *For LEW, the combination of 57 ° C, 7 minutes heat treatment and 5 minutes HHP treatment at 350 MPa provides 4 orders of magnitude reduction of mesophilic aerobic cell count without significant protein denaturation, colour and apparent viscosity change.*
5) *I found that pressure level has a greater effect than holding time of HHP applied in the ratio of protein denaturation in LEP, although denaturation of LEP starts at different pressure levels (LWE> 300 MPa, LEW> 450 MPa, LEY > 150 MPa).*
6) *It was found that the apparent viscosity of egg juices increases significantly after HHP of 5 minutes at 400 MPa and 400 MPa at 5 minutes.*
7) *Brightening of LEP is observed after HHP treatment. Significant colour changes begin at a pressure of 450 MPa in LWE, above 250 MPa in LEW and above 350 MPa in LEY applying 5 minutes holding time.*
8) *I found that the combination of 350 MPa 5 min HHP treatment and 53 ° C 12 min heat treatment decreases the mesophilic aerobic cell count of LEP without significant protein denaturation and brightening. The combination of high hydrostatic pressure and heat treatment, regardless of order, reduces the mesophilic aerobic cell count of LWE. In the case of LEW and LEY, the order of the treatments from a microbiological point of view is preferable first application of heat treatment and second HHP.*

9) *It was found that the rotated central composite experiment design is a helpful tool in modelling of combinations of heat treatment and HHP (53-67 ° C, 12 minutes and 330-470 MPa, 5 minutes) applying the experimental design, b*, apparent viscosity of LWE, L* and mesophyll aerobe cell count of LEY and pH-value, L*, a*, b*, apparent viscosity and mesophyll aerobe cell count of LEW can be predicted.*

3 CONCLUSIONS AND RECOMENDATIONS

In my doctoral dissertation I investigated the effects of high hydrostatic pressure technology alone and in combination with heat treatment on the physical, chemical, microbiological, organoleptic and techno-functional properties of LEP.

LEP lighten with HHP treatment. Overall, the pressure value has a greater effect than the holding time. Significant colour changes begin at a pressure of 450 MPa in the LWE, above 250 MPa in the LEY and above 350 MPa in the b* when treated for 5 minutes. Combined heat and pressure treatment results greater changes than treatment alone. The order of the treatments regarding the change of colour factors is better by first treating the eggs with heat and then pressure (53 ° C, 12 min or 45 °C, 8 h; 350 MPa, 5 min). If preserving the colour of egg juices is an important consideration in the quality of the finished product, I recommend that you use HHP alone at a lower pressure and with a longer holding time.

From a microbiological point of view, increasing the pressure of HHP has a greater effect than increasing the duration of treatment. I found that in the case of LWE, LEW and LEY, the reduction of the total aerobic cell count by 4 orders of magnitude can be achieved by applying a 5 minute high hydrostatic treatment at 450 MPa. During the 5-minute HHP treatment of egg juices, total egg and LEY decreases linearly in the total pressure range of 150-450 MPa, while in the case of LEY, 150-500 MPa increases in pressure. An exponential relationship was found between the holding time and the total mesophilic aerobic cell count in the 0 - 10 minutes holding time at 400 MPa pressure.

The combination of high hydrostatic pressure and heat treatment, regardless of order, reduces the mesophilic aerobic cell count of LWE. In the case of LEW and LEY, the order of treatments is preferable if heat and pressure treatment are used first. Combining the heat and HHP treatments, I achieved 4 orders of magnitude reduction in the total egg juice, 2.5 and a more than 6 orders of magnitude in total mesophilic aerobic cell count. Based on this, it has been found that from a microbiological point of view it is advantageous to perform pressure treatment alone of at least 450 (LWE and LEW) or 500 MPa (LEY). If the preservation of techno-functional properties is an important consideration in addition to reducing the microbial count, it is advisable to combine pressure treatment at pressures of less than 450 MPa with heat treatment.

According to my rheological studies, increasing the pressure value and the holding time both increase the apparent viscosity of the LEP. LWE behaves like a pseudoplastic fluid when the two parameters are changed. In the case of LWE, in all treatments alone or in combination with heat treatment I experienced an increase in the yield point and consistency constant, while the HHP treatments applied alone caused a decrease in the flow index. In contrast, LEY tends to be more pseudoplastic with

pressure increase, whereas with increased holding time and combined heat and pressure treatments, it behaves more like a Herschel-Bulkley fluid. The yield limit and consistency constant of LEY was increased by each treatment applied, while its flow index was decreased.

LEY behaves as a shear-thickening fluid when the pressure is increased, whereas the LEY acts as a pseudoplastic fluid when the holding time is increased. As protein pressure increases, pressure and holding time increase τ_0 and K, while n decreases. A pressure treatment of 550 and 600 MPa for 5 minutes results in aggregate formation such that the LEY cannot be tested by rotation. As a result of the combined treatments, the LEY is characterized as pseudoplastic or Herschel-Bulkley fluid. Overall, the apparent viscosity of total protein and LEY increases with HHP treatment. Significant change in stock occurs with 5-minute treatment above 400 MPa and 400 MPa with treatment longer than 5 minutes.

Based on the centralized, rotational experimental design, the combination of heat and pressure treatment, similarly to the higher pressure HHP treatments used alone, resulted in the formation of gel structures (LWE, LEY) and aggregates (LEY). The gelation of LEY was primarily caused by an increase in the pressure value, especially at pressures greater than 400 MPa, as opposed to an increase in the heat treatment temperature. The combination of heat treatments at temperatures higher than 60 ° C with a HHP pressure of at least 400 MPa resulted in the formation of the largest amount of aggregate in the protein. The higher temperature (65 and 67 ° C) of the LWE resulted in higher aggregate formation.

Based on this, I concluded that it is worth treating egg juices for a longer period at a lower pressure, which has little effect on their rheological properties. When combining HHP with heat, it is advisable to choose a temperature lower than 60 ° C (even with higher pressure values) for the LWE. While a pressure of up to 400 MPa (even at higher temperatures) is preferred for LEW and LEY.

Comparing the effects of pressure and holding time, I found that increasing holding time resulted a smaller decrease in denaturation enthalpy values. For LWE, 600 MPa, 5 minutes treatment denatures about 60% of the protein, while 400 MPa, 10 minutes treatment only 12%. For protein lene, the same treatments caused about 75% and 35% reductions in denatured proteins. In the case of yolk, 600 MPa, 5 minutes treatment resulted in a 66% decrease, while 400 MPa, 10 minutes treatment resulted in a 30% reduction in ΔH.

Compared to the HHP treatment alone, the combined treatments denaturated the LWE to a lesser extent than the increase in pressure (about 60% decrease), but with the same increase in holding time, I experienced almost the same decrease in ΔH (12-15%). The order of the treatments has no effect on the degree of protein denaturation.

The omelette and meringue samples made from pressurized egg juices have a harder texture than untreated egg juices. The pressure holding time has clearly increased the hardness of the finished product. Thus, the time of pressure treatment has a flocking effect on the finished product, not only on the rheological properties of the raw egg juices.

The organoleptic properties of the finished products made from pressure-treated samples ("omelette" and meringue) were considered more favourable by the critics. HHP treatment alone or in combination with heat treatment has a positive effect on the organoleptic quality of the finished product. Samples that have undergone a longer pressure treatment received more favourable scores for most of the criterions. Sensory tests confirmed the results of instrumental measurements. Following the combined treatments, the "omelettes" were perceived by the judges to be more organoleptic than the single heat or pressure treatments. According to the reviews, HHP treatment after heat treatment has a more favourable effect on the individual organoleptic properties.

As a summary of my results, I found that the treatments I applied affect the examined properties of egg juices in different ways. Different pressure treatment parameters or treatment combinations used alone are the best for each of the properties tested. Based on this, I suggest that we do not choose a "relatively favourable" parameter or a combination of parameters, but rather that which is best suited for the research or industrial purpose.

4 PUBLICATIONS RELATED TO THE THESIS

4.1 PUBLICATIONS WITH IMPACT FACTOR

1) **Tóth Adrienn**, Németh Csaba, Jónás Gábor, Zeke Ildikó, Csehi Barbara, Salamon Bertold, Fehér Orsolya, Surányi József, Póti Péter. 2016. "A Tojástermékek Tartósításának Fejlődése Az Elmúlt 25 Évben." Magyar Állatorvosok Lapja 138 (8): 495–502.
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4) **Tóth Adrienn**, Németh Csaba, Tóth Kálmán, Hidas Karina, Ayari Emna, Póti Péter, Pajor Ferenc, Friedrich László (2019). Heat treatment of fermented, spreadable products from egg white. Journal of Hygienic Engineering and Design, Vol. 29, pp. 166-171.
5) Csehi B, Szerdahelyi E, Pásztor-Huszár K, Salamon B, **Tóth A**, Zeke I, Jónás G, Friedrich L Changes of protein profiles in pork and beef meat caused by high hydrostatic pressure treatment ACTA ALIMENTARIA HUNGARICA 45:(4) Pp. 565-571. (2016) IF:0,333
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1) **Tóth Adrienn**, Németh Csaba, Ayari Emna, Pásztorné Huszár Klára, Zeke Ildikó, Hidas Karina, Friedrich László. 2019. "Effects of Minimal Processing and Vitamin C Enrichment on Microbiological Safety and Viscosity of Liquid Egg White." Journal of Engineering & Processing Management 11 (1): 46–50.

2) **Tóth Adrienn**, Palotás Péter, Németh Csaba, Csehi Barbara, Louis Argüello Castillo, Friedrich László, Balla Csaba, Póti Péter Increasing shelf life of fish through high hydrostatic pressure treatment Journal Of Hygienic Engineering And Design 12: pp. 118-122. (2015)
3) **Tóth Adrienn**, Friedrich László, Jónás Gábor, Salamon Bertold, Németh Csaba. 2015b. "Frissen Préselt Narancslé Eltarthatóságának Növelése HHP Technológia Alkalmazásával." Ipari Ökológia 1: 23–35
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