



HUNGARIAN UNIVERSITY OF  
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# **Use of minimal processing technologies in extending shelf-life of egg products**

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The candidate has fulfilled all the conditions prescribed by the Doctoral School of Hungarian University of Agriculture and life sciences, 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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## **Introduction and aims**

Throughout ages, poultry and its products have been one of the important sources of proteins. Their uses and its conservation methods evolved by time to respect its vulnerability as it easily perishable.

The sensitivity of poultry and its products comes from the high level and quality of proteins. Besides the protein value of the whole egg protein which is advised to be 100 and considering the high value of egg proteins, it is considered as standard for measuring nutritional quality of food proteins.

In fact, egg takes a huge part of human diet because of its rich chemical composition of minerals, vitamins, fats, and it boast all the essential amino acids that human body needs. This richness not only enforce nutritional and sensory characteristics, but it emphasizes the functional properties also. These functional qualities remain the main core for food industries. Emulsifying, gelling, coloring, aromatic, and antioxidant properties are the main functional properties spotted in eggs and its products. However, various treatment was applied by food industries to ensure the microbiological safety of egg products although these treatments could be harmful to some properties mainly the functional ones.

Egg products are effortlessly deteriorated during refrigeration storage, making both of consumers and of producers facing difficulties and health issues. According to the literature, many spoilages of food can occur such as physicochemical changes, growth of pathogenic microorganism, and alteration of organoleptic properties. These qualities are correlated with shelf life of egg products and their alteration indicate that the products are not consumable anymore and decrease the period of the shelf life.

The main objective of this study was to highlight the use of active compounds such as acids and bioactive peptides using the minimal processing technologies simultaneously to extend the shelf life and improving the functional properties of refrigerated liquid egg products.

In the first part of the research, heat treatment of egg products with a preliminary addition of acids has been carried out. As it has been mentioned by (Ponce et al. 1998) that the effect of nisin can be enhanced by its synergism with lysozyme, the second part of the work focused on the effect performed by nisin and lysozyme accompanied with high hydrostatic pressure as a minimal process.

The main interests of the study are:

- To investigate the effect of heat treatment on liquid egg products with citric acid and calcium sorbate during storage time on physicochemical, microbiological, and sensory properties of liquid egg products.
- To investigate the effect of heat treatment on liquid whole egg with citric acid or lactic acid during storage time on rheological, physicochemical, microbiological, and sensory properties of liquid egg products.
- To illustrate the effects of HHP treatment on liquid egg products with nisin and lysozyme during storage time on rheological, physicochemical, microbiological, and sensory properties of liquid egg products.

## **Materials and methods**

For all the thesis experiments, the raw liquid egg was supplied from production line of Capriovus Ltd (Szigetcsép, Hungary). To produce different liquid egg, shell whole egg from caged laying hens were disinfected then passed by the breaker-separator egg machine. Three products were generated: raw liquid egg white, raw liquid egg yolk, and raw liquid whole egg. The egg products were directly sent to homogenization in a piston-gap homogenizer at 100 bars.

For the first investigation, final liquid egg products coming from Capriovus Ltd were used. According to the nature of the product, the heat temperature parameter was adjusted. It was regulated to 70 °C, 56 °C, and 67 °C for whole, white and yolk liquid egg respectively with a holding time of 190 seconds (3 minutes approximately). Before pasteurization, citric acid 0.5% and calcium sorbate 0.3% were added to the various products as additives. The samples were stored at 4 °C ± 2 °C in the

refrigerator room. LEW was pasteurized at 65 °C for 10 min for one-time pasteurization and for 20 min for two-time pasteurization. The products then cooled down and transported at 4°C the department. The samples were stored at 4°C for 21 days. The applied measurements are physicochemical (pH, Color), microbiological, foamability for LEW and emulsion for LEY.

In second investigation, liquid raw egg was poured in a big 1000 ml beaker, pH was measured then according to the pH target value, different volume of acidic solution was added. Citric acid, lactic acid, acetic acid, and ascorbic acids were used with 20% concentration each. The target pH values were 5.0, 5.5, 6.0, 6.5 and 7.0. After reaching the appropriate pH, the samples were packed in polyethylene bags. During the hot sealing of the bags, the air was eliminated as much as possible. After the addition of acid to LEW, and sealing the bags, the water bath was preheated at 70 °C. The samples were emerged in the water with a control. The heat treatment lasted for 3 minutes approximately. At the end of the treatment, the samples were directly placed in a sink full of ice to stop any further heat treatment. The moment the samples were cooled down they were transferred to the refrigeration room to be stored for 15 days at  $4 \pm 0.5$  °C. The applied measurements are physicochemical (pH, Color), microbiological, sensorial, and rheological.

Finally, in the third investigation, before HHP treatment the liquid egg products were divided to batches, and for each batch an exact weighted amount of nisin and lysozyme were added. The additives were weighted on glass dishes using a precision balance. The quantity of additives and HHP pressure treatment are defined by a central composite design. After adding the nisin and lysozyme to liquid egg, the sample was mixed with a stainless-steel wood until it was completely dissolved, and no debris remained in the bottom of beakers. Subsequently, the batch of liquid egg with nisin and lysozyme was divided into small quantities by pouring out into polyethylene bags. During the hot sealing of the bags, the air was eliminated as much as possible. The applied measurements are physicochemical (pH, Color),

microbiological, rheological, protein denaturation by DSC, and foamability for LEW.

## **Results and discussion**

### First investigation

Both, one- and two-time pasteurized liquid whole egg showed significant difference of pH values. Results showed that a simple pasteurization was sufficient to maintain the bright yellow color of liquid whole egg. Throughout conservation, one-time pasteurized liquid whole egg showed increasing values while two-time pasteurized liquid whole egg did not only showed lower total plate count, but it retained the microbial growth around 2 Log CFU/ml.

Heat treatment combined with 0.5% citric acid and 0.03% calcium sorbate significantly decreased pH values of liquid whole egg and with this decrease it reduced the microbiological load with 2 log CFU/ml compared to raw liquid whole egg and prevented its growth by maintaining approximately same microbiological load for 21 days, but some discoloration occurred even if they enhanced lightness of liquid whole egg. Thus, the effect of the same amount of citric acid and calcium sorbate is more noticeable on pH values, and on the microbial growth of liquid egg white by decreasing it by 3 log CFU/ml comparing to the control. Additionally, it enhanced the color properties of liquid whole egg and its foaming ability by 35% comparing to raw. Like for other liquid egg products, the combination of heat treatment with 0.5% citric acid and 0.3% calcium sorbate diminished the pH values of liquid egg yolk, enhanced its a\* and b\* values and stabilized its lightness and the color difference between the treated sample and the raw could be noticed by unaided eye. The additives decreased the microbial load by 3 log CFU/ml comparing to the control but during the storage time it decreased and the difference between the treated and non-treated samples was only 1 log CFU/ml in the last day of storage.

## Second Investigation

For heat treated liquid egg products combined with different acids and different starting pH values, it could be noticed that the pH values of liquid whole egg were the least affected ones by acids (citric acid, lactic acid, acetic acid and ascorbic acid 20%) compared to other liquid egg products. For the same acid, acetic acid 20% had the lowest effect on liquid egg products. Combination of heat treatment with citric acid or lactic acid stabilized pH values of samples of liquid whole egg with different starting pHs. Microbial load of treated samples with citric acid reduced the initial microbial load by 2 log CFU/ml for all different starting pH values. Lactic acid reduced the same microbiological load for samples with starting pH value between 6-7 while for samples with low starting pH values (5 - 5.5) it reduced the initial microbiological load by 3 Log CFU/ml. Samples with low starting pH values had discoloration, but it enhanced the color parameters for other starting pH value samples and caused some changes in flow behavior. In sensory analysis, both samples had a favorable ranking comparing to other acids. For heat treated samples with acetic acid or ascorbic acid pH values of samples of liquid whole egg with different starting pH were stabilized after the treatment, and the presence of both acids enhanced the color of liquid whole egg, but samples with low starting pH value greening effect of heat treatment occurred with a change in flow behavior.

## Third investigation

Different pressure of HHP treatment and different concentrations of nisin and lysozyme influenced pH values of liquid whole egg, in the beginning it stabilized pH values of liquid whole egg during 3 weeks for high HHP pressure treated samples, while for lower pressure started to decrease after 2 weeks but it prevented the discoloration. High HHP pressure (350 MPa – 435 MPa) in presence of 3 mg nisin and 1mg lysozyme significantly decreased microbiological population but 435 MPa pressure led to high apparent viscosity. Pressure of 300 MPa and 5 mg nisin

and 1.5 mg of lysozyme showed low microbiological load and its growth was minimal during storage. The combined treatment also stabilized the pH values of liquid whole egg during 3 weeks for high HHP pressure treated samples while for lower pressure values it started to decrease after 2 weeks. Effect of different pressure of HHP treatment with nisin and lysozyme was noticed directly after the treatment. During storage, the effect vanished. Sample treated with 350 MPa HHP pressure in presence of 3 mg nisin and 1 mg lysozyme had minimal microbial growth and less discoloration for 3 weeks of storage, but it had the highest apparent viscosity. Contrary to other liquid egg products, high HHP pressure treated samples of liquid egg yolk in presence of nisin and lysozyme had stable pH value during refrigeration while pH value of other samples started to decrease after 7 days. Like liquid whole egg, effect of different pressure of HHP treatment, nisin and lysozyme on liquid egg yolk samples was noticed directly after the treatment but during storage the effect vanished.

### **Conclusion and recommendations**

Heat treatment combined with 0.5% citric acid and 0.03% calcium sorbate increased the shelf-life of liquid egg products by decreasing the mesophilic total plate count around 2 log CFU/ml for LWE, around 3 Log CFU/ml for liquid egg white and liquid egg yolk during 21 storage days. Simultaneously, the treatment increased the foam ability by 35%, foam expansion by 43%, and foam stability by 4% of LEW.

Heat treatment in presence of citric acid 20% and lactic acid 20% and different starting pH values increased the shelf life of liquid whole egg two weeks by increasing the mesophilic total plate count. In fact, presence of citric acid during the treatment decreases the initial load by 2 Log CFU/ml and the presence of lactic acid and low starting pH values (5-5.5) decreased the initial mesophilic total plate count by 3 Log CFU/ml. It was important to illustrate the sensory profile of the samples to perceive consumer opinion. Accordingly, consumers agreed that the color of muffins made with raw egg was unpleasant where for the muffins made with liquid whole



containing citric acid and lactic acid with low starting pH were considered pleasant. Sample with starting pH value 6.5 was the most pleasant sample for the panelist for its smell, texture, and taste but for color pH 6 was the favorable one for them. Consequently, according to ranking by consumers, egg muffins made liquid whole egg with starting pH value 6.5 in presence of citric or lactic acid were the favorable ones. For samples with low starting pH values (5,5.5, and 6), the flow behavior of the samples changed from pseudoplastic to dilatant behavior.

In general, different HHP pressure had a significant effect on liquid egg products compared to the different quantities of nisin and lysozyme. HHP treatment of liquid whole egg in presence of nisin and lysozyme stabilized pH value and preserve the color of liquid egg. In the other hand, the thermograms shows only one peak which can explain by the denaturation of protein during the treatment. The treatment had a significant impact on mesophilic total plate count of liquid whole egg where lowest microbial growth was noticed in high HHP pressure (400-435MPa) treated samples and the highest one in low HHP pressure (226 MPa).

For liquid egg white, the treatment enhanced the color and prevent the discoloration during the storage time. The thermograms of the samples showed two peaks, the first one suggests the presence of ovo-transferrin and the second one suggest ovalbumin. The treatment had significant impact on the mesophilic total plate count but on the second week only the different HHP pressure had significant impact on the mesophilic total plate count. Significant impact of HHP treatment with nisin and lysozyme was also observed in yield stress of sample treated with low HHP pressure.

Similar to liquid egg white, the HHP treatment in presence of nisin and lysozyme enhanced the color and prevent the greenish effect on liquid egg yolk. The effect of treatment was significant only after the treatment, but the mesophilic total plate count stabilized starting from second week. The treatment significantly affected the mesophilic total plate count of the three samples treated with different HHP pressures but with the same quantity of additives (3mg of nisin and 1mg of

lysozyme). Thermograms of liquid egg yolk illustrated one peak with denaturation temperature around 70-76 °C which may suggest the presence LDL or/and livetins proteins.

To resume, HHP treatment and addition of additives (citric acid with calcium sorbate, citric acid, and lactic acid) reduced microbiological load of liquid egg products but unfortunately, they had some inconvenient effects. For this reason, it is recommended to study the effects of 300-350 MPa pressure of HHP treatment combined with nisin and lysozyme or citric acid or lactic acid since they had a positive impact to sensory qualities according to the panelist.

## New scientific results

1. Pasteurization (65 °C for 10min) and double pasteurization (65 °C for 20 min) had a significant effect on pH values of liquid whole egg during storage. Double pasteurization did not prevent color change (greenish effect) of heat treatment and altered the yellow color comparing of single pasteurization of samples. Double pasteurization showed a stable microbiological load (2 log CFU/ml) for 3 weeks storage comparing to single pasteurization.
2. Combination of heat treatment (70 °C for 3min) with 0.5% citric acid and 0.3% calcium sorbate significantly decreased pH values of liquid whole egg compared to the control which led to the decrease of the microbiological load with 2 log CFU/ml compared to raw liquid whole egg, and it showed a stable microbiological load ( $\approx 3$ log CFU/ml) for 3 weeks storage. The presence of 0.5% citric acid and 0.3% calcium sorbate enhanced the lightness and yellow color of LWE, but it did not prevent the greening caused by heat treatment.
3. Combination of heat treatment (56 °C for 3min) with 0.5% citric acid and 0.3 % reduced pH values of liquid egg white from 9.3 to 5.7 enabling the decrease of microbiological population by 3 log CFU/ml comparing to the raw liquid egg white. Presence of 0.5% citric acid and 0.3% calcium sorbate enhanced color parameters of liquid egg white and improved its foaming ability 35% compared to the control.
4. Combination of heat treatment (67 °C for 3 min) with 0.5% citric acid and 0.3% of calcium sorbate reduced pH values of liquid egg yolk significantly compared to the raw product. This concentration of citric acid and calcium sorbate significantly decreased microbiological load of liquid egg yolk from 5 log CFU/ml to 1 log CFU/ml and enhanced its yellow color, stabilized its L\* and a\* values.
5. Combination of heat treatment with citric acid or lactic acid stabilized pH values of samples of liquid whole egg with different starting pH for two weeks storage. Presence of citric and lactic significantly reduced the mesophilic total plate count

of liquid whole egg minimum by approximately 2 log CFU/ml. Samples with low starting pH value (5-6) had discoloration but it enhanced the color parameters (lightness and yellow color) and caused changes in flow behavior from pseudoplastic to dilatant behavior. Besides, samples with citric acid and lactic acid had a favorable ranking for sensorial parameters comparing to other acids.

6. Combination of HHP treatment with nisin and lysozyme stabilized pH values of liquid whole egg during 3 weeks in case of high pressure (350-435 MPa) treated samples while in case of lower pressure (226-300 MPa) treatments it started to decrease after 2 weeks. High HHP pressure (350-435 MPa) in presence of 3 mg of nisin and 1 mg of lysozyme significantly decreased the microbiological population but 435 MPa pressure led to high apparent viscosity. Pressure of 300 MPa and 5 mg of nisin and 1.5 mg of lysozyme showed low microbiological load and its growth was minimal during storage.
7. Combination of HHP treatment with nisin and lysozyme stabilized pH values of liquid egg white during 3 weeks for high HHP pressure while for lower pressure start to decrease after 2 weeks. Sample treated with HHP pressure 350MPa in presence of 3 mg of nisin and 1 mg lysozyme had the minimal microbiological growth and less discoloration for 3 weeks of storage.
8. Combination of HHP treatment with nisin and lysozyme had a significant effect on pH value of liquid egg yolk where high HHP pressure samples had stable pH value during refrigeration while pH value of other samples started to decrease just after one week. Sample treated with HHP pressure 350 MPa in presence of 3 mg of nisin and 1 mg lysozyme showed minimal microbiological growth, but it had the highest apparent viscosity.

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