Thesis book

Low temperature heat treatment of liquid eggs

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1. BECKGROUND AND AIMS OF THE WORK

Today's modern egg-processing plants have developed several pasteurization technologies. In all cases, two problems must be borne in mind: destroying as many pathogens and spoilage bacteria as possible and simultaneously keeping the essential components of the egg from harm.

By and large, in practice, pasteurization methods have spread in which liquid egg is subjected to heat treatment (periodically or continuously), and the number of living cells is reduced by a few minutes of heat treatment. Following pasteurization and packaging, liquid egg is refrigerated or dried to a powder, a process which ensures that the liquid egg product reaches the consumer in a condition which meets the food safety standards.

Competition in the market demands longer shelf-life for refrigerated liquid egg products. However, to achieve this, more efficient processing measures are called for. One possible solution is warm storage of liquid egg at low temperatures (55°C or less) for a long time (more than 6 hours). If packaged liquid eggs are heat-treated, the danger of post-heat-treat infection is obviated. On the other hand, there is the factor of warm up time to be reckoned with, which means that due to heat shock, bacteria may develop a higher tolerance to heat.

Further, an important question which needs more examination is whether warm storage is possible with pasteurized liquid egg products. Since the heat resistance of microbes in liquid egg is increased by the effects of pasteurization, previously pasteurized and packaged liquid eggs cannot be heat-treated.

It would simplify the application of the technology even further if we could treat egg white, yolk, and whole liquid egg under similar conditions. That is why it is worth examining the variations in lethality of microbes in the various egg preparations.

It is well known that liquid egg products can be stored considerably longer when preservatives are added. But we know little about what happens to the heat stability of their heat-sensitive components, if prior to packaging citric acid, sodium benzoate, or potassium sorbate are added to these egg products. Usually preservatives are mixed into previously heat-treated liquid eggs.

In addition to microbiological stability, the sensory and functional characteristics of the products must be considered, not to mention the foaming ability of egg white and its ability to maintain foam volume.

With all of this in mind, I have searched for answers to the following questions:

1. Can liquid egg products be microbiologically stabilized at limiting temperature (i. e., at the temperature just before egg-white is denatured), using long-term heat-treatment?
2. Does pasteurization prior to heat-treatment increase the risk of food safety hazards?
3. Is there a significant effect on the heat resistance of *Salmonella* spp. during heat-treatment, when using samples of egg white, yolk, and liquid whole egg?
4. Liquid whole egg is used in the largest quantities in liquid egg products. How does the D-value of *Salmonella* Enteritidis, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* change in liquid whole egg as a function of heating time and temperature of heat-treatment?
5. Do the various methods of preservation influence foaming ability and foam stability of egg white?
6. Is it possible to detect with DSC or NIR methods calorimetric or structural changes in egg white by a long term heat treatment at 50 – 55°C?
7. If preservatives are added to raw liquid egg prior to heat-treatment, do they affect the heat stability of heat-sensitive components?

2. MATERIALS AND METHODS

2.1. Influence of pasteurization on the decimal reduction time of Salmonella

Samples of unpasteurized whole liquid egg, liquid egg white, and liquid egg yolk containing 10^2-10^3 CFU/ml of living cells were inoculated with *Salmonella* spp. in the amounts of 10^6-10^7 CFU/ml and examined after 24 hours' heat storage at 55°C to determine the reduction of living cells with both pre-pasteurization and without.

Detailed studies were carried out regarding destruction of bacteria by heat. In the course of the measurements, I used a *Salmonella* isolate derived from egg products acquired from abroad, as well as isolates of *Salmonella enterica subsp. enterica, serotype* Enteritidis of the NCAIM B2052 strain.

An inoculate was derived by incubating *Salmonealla sp*. for 24 hours in a meat broth agar medium, and mixing it with sterile water such that I took two drops from the breeding medium and placed them in two containers each with 10ml sterile water. Next, these were inoculated with 1-1 ml of liquid egg samples and 100-100 ml peptone diluent, respectively. The remaining materials were subjected to similar experiments, and work was performed with 10^6-10^7 CFU/ml of new germs. The inoculation of the microbes on the previously described liquid egg samples took place at 4°C, after which the treated samples, triplicates of liquid egg

white, liquid yolk, and whole liquid egg, and 100 ml of peptone diluent were placed in a thermostat set at 55°C air temperature. Following this, the initial viable cell count was determined by means of alkalising drip on growth medium agar. Viable cell count was taken every three hours. We worked on each of the three samples in parallel for each alkalization. In the case of results below 10^3 CFU/ml, I examined the material on XLD, BPL, and *Salmonella*-selective Bizmut-sulfite medium, to find out whether the effects were caused by *Salmonella spp.* or some kind of background impurities.

Pre-pasteurization was applied on a few samples in order to induce possible changes in heat-resistance. Following inoculation, the egg products and peptone diluent controls were submerged in water bath as a heat treatment (the temperature of the samples was higher than 50 °C for 12 minutes and at 58 °C for a minimum of 7 minutes). Then the samples were cooled with tap water to 20°C room temperature. Next, samples were stored for 30 minutes in a refrigerator and placed in the 55 °C thermostat, as was done with the other ones. Determination of viable cell count was performed in the same way as with the non-heat-treated samples. We compared the rate of thermal death of the given microbes caused by heat shock, that is to say, in relation to decimal reduction time without heat shock.

2.2. Heat resistance of Salmonella Enteritidis, Escherichia coli, Listeria monocytogenes and Staphylococcus aureus during the heating period and in terms of the temperature during treatment.

Whole liquid egg samples were used for the measurements. Samples were artificially inoculated with fresh preparations of *Escherichia coli*, *Salmonella* Enteritidis, *Listeria monocytogenes, and Staphylococcus aureus.*

L. monocytogenes was refrigerated on Brain Heart agar, whereas the other bacteria were stored cool on Nutrient agar at 4°C until they were used. For the measurements, the given strain was used, and it was allowed to multiply at 37°C for 24 hours on agar. An inoculate was prepared with bacteria kept for 24 hours on Brain Heart/Nutrient agar with sterile water, and the bacteria contained a concentration of approximately 10^8-10^9 cells per ml. I inoculated the previously well-homogenized liquid egg samples in 100 ml quantities.

The Central Composite Design system (CCD) was used in the experiment. Response Surface Methodology (RSM) was the analytical method used for certain variables (rate of heating-up, temperature to maintain heat) and for studying the effects occurring during decimal reduction time (D-value). The main advantage of this type of experimental approach is that fewer

experiments are needed in order to achieve statistically acceptable results. I used the approach with a response surface acquired from second-order polynomials.

After reaching the given temperature, samples were kept at that treatment temperature for 20 minutes. Samples were taken immediately upon reaching the treatment temperature and at five-minute intervals thereafter. I prepared a decimating-alkalizing series with sterile water and then determined microbe counts of the samples with Brain Heart/Nutrient agar plate pouring. Plates were incubated at 37°C for 48 hours, and then the number of colonies was determined with a colony counter.

2.3. Egg white foams: a study of their stability

The base material consisted of four types of egg white products: raw liquid egg white, rehydrated egg white powder, low-temperature heat-treated liquid egg white, and pasteurized liquid egg white. 12 foams were prepared using egg white products and sweeteners (sucrose, fructo-oligosaccharide syrup, Isosweet) by means of a hand egg-beater. This material was examined for rheological characteristics, foam stability, quantity and volume, and sensory features.

For the rheological studies, a Physica MCR 51 (Anton Paar Hungary) rotational viscometer CC 27 (with a 27 mm radius cylinder body) and an ST24-2V-2V-2D measuring head were used. I set the rotation at 100-1000 1/second shear rate at a temperature of 20°C. Twenty points were added at each rotation, and the measurement period was 80 seconds.

The freshly made foams were placed in 50 ml measurement cylinders and kept at room temperature for 18 days. The amount of liquid which collected at the bottom of the cylinders was checked every day. A “liquid seepage” curve was set up for certain products, dependent on the storage time. The texture of the foams was described with a Brookfield LFRA Texture Analyser. I used the TexturePro Lite v1.1 Build 4 software to input the data and analyse the texture profile.

Prior to the examination, samples were once again prepared and after being refrigerated, the material was spread on wafers. A 23-member panel of tasters scored based on a 100-point system. The maximum scores allowed for foam texture and taste were 40, whereas smell and general impression could receive no more than 10 scores each.

2.4. ***Checking changes occurred at 50, 55 and 60°C refrigeration with the DSC and NIR methods***

Liquid egg white samples were refrigerated in 100 ml glass test tubes at 50, 55 and 60°C for 24 hours in an aerobic thermostat, and the ensuing changes were observed after 3, 6, 9, 12, and 24 hours.

A MicroDSC III instrument was used to make calorimetric measurements. The mass of the liquid egg samples was 500 mg ±0.1 mg, and distilled water served as a reference fluid. In several instances a second round of measurements was done, but no reversible effect was noted. The evaluation was derived with the help of the Setsoft 2000 program, which belongs to the equipment.

Measurements were concluded using the MetriNIR 10-17 ST reflexive apparatus, and ranged between 700 and 1700 nm with 2 nm distance between the "goalposts." Using 3 separate fillings and measuring at each 90 degree turn, measurements were made with a space homogenizer and by mixing. Using discriminant analysis (DA, software: SPSS 15.0) we completed the study with a polar qualification system (PQS), applied software: PQS 1.56. .

The PLS (partial least squares) method was used to model the egg white samples in the study regarding both their spectrum and enthalpy values, which were derived from the heat denaturation tests. For the modelling, the average spectrum of samples kept at 50 and 60°C were used.

2.5. Influence of preservatives on calorimetric characteristics of liquid eggs

Liquid raw egg white, egg yolk, and whole egg were observed. Sodium benzoate and potassium sorbate were added to samples in such a way that 0.1; 0.3; and 0.5 g/l concentrations were produced. To acquire various pH-values (5.5; 5.0; 4.5) of liquid egg products, citric acid was used.

Adjustment of pH by citric acid was checked with a CONSORT C831 liquid pH meter. I made calorimetric measurements with the DSC method on liquid egg samples with precisely set preservative concentration and pH-values.

Finally, calorimetric measurements were carried out with a MicroDSC III apparatus. In every case, liquid egg samples were heated from 20°C to 95°C at a rate of 1.5 °C/minute.

The liquid egg samples were 500 mg ±0 mg by volume, and the reference liquid used was distilled water. In a few cases a second round of measurements was done, but no reversible characteristic was observed.

Evaluations were performed using a Setsoft2000 program, which was part of the apparatus. Using the program, I was able to calculate among the calorimetric parameters the temperature (°C) and enthalpy (J/g) of denaturation.

3. RESULTS

3.1. Effect of pasteurization on decimal reduction time of Salmonella

Having determined the reduction of viable cell count, I concluded that when pasteurization precedes post-packaging heat treatment at 55°C, the heat resistance of *Salmonella* spp. increases. This phenomenon does not always appear significantly, however.

The more striking influence with prior pasteurization was the slow warming of the samples. After being placed in the thermostat, the S*almonella*-inoculated liquid egg and peptone diluent needed more than 60 minutes to reach 55°C. During this time, the *Salmonella* bacteria conceivably suffered heat shock, which led to their heat tolerance being increased many times.

In spite of the heat shock reaction, my experiments showed a more favourable result (5 times the amount of reduction) without prior pasteurisation than with it, when 24-hour, 55°C heat treatment was applied to *Salmonella* spp. Likewise, the various liquid egg samples kept for 24 hours at 55°C, the viable cell count was undetectable, if the bacteria had not acquired ability to develop resistance to the influence of sub-lethal heat.

3.2. Heat resistance dependence of Salmonella Enteritidis, Escherichia coli, Listeria monocytogenes and Staphylococcus aureus on warm-up time and treatment temperature

Based on the results of my calculations, all of the microbes examined (*Salmonella* Enteritidis, *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus*) were influenced by the rate of warming-up in their decimal reduction time ($p<0,15$), in the range of 1 – 5.1°C·min^{-1}. In the 48.96 – 56.04°C temperature range, only the D-value of *Listeria monocytogenes* did not show any significant change.

According to these results, where liquid whole eggs must be heated over a fairly long time, one must keep in mind that certain microbes' decimal reduction time may increase manyfold, which mainly must be reckoned with low treatment temperatures.

3.3. Examination of egg white foam stability

I established that from the foam stability standpoint, the best sample was the one prepared with egg powder and sucrose. That is because liquid drip was not observed during storage experiments, and when freshly prepared, it was more firm than the other samples. Further, it showed an extremely good result in the rheological test. Egg powder-Isosweet samples also had especially good results, where hardness and adhesive qualities rated high along with low drip values, although rheological test results were not as favourable.

The sample prepared with raw liquid egg white and sucrose, when freshly prepared, displayed hardness and adhesive values, and amount of liquid drip was also favourable. The rheological test result for this sample yielded the highest of all viscosity values.

Heat-treated liquid egg white mixed with Isosweet, and pasteurized liquid egg white with sucrose had not especially impressive parameters, but due to their low liquid drip values, they were appropriate from the aspect of storage.

The best rheological result came from the pasteurized liquid egg white-sucrose sample, and this product was the most convincing according to the flow curve.

Sensory test: the 23-member panel evaluated products with a score system, 100 points being the maximum. In the opinion of the panellists, there were no real sensory differences between the various products, and they did not find any unpleasant after-taste.

In conclusion, liquid egg white products treated at 55°C for 24 hours possessed the technical and functional characteristics which are suited for confectionery applications..

3.4. Study of changes occurring during storage at 50, 55 and 60°C, using DSC and NIR methods

I observed that at 50 and 55°C, there is no relationship between heat treatment and organic changes in liquid egg white. Thus, using the NIR and DSC measurements, I concluded that 50-55°C produced neither noticeable denaturation nor organic changes. Therefore, at these temperatures such technological processing is possible, where the viable cell count is drastically reduced while the product retains its native egg characteristics.

At 60°C a correlation was detected between the PQS quality points of liquid egg white and the duration of heat treatment. This proves that the NIR method is right for finding denaturation in egg white. Moreover, the conclusion was supported by both NIR base spectrums prepared for liquid egg white products heated up for various lengths of time to 60°C; and comparison of the DSC results, that only negligible changes occur in liquid egg

white when kept at 50 and 55°C, and there is no significant denaturation compared to the 60°C storage.

Although various sample groups differed from each other when stored at 50-55°C, I failed to find a viable reason for the variations. Reasons for changes detected in the infrared area for these temperatures (50-55°C) were probably unrelated to the denaturing of proteins (e.g. formation of lysosome-ovomucin complex, slight denaturation of secondary proteins).

3.5. The influence of preservatives on calorimetric characteristics of liquid eggs

When I reduced pH to 5.0, my calculations showed that significant changes occurred in the liquid egg samples' calorimetric parameters. Along with denaturation-related enthalpy caused by acidification of the samples, reduced denaturation temperature was observed in the protein. Whereas in native state protein began to emerge at 60°C by then, with a 5.0 pH value, denaturation had already begun at 54.5°C. Addition of sodium benzoate and potassium sorbate to liquid eggs produced a significant difference according to the calorimetric values only when preservative was present in the amount of 0.5 g/l.

My tests proved that the calorimetric characteristics of liquid egg products, such as initial denaturation temperature, may be changed by preservatives which are added in approved concentrations.

4. NEW SIENTIFIC RESULTS

1. I have established that the 55°C, 24-hour heat treatment is applicable for production of *Salmonella* Enteritidis-free liquid egg products.
2. I have determined that the rate of heating-up for heat-treatment of liquid eggs had a significant influence ($P<0,05$) on the heat tolerance of *Salmonella* Enteritidis, *Staphylococcus aureus*, *Echerichia coli* and *Listeria monocytogenes* present in the samples. Heating time of more than 15 minutes may increase the D_{50-55} value of these microbes by as much as three to four times, and a previous heat treatment (58°C, 10 minutes) can further enhance this effect.
3. I have ascertained that thermal decimation of *Salmonella* spp. in liquid egg yolk, whole liquid egg, and liquid egg white differed considerably during storage at 55°C for 24 hours. The D_{55} value of *Salmonella* spp.- when kept under heat for 24 hours - was smallest in liquid egg white, and largest in liquid egg yolk.
4. Using the DSC method, I have discovered that protein denaturation did not occur during heat treatment of liquid egg white at 55°C. Further, the rheological, foaming, and foam-stability of 55°C, 24-hour treated liquid egg white were not significantly different from the raw, traditionally treated (at 64°C for 5 minutes) liquid egg white.
5. My experiments proved that concentrations of preservative additives (citric acid, sodium benzoate, potassium sorbate) within limits set by the Hungarian Codex Alimentarius may change calorimetric characteristics (e.g. initial denaturation temperature) of liquid egg products. At lower than 55°C, protein denaturation must be taken into account when changing heat treatment parameters.

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