# A NEW LIQUID EGG PRODUCT

***Csaba Németh, Laszló Friedrich, Ildikó Zeke, Csaba Balla***
Department of Refrigeration and Livestock Products Technology, Faculty of Food Science, Corvinus University of Budapest, 1118 Budapest, Ménesi út 43-45, Hungary
e-mail: csaba.nemeth@uni-corvinus.hu

**ABSTRACT**

Nowadays, pre-processed egg products are preferred as raw materials rather than shell eggs by the food industry. These are sold as pasteurized liquid egg or egg powder products. Pasteurisation of egg products means a heat treatment of several minutes at about 60 °C. Two important issues shall be kept in mind: to destroy as much contaminating micro-organisms as possible and at the same time not to damage the valuable egg proteins. Research data show that the number of micro-organisms in pasteurized liquid eggs is between 100-1000 cfu/ml and *Salmonella* strains can also be found among the survivors.
Microbiological examinations were carried out to develop an alternative pasteurization method, which can be used in the manufacture of egg products. The effect of 24-hour incubation at 55 °C was studied.
The samples were artificially contaminated with strains belonging to *Enterobacteriaceae* family, which are the most frequently occurring contaminants in egg products. The samples were raw liquid whole egg, liquid egg white and liquid egg yolk produced by an egg products manufacturing plant. Liquid egg samples were inoculated with *Serratia marcescens*, *E. coli*, and *Salmonella* spp. During incubation at 55 °C , reduction in viable cell counts were determined.
Having determined the reduction in viable cell counts against time, the data obtained were in good agreement with literary ones, as the destruction of bacteria is faster in liquid egg white than in products containing egg yolk. In the case of all three test strains, experience shows that during the incubation for 12 hours the initial cell count decreased by 4-5 log cycles. Our results showed that a 24 hours incubation at 55 °C can provide microbiologically safe, pasteurized products.
The heat treatment was compared to the widely used pasteurization procedure for these products. Marked differences were detected, i.e., while pasteurization only slightly decreased cell counts, after 24 hours of incubation at 55 °C, no viable cells were detected.

## 1. INTRODUCTOUN

During the processing of egg products (liquid egg and egg powder), which are widely used as raw materials in the food industry, after breaking shell eggs a pasteurisation step is applied in the technology. In the pasteurisation process of liquid egg several minutes long heat treatment takes place at about 60 °C (USDA, 1980; JONES at al. 1983) in a heat exchanger, during which two aspects must be take into consideration: to destroy as much contaminating microorganisms as possible and in the same time not to damage the valuable components of the egg, mainly proteins (FRONING at al., 2002). Hygienic control measurements show that the number of microorganisms in pasteurised liquid egg can be up to $10^2$-$10^3$ cfu/ml and sometimes *Salmonella* sp. can be found among the survivors. For this last one the regulation is 0 viable cell in 25 g of food.
The main group of microorganisms most frequently infecting egg products, are Enterobacteriaceae family members entering into liquid egg from the shell. Their optimal growth temperature is 37 °C but most of them grow between 10-45 °C in good culture-medium. As they are asporogenous species they can be relatively well destroyed (ADAMS & MOSS, 1995) by heat.
Widely used parameter for cell destruction in the food industry is the decimal reduction time (D) or D-value. It is the time required to kill 90% of the microorganisms or spores in

a sample at a specified temperature. Literature data show that e.g. different *Salmonella* species can have different D-values (PALUMBO et al., 1996), but their thermal resistance can also be strongly influenced by the medium the cells are in. According to experiments by Jin et al. (2008) the investigated *Salmonella enteritidis* and *E. coli* strains have greater thermo tolerance in liquid whole egg than in liquid egg white. In earlier experiments the same has already been found by Michalski et al. (1999), who attributed this to the probable differences in pH, water activity and composition of egg white and egg melange (liquid whole egg); e.g. *S. enteritidis* is not able to tolerate the high pH value of liquid egg white whereas the neutral pH of liquid whole egg is appropriate for it. Moreover, several proteins and lipids were found in egg yolk, which help to stabilize the cell membrane against thermal effects (MURIANA et al., 1996; NIEDHART & VANBOGELEN, 1987).
Bunning and his colleagues (1990) examining the thermo tolerance of *Salmonella typhimurium* and Kumar (KUMAR A. & KUMAR, S., 2003) studying *Salmonella senftenberg* have found, that a preliminary 30-minute heat shock at 52°C or at 55°C enhances the thermo tolerance of these bacteria. Other authors examining *S. enteritidis* have found that after 2-3 cell cycles the effect of heat shock was not significant on bacterial grown at optimal temperature (SHAH et al,. 1991; MACKEY & DERRICK, 1986).
Taking all of this into account such pasteurisation technique is needed, which results in a lower viable cell count than the current one and guarantees that the products are free from *Salmonella* sp in every case and under all circumstances.

In order to reach this aim, various technological solutions have been tried, such as ultra pasteurisation of liquid eggs, pasteurisation of shell eggs, pasteurisation of liquid eggs with electrical heating, pasteurisation of separated egg white and egg yolk by electric current or our investigations on incubation at lower temperature than the currently known pasteurisation temperature.

## 2. MATERIALS AND METHODS

In our work, microbiological examinations were carried out to facilitate developing an alternative pasteurisation method which can be used in processing eggs. The effect of 24 hours incubation at 55 °C on reduction of viable cell count was studied in non-processed liquid egg.
Samples of non pasteurised liquid whole egg, liquid egg white and liquid egg yolk were coming from an egg products manufacturing plant.
First we investigated how this storage influence the original microflora of liquid egg, after this we carried out experiments with artificially infected samples. Artificial infection was made with some Enterobacteria, *Serratia marcescens* frequently occurring as a contaminating agent in food, *E. coli* an important indicator of faecal contamination and some strains of *Salmonella* which are the greatest hazard to egg products. *Serratia marcescens* and *E.coli* were isolated earlier and identified by the API 20E System, one *Salmonella* species was isolated from egg powder and the other *Salmonella enterica subsp. enterica serotype Enteritidis* B2052 originated from the NCAIM.
Bacteria grown for 24 hours on nutrient agar slope were used for inoculum in a suspension at about $10^7$-$10^8$ cell/ml concentration with sterile water and transferred 1-1 ml to 100 ml of each liquid egg sample as well as to control peptone water per experiment. The infected samples were put into a thermostat set at 55 °C and the viable cell count was determined in every 3 hours by the pour plate method. Three parallel experiments were made from each sample.

Moreover, preliminary heat shock experiments with both strains of *Salmonella* were carried out. Egg products and the control peptone water were infected according to the above procedure. Subsequently samples were exposed to heat treatment for 10 minutes at 58 °C, then cooled to room temperature with tap water. Then the samples were put into a thermostat set at 55 °C. The viable cell count was determined in the same way as it was done with samples not exposed to heat shock (see above).

## 3. RESULTS AND DISCUSSION

Having compared the efficiency of pasteurisation in egg processing plants and the 24 hours incubation at 55 °C, significant differences were found. The positive effect of incubation was striking in the case of liquid whole egg, where the number of microorganisms slightly decreased after pasteurisation, while at 55 °C it was 0 CFU·ml$^{-1}$0 after 24 h (Fig. 1).

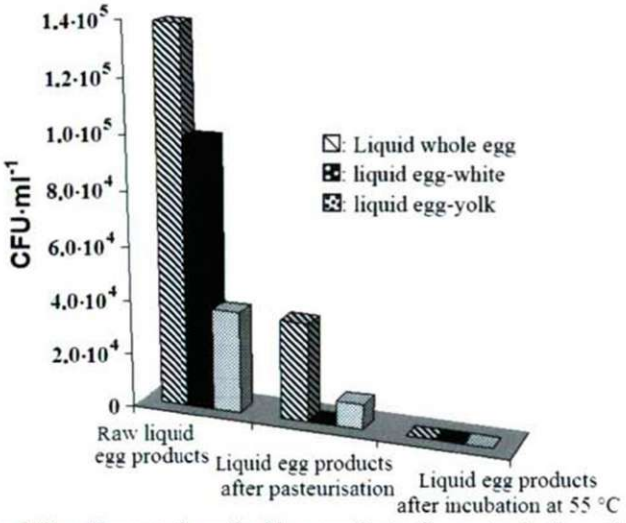


***Fig. 1. Reduction in viable cell count of raw liquid egg products after pasteurisation or incubation at 55 °C.***

After 24 h of heat treatment, none of the artificially infected samples contained detectable viable microorganisms. Consequently, at 55 °C the procedure is equally effective for the examined strains of *Serratia marcescens, E. coli* and the two *Salmonella* species.
In the case of *E. coli* and our *Salmonella* isolate strain, the viable cell count linearly decreased in egg white within 9 h. However, *Serratia marcescens* and *Salmonella enterica* B2052 showed similar decrease in viable bacteria count in each of the different liquid egg products.
It can be seen in the graphs that the decrease in the number of micro-organisms was relatively slow during the first three hours of incubation, then it accelerated between the third and the ninth hours and within this time period the decimal reduction time value (D-value) was approximately constant. The initial slight decrease in the number of micro-organisms can be explained by the fact that it takes time for the samples to reach the ambient temperature. Thermometer cards were placed into one group of the samples and according to our measurements the samples reached 48 °C in about fifty minutes, which was already an unfavourable temperature for multiplication of mesophilic bacteria

(MEMBRÉ et al., 2005). Within this time the rise in the number of micro-organisms does not have to be taken into account since the reproduction cycle of *Enterobacteriaceae* takes on the average 1,5 hours even under optimal conditions.
It was found in some measurements that the cell-destruction rate slowed down after the decrease of viable cell count to $10^3$-$10^4$ CFU·ml$^{-1}$ value. One of the possible reasons of this can be that even micro-organisms, belonging to the same strain, have different thermal resistance, and at this stage of heat treatment only the ones with high heat resistance can survive. This effect can be enhanced by the fact that if there are less heat sensitive strains among the micro-organisms comprising the stable micro flora of egg liquids than the inoculated ones, the ratio of these will become more and more dominating and thus they will influence the heat destruction curve.
According to our measurements the most rapid destruction of microorganisms was found in liquid egg white (Table 1) in most cases. Literature data show that there are several proteins in liquid egg white that reduce the number of microorganisms or inhibit their growth; such as lysozyme (PARK et al., 2006) that can lyse the bacterial cell wall, avidin (ELO et al, 1980) that is able to bind biotin (inhibiting the multiplication of Gram(-) bacteria), conalbumin (IBRAHIM et al., 2000) that is able to bind $Fe^{++}$ (also inhibiting the multiplication of Gram(-) bacteria). Moreover, the pH value of egg white is about 9 and at this pH value egg-contaminating bacteria are not able to multiply (BOARD & FULLER, 2008). Egg yolk, however, in accordance with our measurements (Table 1) and on the contrary to egg white, has a protective effect attributed to e.g. lecithin (CHHABRA et al., 2002). In most of our measurements due to this fact the slowest destruction of micro-organisms was found in egg yolk among egg products.

***Table 1. D-value (min) during incubation for 12 h at 55 °C***

| Microorganism | Liquid egg product | D-value |
|---|---|---|
| *Serratia marcescens* | whole | 106.5±9.2 |
| | white | 110.9±11.3 |
| | yolk | 102.7±7.4 |
| *Escherichia coli* | whole | 284.3±9.0 |
| | white | 95.5±6.7 |
| | yolk | 271.8±14.7 |
| *Salmonella* isolated | whole | 114.5±8.1 |
| | white | 47.4±5.3 |
| | yolk | 189.6±10.7 |
| *Salmonella enterica* | whole | 175.2±2.1 |
| | white | 168.3±6.9 |
| | yolk | 182.4±7.3 |

In the case of our *Salmonella* isolate even after 12 hours of incubation at 55 °C, $10^2$-$10^3$ CFU·ml$^{-1}$ detectable viable cells remained in all the three liquid egg samples as well as in the control peptone water. Although preliminary heat shock can significantly increase the thermal resistance of *Salmonella* sp., this not always can be experienced.

## 4. CONCLUSION

In the case of all three Enterobacteria, experiences show that during incubation for 12 hours at 55 °C the initial cell count decreased by 4-5 log cycles and within 24 hours in not any of the liquid egg products could be found any viable cells.

Having determined the reduction in viable cell counts against time, data were in good agreement with literature ones, as the destruction of bacteria is often quicker in liquid egg white than in products containing egg-yolk. Nevertheless, all the datas show that more than 12 hours incubation at 55 °C is needed to obtain certainly germ-free product, particularly in products containing egg yolk.

In the case of *Salmonella* species isolated from egg powder the preliminary heat shock enhanced the thermo-tolerance of the bacterium. However, this phenomenon does not appear definitely in all species, e.g. for *S. enterica* B2052 we have not found anything similar.

According to our experiments, the incubation treatment proved to be more effective than the usual pasteurisation procedure for liquid egg. After 24 hours incubation at 55 °C the bacteria under investigation were destroyed. Simultaneously we did not find any significant changes in the consistency of these liquid egg products, apart from certain increase in viscosity. This warm incubation technology, carried out immediately after packaging in aseptic boxes could eliminate also post-infection coming from the surroundings of the plant.

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