Microbiological Measurements for the Development of a New Preservation Procedure for Liquid Egg

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Abstract

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Since recently, the food industry has been increasingly using ready-to-process egg products as the basic materials instead of shell eggs. Subsequent to breaking shell eggs and completing pasteurisation, they are put on the market as liquid egg products or in powdered form as dried eggs. Consumers prefer liquid eggs which better preserve the advantageous properties of natural eggs, however, their shelf life is short with quick spoilage. We have examined, how long heat treatment is needed at temperatures below pasteurisation to influence the microbiological status of liquid egg products and in this way also their shelf life. A significant difference was found between the microorganism reducing effects of the commonly used pasteurisation process and that of keeping liquid eggs at 55°C for 24 hours. While pasteurisation can only "considerably" reduce the viable cell count in liquid egg products, keeping the product at 55°C for 24 h would very probably result in no or very low viable cell count.

Keywords: egg product; microorganism; Enterobacteriaceae family; incubation

During the processing of egg products (liquid eggs and egg powder), which are widely used as raw materials in the food industry, subsequent to breaking shell eggs a pasteurisation step is applied in the technology. In the pasteurisation process of liquid eggs, a several minutes long heat treatment takes place at about 60°C (USDA 1980; Jones *et al*. 1983) in a heat exchanger, during which two aspects must be taken into consideration: the destruction of as many contaminating microorganisms as possible while not damaging the valuable components of the egg, mainly proteins (Froning *et al*. 2002). Hygienic control measurements show that the number of microorganisms in pasteurised liquid eggs can be up to 10^2–10^3 CFU/ml and sometimes *Salmonella* sp. can be found among the survivors. For these, the regulation gives 0 viable cells in 25 g of food (EC Regulation 2073/2005).

The main group of microorganisms most frequently infecting the egg products are the members of the Enterobacteriaceae family entering into the 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 non-spore forming species, they can

be relatively well destroyed by heat (Adams & Moss 1995; Miller *et al*. 2010).

A widely used parameter for the 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 in the 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 cell medium. According to the experiments by Jin *et al*. (2008), the investigated *Salmonella enteritidis* and *Escherichia coli* strains have a higher thermo-tolerance in liquid whole egg than in liquid egg white. In earlier experiments, the same result was already obtained by Michalski *et al*. (1999), who attributed this to the probable differences in the 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 stabilise the cell membrane against thermal effects (Niedhart & Van Bogelen 1987; Muriana *et al*. 1996).

Bunning *et al*. (1990) examining the thermo tolerance of *Salmonella typhimurium*, and Kumar and Kumar (2003) studying *Salmonella senftenberg*, have found that a preliminary 30-min 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 to 3 cell cycles the effect of the heat shock was not significant in view of the bacterial growth at optimal temperature (Mackey & Derrick 1986; Shah *et al*. 1991).

Taking all this into account, a new pasteurisation technique is needed which would result in a lower viable cell count than the current one and guarantee that the products are free from *Salmonella* sp. in every case and under all circumstances.

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 into the incubation at a lower temperature than the currently known pasteurisation temperature.

We therefore conducted microbiological examinations to develop an alternative pasteurisation method which could be used in the manufacture of egg products.

MATERIAL AND METHODS

Raw, non pasteurised, homogenised, liquid whole egg, liquid egg white, and liquid egg yolk samples coming from an egg products manufacturing plant, as well as peptone water as a control (dehydrated pre-prepared culture-medium that was re-hydrated and sterilised according to the manufacturer's instruction at 0.5 bar overpressure for 15 min in an autoclave), were inoculated by *Enterobacteria* agents. The infection was induced by *Serratia marcescens*, a frequently occurring contaminant in the egg products, *Escherichia coli* as an important indicator of faecal contamination and two strains of *Salmonella* bacteria, which pose the greatest hazards to the egg products. *Serratia marcescens* and *Escherichia coli* bacteria used for infection were isolated by API 20E System in the course of earlier investigations into liquid egg products. One of the *Salmonella* strains was isolated from egg powder and the other one, *Salmonella enterica* subsp. *enterica* serotype Enteritidis B2052, was obtained from the National Collection of Agricultural and Industrial Microorganisms. The stable micro-flora of liquid egg products was always present, since if we had intended to sterilise the products, this would have modified the nutritional value of the samples and thus we would have made the measurements under the plant conditions.

We prepared the inoculate from the culture of the given bacteria made on liquid meat agar slope and aged 24 h in such a way that two portions of the respective culture on the inoculation loop were put into 10 ml of sterile water, and 1 ml of the inoculum was transferred into 100 ml of each liquid egg sample as well as into the control peptone water. Using this method, we worked with various samples whose initial viable cell counts were approximately similar to one another, namely 10^6–10^7 CFU/ml. The inoculation of the samples was carried out at a storage temperature specified according to the egg products such as 4°C, and 100 ml of the 3 parallels of each infected sample comprising liquid whole egg, liquid egg white, liquid egg yolk, and peptone water were put into a glycerine thermostat, with a capacity of 150 l, set at 55°C. Subsequently, the initial viable cell count was determined on nutrient agar (18 g/l agar-agar, 5 g/l peptone, 5 g/l glucose, 3 g/l

meat extract, 0.5 g/l sodium-chloride) by diluted plate pouring. During our measurements, the viable cell count was measured every 3 hours. We carried out three determinations on each sample.

To induce a possible change in thermal resistance, a preliminary heat shock was applied to some samples. The egg products spiked as described above and control peptone water were heat treated in a water bath after infection (the temperature of the samples exceeded 50°C for 12 min and was kept at 58°C for at least 7 min), and cooled down to room temperature (20°C) with tap water. Subsequently, the samples were stored in a refrigerator for 30 min and then placed into an incubator at 55°C, similarly to those samples that were not subjected to the preliminary heat treatment. The total plate counts were measured in the same way as with the samples without the heat treatment. The decimal reduction rate (D-value) was calculated from the slope of the curve fitted on the viable cell number of the heat treated samples. The heat destruction results of the microorganisms tested with or without the preliminary heat shock were compared.

In our measurements, we did not investigate the organoleptic changes occurring in the liquid egg products during the heat treatment. However, we plan to perform an objective measurement of the changes in the taste, colour, odour, and consistency occurring in liquid egg sample, since we did not experienced any considerable change in texture at the primary heat-shock (58°C).

RESULTS AND DISCUSSION

The comparison between the efficiency of conventional pasteurisation in egg processing plants and that of 24 h incubation at 55°C revealed significant differences. The positive effect of a long 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 after 24 h (Figure 1).

After 24 h of heat treatment, none of the artificially infected samples contained detectable viable microorganisms. Consequently, at 55°C the procedure was 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 strains, the viable cell count linearly decreased in egg white within 9 h (Figures 2b and 2c). However, *Serratia marcescens* and *Salmonella enterica* B2052 showed a similar decrease in viable bacteria count in each of the different liquid egg products (Figures 2a and 2d).

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 a 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 the multiplication of mesophilic bacteria (Membré *et al.* 2005). Within this time period, the rise in the number of micro-organisms did not have to be taken into account since the reproduction cycle of *Enterobacteriaceae* takes on the average 1.5 h even under optimal conditions.

It was found in some measurements (Figures 2a and 2d) that the cell-destruction rate slowed down after the decrease of viable cell count to 10^3–10^4 CFU/ml value. One of the possible reasons for this can be that even the micro-organisms, belonging to the same strain, have different thermal resistance, and at this stage of the heat treatment only those with a high heat resistance can survive. This effect can be en-

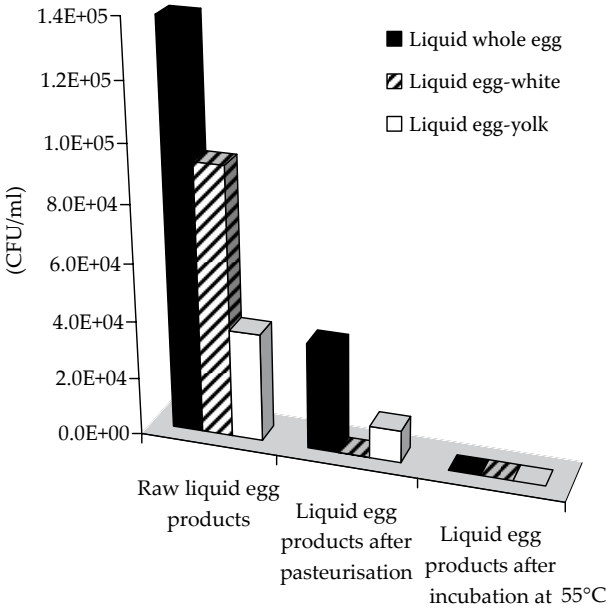

Figure 1. Reduction in viable cell count of raw liquid egg products after pasteurisation or incubation at 55°C

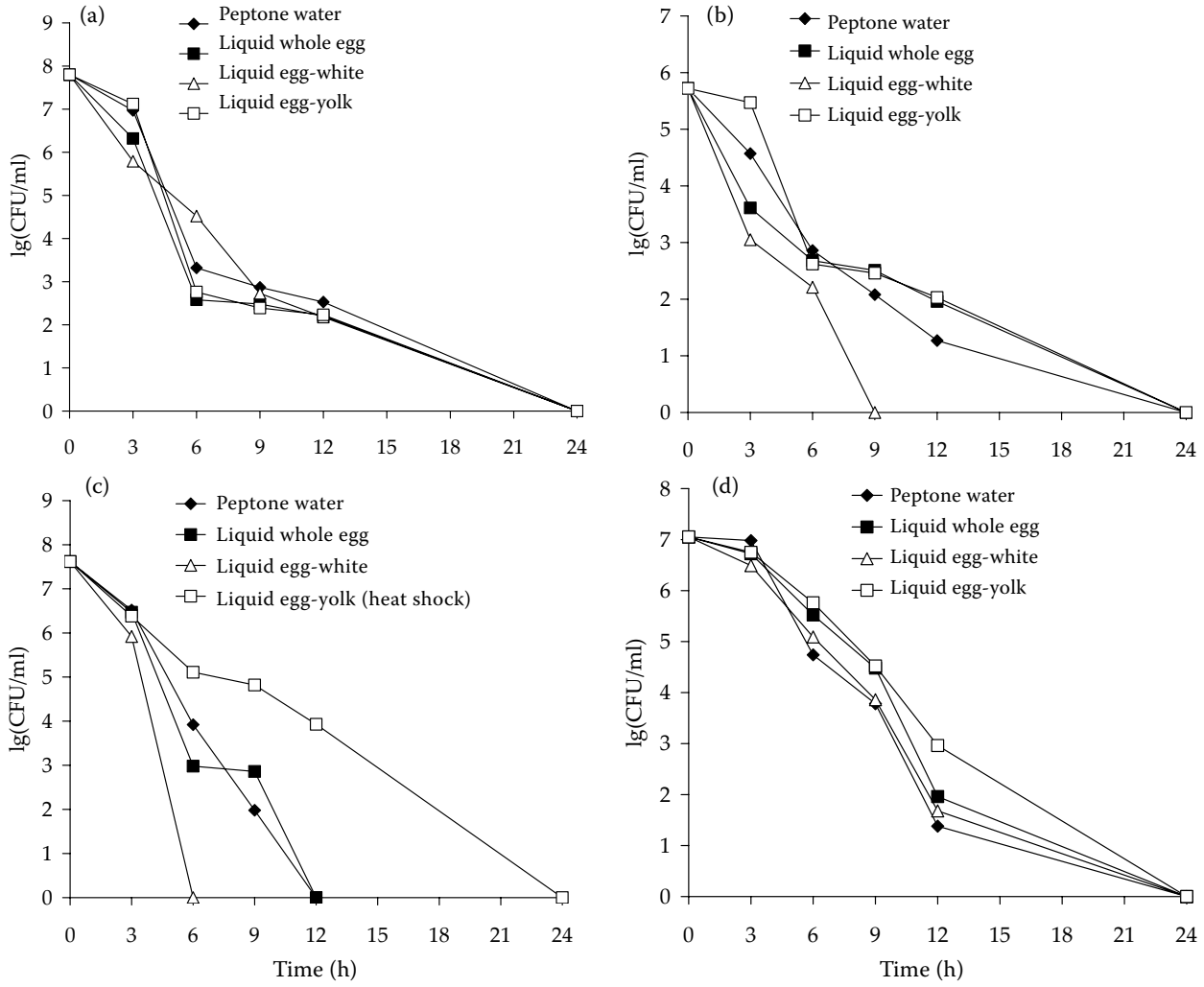

Figure 2. Changes in viable cell count of liquid egg products and peptone water at 55°C infected by *Serratia marcescens* (a), *Escherichia coli* (b), *Salmonella* isolate from egg powder (c), and *Salmonella enterica* B2052 (d)

hanced by the presence among the micro-organisms representing the stable micro flora of egg liquids of strains less heat sensitive than the inoculated ones, due to which the proportion of the former will become more and more prevailing and thus they will influence the heat destruction curve.

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

Table 2. Changes in D-value (min) using a preliminary heat shock for 10 min at 58°C

Micro-organism	Liquid egg product	D-value		
		(A)	(B)	(B)/(A)
Salmonella isolated	whole	114.5	223.1	1.95
	white	47.4	102.8	2.17
	yolk	189.6	244.8	1.29
Salmonella enterica	whole	175.2	187.3	1.07
	white	168.4	169.2	1.00
	yolk	182.4	230.4	1.26

D-value(A) – no preliminary heat shock; D-value(B) – preliminary heat shock for 10 min at 58°C

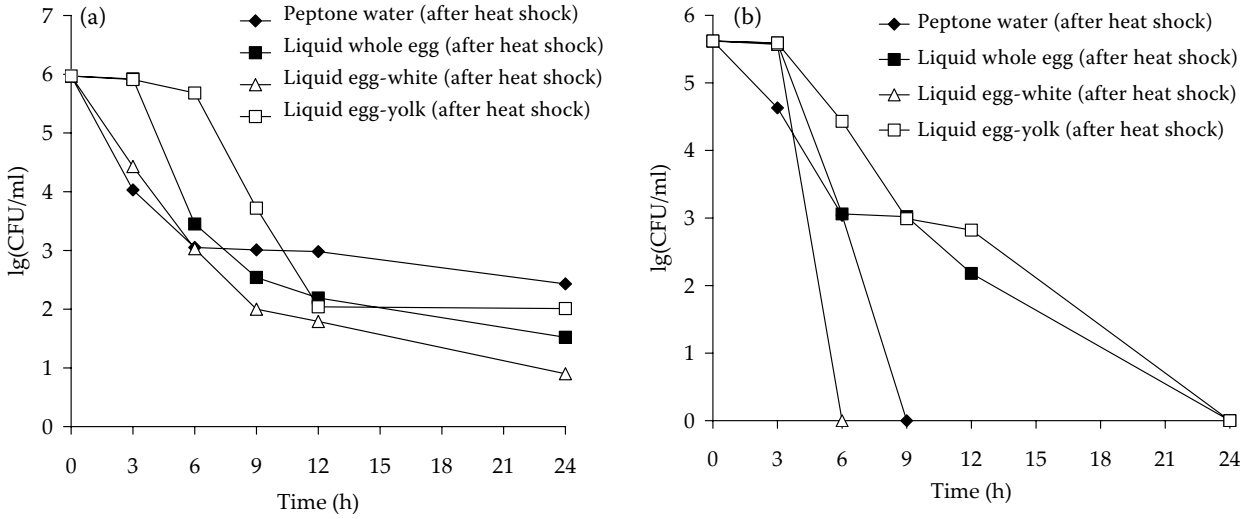

Figure 3. Changes in viable cell count of liquid egg products and peptone water at 55°C infected by *Salmonella* isolate from egg powder (a) and *Salmonella enterica* B2052 (b); using a preliminary heat shock for 10 min at 58°C

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; avidin (Elo *et al*. 1980) that is able to bind biotin (inhibiting the multiplication of Gram-negative bacteria), conalbumin (Ibrahim *et al*. 2000) that is able to bind Fe^{2+} (also inhibiting the multiplication of Gram-negative 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 unlike egg white, has a protective effect attributed to e.g. lecithin (Chhabra *et al*. 2002). In most of our measurements, the slowest destruction of micro-organisms among egg products was found in egg yolk due to this fact.

In the case of our *Salmonella* isolate (Figure 3a), even after 12 h of incubation at 55°C, 10^2–10^3 CFU/ml detectable viable cells remained in all the three liquid egg samples as well as in the control peptone water. Although a preliminary heat shock can significantly increase the thermal resistance of *Salmonella* sp., this can not be always experienced (Figure 3b, Table 2).

CONCLUSIONS

In the case of all three Enterobacteria, the experience shows that during the incubation for 12 h at 55°C the initial cell count decreased by 4–5 log and within 24 h no viable cells could be found in any of the liquid egg products.

In the determination of the reduction in viable cell counts against time, the data proved to be in a good agreement with the literature ones, as the destruction of bacteria is often more rapid in liquid egg white than in the products containing egg-yolk. Nevertheless, all the figures show that more than 12 h of incubation at 55°C is needed to obtain definitely germ-free product, particularly with the products containing egg yolk.

In the case of *Salmonella* species isolated from egg powder (Figure 6), the preliminary heat shock increased the thermo-tolerance of the bacterium. However, this phenomenon did not appear definitely in all species, e.g. for *S. enterica* B2052 we did not find anything similar.

According to our experiments, the incubation treatment proved to be more effective than the usual pasteurisation procedure for liquid eggs. After 24 h of 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 a certain increase in viscosity (based on our results measured by Rheomat 115 rotational viscometer, the dynamic viscosity of albumen increased from 15.5 ± 0.4 mPa·s to 16.7 ± 0.5 mPa·s). This warm incubation technology carried out immediately after packaging in aseptic boxes could eliminate also the post-infection coming from the surroundings of the plant.

References

Adams M.R., Moss M.O. (1995): Food Microbiology, Bacterial Agents of Foodborne Illnesses, Chapter 7. The Royal Society of Chemistry, Cambridge: 182–189.

Board R.G., Fuller R. (2008): Non-specific antimicrobial defences of the avian egg, embryo end neonate. Biological Reviews, **49**: 15–49.

Bunning V.K., Crawford R.G., Tierney J.T., Peeler J.T. (1990): Thermotolerance of *Listeria monocytogenes* and *Salmonella typhimurium* after sublethal heat shock. Applied and Environmental Microbiology, **56**: 3216–3219.

Chhabra A.T., Carter W.H., Linton R.H., Cousin M.A. (2002): A predictive model that evaluates the effect of growth conditions on the thermal resistance of *Listeria monocytogenes.* International Journal of Food Microbiology, **78**: 235–243.

Elo H.A., Räisänen S., Tuohimaa P.J. (1980): Induction of an antimicrobial biotin-binding egg white protein (avidin) in chick tissues in septic *Escherichia coli* infection. Cellular and Molecular Life Sciences, **36**: 312–313.

EC Regulation No. 2073/2005 on Microbiological criteria for food staff. Official Journal of the European Communities on 22 December 2005.

Froning G.W., Peters D., Muriana P., Eskridge K., Travnicek D., Sumner S.S. (2002): International Egg Pasteurization Manual. United Egg Association, Alpharetta: 5–12.

Ibrahim H.R., Sugimoto Y., Aoki T. (2000): Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism. Biochemica and Biophysica Acta, **1523**: 196–205.

Jin T., Zhang H., Boyd G., Tang J. (2008): Thermal resistance of *Salmonella enteritidis* and *Eschericha coli* K12 in liquid egg determined by thermal-death-time disk. Journal of Food Engineering, **84**: 608–614.

Jones J.M., Monsey J.B., Payne J. (1983): Egg pasteurisation. Nutrition and Food Science, **83**: 18–19.

Kumar A., Kumar S. (2003): Survival kinetics of *Salmonella enterica* serotype *senftenberg* (*S. senftenberg*) after heat and acid stress. World Journal of Microbiology and Biotechnology, **19**: 985–987.

Mackey B.M., Derrick C.M. (1986): Elevation of the heat resistance of *Salmonella typhimurium* by sublethal heat shock. Journal of Applied Bacteriology, **61**: 389–393.

Membré J.-M., Leporq B., Vialette M., Mettler E., Perrier L, Thuault D., Zwietering M. (2005): Temperature effect on bacterial growth rate: quantitative microbiology approach including cardinal values and variability estimates to perform growth simulations on/in food. International Journal of Food Microbiology, **100**: 179–186.

Michalski C.B., Barckett R.E., Hung Y.-C., Ezeike G.O.I. (1999): Use of capillary tubes and plate heat exchanger to validate US Department of Agriculture pasteurization protocols for elimination of *Salmonella enteritidis* from liquid egg products. Journal of Food Protection, **36**: 523–526.

Miller P., Haveroen M.E., Solichová K., Merkl R., McMullen L.M., Míková K., Chumchalová J. (2010): Shelf life extension of liquid whole eggs by heat and bacteriocin treatment. Czech Journal of Food Sciences, **28**: 280–289.

Muriana P.M., Hou H., Singh R.K. (1996): A flow-injection system for studying heat inactivation of *Listeria monocytogenes* and *Salmonella enteritidis* in liquid whole egg. Journal of Food Protection, **59**: 121–126.

Niedhart F.C., Van Bogelen R.A. (1987): Heat shock response. In: Neidhardt F.C., Ingraham J., Low K.B., Magasanik B., Schaechter M., Umbarger H.E (eds): *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. Vol. 2. American Society for Microbiology, Washington: 1334–1345.

Palumbo M.S., Beers S.M., Bhaduri S., Palumbo S.A. (1996): Thermal resistance of *Listeria monocytogenes* and *Salmonella* spp. in liquid egg white. Journal of Food Protection, **59**: 1182–1186.

Shah D.B., Bradshaw J.G., Peeler J.T. (1991): Thermal resistance of egg-associated epidemic strains of *Salmonella enteritidis.* Journal of Food Science, **56**: 391–393.

USDA (1980): Regulations governing inspection of egg products. 7 CFR, Part 2859.

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