

Full Length Research Paper

Thermal destruction of *Listeria monocytogenes* in liquid egg products with heat treatment at lower temperature and longer than pasteurization

CS. Nemeth^{1*}, L. Friedrich¹, K. Pásztor-Huszár¹, E. Pipoly², Á. Suhajda² and Cs. Balla¹

¹Department of Refrigeration and Livestock Products Technology, Faculty of Food Science, Corvinus University of Budapest, Hungary.

²Department of Applied Biotechnology and Food Science, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Hungary.

Accepted 28 February, 2011

Today food manufacturing plants prefer „ready-for-use” liquid egg products to whole eggs. These are easier to use since one should not deal with breaking and storing the egg shell contaminated by faeces. However the shelf life of liquid egg products containing no preservatives is relatively short, there is a need to elaborate new technologies – like the long term (6 to 24 h) heat treatment of liquid egg products on 53 to 55°C. In our measurements we infected non-heat treated liquid egg samples from food industrial plant (liquid egg white, liquid egg yolk, liquid whole egg) with *Listeria monocytogenes* strain (NCAIM B1371) and incubated at 53 and 55°C. Then dilution plate pouring was performed with Brain Heart Infusion agar for live germ count measurement every 30 min for 6 h and then after 24 h. We observed live germ count reduction of more than 6 orders of magnitude in all liquid egg products even after 6-hour treatment. It was investigated if the new technology can be used to reduce live *L. monocytogenes* count also in products already heat treated or heat treated and stored in refrigerator. Our results have shown that preliminary pasteurization or pasteurization and storing in refrigerator only slightly increased the thermal resistance of *L. monocytogenes*.

Keywords: *Listeria*, liquid egg, heat treatment.

INTRODUCTION

Food manufacturers using egg as a raw material such as bakeries, confectioneries, large scale pastry manufacturers prefer egg products (liquid whole egg, liquid egg white, liquid egg yolk as well as the powdered forms) to shell egg due to easier handling and lower microbiological risk. Considering egg products they prefer liquid egg products because these better preserve the favourable characteristics of the native egg. However, the shelf-life of liquid egg products is short and such products deteriorate rapidly (Foegending and Stanley, 2006).

The deteriorating and pathogenic microbes may contaminate the inner part of the egg during the breaking procedure where they can easily proliferate due to the excellent nutrient features of the egg. When the egg comes out from hen the egg shell is contaminated with faeces (*Escherichia coli*, *Salmonella spp.*) and through its contact with the nest micro-organisms can attach to the surface (*Staphylococcus aureus*). To reduce the number of deteriorating micro-organisms contaminating liquid egg and inactivate pathogens heat treatment is required during the preparation of egg products (Board, 1966; Henry and Garibaldi, 1975; Musgrove et al., 2009).

For heat treatment of liquid eggs the temperature and duration of treatment should be selected to reduce the number of living contaminating micro-organisms

*Corresponding author. E-mail: csaba.nemeth@uni-corvinus.hu.
Tel: +3614826059. Fax: +3614826321.

sufficiently while avoiding impairment of the egg content (proteins, vitamins) (Ferreira et al., 1997; Froning et al., 2002). In the pasteurization procedures widely used in practice the egg white passes through intermittent or continuous heat exchanger where it is exposed to temperatures of 57 to 60°C for 5 to 10 min (USDA, 1980; Jones et al., 1983).

Results from international microbiological monitoring measurements have shown that pasteurization of eggs is not a sufficiently procedure to reduce the germ count; survival micro-organisms, sometimes pathogens can be found in the liquid egg product after pasteurization (Rivoal et al., 2009). The direction of development is to find a more efficient germ reducing technology. A solution may include the treatment of liquid egg at relatively lower temperature (53 to 55°C) for long time (6 to 24 h), that is, thermostation during which the live germ count of specific bacteria can be reduced significantly or completely destroyed without damaging egg proteins demonstrated by our experiments with *Serratia marcescens*, *E. coli* and various *Salmonella enteritidis* strains.

Occasionally *Listeria monocytogenes* may also be found in poultry and egg products (Lawrence and Gilmour, 1994). The conditions in soil, surface waters, waste waters, on plants, in animal faeces and feed, that is, the environment of the egg or the associated animal are favourable for proliferation (Farber and Peterkin, 1991). This opportunistic pathogen viable within a wide temperature range and infectious through the digestive system is resistant to alkaline pH (egg white) and high salt concentration (raw materials for omelette) that increases its significance in regards of egg products (Berche et al., 1988; Hwang et al., 2006).

As with other bacteria, the heat sensitivity of *L. monocytogenes* depends on the age, growth conditions of the bacterial culture, the characteristics of food matrix, example, pH, salt, acid content, water activity and the presence of potential inhibitors (Palumbo et al., 1995; Palumbo et al., 1996). In general, the D values characteristic to the heat sensitivity of the bacterium: $D_{60}=3$ to 4 min and $D_{70}=9-10$ s, that is, at 60°C it takes a few minutes that 90% of live cells are destroyed while at 70°C a few seconds is enough for this (Adams and Moss, 1995; Doyle et al., 2001). Previous research has reported that environmental stress affecting the micro-organisms (example, change of pH, temperature, water activity, etc.) may increase the thermal resistance of the micro-organisms. In case of *L. monocytogenes* following the first heat shock treatment an increase in thermal resistance can be observed, during treatment the cells produce heat shock proteins (HSPs) protecting the other proteins from damage and facilitating the repair of damaged cells as well as preventing further damages (Bunning et al., 1990; Van der Veen et al., 2007). Thus the cells surviving pasteurization follow other destruction kinetics compared to the cells in the raw samples. This may be important in cases when the new procedure aims

to increase the shelf-life of already pasteurized liquid egg products.

The risk of listeriosis is increased by the capability of *L. monocytogenes* to grow at 4°C unlike the pathogenic micro-organisms occurring in the most egg products (Hwang and Marmer, 2007). Since there are several fractions of liquid egg products sensitive to freezing such treatment would damage the quality and functional characteristics of native egg. Thus it is an important criterion for a technology suitable for production of liquid egg products with long shelf-life that it must not contain pathogens capable of growing during refrigerated storage.

In our work our purpose is to develop a model for a new long term (12 to 24 h) heat treatment at temperatures lower than the conventional pasteurization (53 to 55°C) in laboratory circumstances and to find out if the live *L. monocytogenes* count can be reduced by this procedure. Additional, our purpose is to find out if this procedure can be used both for the treatment of raw products and increasing the shelf-life if already pasteurized liquid egg products.

MATERIALS AND METHODS

The samples used for measurement (homogenized liquid whole egg, egg yolk, egg white) were obtained on the day before the testing from a Hungarian egg processing plant. Eggs were stored in a refrigerator until testing. During the experiments the samples were deliberately infected with a *L. monocytogenes* strain (NCAIM B1371) isolated previously. Bacteria were maintained on Brain Heart agar (LabM) and stored at 4°C in a refrigerator. For the incubation testing we used 24 h fresh culture of the strain grown on Brain Heart agar at 37°C.

We prepared inoculum with concentration of approx. 10^{10} cell/ml with sterile water from the culture made on Brain Heart agar slant, 1 ml of which was inoculated into 100 ml of each liquid egg samples (from each type of sample-homogenized liquid whole egg, egg yolk and egg white (100 ml raw samples) are measured into a beaker and infected).

Liver germ count of egg samples were measured before deliberate infection (own microflora) and then following inoculation of samples with *L. monocytogenes* the baseline germ count was determined.

Live germ count of raw samples was below the order of magnitude of 10^4 CFU/ml in each case, that is, the deliberately inoculated *L. monocytogenes* bacterium count was higher by at least 10^4 orders of magnitude compared to the number of natural micro-organisms. Thus, it can be concluded that in our measurement we could investigate the destruction kinetics of *L. monocytogenes* with good approximation.

The deliberately infected samples were incubated in thermostat (Labor-Mix – type LP-102 – laboratory thermostat cabinet with glycerol filling in the jacket which is more suitable for maintenance of 50 to 60°C than water) with airspace at 53°C and 55°C (for modelling of practical application of the technology) and then dilution plate pouring was performed for each sampling with PALCAM Listeria Selective agar for live germ count testing. Change of live germ count was measured in each beaker with three parallels for each dilution. The plates prepared from the original liquid egg samples were incubated at 30°C and the plates prepared from the samples inoculated with the bacterium were incubated at

Table 1. Change of temperature of samples and airspace.

Time (min)	Temperature of samples (°C)						airspace
	Liquid egg white		liquid whole egg		liquid egg yolk		
	T(core)	T(cortex)	T(core)	T(cortex)	T(core)	T(cortex)	
0	4.0	4.4	4.0	4.3	4	4.2	45.3
10	22.3	25.2	21.4	23.8	19.6	22.6	53.6
20	36.2	39.8	34.6	37.7	33.8	36.5	54.8
30	43.3	46.2	41.4	44.7	39.7	42.5	55.1
40	46.9	49.3	44.8	47.3	43.8	46.7	55.0
50	49.9	51.7	48.2	50.3	47.0	49.8	55.1
60	51.8	53.9	50.0	51.7	48.6	51.5	54.8
70	54.2	55.0	52.2	53.0	49.8	52.4	55.0
80	54.5	54.8	53.3	53.9	51.7	52.9	54.6
90	54.8	55.0	53.6	54.1	52.4	53.1	55.2
120	55.0	55.2	54.2	54.7	53.9	54.3	55.1
150	55.1	55.0	54.9	55.1	54.9	54.9	54.9
180	54.9	54.9	55.0	55.0	55.0	54.9	54.8
240	55.1	55.0	55.0	55.0	55.0	55.0	54.9
300	55.1	55.0	55.1	55.1	55.1	55.1	55.2
360	54.8	54.8	54.9	54.9	55.0	54.9	54.7

37°C for 48 h and then the grown colonies are counted with a colony counter.

For modelling of pasteurization prior to incubation heat shock experiment was also performed with *L. monocytogenes* strain. The infected samples and peptone water were incubated in water bath at 58°C for 10 min and cooled down to room temperature by tap water, then the samples were placed into the thermostat either after heat treatment or following 12 h storage in a refrigerator. The live germ count was measured in the same way as with the samples without heat shock.

For measurement of the temperature of the airspace of the thermostat and the liquid egg products we used Testo 454 instrument and the associated software. We measured the core temperature of liquid egg samples as well as the temperature in the "cortex" close to the surface being in contact with air (in 5 mm distance from the glass wall).

RESULTS AND DISCUSSION

In this case the temperature of samples was constantly increasing in the first phase of measurement since it took some time until the different liquid egg samples warmed up to the temperature of the airspace of the thermostat (Table 1). It is obvious from the table that at the beginning of our tests the samples did not reach the lethal temperature.

Our results have also shown that although there could be temperature fluctuations of $\pm 0.5^\circ\text{C}$ within the airspace of the thermostat due to the defect of the regulation system, the temperature of liquid egg products was fluctuated only very slightly due to their thermodynamic inertia ($\pm 0.1^\circ\text{C}$).

Based on our results it can be concluded that *L.*

monocytogenes bacterium in the liquid egg samples was completely destroyed during 24 h. Table 1 has shown that in the first 2 h the reduction of live germ count was relatively low and the destruction of micro-organisms reached the exponential phase only after 2 to 2.5 h. It can be explained by the fact that the samples warmed up from 4 to 50°C in 60 to 70 min (they warm up to the temperature of the thermostat in 90 to 12 min).

The reduction of live germ count started the most rapidly in the liquid egg white. In addition to the alkaline pH of egg white ($\text{pH}=8.9\pm 0.3$ in the samples tested by us) it can be due to the proliferation inhibiting and cell destroying effects of lysozyme, conalbumin and avidin (Ibrahim et al., 2000; Castellano et al., 2001; Park et al., 2006; Board and Fuller, 2008).

From the liquid egg products the reduction of live *L. monocytogenes* count at 55°C was the slowest in the egg yolk. In addition to the absence of the effect of molecules present in the mentioned egg white this is probably due to the fact that the egg yolk contains ingredients facilitating heat resistance such as lecithin (Muriana et al., 1996; Chhabra et al., 2002). The cell destruction kinetics of *L. monocytogenes* was different in the various samples and by the end of 24 h incubation the live germ count has reduced below the detectable level in all samples (Figure 1).

Since the destruction of bacterial cells was relatively fast at the treatment temperature of 55°C we investigated if the preservation of liquid egg samples with incubation at fix temperature is possible at lower temperature. For these experiments we decreased the temperature of thermostat to 53°C (industrial application of technology is

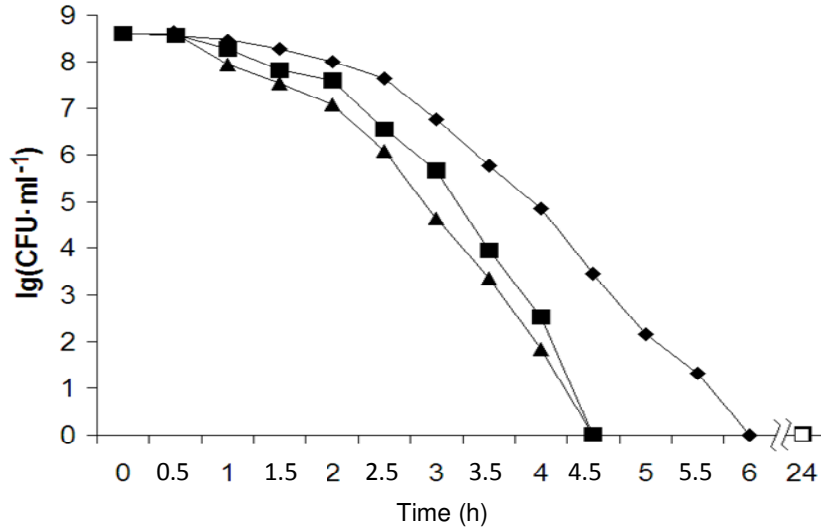


Figure 1. Heat destruction of *Listeria monocytogenes* at 55°C in ▲ liquid egg white, ■ liquid whole egg, ◆ liquid egg yolk.

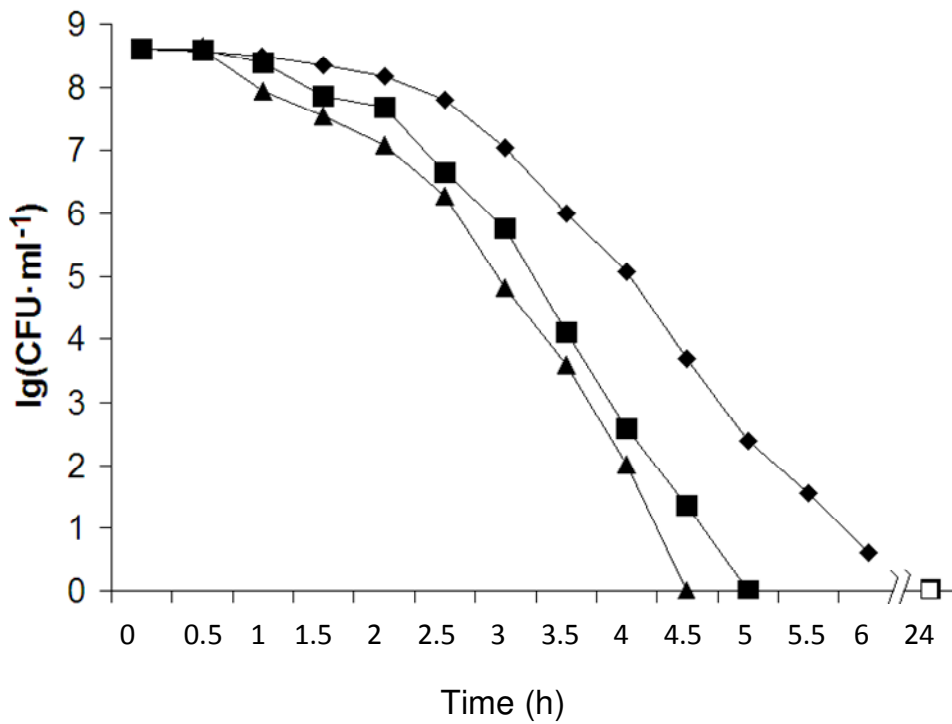


Figure 2. Heat destruction of *Listeria monocytogenes* at 53°C in ▲ liquid egg white, ■ liquid whole egg, ◆ liquid egg yolk.

simpler at this temperature since the lower fluctuation of product temperature does not cause protein denaturation).

As it is shown in Figure 2 at 53°C-on the destruction of micro-organisms starts slower than at 55°C and in the exponential phase the decimation time was slightly

increased. In case of liquid egg white the decimation time increased from 41.6 ± 0.9 min measured at 55°C (the correlation coefficient of regression line for averages determining D value: $r^2=0.9989$) to 44.7 ± 1.3 min ($r^2=0.9977$). In the whole egg the D value of *L. monocytogenes* increased from 42.4 ± 1.5 min ($r^2=0.9860$)

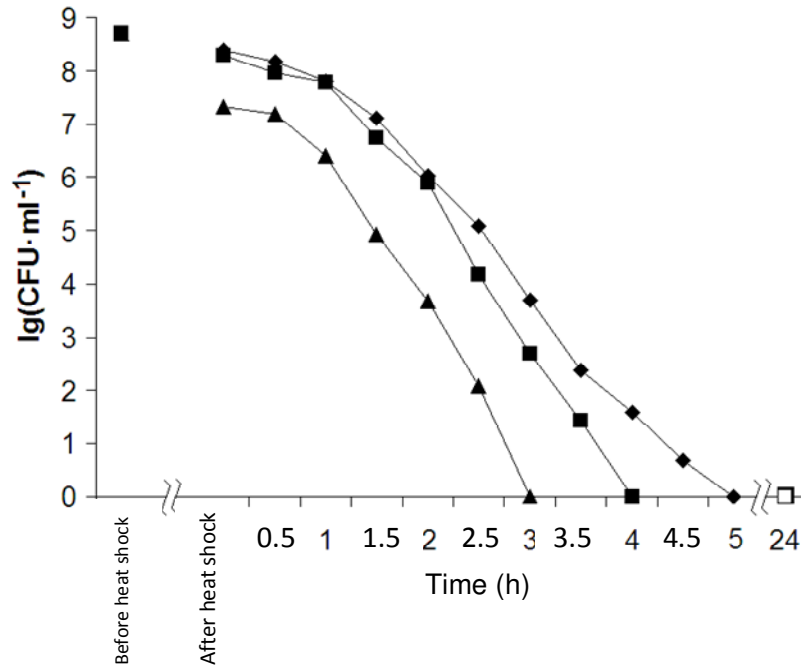


Figure 3. Heat destruction of *Listeria monocytogenes* at 53°C after heat shock at 58°C for 10 min in ▲ liquid egg white, ■ liquid whole egg, ◆ liquid egg yolk.

to 45.6 ± 1.9 min ($r^2=0.9951$), while in the liquid egg yolk from 53.3 ± 2.1 min ($r^2=0.9939$) to 58.2 ± 1.1 min ($r^2=0.9951$).

No *L. monocytogenes* could be detected in any samples even after incubation for 24 h at 53°C.

Heat treatment prior to incubation at 58°C for 10 min significantly reduced the live germ count of liquid egg white ($P<0.001$), liquid whole egg ($P<0.05$) and liquid egg yolk ($P<0.05$).

Figure 3 shows that the reduction of live germ count reached the exponential phase faster compared to the previous measurements. It can be explained by the fact that following treatment at 58°C for 10 min the samples were cooled down with tap water only to room temperature (20°C) and therefore less time was needed to reach the temperature of the airspace of the thermostat compared to the previous measurements.

In case of liquid egg white D value did not change significantly in the exponential phase (42.3 ± 2.5 min), however, in case of liquid whole egg (51.9 ± 4.2 min) and liquid egg yolk (64.6 ± 3.2 min) it increased slightly ($P<0.05$).

When we applied 12 h storage at 4°C in a refrigerator after heat shock at 58°C for 10 minutes, a slight increase in live germ count was observed in the liquid egg yolk and liquid whole egg ($P<0.05$) confirming literature data indicating that *Listeria monocytogenes* can grow at low temperature.

Decimation time in the liquid egg white in the exponential phase was 48.4 ± 1.2 minutes (Figure 4), representing

significant increase in heat resistance ($P<0.05$) even compared to the results measured by using heat shock. This additional increase in heat resistance can be the so called “cold shock effect” that may facilitate the resistance of *L. monocytogenes* to among others heat treatment (Miller et al., 2000; Hayman et al. 2008).

In the liquid whole egg D value (51.3 ± 2.0 min) did not significantly differ from that of the liquid whole egg samples treated directly after heat shock. The decimation time in liquid egg yolk (55.5 ± 3.4 min) reduced back to the value without use of heat shock during the 12 h incubation.

Incubation for 24 h reduced the live germ count below the detectable level also in the samples preliminary exposed to heat shock and then stored in a refrigerator for 12 h.

In our previous measurement we tested the effect of long term (24 h) procedure at temperature below the temperature of pasteurization used today on the enterobacteria (*S. marcescens*, *E. coli*, *S. enterica*) important in terms of preservation of egg. Our studies have shown that in majority of cases decimation time of Gram negative pathogens in liquid egg products was twice higher than the decimation time of *L. monocytogenes* (Table 2) unlike the most literature data indicating that in liquid matrix most of these bacteria are less resistant to heat than *L. monocytogenes* (Sörqvist, 2003).

Studying the quotient of various enterobacteria and *L. monocytogenes* the relatively high differences were also due to the fact that the heat resistance of various

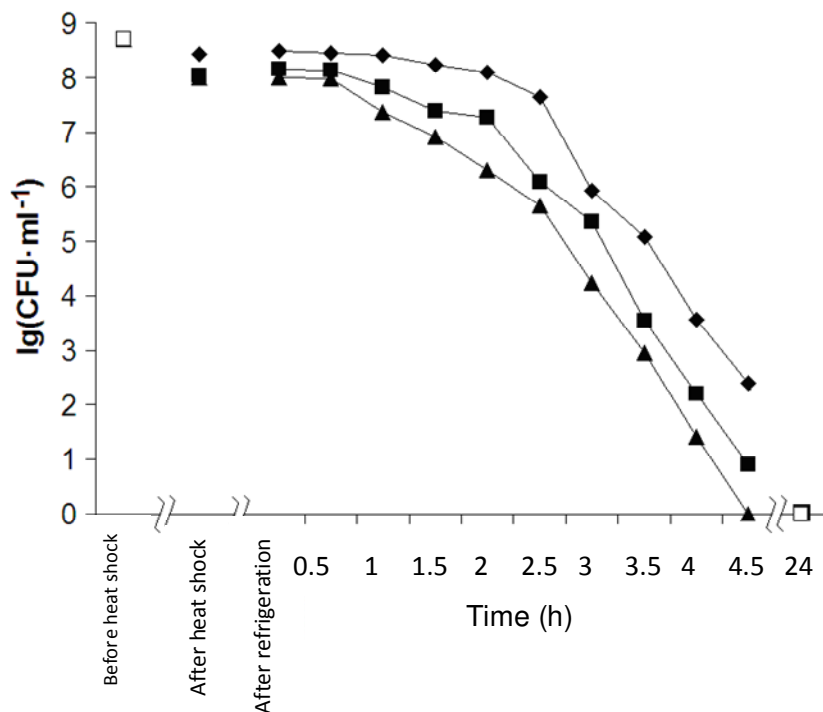


Figure 4. Heat destruction of *Listeria monocytogenes* at 53°C after heat shock at 58°C for 10 min and storing in a refrigerator for 12 h in ▲ liquid egg white, ■ liquid whole egg, ◆ liquid egg yolk

Table 2. Comparison of decimation time of enterobacteria and *Listeria monocytogenes* measured in the exponential phase during incubation at 55°C.

Enterobacterium	Sample	D value (ENT)*	D value (LIS)*/D value (ENT)
<i>S. marcescens</i>	Liquid whole egg	106.5	0.39
	Liquid egg white	110.9	0.38
	Liquid egg yolk	102.7	0.52
<i>E. coli</i>	Liquid whole egg	284.3	0.15
	Liquid egg white	95.5	0.44
	Liquid egg yolk	271.8	0.20
<i>S. enterica</i>	Liquid whole egg	175.2	0.24
	Liquid egg white	168.3	0.25
	Liquid egg yolk	182.4	0.29

* D value enterobacterium, ** D value of *L. monocytogenes*.

Microorganism in case of incubation at 55°C was related to the medium into which we inoculated them, that is, the type of liquid egg sample in different extents. For example the heat resistance of *S. enterica* increased in less extent (D value increased by 1.08 in the yolk compared to the white) compared to the heat resistance of *L. monocytogenes* (D value increased by 1.28 in the yolk compared to the white) and the difference in heat resistance was the most prominent in case of *E. coli* (D

value increased by 2.84 in the yolk compared to the white)

Conclusions

Reduction of live germ count of *L. Monocytogenes* (NCAIM B1371) strain in long term incubation at 53 and 55°C was significantly different in the various liquid egg

products. Cell destruction was the fastest in the liquid egg white followed by the liquid whole egg. From the liquid egg products the reduction of live germ count was the slowest in the liquid egg yolk in each case.

Our measurements have shown that in the new (24 h) incubation technology the live germ count of *L. monocytogenes* bacteria reduced below the detectable level in all samples irrespectively of the treatment temperature (53 and 55°C), the previous life of bacteria (heat shock for 10 min at 58°C and/or storage at 4°C for 12 h). Since *L. monocytogenes* may not occur in raw liquid egg products in such a high concentration it has been demonstrated that the new technology is suitable produce *L. monocytogenes* free products.

Comparing our results with the preliminary measurements it has been concluded that *L. monocytogenes* is less resistant to heat in the liquid egg product during incubation at 55°C than the enterobacteria tested previously (*S. marcescens*, *E. coli*, *S. enterica*).

REFERENCES

- Adams MR, Moss MO (1995). Food Microbiology, Bacterial Agents of Food borne Illnesses, Chapter 7: The Royal Society of Chemistry, Cambridge, UK
- Berche P, Gaillard JL, Richard S (1988) Invasiveness and intracellular growth of *Listeria monocytogenes*, *Infection*, 16: 145-148.
- Board RG (1966): Review article, the course of microbial infection of the hen's egg, *J. Appl. Bacteriol.*, 29: 319-341.
- Board RG, Fuller R (2008): Non-specific antimicrobial defences of the avian egg, embryo end neonate, *Biol. Rev.*, 49: 15-49.
- Bunning VK, Crawford RG, Tierney JT, Peeler JT (1990). Thermotolerance of *Listeria monocytogenes* and *Salmonella typhimurium* after sublethal heat shock, *Appl. Environ. Microbiol.*, 56(10): 3216-3219.
- Castellano P, Fariás ME, Holzappel W, Vignolo G (2001). Sensitivity variations of *Listeria* strains to the bacteriocins, lactocin 705, enterocin CRL35 and nisin, *Biotechnol. Lett.*, 23: 605-608.
- Chhabra AT, Carter WH, Linton RH, Cousin MA (2002). A predictive model that evaluates the effect of growth conditions on the thermal resistance of *Listeria monocytogenes*, *Int. J. Food Microbiol.*, 78: 235-243.
- Doyle ME, Mazotta AS, Wang T, Wiseman DW, Scott VN (2001). Heat resistance of *Listeria monocytogenes*; *J. Food. Prot.*, 64:410- 429.
- Farber JM, Peterkin PI (1991). *Listeria monocytogenes*, a food-borne pathogen, *Microbiol. Rev.*, 55: 476-511.
- Ferreira M, Hofer C, Raemy A (1997). A calorimetric study of egg white proteins, *J. Therm. Anal. Calorim.*, 48: 683-690.
- Foegending PM, Stanley NW (2006): Growth and inactivation of microorganisms isolated from ultrapasteurized egg, *J. Food. Sci.*, 52: 1219-1227.
- Froning GW, Peters D, Muriana P, Eskridge K, Travnicsek D, Sumner SS (2002). International egg pasteurization manual, United Egg Association, Alpharetta, GA.
- Hayman M, Anantheswaran M, Ramaswamy C, Knabel SJ (2008). Heat shock induces barotolerance in *Listeria monocytogenes*; *J. Food Protect.*, 71: 426-430.
- Henry NG, Garibaldi JA (1975). Death of *Staphylococcus aureus* in liquid whole egg near pH 8, *Appl. Microbiol.*, 29, 782-786.
- Hwang CA, Marmer BS (2006). Growth of *Listeria monocytogenes* in egg salad and pasta salad formulated with mayonnaise of various pH and stored at refrigerated and abuse temperatures, *Food Microbiol.*, 24: 211-218.
- Ibrahim HR, Sugimoto Y, Aoki T (2000). Ovotransferrin antimicrobial peptide (OTAP-92) kills bacteria through a membrane damage mechanism, *Biochim. Biophys. Acta*, 1523: 196-205.
- Jones JM, Monsey JB, Payne J (1983). Egg pasteurisation. *Nutr. Food. Sci.*, 83: 18-19.
- Lawrence LM, Gilmour A (1994). Incidence of *Listeria* spp. and *Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR., *Appl. Environ. Microbiol.*, 60: 4600-4604.
- Miller AJ, Bayles DC, Eblen BS (2000). Cold shock induction of thermal sensitivity in *Listeria monocytogenes*; *Appl. Environ Microbiol.*, 66: 4345-4350.
- Muriana PM, Hou H, Singh RK (1996). A flow-injection system for studying heat inactivation of *Listeria monocytogenes* and *Salmonella enteritidis* in liquid whole egg. *J. Food. Prot.*, 59: 121-126.
- Musgrove MT, Jones DR, Shaw JD, Sheppard M, Harrison MA (2009). Enterobacteriaceae and related organisms isolated from nest run cart shelves in commercial shell egg processing facilities, *Poultry Sci.*, 88: 2113-2117.
- Palumbo MS, Beers SM, Bhaduri S, Palumbo SA (1995). Thermal Resistance of *Listeria monocytogenes* and *Salmonella* spp. in liquid egg yolk and egg yolk products, *J. Food. Prot.*, 58: 960-966.
- Palumbo MS, Beers SM, Bhaduri S, Palumbo SA (1996). Thermal resistance of *Listeria monocytogenes* and *Salmonella* spp. in liquid egg white, *J. Food. Prot.*, 59: 1182-1186.
- Park SI, Daeschel MA, Zhao Y (2006). Functional properties of antimicrobial lysozyme-chitosan composite films, *J. Food. Sci.*, 69: 215-221.
- Rivoal K, Protais J, Quéguiner S, Boscher E, Chidaine B, Rose V, Gautier M, Baron F, Grosset N, Ermel G, Salvat G (2009). Use of pulsed-field gel electrophoresis to characterize the heterogeneity and clonality of *Salmonella* serotype Enteritidis, Typhimurium and Infantis isolates obtained from whole liquid eggs, *Int. J. Food Microbiol.*, 129: 180-186.
- Sörqvist S. (2003). Heat resistance of liquids of *Enterococcus* spp., *Listeria* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Salmonella* spp. and *Campylobacter* spp., *Acta Vet. Scand.*, 44: 1-19.
- USDA (1980). Regulations governing inspection of egg products. 7CFR, Part 2859
- Van der Veen S, Hain T, Wouters JA, Hossain H, Vos WM, Abee T, Chakraborty T, Wells-Bennik MHJ (2007). The heat-shock response of *Listeria monocytogenes* comprises genes involved in heat shock, cell division, cell wall synthesis, and the SOS response, *Microbiology-Engl. TR.*, 153: 3593-3607.