ISSN 1330–9862
(FTB-1405)

original scientific paper

Survival of *Escherichia coli* O157:H7 in Milk Exposed to High Temperatures and High Pressure**

Irena Usajewicz and Beata Nalepa*

Department of Industrial and Food Microbiology, Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Pl. Cieszyński 1, PL-10-975 Olsztyn, Poland

Received: September 22, 2004
Revised version: September 5, 2005
Accepted: September 21, 2005

Summary

The objective of the present study was to determine the survival of two enterohemorrhagic *Escherichia coli* O157:H7 strains (no. 94 and 402) and a saprophytic *E. coli* 1 strain at temperatures of 55 and 60 °C, and under the pressure of 300 to 600 MPa at ambient temperature (about 20 °C). The strains, in populations of 10^6–10^7 CFU/mL, were introduced into the skim milk and broth. The survival of test strains at high temperatures and high pressure depended to a high degree ($p<0.05$) on the type of medium in which the cells were suspended. At 55 °C the inactivation of *E. coli* cells was recorded after 60 to 120 min in the broth, and after 180 min in the milk. At 60 °C the time required for their thermal death was 15 to 30 min in broth. In milk only *E. coli* 1 cells died after 30-minute heating; the other strains survived in populations of about 40 CFU/mL. In the broth, a pressure of 550 MPa, applied for 20 min at ambient temperature, killed the entire populations of *E. coli* 94 and *E. coli* 402, and all *E. coli* 1 cells died at 600 MPa, also applied for 20 min at ambient temperature. In the milk live cells of all pressurized strains survived in the quantities of 10^2–10^3 CFU/mL, so their reduction by 5 log cycles was not achieved. Damaged cells were found in the majority of samples exposed to heating and high pressure. These cells did not form colonies on nutrient agar, but were able to repair damage and grow in nutrient broth at 37 °C.

Key words: *Escherichia coli*, survival, high temperature, high pressure

Introduction

Escherichia coli bacteria are major sanitation indicators in food production. Their presence in processed foods results from reinfection, because these bacteria usually do not survive food preservation processes. The main reasons for the presence of *E. coli* in food products are nonobservance of relevant technological regimes, incompliance with recommended process standards, and the lack of personal hygiene.

The majority of *E. coli* rods do not constitute a serious health hazard, but some serotypes can cause food poisonings and alimentary intoxications. Today the most dangerous among them are enterohemorrhagic *E. coli* strains, especially serotype O157:H7. Diseases caused by these strains may attack humans of all ages, but first of all elderly people and children (*1–3*). The sources of infections with enterohemorrhagic *E. coli* strains are mostly meat products, especially underdone steaks and hamburgers, but also such foodstuffs as apple cider, fruit juices, mayonnaise or yogurt, which under normal conditions rarely cause food poisonings due to high acidity (*2,4,5*). The infective dose of enterohemorrhagic *E. coli* strains is

*Corresponding author; Phone: ++48 89 52 33 729; Fax: ++48 89 52 34 615; E-mail: bena@uwm.edu.pl
**This paper was presented at the 19th International Symposium Food Micro 2004 in Portorož, Slovenia, September 12–16, 2004

very low, in contrast with other pathogenic strains of this species, and varies from several to 100 cells consumed with a food ration (*6,7*).

Until quite recently *E. coli* rods had been believed to be sensitive to most food preserving factors, but then Weagant *et al.* (*8*) demonstrated that some *E. coli* O157:H7 strains may survive for a relatively long time in foods with pH=3.7–4.4. Hill *et al.* (*9*) proved that these bacteria can easily adapt to strongly acid environments. According to Rowe and Kirk (*10*), the process of acquiring resistance to a single factor, *e.g.* low pH, may be accompanied by an increase in resistance to other environmental factors, like high temperature or high salt concentration.

One of modern food preservation methods is high-pressure technology, still applied on a limited scale only (*11,12*). The microorganisms considered most sensitive to high pressure are, among others, Gram-negative bacteria, usually inactivated at 300–400 MPa. However, results of recent studies (*13–15*) show that some Gram-negative strains are much more resistant to high pressures. An example may be *E. coli* O157:H7 strain no. NCTC 12079 (*13,15*), which can survive a pressure up to 700 MPa not only in milk, but also in phosphate buffer.

The objective of the present study was to determine the survival of three *E. coli* strains at temperatures of 55 and 60 °C, and under the pressure of 300 to 600 MPa.

Materials and Methods

The experimental material comprised two enterohemorrhagic *Escherichia coli* strains, no. 94 and 402 (serotype O157:H7), obtained from the collection of the Department of Animal Product Hygiene, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland, and a saprophytic *E. coli* 1 strain from our own collection. Test strains were cultured on nutrient agar slants with glucose (BTL, Łódź, Poland) at 37 °C for 24 h, and then stored at 4 °C. The studies on thermoresistance were performed on 24-hour glucose broth cultures incubated at 37 °C. Survival under high pressure was also tested using cultures multiplied in a glucose nutrient broth at 37 °C, which after 24-hour incubation at 37 °C were stored at 4 °C for 24 h.

Survival at high temperatures

The experiment was performed in reconstituted skim milk (pH=6.9–7.0) and nutrient broth (pH=7.0). Portions of milk or broth were inoculated with single *E. coli* strains, so as to obtain an initial cell population of 10^6–10^7 CFU/mL. A volume of 2 mL of the mixture was poured into sterile tubes, 6 mm in diameter, and heated in a thermo-regulated water bath at 55 and 60 °C. Heating time was 0.5, 1, 2 and 3 h at 55 °C, and 1, 5, 10, 15 and 30 min at 60 °C. A temperature of 55 °C was obtained after 2.5 min, and 60 °C after 3.5 min. Each experimental variant was performed in three parallel replications. Immediately after heating the samples were cooled and the count of live cells was determined by surface plating of 0.1 mL of respective dilutions onto glucose nutrient agar in Petri dishes, 9 cm in diameter. Undiluted samples, in the quantity of 1 mL, were cultured in Petri dishes, 14 cm in diameter. Incubation was carried out at 37 °C for 48 to 72 h. The remaining material (parallel samples) was stored at 37 and 7 °C for 7 days to confirm the results of quantitative cultures if no colonies were found on the agar, because it is a well-known fact that sublethally damaged cells left after pasteurization do not form colonies on agar media. However, under appropriate conditions such cells are able to repair damage and continue growth (*16*). In the samples stored for seven days after pasteurization cell survival was assessed on the basis of turbidity (in broth), or, in the case of milk samples, in brilliant green bile broth cultures (Danisco, Olsztyn, Poland).

Survival under high pressures

The effect of high pressures was analyzed in a Laboratory Hydraulic Press U 101 (Warszawa, Poland), in a high-pressure chamber Liquid Vessel LV/30/16, with extraction naphtha III as a pressure transfer medium. Cultures of *E. coli* 1, 94 and 402, in populations of 10^6–10^7 CFU/mL, were introduced into skim milk and broth. The samples were transferred to 5-mL sterile teflon vials, stored at 4 °C before pressurization and then exposed to the pressure of 300–600 MPa for 20 min at ambient temperature (about 20 °C). Pressure growth rate was approx. 350 MPa/min, and the maximum decompression time was 45 s. Pressurization time did not include these values. Immediately after pascalization the count of live cells was assessed by surface plating onto nutrient agar and violet red bile lactose (VRBL) agar (Merck, Darmstadt, Germany). The cultures were incubated at 37 °C for 48 h. The results obtained for both media provided the basis for calculating the percentage of sublethally damaged cells in populations, incapable of growth in selective media, according to the formula:

$$[(\text{no. colonies on nutrient agar} - \text{no. colonies on VRBL agar}) / \text{no. colonies on nutrient agar}] \cdot 100\ \%.$$

To check whether pressurized cells were unable to form colonies on agar, like in the case of high temperature, the sample residues (approx. 1 mL) were transferred to glucose broth and incubated at 37 °C for 7 days.

All experiments were performed in at least three replications. The results were subjected to multifactorial analysis of variance (ANOVA) by the Duncan test.

Results and Discussion

Survival at high temperatures

The rate of thermal death of test strain cells in the temperature range examined was significantly affected ($p<0.05$) by the medium, and was slower in milk than in broth. In milk heated at 55 °C (Fig. 1), death of the entire cell populations (10^6–10^7 CFU/mL) of all strains (lack of colony growth in 1-mL sample on nutrient agar media) was recorded after 3 h of heating. At 60 °C (Fig. 2) only *E. coli* 1 cells underwent inactivation in milk after 30 min of heating (reduction by 6.53 log units), whereas *E. coli* 94 and *E. coli* 402 strains survived at levels of 28–29 CFU/mL (reduction by approx. 5.5 log units). In broth, cell inactivation of the entire population took place after one hour (*E. coli* 1 and *E. coli* 94) or two hours (*E. coli* 402)

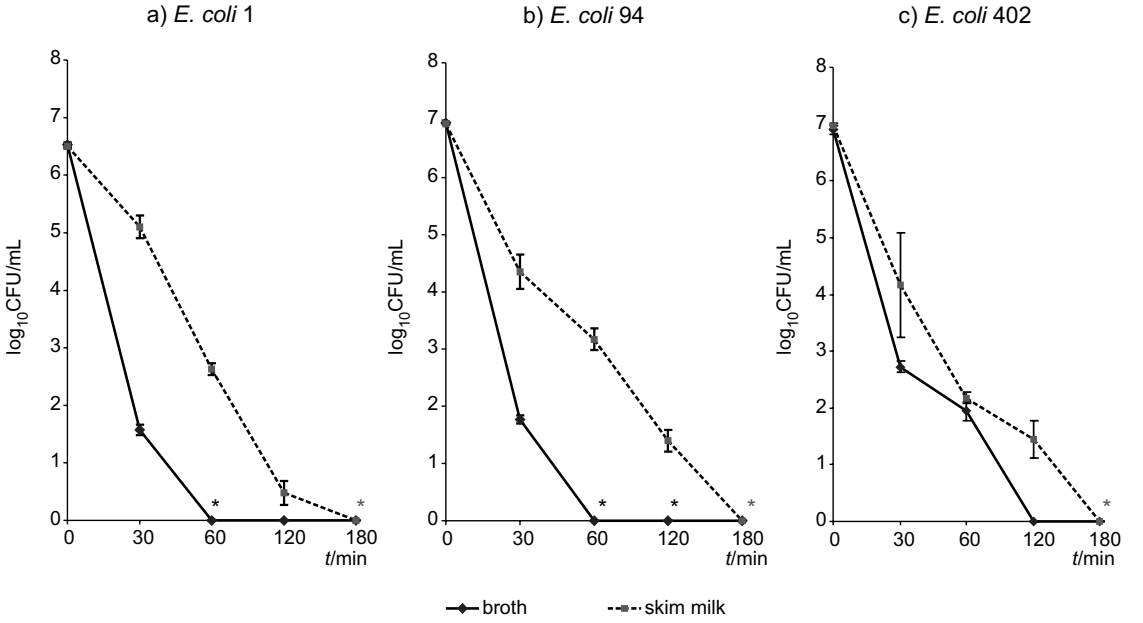

Fig. 1. Survival of *Escherichia coli* strains at 55 °C in milk and broth
*presence of damaged cells not forming colonies on nutrient agar; detection limit 1 CFU/mL

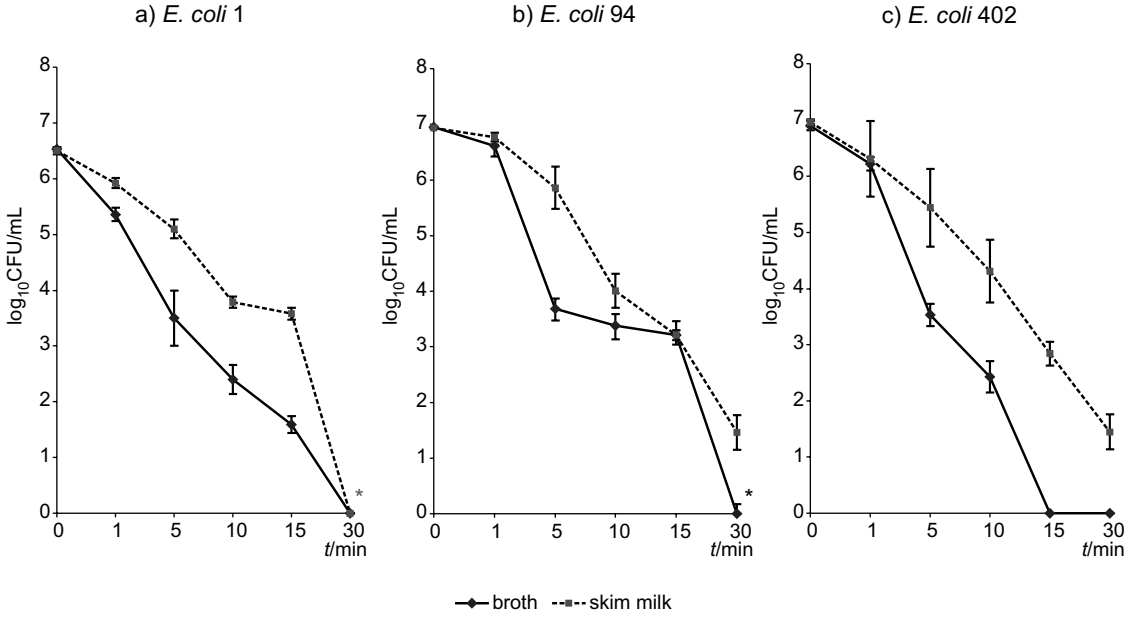

Fig. 2. Survival of *Escherichia coli* strains at 60 °C in milk and broth
*presence of damaged cells not forming colonies on nutrient agar; detection limit 1 CFU/mL

at 55 °C, and after 15 (*E. coli* 402) or 30 min (*E. coli* 1 and *E. coli* 94) at 60 °C. These results indicate that *E. coli* O157:H7 strains no. 94 and 402 are slightly more resistant to heating than the saprophytic strain *E. coli* 1.

Damaged cells were found in the majority of samples containing no live cells capable of forming colonies on nutrient agar (<1 CFU/mL) immediately after heating at 55 and 60 °C. Their presence (* in Figs. 1 and 2) was confirmed only when the experimental material was incubated at 37 °C. After 3–5 days of incubation (depending on the sample) the growth of bacterial strains was observed in broth, and their proliferation in milk was confirmed in brilliant green bile broth. Damaged cells were detected in milk in the samples of all test strains heated at 55 °C for 180 min, and in the sample of *E. coli* 1 heated at 60 °C for 30 min. In the broth, damaged cells were found in *E. coli* 1 suspension heated at 55 °C for 60 min, and in *E. coli* 94 suspension heated at 55 °C for 60 to 120 min and at 60 °C for 30 min. In the samples stored at 7 °C (minimal temperature required for strain growth) there were no cells capable of growth even after a 7-day incubation. This suggests that thermally damaged cells

of *E. coli* test strains are able to regenerate and continue growth under optimum conditions only.

Survival of *E. coli* strains at high temperatures has been studied by many authors in various media and by different research methods. Buchanan and Edelson (*17*) reported that the thermoresistance of *E. coli* O157:H7 strain heated at 58 °C in milk or chicken broth was significantly related to the final pH of the culture medium. In milk the value D_{58} determined for *E. coli* cells cultured in a medium whose final pH was 4.6–4.7 was by almost 3-fold higher, and the time required for their reduction by 5 log cycles (40.6 min) was 3.6-fold longer, compared with cells cultured in a medium whose final pH was 7.0–7.2. An increase in the resistance of *E. coli* O157:H7 strains to high temperatures, following cell adaptation to the acid environment, is referred to as cross protection (*10*). The thermoresistance of enterohemorrhagic *E. coli* strains has been studied primarily in meat, which is natural due to epidemiological reasons. For instance, Abdul-Raouf *et al.* (*18*) reported that the counts of *E. coli* O157:H7 in ground beef heated at 56 °C decreased by 6 log cycles as quickly as after 14 min. Duffy *et al.* (*19*) demonstrated that in minced salami (pH=4.7–5.1) the cell counts of three *E. coli* O157:H7 strains reduced by 6 to 7 log cycles after heating at 55 °C for 105–120 min. Heating at 60 °C for 7 min resulted in a reduction in the cell count of one of the strains by 3 log units, and complete inactivation of the other two strains. The populations that survived heating at both temperatures were dominated by damaged cells, unable to grow on selective agar. We found that the experimental material heated at 55 and 60 °C may contain even more damaged cells, unable of growth in selective or nutrient agar, and that cells can repair the damage under favourable conditions. A similar phenomenon was observed by Dabbah *et al.* (*16*) who studied the survival of *Pseudomonas* sp. in tryptose soy broth heated at 55 °C.

The above data indicate that Gram-negative bacteria, considered sensitive to high temperatures, do not always undergo complete inactivation during heating. Thermally damaged cells are not detected during routine food analyses, which in the case of pathogenic strains, *e.g. E. coli* O157:H7, may pose a serious threat to consumers' health.

Survival under high pressures

The value of pressure enabling to destroy test strain populations of the order of 10^6–10^7 CFU/mL depended significantly ($p<0.05$) on the growth medium. The lethal effect of pressure of 300–550 MPa (20 min at about 20 °C) on *E. coli* O157:H7 no. 94 and 402 was stronger in nutrient broth than in skim milk (Fig. 3). In the case of *E. coli* 1 this relationship was recorded only at 500 to 600 MPa, and at 350–450 MPa the degree of population inactivation was similar in both media. In broth, at 400 MPa, the population size of strains *E. coli* 1, *E. coli* 94 and *E. coli* 402 reduced by 1.4, 4.7 and 5.3 log cycles, respectively. At 500 MPa the counts of *E. coli* 94 and 402 decreased by about 7 log cycles, so it seems that complete inactivation of both strains was achieved, because immediately after pressurization no cells that were able to form colonies on nutrient agar were found in 1 mL of broth (<1 CFU/mL). When the same samples were transferred to fresh nutrient broth and incubated at 37 °C, growth of bacterial strains was observed as soon as after 24 h, which suggests that damaged cells capable of regeneration under appropriate conditions survived under the pressure of 500 MPa. No such cells were found in the broth pressurized at 550 MPa. Complete inactivation of *E. coli* 1 in broth (reduction by 6.2 log cycles and the lack of damaged cells) occurred at 600 MPa. In milk, at 400 MPa, the counts of live cells of *E. coli* 1, 94 and 402 decreased by 1.5, 2.7 and 2.1 log cycles, respectively. At the highest pressure in skim milk, *i.e.* 550 (*E. coli* 94 and 402) or 600 MPa (*E. coli* 1), the cells survived in populations of 10^2 and 10^3 CFU/mL, respectively. Similarly as in the broth, *E. coli* 1 showed the highest pressure resistance, since a reduction by 2.7 and 2.9 log cycles was achieved at 550 and 600 MPa, respectively. The populations of *E. coli* 94 and *E. coli* 402 were reduced by respectively 4.6 and 4.0 log cycles at 550 MPa. The protective effect of milk on bacterial cells treated with high hydrostatic pressure was also reported by other authors. Garcia-Graells *et al.* (*20*) stated that one of *E. coli* strains survived in milk at 600 MPa (15 min at 20 °C) in quantities of 5 log units greater than in phosphate buffer. Also in experiments performed by Patterson *et al.* (*13*) and Patterson and Kilpatrick (*21*), *E. coli* O157:H7 strains survived in milk at 600–700 MPa in populations greater by several log cycles than in phosphate buffer or poultry meat. Higher pressure resistance of bacteria in milk results from the presence of protective substances, mostly proteins, carbohydrates and fat (*22*).

Results of numerous studies show that one of the key factors deciding on survival of bacteria under high pressures is strain resistance. Gram-negative bacteria are generally more sensitive to high pressures than Gram-positive bacteria. Until quite recently it was believed that a pressure of 300–400 MPa was sufficient for successful inactivation of many Gram-negative bacteria (*23*). Examples of so sensitive bacteria can be found in a few publications only. For instance, Arroyo *et al.* (*24*) demonstrated that a pressure of 350 MPa applied at 10 °C for 20 min was sufficient to kill *E. coli* populations of 10^8 CFU/mL in tryptose soy broth, and that the same pressure applied at 20 °C for 10 min caused a reduction in the bacterial cell count by 6 log units. Gervilla *et al.* (*25*) reported that an *E. coli* strain was completely inactivated (reduction by 6 log cycles) in ovine milk at 400 MPa after 15 min (at 25 °C), and a *P. fluorescens* strain was reduced by 6 to 7 log cycles at 300 MPa after 10 to 15 min. However, results obtained by other authors indicate that Gram-positive and Gram-negative bacteria differ considerably in strain sensitivity to high hydrostatic pressure. Garcia-Graells *et al.* (*20*), Wuytak *et al.* (*14*) and Bozoglu *et al.* (*26*) proved that some *E. coli* and *Salmonella* spp. strains can survive under higher pressures than *Staphylococcus aureus*, *Listeria monocytogenes* or *Leuconostoc dextranicum* strains. Distinct differences in pressure sensitivity were also observed among *E. coli* strains. Benito *et al.* (*27*) studied the survival of five *E. coli* O157:H7 strains in phosphate buffer and found that at 500 MPa the cell count of sensitive strains decreased by 6 log units as soon as after 15–20 min, and of resistant strains by 1 to 1.5 log units after 30 min. Garcia-Graells *et al.* (*20*) and Linton *et al.* (*15*) demonstrated low sensitivity of *E. coli* strains to the pressure of 200–400 MPa in milk, whereas at higher pressures the cells of resistant strains survived in quan-

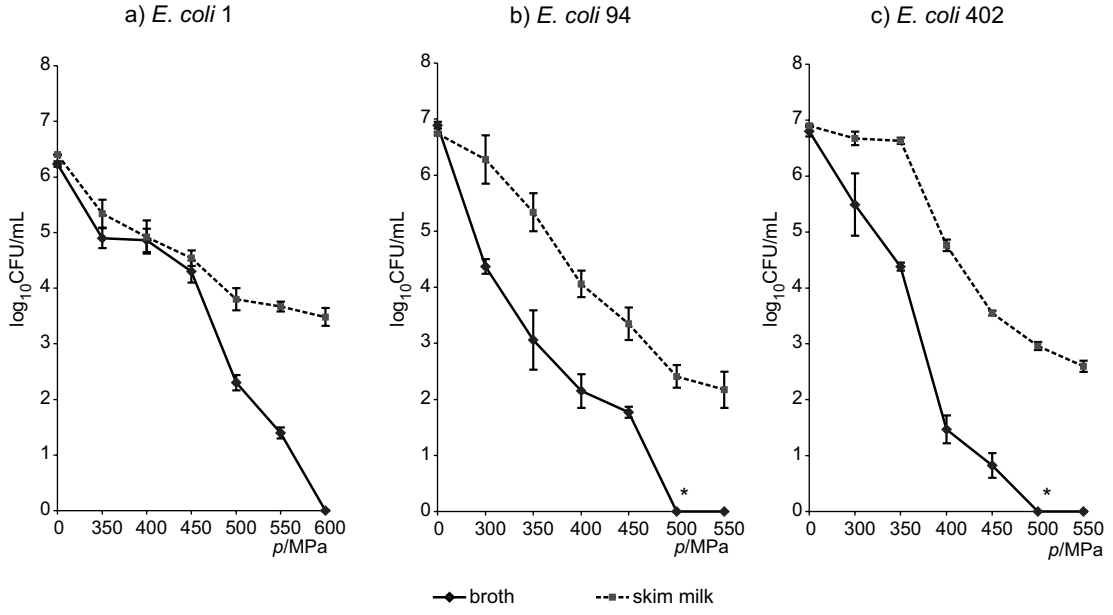

Fig. 3. Survival of *Escherichia coli* under high pressure in milk and broth
*presence of damaged cells not forming colonies on nutrient agar; detection limit 1 CFU/mL

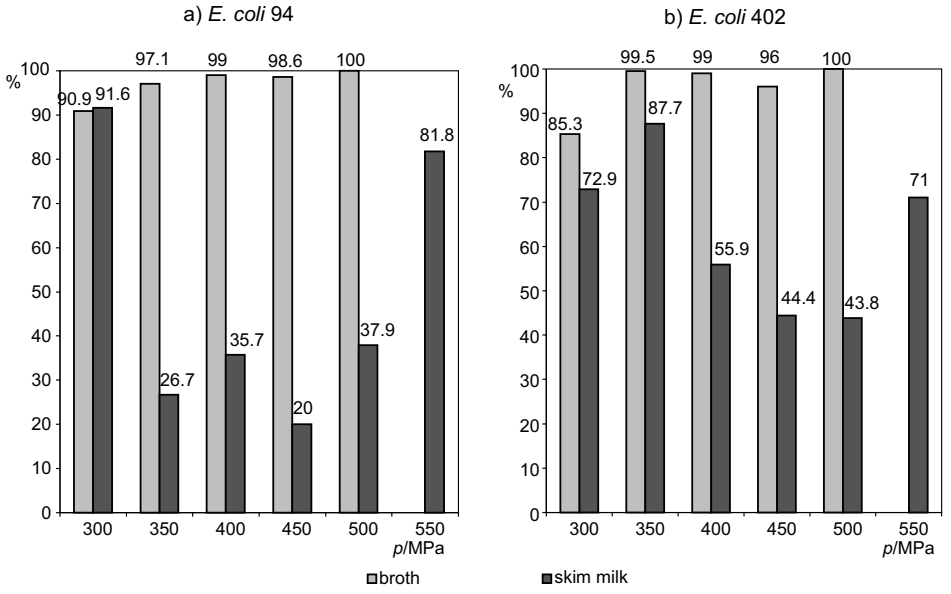

Fig. 4. Percentage of damaged cells (not forming colonies on VRBL agar) in populations that survived under high pressures

tities greater by about 3 log cycles than the cells of sensitive strains. In our study the most pressure resistant *E. coli* 1 strain survived in broth at 400–550 MPa applied for 20 min in quantities greater by 1.4–2.7 and 1.4–3.4 log units than *E. coli* 94 and *E. coli* 402 strains, and in milk in quantities grater by 0.9–1.4 and 0.16–0.99 log cycles, respectively.

Food preservation with high pressures (300–600 MPa) is considered an alternative to pasteurization and is sometimes referred to as high-pressure pasteurization, especially with respect to milk (*11,20*). In our opinion the use of this term is not fully justified. The processes of pasteurization, *i.e.* food product heating at 65–95 °C, cause complete inactivation of vegetative cells of pathogens, regardless of their count in raw materials. High pressures, even 600 or 700 MPa, are not equally efficient at ambient temperature (20 °C), as confirmed by our findings and those of other authors (*13,15,20,21*).

Another problem are cells damaged by high hydrostatic pressure, which are not detected in routine microbiological analyses. We found that samples of pressurized *E. coli* O157:H7 strains no. 94 and 402 contained two groups of cells showing various degrees of damage. Cells with minor damages did not form colonies on VRBL agar, but could grow on nutrient agar. Cells with major damages did not form colonies even on nutrient

agar, but were capable of regeneration in liquid media. Fig. 4 presents the percentage of damaged cells, unable to grow on VRBL agar, in the populations that survived pressurization. Such cells dominated in the majority of samples, accounting for 85.3 to 100 % of the populations that survived under 300–500 MPa in broth. In milk, damaged cells made up 81.8 to 90.9 % of the *E. coli* 94 population under 300 and 550 MPa, and over 70 % of the *E. coli* 402 population under 300–400 and 550 MPa. The differences in the cell count determined on nutrient agar and VRBL agar ranged from 0.83 to 2.28 log units in broth, and from 0.1 to approx. 1 log unit in milk. Similar damage of pressurized *E. coli* cells and their inability to grow on selective media were also described by other authors (*13,21,26*). Patterson *et al.* (*13*) reported that the count of *E. coli* O157:H7 cells that survived at 600 MPa for 15 min in milk and poultry meat, determined on tryptose soy agar with 2 % NaCl, was by 3.03 and 4.54 log units respectively lower than their count determined on tryptose soy agar without NaCl. The presence of cells with major damages, unable to grow on nutrient agar, was recorded in suspensions of *E. coli* strains no. 94 and 402 in broth at 500 MPa (* in Fig. 3). In the cultures of these samples, made immediately after pressurization, colony growth was observed neither on nutrient agar nor on VRBL agar (<1 CFU/mL), so it seemed that both strains had been completely inactivated. However, in parallel nutrient broth cultures of the experimental material the growth of both strains was observed after one-day incubation at 37 °C, which indicates that the above strains are capable of repairing even major cellular damage. The occurrence of the same two types of sublethal cellular damage caused by high pressure was also described by Bozoglu *et al.* (*26*) and Benito *et al.* (*27*). These authors examined the effect of the pressure of 350–550 MPa at 45 °C on the following strains: *E. coli* O157:H7, *S. enteritidis*, *L. monocytogenes* and *S. aureus*. Damage repair usually took place after one or two days of storage of pressurized samples at 22 and 30 °C. The process took longer at 4 °C, and the *E. coli* O157:H7 strain was not able to repair damage of the second type. According to Chilton *et al.* (*28*), damage to *E. coli* cells treated with a pressure of 400 MPa included outer membrane and cytoplasmatic membrane damage. The cells were capable of repairing the damage done to both membranes during incubation at 37 °C in tryptose soy broth. This indicates that cytoplasmatic membrane damage is not always followed by cell inactivation. According to Alpas *et al.* (*29*), the main cause of cell high pressure and thermal death may be ribosome destruction. These authors demonstrated that the mechanism of ribosome destruction under conditions of high pressure and high temperature are different, which may explain different sensitivity of bacteria to these two factors, observed also in our study.

Conclusions

Two *E. coli* O157:H7 strains (no. 94 and 402) examined in the study were characterized by high thermoresistance. They survived 30-minute heating in milk at 60 °C in populations reduced by 5.5 log cycles. The *E. coli* 1 strain, inactivated at 60 °C, showed higher pressure resistance than *E. coli* O157:H7 strains (no. 94 and 402). A pressure of respectively 550 and 600 MPa was not sufficient to reduce the cell count of the tested strains in milk by 5 log cycles. High temperatures and high pressures resulted in cellular damage in test strains. The cells were able to repair damage only under optimum conditions (*i.e.* at 37 °C), whereas the storage of samples at 7 °C inhibited their regeneration and growth. The presence of sublethally damaged, but capable of regeneration, pathogenic cells in preserved foods may be dangerous, especially in the case of such bacteria as *E. coli* O157:H7, whose infective dose is very low.

Acknowledgement

This work was financially supported by WAMADA-IREC.

References

1. D. Law, Virulence factor of *Escherichia coli* O157 and other Shiga toxin-producing *Escherichia coli*, *J. Appl. Microbiol. 88* (2000) 729–745.
2. N.V. Padhye, M.P. Doyle, *Escherichia coli* O157:H7: Epidemiology, pathogenesis, and methods for detection in food, *J. Food Prot. 55* (1992) 555–565.
3. K. Takahashi, Microbicidal effect of hydrostatic pressure on satsuma mandarin juice, *Int. J. Food Sci. Technol. 28* (1993) 95–102.
4. P.S. Mead, P.M. Griffin, *Escherichia coli* O157:H7, *Lancet*, *352* (1998) 1207–1212.
5. L.G. Miller, C.W. Kaspar, *Escherichia coli* O157:H7 acid tolerance and survival in apple cider, *J. Food Prot. 57* (1994) 460–464.
6. S.H. Choi, D.J. Baumler, C.W. Kaspar, Contribution of *dps* to acid stress tolerance and oxidative stress tolerance in *Escherichia coli* O157:H7, *Appl. Environ. Microbiol. 66* (2000) 3911–3916.
7. J.M. Li Wan Po, P. Piyasena, R.C. McKellar, F.M. Bartlett, G.S. Mittal, X. Lu, Influence of simulated apple cider composition on the heat resistance of *Escherichia coli* O157:H7, *Lebensm. Wiss. Technol. 35* (2002) 295–304.
8. S.D. Weagant, J.L. Brylant, D.H. Bark, Survival of *Escherichia coli* O157:H7 in mayonnaise and mayonnaise-based sauces at room and refrigerated temperatures, *J. Food Prot. 57* (1994) 629–631.
9. C. Hill, B. O'Driscoll, I. Booth, Acid adaptation and food poisoning microorganisms, *Int. J. Food Microbiol. 28* (1995) 245–254.
10. M.T. Rowe, R. Kirk, An investigation into the phenomenon of cross-protection in *Escherichia coli* O157:H7, *Food Microbiol. 16* (1999) 157–164.
11. J.P.P.M. Smelt, Recent advances in the microbiology of high pressure processing, *Trends Food Sci. Technol. 9* (1998) 152–158.
12. A. Suzuki, High pressure-processed foods in Japan and the world, *Trends High Press. Biosci. Biotechnol. 19* (2002) 365–374.
13. M.F. Patterson, M. Quinn, R. Simpson, W. Gilmour, Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate–buffered saline and foods, *J. Food Prot. 5* (1995) 524–529.
14. E.Y. Wuytak, A.M.J. Diels, C.W. Michiels, Bacterial inactivation by high-pressure homogenization and high hydrostatic pressure, *Int. J. Food Microbiol. 77* (2002) 205–212.
15. M. Linton, J.M.J. McClements, M.F. Patterson, Inactivation of pathogenic *Escherichia coli* in skimmed milk using high

hydrostatic pressure, *Innov. Food Sci. Emerg. Technol. 2* (2001) 99–104.

16. R. Dabbah, W.A. Moats, J.F. Mattick, Factors affecting resistance to heat and recovery of heat-injured bacteria, *J. Dairy Sci. 52* (1969) 608–614.
17. R.L. Buchanan, S.G. Edelson, Effect of pH-dependent, stationary phase acid resistance on the thermal tolerance of *Escherichia coli* O157:H7, *Food Microbiol. 16* (1999) 447–458.
18. U.M. Abdul-Raouf, L.R. Beuchat, M.S. Ammar, Survival and growth of *Escherichia coli* O157:H7 in ground, roasted beef as affected by pH, acidulants, and temperature, *Appl. Environ. Microbiol. 59* (1993) 2364–2368.
19. G. Duffy, D.C.R. Riordan, J.J. Sheridan, B.S. Eblen, R.C. Whiting, I.S. Blair, D.A. McDowell, Differences in thermotolerance of various *Escherichia coli* O157:H7 strains in a salami matrix, *Food Microbiol. 16* (1999) 83–91.
20. C. Garcia-Graells, C. Valckx, C.W. Michiels, High-pressure inactivation and sublethal injury of pressure-resistant *Escherichia coli* mutants in fruit juices, *Appl. Environ. Microbiol. 66* (2000) 4173–4179.
21. M.F. Patterson, V. Kilpatrick, The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry, *J. Food Prot. 61* (1998) 432–436.
22. M.F. Patterson, M. Quinn, R. Simpson, A. Gilmour: Effects of High Pressure on Vegetative Pathogens. In: *High Pressure Processing of Food*, University Press, Nottingham (1995) pp. 110–117.
23. A. Carlez, J.F. Rosec, N. Richard, J.C. Cheftel, High pressure inactivation of *Citrobacter freundii*, *Pseudomonas fluorescens* and *Listeria innocua* in inoculated minced beef muscle, *Lebensm. Wiss. Technol. 26* (1993) 357–363.
24. G. Arroyo, P.D. Sanz, G. Prestamo, Effect of high pressure on the reduction of microbial populations in vegetables, *J. Appl. Microbiol. 82* (1997) 735–742.
25. R. Gervilla, X. Felipe, V. Ferragut, B. Guamis, Effect of high hydrostatic pressure on *Escherichia coli*, *J. Dairy Sci. 80* (1997) 2297–2303.
26. F. Bozoglu, H. Alpas, G. Kaletunç, Injury recovery of foodborne pathogens in high hydrostatic pressure treated milk during storage, *FEMS Immunol. Med. Microbiol. 40* (2004) 243–247.
27. A. Benito, G. Ventoura, M. Casadei, T. Robinson, B. Mackey, Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses, *Appl. Environ. Microbiol. 65* (1999) 1564–1569.
28. P. Chilton, N.S. Isaacs, P. Manas, B.M. Mackey, Biosynthetic requirements for the repair of membrane damage in pressure-treated *Escherichia coli*, *Int. J. Food Microbiol. 71* (2001) 101–104.
29. H. Alpas, J. Lee, F. Bozoglu, G. Kaletunç, Evaluation of high hydrostatic pressure sensitivity of *Staphylococcus aureus* and *Escherichia coli* O157:H7 by differential scanning calorimetry, *Int. J. Food Microbiol. 87* (2003) 229–237.

Preživljavanje bakterije *Escherichia coli* O157:H7 u mlijeku izloženom visokim temperaturama i tlaku

Sažetak

Ispitano je preživljavanje dvaju enterohemoragijskih sojeva bakterije *Escherichia coli* O157:H7 (broj 94 i 402) i saprofitnoga soja *Escherichia coli* 1 pri temperaturi od 55 i 60 °C, te pod tlakom od 300 do 600 MPa pri približno 20 °C. Obrano mlijeko i mesni bujon inokulirani su pojedinim sojem *E. coli* kako bi se postigla početna populacija od 10^6 do 10^7 CFU/mL. Preživljavanje sojeva pri visokim temperaturama i tlaku ovisilo je uvelike ($p<0,05$) o vrsti medija u kojem su stanice suspendirane. Pri 55 °C opažena je inaktivacija stanica *E. coli* u bujonu nakon 60–120 min, a u mlijeku nakon 180 min. Pri 60 °C vrijeme potrebno za toplinsko uništenje u bujonu iznosilo je 15–30 min. U mlijeku su nakon 30 min uništene samo stanice soja *E. coli* 1, dok su ostali sojevi preživjeli u populaciji od približno 40 CFU/mL. U bujonu je izlaganjem tlaku od 550 MPa tijekom 20 min pri 20 °C uništena cijela populacija *E. coli* 94 i *E. coli* 402, dok su pri istoj temperaturi sve stanice *E. coli* 1 uginule pri 600 MPa tijekom 20 min. U mlijeku je količina od 10^2 do 10^3 CFU/mL živih stanica svih sojeva preživjela pri tlaku od 550 do 600 MPa, pa se nije postiglo njihovo smanjivanje za 5 logaritamskih ciklusa. U većini uzoraka izloženih povišenoj temperaturi i tlaku nađene su oštećene stanice. One nisu mogle stvarati kolonije na hranjivom agaru, ali su bile sposobne popraviti oštećenja i rasti u hranjivom bujonu na 37 °C.