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Death Kinetics of *Escherichia coli* in Goat Milk and *Bacillus licheniformis* in Cloudberry Jam Treated by Ohmic Heating*

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The influence of ohmic heating on the death kinetic parameters of *Escherichia coli* ATCC® 25922 in goat milk and spores of *Bacillus licheniformis* ATCC® 14580 in cloudberry jam was investigated and compared with that of conventional heating. Ohmic treatment of goat milk shortened the decimal reduction time D in comparison with the D values obtained at conventional treatment. Similarly, the z value, increase of temperature required for a ten-fold reduction of D, was also lower at ohmic treatment. The death kinetics of *Bacillus licheniformis* ATCC® 14580 spores in cloudberry jam was also studied employing both types of heat treatment. Similar conclusions were obtained for the D values as in the case of goat milk. However, the differences between the z values obtained for ohmic and conventional heating were not significant.

Keywords: ohmic heating, *Escherichia coli*, *Bacillus licheniformis*, spores, death kinetics

INTRODUCTION

Presently, the most commonly practiced technology for preservation of foods is thermal processing, which includes *e.g.* cooking, pasteurizing, drying, distillation, and evaporation. The application of these technologies, relying on indirect mechanisms of heat transfer to particulate foods, is limited by the time required to conduct sufficient heat into the centre of large particles to ensure sterilization. In most cases the price of the safety and long-term stability is the loss of fresh volatile flavours, vitamins, and physicochemical characteristics [1]. Recently, electromagnetic technologies in food processing have gained increased industrial interest as alternatives to conventional pasteurization techniques in an effort to produce new, high-quality and shelf-stable food products.

Ohmic heating (also called Joule heating) is one of the earliest applications of electricity in food pasteurization and is based on the passage of electrical current through a food product, which serves as an electrical resistance [2]. Indeed, the food behaves as a resistor in an electrical circuit and the passage of an alternating current creates an oscillatory movement of ions, which generates the heat dissipation underlying the resistance-heating phenomenon [3]. A major advantage claimed for ohmic heating is its ability to heat materials rapidly and uniformly, including products containing particulates, resulting in less thermal damage to the product in comparison to conventional heating [4]. In the aseptic systems of food processing, ohmic heating is seen as a potential alternative to conventional heating processes because it provides uniform heating of both large solid particles and the liquid phase [5, 6]. Presently, the discussion is focused on the application of ohmic heating for enzyme and microbial control. Recently, the interaction of microorganisms with electric field dealt with microbial inactivation [5—7]. In ohmic heating the principal mechanisms of microbial inactivation are thermal in nature; however, recent research [8, 9] suggests that a mild electroporation mechanism may contribute to cell death, bringing a non-thermal effect to inactivation. Another study [10], where conventional and ohmic heating were conducted to ensure the same temperature histories in order to discard the influence of thermal effects, indicates that ohmic heating can accelerate kinetics of inactivation of *Bacillus subtilis* spores.

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However, according to *Leizerson* and *Shimoni* [7] differences between the conventional and ohmic heating treatment were not detected for the inactivation of *Bacillus subtilis* in fresh orange juice. Although the possibility of non-thermal killing effects of the ohmic heating is suspected, further research is needed to understand inactivation mechanisms of various microorganisms in the different types of foodstuffs. Thermal destruction of microorganisms tends to follow the first-order reaction kinetics and traditionally is described by the time required to cause 90 % reduction in a microbial population at a given temperature. This value is referred to as the D value, or decimal reduction time, which also follows exponential relationship. The temperature increase required to reduce a microorganism's D value by 90 % is referred to as the z value. For thermal processes, understanding a microorganism's D and z values allows to assess the extent of microbial destruction caused by the process [4]. The industrial application of the ohmic heating technology is fully dependent on its validation with experimental data to evaluate the effects of the electric field on enzymes, biological tissues, and microorganisms [11].

In the present work, samples of cloudberry jam and goat milk were artificially contaminated with spores of *Bacillus licheniformis* ATCC® 14580 and *Escherichia coli* ATCC® 25922, respectively. These strains are known to be a microbiological concern for the companies producing low-acid foods (namely fruit jams) and cheese. Experiments were performed using a static ohmic heater and matching heating histories were applied for both conventional and ohmic heating in order to examine the inactivation effect of the electrical current. Heat resistance is an important characteristic of *Bacillus* genus spores, which are highly resistant to HTST (high-temperature short-time) treatments. Species such as *B. licheniformis* commonly isolated from fruit preparations have been associated with food poisoning [12]. Due to its high water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms such as *Escherichia coli*, which frequently contaminates dairy products when their manufacture conditions are unsanitary.

The objectives of this investigation were to determine the influence of ohmic heating on the heat resistance (D and z values) of the microorganisms under study in a wide range of temperature treatments and compare it to that of conventional heating.

EXPERIMENTAL

In the present study two different food products were analyzed, goat milk and cloudberry jam, obtained directly from jam and goat cheese producers. Milk was collected immediately before industrial pasteurization and transported under refrigerated conditions to the laboratory. Experiments were conducted promptly in order to avoid microbiological deterioration of the products, which might affect the results. The jam and milk used in the experiments had an initial pH value of 3.83 ± 0.03 and 6.59 ± 0.04, respectively. Further preparation was necessary for the samples of cloudberry jam, which were homogenized and smashed using a Moulinex Commercial Turbo Blender. The seeds present in the jam were drained off and the remnant was centrifuged (5 min at 10000 min^{-1}). This procedure was necessary to liquefy the sample. The homogenates were sterilized in order to eliminate contaminations during the sample preparation stages.

The strain of *Escherichia coli* ATCC® 25922 used in this work was purchased from Oxoid (Basingstoke, U.K.) as a Culti-loop®. For the preparation of a suspension of cells, a 1 mL loop of stock culture was transferred to 10 mL of Tryptic Soy Broth (TSB) (ref. 211825, Becton, Dickinson and Company, Sparks, USA) and aerobically incubated at 37 °C for 18 h. The population density in each inoculum was enumerated using the pour-plate procedure.

The strain of *B. licheniformis* ATCC® 14580 (Spanish Type Culture Collection 20) was maintained through monthly transfers on Plate Count Agar (PCA) (ref. 247940, Becton, Dickinson and Company, Sparks, USA) slants and stored at 4 °C. Cultures were then transferred and spread on the surface of PCA and incubated at 35 °C for 14 days. Sporulation was checked by the phase contrast microscopy and green malachite staining technique. The spores were harvested with a glass spatula, washed with sterile peptone water, and concentrated by centrifugation four times at 2500 g for 15 min [13]. After the last centrifugation and resuspension in sterile distilled water, spore suspension was heated (80 °C for 10 min) to kill vegetative cells and stored at 4 °C until use.

For thermal inactivation by conventional heating, Eppendorf tubes (9 mm of internal diameter and 40 mm height) containing the inoculated sample were completely immersed in a temperature-controlled bath during the heating cycle. Timing was started when the tubes reached the test temperature. At the same time, a sample (two tubes) was taken to measure the actual initial count. Pairs of tubes were subsequently removed at appropriate time intervals. All tubes containing the samples were immediately transferred to an ice bath until further analyses were performed. The thermal history of the samples was monitored by the introduction of the K-type thermocouple connected to a data acquisition system in one Eppendorf tube which served as a control. This procedure was repeated for all temperatures.

The ohmic heater consisted of a cylindrical glass tube of 30 cm total length and 2.3 cm inner diameter. Three thermocouple openings were provided; two at an equal distance of the centre of the tube and one at the centre, where the K-type thermocouple

was placed. Two platinized titanium electrodes with teflon pressure caps were placed at each end of the tube. For each type of microorganism, samples of approximately 25 mL were heated using an alternating current source of 50 Hz frequency and variable amplitude. For the ohmic heating treatment the inoculated sample was transferred to the ohmic chamber. Like in conventional inactivation, timing was started and two samples were removed when the sample reached the desired test temperature. Each test temperature was maintained constant, during the prescribed time interval, by the control of voltage applied to the ohmic heating unit. Pairs of samples were subsequently removed with a micropipette at the established time intervals. All the samples were immediately transferred to an ice bath until further analyses were performed.

A set of experiments was conducted to determine the effect of the field strength on the electrical conductivity changes during the ohmic heating. The goat milk and cloudberry jam samples were heated up to 90 °C using four different field strengths ranging from 20 V cm^{-1} to 54 V cm^{-1} (Fig. 1) with a 2 cm gap between electrodes. During the experiments the electric field was varied by a rheostat to adjust the supplied voltage and simulate the thermal history of samples observed during the experiments with conventional heating.

Thermal resistance of *E. coli* was assessed using 50 mL of homogenized goat milk that was heated twice to eliminate background microflora. The *E. coli* inoculum (1 mL) was added to the goat milk in order to achieve a final concentration of colony-forming units (CFU) of approximately 10^8—10^9 mL^{-1}. For the experiments with conventional heating the inoculated samples were homogenized again, placed in sterile Eppendorf tubes (1 mL), and subjected to the test temperatures of 55 °C, 63 °C, 65 °C, 67 °C, and 70 °C in a temperature-controlled water bath. For ohmic heating assays, approximately 25 mL of the inoculated sample was placed in the ohmic heater and heated at 55 °C, 60 °C, 63 °C, and 65 °C.

Thermal resistance of the spores of *B. licheniformis* was determined in the suspension by diluting the extract of previously sterilized sample (121 °C for 15 min) of cloudberry jam. Initial concentrations of spores in suspensions varied from 10^4 mL^{-1} to 10^7 mL^{-1}. Volumes of 2 mL of cloudberry jam were placed in sterile Eppendorf tubes and heated at 70 °C, 75 °C, 80 °C, 85 °C, and 90 °C in a temperature-controlled water bath. In the ohmic heater the 25 mL samples were heated at 70 °C, 75 °C, and 80 °C.

To enumerate the count of surviving microorganisms, appropriate serial dilutions (1 : 10) were carried out with sterile peptone water (0.1 %) and were plated in triplicate. The number of surviving spores of *B. licheniformis* was determined using PCA after incubation at 37 °C for 24 h. The number of viable colonies of *E. coli* was counted in MacConkey agar

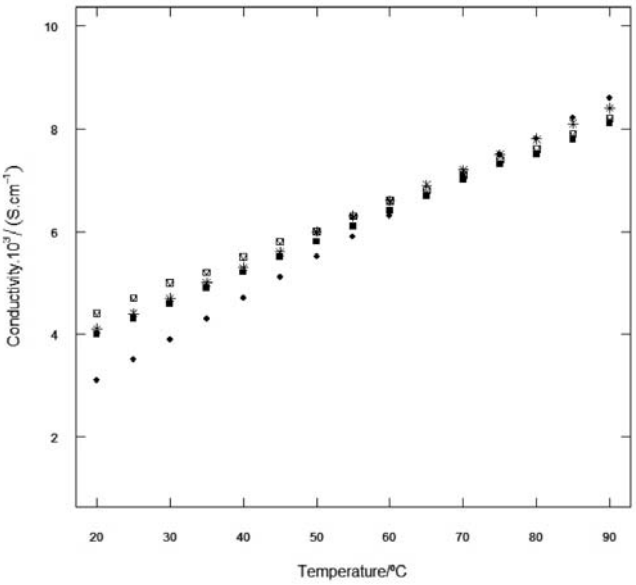

Fig. 1. Electrical conductivity of goat milk for the field strength of 20.6 V cm^{-1} (□), 28.9 V cm^{-1} (■), 37.5 V cm^{-1} (◆), and 53.8 V cm^{-1} (∗).

No. 3 (CM 0115, Oxoid, Basingstoke, U.K.) after incubation at 36 °C for 18—24 h.

For each temperature, media, and strain, the decimal reduction time D was determined by the linear regression from the expression of *Singh* and *Heldman* [14]

$$\log N = \log N_0 - t/D \qquad (1)$$

where N is the count value at the time t and N_0 the initial count value. The number of survivors in the samples exposed to the heat treatment for defined time intervals was determined by the plate count. The temperature increase necessary to reduce the value of D by 90 %, z, was determined from the slope of the regression line obtained by plotting the D values *vs.* their corresponding heating temperatures.

To evaluate the significance of differences found in the kinetic parameters determined for the conventional and ohmic heating treatments, Student's t-test was performed at a significance level of 5 %. The whole statistical comparison was carried out using the SPSS software package (version 14.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

In order to eliminate the influence of the temperature difference on the kinetic parameters obtained for both kinds of thermal treatments, the ohmic heating experiments were adapted to simulate the sample temperature changes during the conventional heating. Comparison of the typical thermal history of the samples treated by conventional and ohmic heating is pre-

Table 1. D and z Values for *E. coli* Cells Inactivation when Submitted to Conventional and Ohmic Heating

Temperature/ ℃	Conventional heating			Ohmic heating		
	D/min	z/ ℃	r^2	D/min	z/ ℃	r^2
55	10.9 ± 1.8	23.1	0.98	14.2 ± 0.2	8.4	0.99
60	–			4.2 ± 0.6		
63	3.9 ± 0.5			1.9		
65	3.5 ± 0.2			0.86		
67	2.8			–		
75	1.5			–		

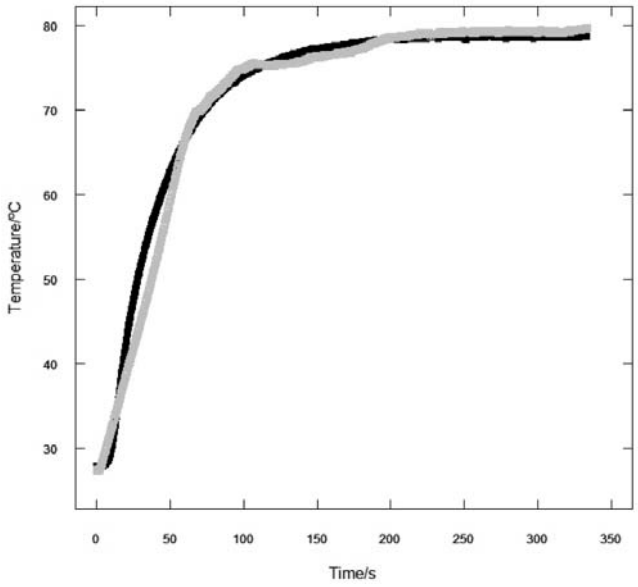

Fig. 2. Thermal history of samples of cloudberry jam processed by conventional (black line) and ohmic (grey line) heating.

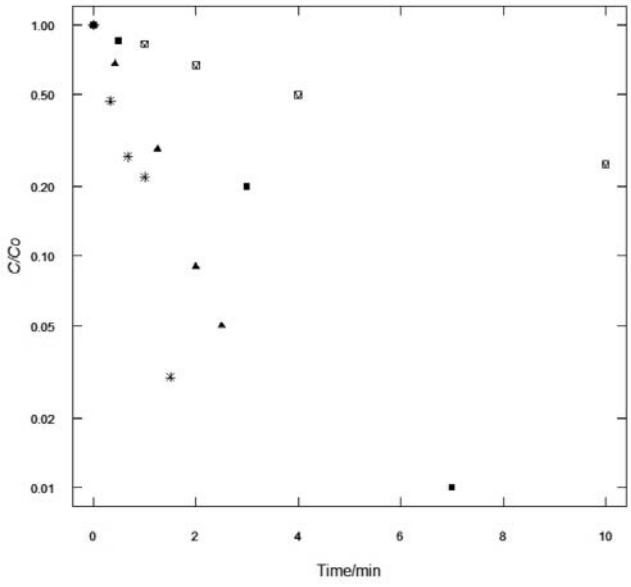

Fig. 3. D Values for spores of *E. coli* ohmically heated at 55 ℃ (□), 60 ℃ (■), 63 ℃ (▲), and 65 ℃ (∗).

sented in Fig. 2. A close coincidence of the temperature profiles during the sample heating phase was the necessary condition permitting to evaluate the non-thermal effects of the ohmic processing.

Possible non-thermal effects of the ohmic heating caused by chemical reactions at the surface of electrodes were avoided by the use of platinized titanium electrodes, which are relatively inert applying commonly available low-frequency alternating currents in the whole range of pH values [15]. For this reason, the possible differences in the results obtained should be related to the effect of the electric field.

The results of the thermal inactivation of *E. coli* are summarized in Table 1 showing that the D values for ohmic heating were considerably lower than those calculated for conventional heating, especially at higher temperatures. The only exception was found when the experiments were carried out at 55 ℃.

According to the previous studies [16], ohmic heating performed at temperatures similar to the optimum growth temperature of the tested microorganism, enhanced the early stages of its growth. Based upon this fact, the ohmic treatment of the contaminated goat milk at 55 ℃ could have stimulated the growth of *E. coli*, thus contributing to its higher resistance against inactivation. However, the overall tendency observed in Figs. 3 and 4 is that the ohmic heating provided higher inactivation rate compared with the conventional heating. This fact was also reflected when evaluating the z value (Fig. 5). The results obtained for the two inactivation treatments carried out at different temperatures were not compared.

The z value for conventionally heated samples contaminated with cells of *E. coli* was substantially higher ($z = 23.1$ ℃) than that obtained for samples treated by the ohmic heating ($z = 8.2$ ℃). Therefore, noticeable additional killing effect of the electrical current on *E. coli* was observed at almost all temperatures studied. Similar results were found elsewhere [17—21] reporting the sublethal injury of *E. coli* cells by electrical current.

Kinetic parameters corresponding to the decay of *B. licheniformis* spores are summarized in Table 2.

Table 2. D and z Values for *B. licheniformis* Spores Inactivation when Submitted to Conventional and Ohmic Heating

Temperature/ ℃	Conventional heating			Ohmic heating		
	D/min	z/ ℃	r^2	D/min	z/ ℃	r^2
70	85.3 ± 6.8	11.4	0.98	59.6 ± 3.5	11.8	0.99
75	51.0 ± 2.3			25.2 ± 1.2		
80	18.1 ± 1.1			11.1 ± 1.3		
85	6.02 ± 0.11			–		
90	1.57 ± 0.36			1.19 ± 0.01		

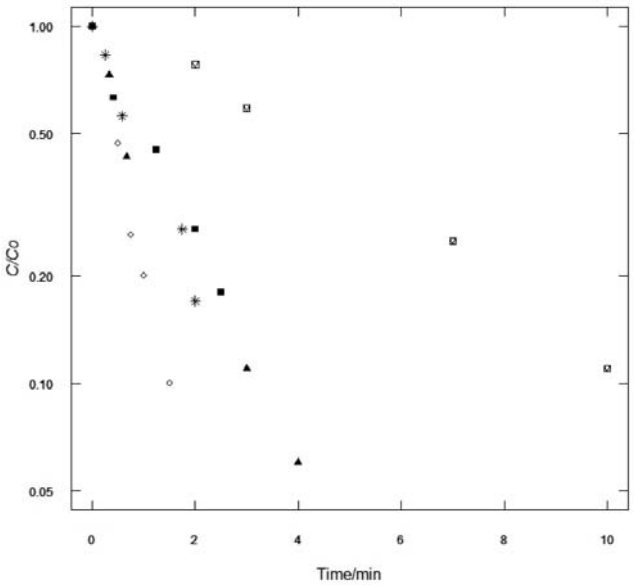

Fig. 4. D Values for spores *E. coli* conventionally heated at 55 ℃ (□), 63 ℃ (■), 65 ℃ (▲), 67 ℃ (∗), and 75 ℃ (◇).

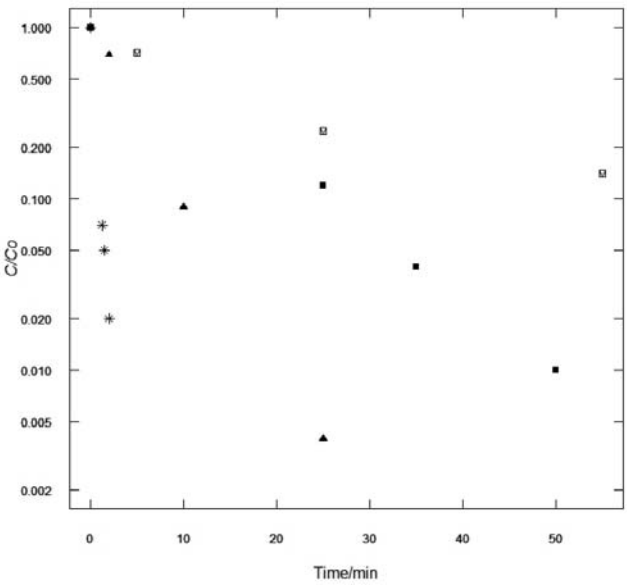

Fig. 6. D Values for the spores of *B. licheniformis* ohmically heated at 70 ℃ (□), 75 ℃ (■), 80 ℃ (▲), and 90 ℃ (∗).

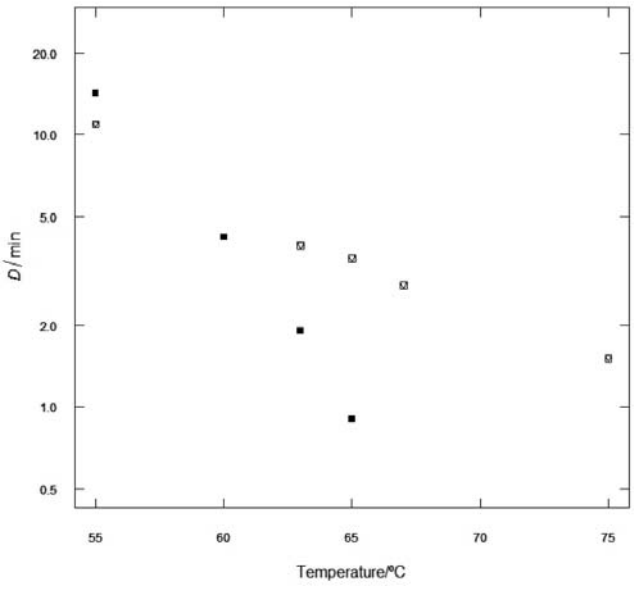

Fig. 5. Death kinetics of *E. coli* when submitted to conventional (opened symbols) and ohmic (full symbols) heating.

In general, experimental D values were smaller in the case of food samples treated ohmically compared to those calculated for pasteurization by conventional heating. Consequently, the microorganism inactivation was faster when the ohmic heating was used (Figs. 6 and 7). Differences between the decimal reduction times obtained for the two thermal treatments were statistically significant when the treatment was carried out at 70 ℃, 75 ℃, and 80 ℃, while the opposite was true ($p > 0.05$) for the pasteurization of food samples at 90 ℃.

The z values obtained by the linear regression were 11.4 ℃ and 11.1 ℃ for conventional and ohmic treatment, respectively. According to *Cho et al.* [10] these results could indicate that the electrical current affected the death rate, but did not affect the temperature variation of the spores inactivation process (Fig. 8). No data regarding the influence of ohmic heating on *B. licheniformis* spores inactivation have been published yet; however, the z value calculated for the conventionally treated samples is comparable with those found in literature [22, 23] and concerning inactivation of *B. licheniformis* spores in specific

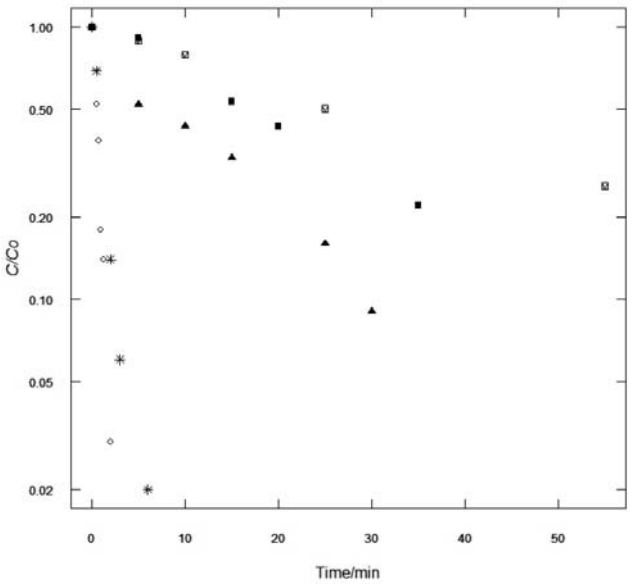

Fig. 7. *D* Values for the spores of *B. licheniformis* conventionally heated at 70 °C (□), 75 °C (■), 80 °C (▲), 85 °C (∗), and 90 °C (◇).

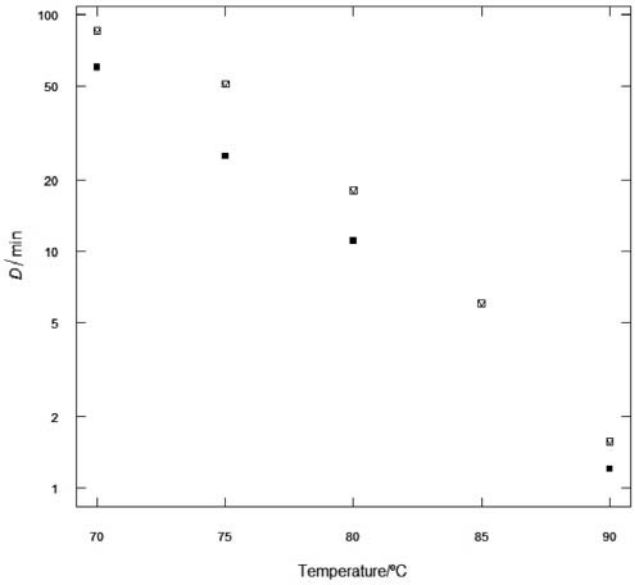

Fig. 8. Death kinetics of the spores of *B. licheniformis* when submitted to conventional (opened symbols) and ohmic (full symbols) heating.

homogenized food products and buffers with similar range of pH.

The thermal inactivation profiles of both microorganisms present in the food samples treated by the conventional and ohmic heating followed the first-order kinetics (Tables 1 and 2). It should be noted that the regression coefficients were in all cases very close to unity.

Considering both studied microorganism strains, for the same degree of inactivation the time required for the thermal treatment was reduced when ohmic heating was used, indicating that in addition to the thermal effect the presence of an electric field provided a non-thermal killing effect over vegetative cells of *E. coli* and bacterial spores of *B. licheniformis* in goat milk and cloudberry jam, respectively. By reducing the time required for inactivation of microorganisms, the use of ohmic heating in the food industry could diminish negative thermal effects of pasteurization on the food products in question, opening a new perspective for shorter, less aggressive processing.

REFERENCES

1. Rastogi, N. K., *Food Rev. Int. 19*, 229 (2003).
2. Icier, F. and Ilicali, C., *Eur. Food Res. Technol. 220*, 406 (2005).
3. de Halleux, D., Piette, G., Buteau, M.-L., and Dostie, M., *Canadian Biosystems Engineering/Le génie des biosystèmes au Canada 47*, 3.41 (2005).
4. *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies*. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, 2000.
5. Yildiz, H. and Baysal, T., *J. Food Eng. 75*, 327 (2006).
6. Qin, B. L., Chang, F. J., Barbosa-Canovas, G. V., and Swanson, B. G., *Food Sci. Technol. 28*, 564 (1995).
7. Leizerson, S. and Shimoni, E., *J. Agric. Food Chem. 53*, 3519 (2005).
8. Imai, T., Uemera, K., Ishida, N., Yoshizaki, S., and Noguchi, A., *Int. J. Food Sci. Technol. 30*, 461 (1995).
9. Wang, W. C. *Ohmic Heating of Foods: Physical Properties and Applications*. The Ohio State University, Columbus, 1995.
10. Cho, H.-Y., Sastry, S. K., and Yousef, A. E., *Biotechnol. Bioeng. 62*, 368 (1999).
11. Castro, I., Macedo, B., Teixeira, J. A., and Vicente, A. A., *J. Food Sci. 69*, C696 (2004).
12. Iurlina, M., Saiz, A., Fuselli, S., and Fritz, R., *LWT Food Sci. Technol. 39*, 105 (2006).
13. Mazas, M., Gonzalez, I., Lopez, M., Gonzalez, J., and Martin, R., *Int. J. Food Sci. Technol. 30*, 71 (1995).
14. Singh, R. P. and Heldman, P. R., *Introduction to Food Engineering*. Academic Press, New York, 1993.
15. Samaranayake, C. P. and Sastry, S. K., *J. Electroanal. Chem. 577*, 125 (2005).
16. Cho, H.-Y., Sastry, S. K., and Yousef, A. E., *Biotechnol. Bioeng. 49*, 334 (1996).
17. Shimada, K. and Shimahara, K., *Agric. Biol. Chem. 45*, 1589 (1981).
18. Shimada, K. and Shimahara, K., *Agric. Biol. Chem. 46*, 1329 (1982).
19. Shimada, K. and Shimahara, K., *Agric. Biol. Chem. 47*, 129 (1983).
20. Shimada, K. and Shimahara, K., *Agric. Biol. Chem. 49*, 405 (1985).
21. Shimada, K. and Shimahara, K., *Agric. Biol. Chem. 49*, 423 (1985).
22. Palop, A., Raso, J., Pagh, R., Condh, S., and Sala, F. J., *Int. J. Food Microbiol. 29*, 1 (1996).
23. Raso, J., Palop, A., Bayarte, M., Condón, S., and Sala, F. J., *Food Microbiol. 12*, 357 (1995).