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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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## Abstract

In recent years, the world's food industry has focused increasing attention on electrical techniques of food processing. Ohmic heating is one of these techniques that can be considered as a high temperature short time and a purely bulk heating method, having potential applications in processes such as blanching, evaporation and pasteurization in the food industry. However such technology would have to assure the microbiological safety obtained by the conventional cooking methods. Concerning this, the influence of heat treatment by ohmic and conventional technology on death kinetic parameters ($D$ and $z$ values) of *Escherichia coli* ATCC® 25922 was studied in goat milk. In ohmic treatment lower $D$ values were obtained ($D_{60°C} = 4.2$ min, $D_{63°C} = 1.9$ min, $D_{65°C} = 0.86$ min) as compared to conventional treatment ($D_{63°C} = 3.9$ min, $D_{65°C} = 3.5$, $D_{67°C} = 2.8$ min, $D_{75°C} = 1.5$ min). The increase of temperature required for a ten fold decrease in $D$ value was also lower in the ohmic inactivation ($z = 8.4$ °C) comparing with the conventional inactivation ($z = 23.1$ °C). The death kinetics for *Bacillus licheniformis* ATCC® 14580 spores in cloudberry jam were also studied under both types of heat inactivation (ohmic and conventional) and similar conclusions were drawn for the $D$ values; lower $D$ values were also obtained for ohmic treatment ($D_{70°C} = 57.1$ min, $D_{75°C} = 25.2$ min, $D_{80°C} = 7.2$ min) as compared to conventional treatment ($D_{70°C} = 85.3$ min, $D_{75°C} = 51.0$, $D_{80°C} = 18.1$ min, $D_{85°C} = 6.0$ min, $D_{90°C} = 1.6$ min). However, between the $z$ values obtained for those treatments ($z_{ohmic} = 11.1$ °C and $z_{conventional} = 11.4$ °C) the differences were not significant. In general the results of present work indicate that the ohmic heating provides quicker death kinetics. This opens the perspective for shorter, less aggressive treatments.

**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 sterilisation. 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 industry interest as alternatives to 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 which consists in the direct passage of electric currents through the food products with the main purpose of heating them by internal heat generation as result of electrical resistance [2]. 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 [3]. In the aseptic processing of food systems, ohmic heating is seen as a potential alternative to conventional heating processes because particles may heat faster than liquid [4,5]. Presently the discussion is focused in the application of ohmic heating for microbial control. Most of the previous studies [5-7] on the interaction of microorganisms with electric field dealt with microbial inactivation. In ohmic heating the principal mechanisms of microbial inactivation are thermal in nature however recent research [8-10] suggests that a mild electroporation mechanism may contribute to cell inactivation, bringing a non-thermal effect to inactivation. Another recent study [11], where conventional and ohmic heating were conducted to ensure the same temperature histories in order to discard the influence of thermal effects, indicates that kinetics of inactivation of *Bacillus subtilis* spores can be accelerated by ohmic heating. Lee and Yoon [12] also reported that the leakage of intracellular constituents of *Saccharomyces cerevisae* was enhanced under ohmic heating, as compared with conventional heating, although in this study does not clearly show whether the temperature effects were eliminated or not. In a different work, where the inactivation of *Bacillus subtilis* spores was studied, Leizerson and Shimoni [13] did not detect differences between conventional and ohmic heating treatment of fresh orange juice. While some evidences suggest the existence of non-thermal killing effects in ohmic heating, more research is needed to understand inactivation mechanisms of various microorganisms in the different types of foodstuffs. 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 [14].

In the present work, samples of cloudberry jam and goat milk were artificially contaminated with *Bacillus licheniformis* ATCC® 14580 spores 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* spp. spores, which are highly resistant to HTST (high temperatures short time) treatments. Species such as *B. licheniformis* are commonly isolated from fruit preparations and according to Fields et al [15], this micro-organism can facilitate the germination of *Clostridium botulinum* which survives heat treatment increasing the risk of a botulism outbreak. Due to its high water activity milk serves as an excellent culture medium for the growth and multiplication of many kinds of microorganisms. Among all micro-organisms *Escherichia coli* is frequently a contaminating organism, and is a reliable indicator of faecal pollution generally found in water, food, milk and other dairy products in unsanitary conditions [16]. The objectives of this investigation were to determine the influence of ohmic heating on 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.

## Materials and methods

### Food products

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 pasteurisation and was 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 $3.83 \pm 0.03$ and $6.59 \pm 0.04$, 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 minutes at 10000 rpm). This procedure was necessary to liquefy the sample. The homogenates were sterilized in order to eliminate contaminations during sample preparation stages.

### Microorganisms.

*Escherchia coli.* The strain of *Escherchia 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 a 37 ºC for 18 h. The population density in each inoculum was enumerated using the pour plate procedure.

*Bacillus licheniformis.* 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 ºC. Cultures were then transferred and spread on the surface of PCA and incubated at 35 ºC for 14 days. Sporulation was checked by phase contrast microscopy and green malachite staining technique. The spores were harvested with a glass spatula and sterile peptone water and then washed and concentrated by centrifugation four times at 2500g for 15 min with sterile peptone water [17]. After last centrifugation and re-suspension in sterile distilled water, spore suspension was heated (80 ºC for 10 min) to kill vegetative cells and stored at 4 ºC until use. During storage no variations in heat were observed.

**Heating phase**

**Conventional heating.** 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 (time = 0) when the tubes reached the test temperature, being a sample taken at that time (two tubes) 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 a thermocouple connected to a data acquisition system in one Eppendorf tube which served as a control. This procedure was repeated for all temperatures.

**Ohmic heating**. The ohmic heater (see Figure 1) consisted of a cylindrical glass tube of 30 cm total length and 2.3 cm inside diameter. Three thermocouple openings were provided; two at an equal distance of the centre of the tube and one at the centre, where the thermocouple was placed. Two 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. Temperature was continuously monitored using type K thermocouples, set at the geometrical centre of the chamber. The power source was turned on and the electric field was varied through the use of a rheostat to simulate the conventional thermal history of the samples. An example of comparison of the thermal history of the samples heated by conventional and ohmic heating is presented in Figure 2. It is very important to have coincidence in the heating phase of both processes because the objective is to evaluate the non-thermal effects of the ohmic processing, thus the thermal effects should be made equal. For the ohmic heating, the inoculated 25 mL 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 temperature test was maintained constant, during the prescribed time interval, by the control of voltage applied to the ohmic heating unit. Also as described before, pairs of samples were subsequently removed with a micropipette at the pre-established time intervals. All the samples were immediately transferred to an ice bath until further analyses were performed.
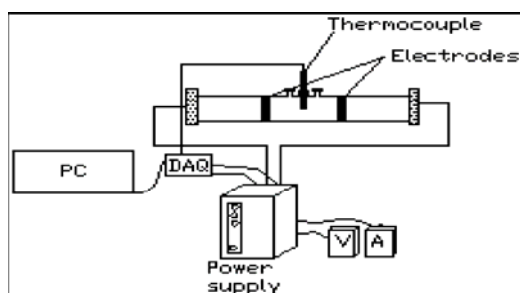
**Figure 1-Ohmic heater and data acquisition system**

## Determination of thermal resistance

**Spores of *B. licheniformis***. The suspension of spores was diluted before the experiments using the extract of cloudberry jam that was previously sterilized (121 ºC for 15 min). Initial concentrations of spores in suspensions varied from $10^4$ to $10^7$ ml$^{-1}$. Volumes of 2 ml of cloudberry jam were placed in sterile Eppendorf tubes and heated at 70 ºC, 75 ºC, 80 ºC, 85 ºC and 90 ºC in a temperature-controlled water bath. In the ohmic heater the 25 mL samples were heated at 70 ºC, 75 ºC and 80 ºC.

***E. coli***. For each experiment, 50 mL of goat milk were homogenized and 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 approximately $10^8–10^9$ CFU/mL. For the conventional heating the inoculated samples were again homogenized, placed in sterile Eppendorf tubes (1 mL) and subjected to test temperatures of 55 ºC, 63 ºC, 65 ºC, 67 ºC and 70 ºC in a temperature-controlled water bath. For ohmic heating approximately 25 mL of inoculated sample was placed in the ohmic heater and heated at 55 ºC, 60 ºC, 63 ºC and 65 ºC.

## Enumeration of surviving microorganisms

For enumeration purposes appropriate serial dilutions (1:10) were made 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 ºC for 24 h. The number of viable colonies of *E. coli* was counted in MacConkey agar No.3 (CM 0115, Oxoid, Basingstoke, U.K.) after incubation at 36 ºC for 18-24h.

## Determination of heat resistance parameters (*D* and *z*)

For each temperature, media, and strain, *D* values (in minutes) were determined by regression from the expression of Singh and Heldman [18]:

$$\textbf{Log } N = \textbf{log } No - \textbf{t/D} \tag{3}$$

where *N* is the experimental count value at time *t* and *No* is the initial count value. The z values were determined from the slope of the regression line obtained by plotting log *D* versus their corresponding heating temperatures.

# Results and Discussion

In this study, the temperature as a variable was eliminated by simulating the conventional heating profile during the ohmic heating phase (see Figure 2); as so, the possible differences in the results obtained must be related to the effect of the electric field.
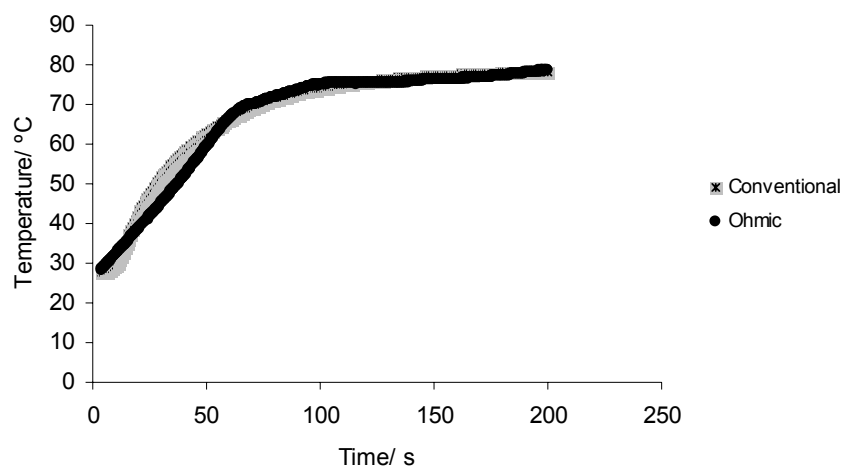
**Figure 2. Thermal history of samples of cloudberry jam processed by conventional and ohmic heating.**

The thermal inactivation profiles of conventional and ohmic heating followed 1st order kinetics for all tested microorganisms. This can be concluded from the results presented in Table 1 to 4 and also from Figures 3 and 4. It should be noted that the linear regression coefficients were in all cases very close to 1. Treatments were applied at different temperatures in ohmic and conventional heating (see e.g. Table 1 and 2), therefore mean comparisons were not performed on these data points.
For the thermal inactivation of *E. coli* the analysis of the results (Tables 1 and 2 and Figure 3) show that $D$ values for ohmic heating were considerably lower than conventional heating, especially at higher temperatures, which reflected in the $z$ values.

**Table 1.** *D* and *z* values of *E. coli* when submitted to conventional heating.

| | Temperature / °C | | | | |
|---|---|---|---|---|---|
| | 55 | 63 | 65 | 67 | 75 |
| $D_{conventional}$ / min | $10.9 \pm 1.8$ | $3.9 \pm 0.5$ | $3.5 \pm 0.2$ | 2.8 | 1.5 |
| $z_{conventional}$ / °C | | 23.1 ($r^2 = 0.98$) | | | |

**Table 2.** *D* and *z* values of *E. coli* when submitted to ohmic heating.

| | Temperature / °C | | | |
|---|---|---|---|---|
| | 55 | 60 | 63 | 65 |
| $D_{ohmic}$ / min | $14.2 \pm 0.2$ | $4.2 \pm 0.6$ | 1.9 | 0.86 |
| $z_{ohmic}$ / °C | | 8.4 ($r^2 = 0.99$) | | |

The $z$ value for conventionally heated cells of *E. coli* was substantially higher ($z = 23.1$ °C) than that obtained for cells treated by ohmic heating ($z = 8.2$ °C). There was a noticeable additional killing effect caused by the electric current in the majority of experiment temperature and these results are in accordance with previous studies [19-23] that reported sublethal injury of *E. coli* cells due to electrical current.
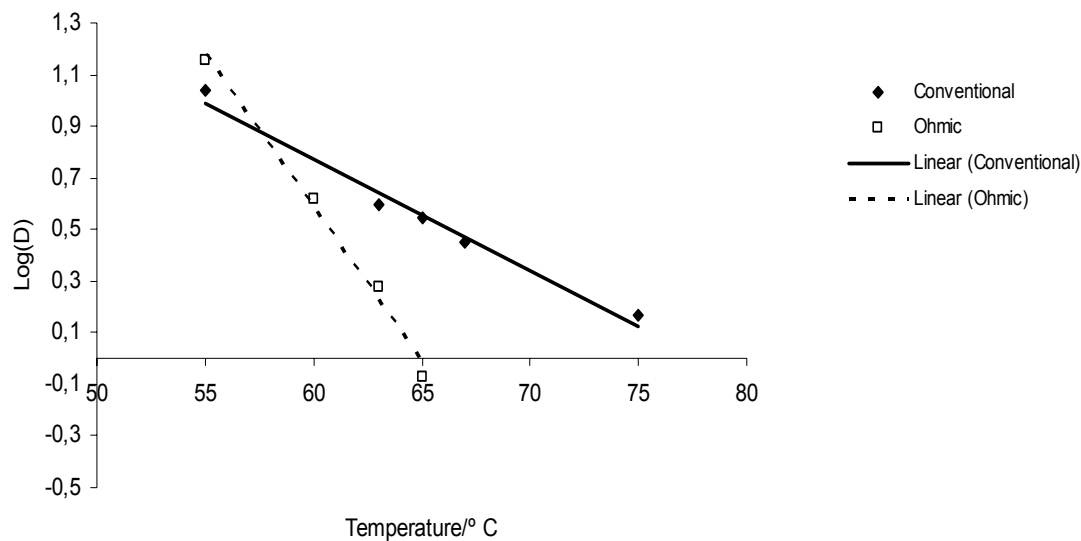
**Figure 3. Death kinetics of *E. coli* when submitted to conventional and ohmic heating; determination of the *z* values.**

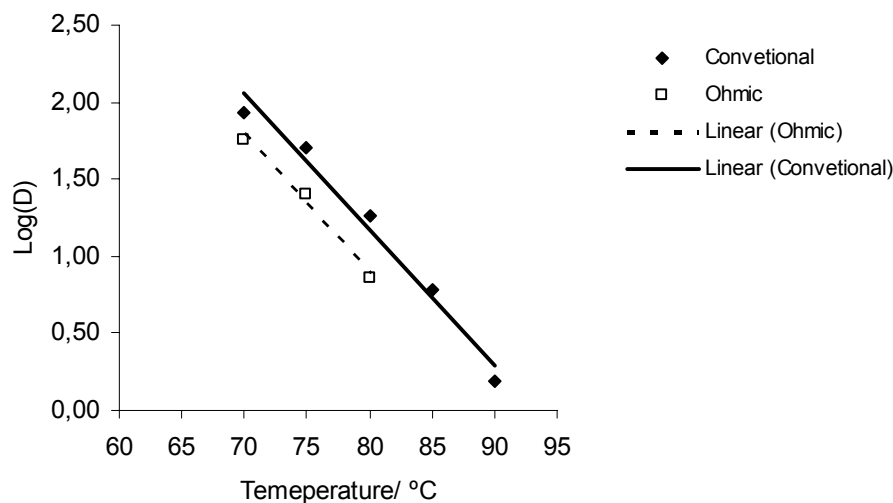Figure 4 illustrates the survival curve for *B. licheniformis* spores during conventional and ohmic heating.



**Figure 4. Death kinetics of *B. licheniformis* when submitted to conventional and ohmic heating; determination of the *z* values.**

The *D and z* values corresponding to Figure 4 are shown in Tables 3 and 4, respectively. At each temperature experiment *D* values were generally smaller for ohmic than conventional heating. The results of analysis of variance performed on data representing treatments at 75 °C show that differences found were significant. Spores heated at 75 °C had significantly ($p < 0.01$) smaller *D* values when heated using ohmic than conventional treatment. However, the *z* value obtained by the linear regression of *D* values versus temperature was statistically similar in both kind of treatments ($z_{conventional} = 11.4$ °C; $z_{ohmic} = 11.1$ °C). In agreement with Cho et al. [11] these results could indicate that in this experiment electrical current affected the death rate, but did not affect the temperature dependency of the spore inactivation process. No data have been published on the influence of ohmic heating treatment on *B.*

*licheniformis* spore inactivation with which the results of this investigation can be compared. However the *z* value of conventional treatment is comparable to that found in literature [24, 25] of inactivation of *B. licheniformis* spores in specific homogenised food products and buffers with similar range of pH of cloudberry jam.

**Table 3. *D* and *z* values of *B. licheniformis* spores when submitted to conventional heating.**

| | Temperature / ºC | | | | |
|---|---|---|---|---|---|
| | 70 | 75 | 80 | 85 | 90 |
| $D_{conventional}$ / min | 85.3 ± 6.8 | 51.0 ± 2.3 | 18.1 ± 1.1 | 6.0 ± 0.1 | 1.6 ± 0.4 |
| $z_{conventional}$ / ºC | 11.4  ($r^2 = 0.98$) | | | | |

**Table 4. *D* and *z* values of *B. licheniformis* spores when submitted to ohmic heating.**

| | Temperature / ºC | | |
|---|---|---|---|
| | 70 | 75 | 80 |
| $D_{ohmic}$ / min | 57.1 | 25.2 ± 1.2 | 7.2 |
| $Z_{ohmicl}$ / ºC | 11.1 ($r^2 = 0.99$) | | |

## Conclusion

In both strains of microorganisms studied for the same inactivation degree, the time required for thermal treatment was reduced with ohmic heating treatment, indicating that in addition to the thermal effect the presence of an electric field in ohmic heating 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. However, in the case of *B. licheniformis*, that difference cannot be reported to be statistically relevant. By reducing the time required for inactivation of microorganisms, the use ohmic heating in the food industry will diminish negative thermal effects in the food products in question, opening a new perspective for shorter, less aggressive processing treatments.

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