HIGH INTENSITY PULSED ELECTRIC FIELD FOR PASTEURIZATION OF LIQUID EGGS UTILIZING *Staphylococcus aureus* AS A PROCESS INDICATOR

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A taxa da sobrevivência do *Staphylococcus aureus* foi estudada como indicador no processo de campo elétrico pulsado de alta freqüência (PEF) para a pasteurização de ovos líquidos. A taxa da sobrevivência depende do tempo total de tratamento sob tensão e da intensidade do pico de tensão. A pasteurização envolveu a aplicação de um pulso curto de alta tensão para atingir os ovos posicionados entre dois eletrodos. Dois tratamentos diferentes foram conduzidos em temperatura ambiente: 20 e 40 pulsos, permanecendo as condições de intensidade de pico de campo elétrico em 32 kV/cm, elevação de pulso de 1,2 µs, duração de pulso de 50 µs, velocidade de aplicação de pulso de 6/min para ambos os testes. A exigência de energia na pasteurização das amostras por PEF foi minimizada. As amostras tratadas por PEF mantiveram as características físico-químicas, nutricionais e de frescor, manifestando vida de prateleira satisfatória a temperatura ambiente.

1 INTRODUCTION

The application of short-duration high intensity pulsed electric fields is a promising technology for non-thermal pasteurization of liquid foods. Thermal processing methods are commonly used in the food industry to

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increase the shelf-life and to maintain food safety by inactivation of spoilage and pathogenic microrganisms. However, heat treatment can adversely affect the flavor, taste, and nutrients of foods.

With the increasing consumer demand of minimally processed food products, there is a growing interest in non-thermal preservation techniques, including the addition of preservatives to control microbial growth, irradiation to inactive bacteria, physical treatments to remove microrganisms, cold storage to inhibit microbial growth, and a combination of these related methods.

Several new techniques have been explored as potential alternatives to the thermal methods, including the utilization of pulsed electric fields, oscillatory magnetic fields, high-hydrostatic pressure, intense light pulses, irradiation, antimicrobials, and bactericins (MERTENS & KNORR, 1992; POTHAKAMURY *et al.*, 1993; DUNN *et al.*, 1995).

High-intensity pulsed electric fields (PEF) pasteurization involves the application of short burst of high voltage to foods placed between two electrodes. Treatment is performed at room temperature for less than 1 second, and energy loss due to heating of foods is, thus, minimized. The PEF-treated samples retain physical, chemical, and nutritional characteristics of fresh foods and possess a satisfactory ambient shelf-life.

According to QIN *et al.* (1995) microbial and chemical analyses and shelf-life studies were carried out on PEF-treated liquid eggs. The reached shelf-life was 4 weeks and there were no apparent changes in most of the physical and chemical properties of eggs except viscosity and color. There was a decrease in viscosity to 19.2 +- 1.7 cP, and the color increased to 11.5 +- 0.4 μg/g β-carotene. A sensorial analysis found no significant differences between fresh eggs and PEF-treated eggs.

Studies have demonstrated that inactivation of microorganisms was due to the electric field and not to the products of electrolysis, or to the temperature increases. The PEF lethal effect as a function of field intensity, treatment time (pulse duration and number of pulses) and a model constant determined by the microrganism and its physiological status (QIN et al, 1995).

The effect is explained by the dielectric rupture theory. The external electric field induces an electric potential over the membrane, which, in turn, causes separation of the charged molecules in the cellular membrane. When the transmembrane potential exceeds a critical value of approximately 1 volt, the repulsion between charge-carrying molecules causes the growth of pores in the cellular membrane. The critical transmembrane potential, and consequently the critical external field strength, depends upon the kind of biological cell, cellular diameter and

shape, and medium conditions. When the external electric field strength is equal to or slightly superior to the critical value, the increase of permeability of the membrane is reversible. When the critical field strength is greatly exceeded the pores will become irreversible and cellular membranes will be destroyed (MERTENS & KNORR, 1992).

Studies have been carried out on two types of bacteria: Gram-negative and Gram-positive (*S. aureus*) with several conditions of PEF. The related microrganisms are widely found in foods, causing decomposition and toxin production, which may lead to food poisoning (MAZUREK *et al.*, 1995)

The influence of high-voltage (HV) pulses on microrganisms has been measured as a survival rate ($s=N/N_o$, where N and N_o are numbers of active microrganisms per 1 mL after and before the treatment, respectively).

The survival rate is a function of the peak voltage. It was observed that the survival rate of *S. aureus* decreases by two orders of magnitude when used the peak voltage of 45 kV and 20 pulses, and about four orders when applied peak voltage higher than 60 kV. According to MAZUREK *et al.* (1995) there is no significant effect of the rising time within the range of 500 to 1100 ns on the lethality rate of some tested bacteria, however, it is verified a decrease from 10^{-1} to 10^{-2} for *S. aureus*.

The survival rate of *Staphylococcus aureus* was studied as a process indicator of the high-intensity pulsed electric field (PEF) for pasteurization of liquid eggs.

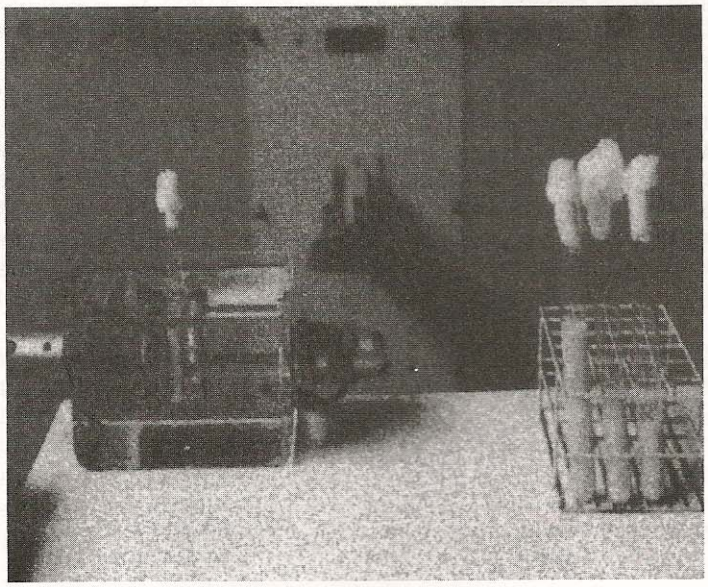
2 MATERIAL AND METHODS

A high-voltage pulse generator, model Haefely P35, produced high-intensity pulsed electric field. The samples submitted to PEF treatment were poured down into a 10 x 1 cm glass tube, between two brass electrodes distanced 1 cm, maintained into an acrylic chamber - as shown in the Figure 1 - containing oil as tension regulator. Figure 2 shows the laboratory-scaled prototype of the treatment system. The viscosity was measured using Hoeppler Viscometer.

Fresh eggs purchased from a local supermarket were cracked and mixed using a sterile beater. The samples submitted to shelf life and viscosity evaluation received 0.15% w/v citric acid.

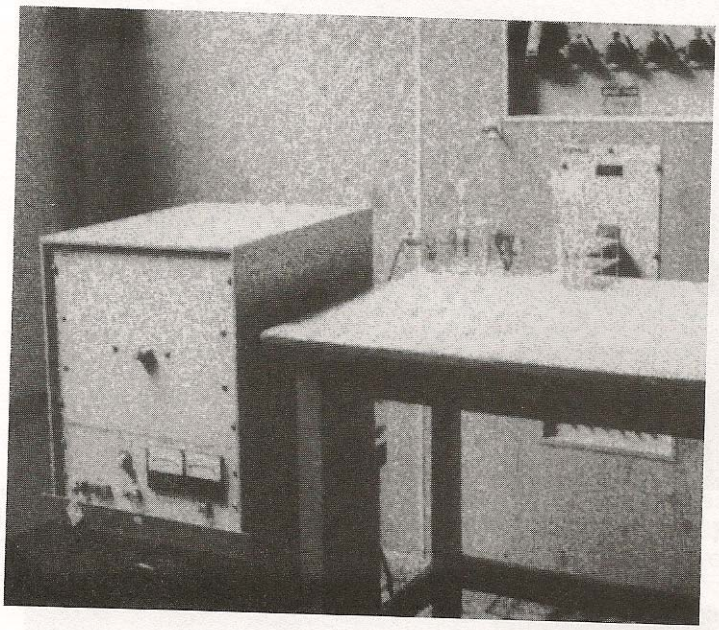
S. aureus from CEPPA/UFPR (Center of Research and Processing of Foods) was grown on nutritive broth at 37 °C of temperature for 24 hours. The microorganism was dispersed in beaten eggs and kept refrigerated at 4 °C awaiting the PEF treatment.

FIGURE 1 - ACRYLIC CHAMBER WITH SAMPLE DISPOSED BETWEEN THE ELECTRODES



The assay with beaten eggs inoculated with *S. aureus* was performed in two distinct treatments at room temperature: 20 and 40 pulses, remaining the same conditions of electric field peak intensity of 32 kV/cm, pulse elevation time elevation of 1.2 µs, pulse duration of 50 µs, and velocity of pulse application of 6/min for both tests.

FIGURE 2 - LABORATORY-SIZED PROTOTYPE OF THE TREATMENT SYSTEM



Microbiological analyses after PEF treatment were carried out with Baird Parker Agar and Plate Count Agar at temperature of 37 °C and period of 24 hours. Microbiological, shelf-life, and viscosity studies were also performed, analyzing refrigerated eggs after the mentioned PEF treatments.

3 RESULTS AND DISCUSSION

3.1 RESULTS

Typical colonies of *S. aureus* were observed in the samples tested into the Baird Parker Agar medium. Figures 3A, 3B and 3C illustrate a decrement of the number of colonies for PEF-treated samples.

FIGURE 3A - TYPICAL COLONIES OF *S. aureus* FOR NON-TREATED SAMPLE

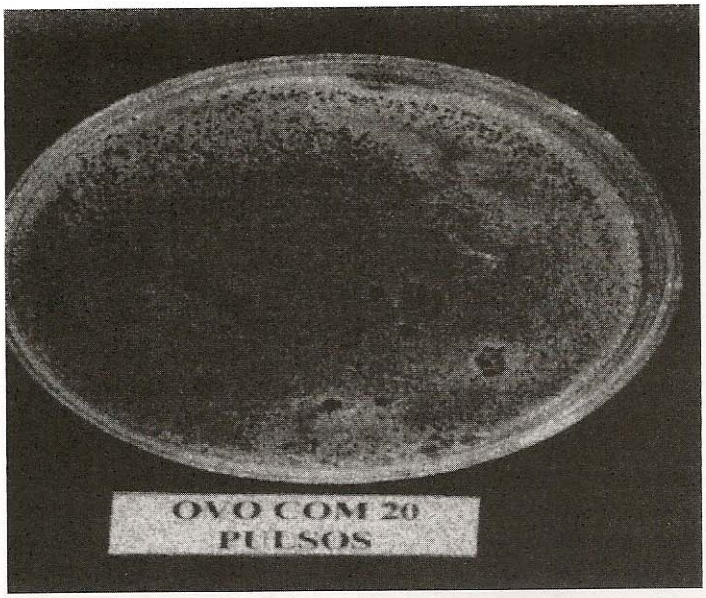


FIGURE 3B - TYPICAL COLONIES OF *S. aureus* FOR 20-PULSE TREATED SAMPLE

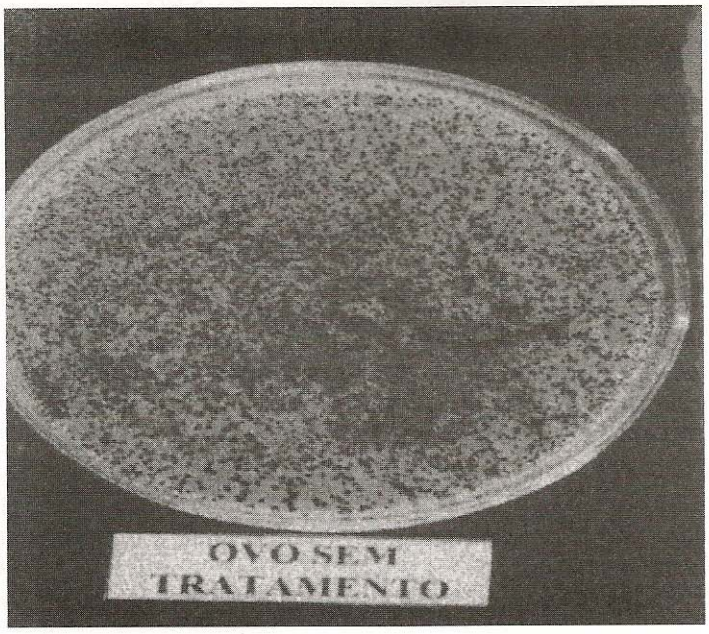


FIGURE 3C - TYPICAL COLONIES OF *S. aureus* FOR 40-PULSE TREATED SAMPLE

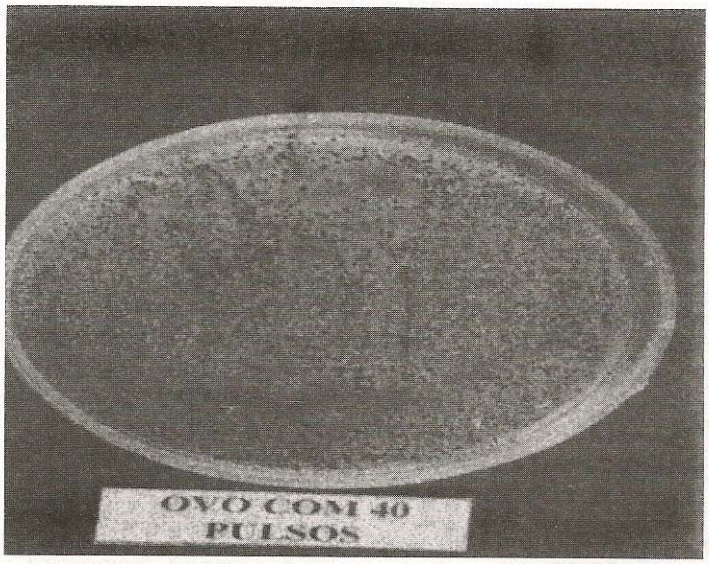
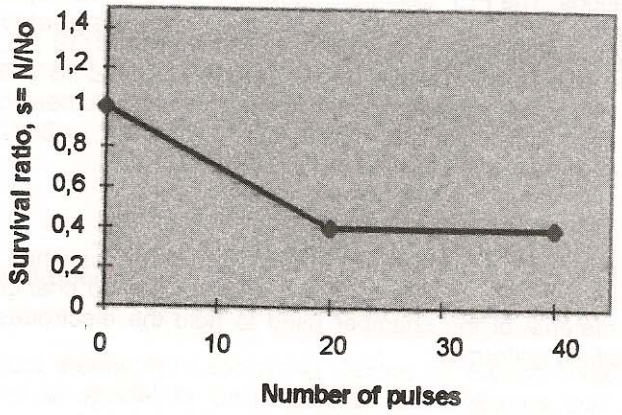


Figure 4 relates the results of the survival ratio, $s=N/N_0$, for non-treated and treated samples. The initial amount of microrganisms N_0 was approximately 10^7 CFU/mL. The effect of the applied number of pulses was not observed within the range of n=20 to n=40 pulses.

FIGURE 4 - SURVIVAL RATIO OF THE *S. aureus VS.* NUMBER OF PULSES WITH 32 KV/CM OF PEAK INTENSITY



The shelf-life evaluation analyzed liquid eggs with the addition of 0,15% w/v citric acid. There were no apparent changes in the properties of liquid eggs, except for viscosity, within a period of three weeks. The results for viscosity are shown in the Table 1.

TABLE 1 - VALUES OBTAINED FOR VISCOSITY OF TREATED AND NON-TREATED EGGS

Sample	Initial Viscosity, cP	Final Viscosity *, cP
Non-treated eggs	2,0216	1,0002
Treated eggs	1,2102	1,1877

* Viscosity evaluated after 3 weeks at 4 °C.

3.2 DISCUSSION

There was no difference in the survival ratio of *S. aureus* within the range of 20 and 40 pulses, although it has been discussed that the effect caused by high-intensity pulses on microrganisms depends upon the number of pulses applied. It has observed that the inactivation of *S. aureus* declined only one order of magnitude, in the tested conditions.

According to other authors, probably the peak intensity applied was not sufficient for a higher inactivation. However, the experimental conditions involved a glass chamber that may affect the uniformity of the electric field, causing deviations. The PEF treatment showed another effect, by which the non-treated sample had its viscosity decreased, while the treated sample maintained its viscosity lightly constant.

4 CONCLUSION

The survival ratio of *S. aureus* demonstrated no differences when used 20 or 40 pulses in the PEF treatment. It has been suggested changes in the construction material of the chamber used to hold the electrodes, aiming better electric field uniformity.

Abstract

The survival rate of *Staphylococcus aureus* was studied as a process indicator of the high-intensity pulsed electric field (PEF) for pasteurization of liquid eggs. The survival rate depends upon the total time voltage treatment and intensity of the peak voltage. The pasteurization involved the applications of a short burst of high voltage to beat the eggs placed between two electrodes. Two different treatments were conducted at room temperature: 20 and 40 pulses, remaining the same conditions of electric field peak intensity of 32 kV/cm, pulse elevation time of 1.2 μs, pulse duration of 50 μs, velocity of pulse application 6/min for both tests. The requirement of energy to pasteurize samples by PEF was minimized. The PEF-treated samples kept physical, chemical, and nutritional freshness characteristics and manifested a satisfactory room temperature shelf-life.

REFERENCES

1 ABIA. Compêndio da Legislação de Alimentos. **Consolidação das normas e padrões de alimentos**. São Paulo, 1991. v. 1.

2 DUNN, J., OTT, T., CLARK, W. Pulsed-light treatment of food and packaging. **Food Technology,** v. 49, n. 9, p. 95-98, 1995.

3 MAZUREK, B., LUBICKI, P., STARONIEWICZ, Z. Effect of short hv pulses on bacteria and fungi. **IEEE**, v. 2, n. 3, p. 418-425, 1995.

4 MERTENS, B., KNORR, D. Developments of nonthermal processes for food preservation. **Food Technology,** v. 46, n. 5, p. 124-132,1992.

5 POTHAKAMURY, U.R., CÁNOVAS, G.V.B., SWANSON, B.G. Magnetic field inactivation of microorganisms and generation of biological changes. **Food Technology,** v. 47, n. 12, p. 85-93, 1993.

6 QIN, B., POTHAKAMURY, U.R., VEGA, H., MARTIN, O, CÁNOVAS, G.V.B., SWANSON, B.G. Food pasteurization using high-intensity pulsed electric fields. **Food Technology,** v. 49, n. 12, p. 55- 60, 1995.

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