Influence of pulsed electric field on the survival of *Yersinia enterocolitica* in minced beef meat

Assessment of microbiological activity of selected cell lines of bacteria under influence of physical-chemical factors

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Keywords: *Yersinia enterocolitica*, foodborne pathogens, pulsed electric field, inactivation

ABSTRACT

The main purpose of this study was to examine the efficacy of the pulsed electric field (PEF) on inoculated *Yersinia enterocolitica* ATCC 35669 in minced beef meat. The pulse frequencies ranged from 28 to 2800 MHz. The samples were examined after 3 days of storage in temperature of +4 ^{0}C and after 30 days of storage in temperature of – 20 ^{0}C. Inactivation of *Y. enterocolitica* increased with the pulse frequencies. The treatment of meat with the electric field with the pulse frequencies of 28 MHz occurred to be ineffective towards *Y. enterocolitica.* The treatment of meat with the electric field with the pulse frequencies of 280 MHz occurred to be effective only towards *Y. enterocolitica* contained in beef meat stored at -20 ^{0}C for 30 days. The treatment of meat with the electric field with the pulse frequencies of 2800 MHz occurred to be highly effective towards *Y. enterocolitica* contained in meat stored both +4 ^{0}C and -20 ^{0}C.

Wpływ pulsacyjnego pola elektrycznego na przeżywalność *Yersinia enterocolitica* w mielonym mięsie wołowym

Badanie aktywności mikrobiologicznej wybranych linii komórkowych bakterii pod wpływem czynników fizyko-chemicznych

Słowa kluczowe: *Yersinia enterocolitica*, patogeny, impulsowe pole elektryczne, inaktywacja

STRESZCZENIE

Głównym celem tego badania była ocena skuteczności impulsowego pola elektrycznego (PEF) wobec *Yersinia enterocolitica* ATCC 35669 namnożonej w mielonym mięsie wołowym. Częstotliwość impulsów mieściła się w przedziale od 28 do 2800 MHz. Próbki badano po 3 dniach przechowywania w temperaturze +4 ^{0}C i po 30 dniach przechowywania w temperaturze –20 ^{0}C. *Y. enterocolitica* wykazała wrażliwość na zwiększenie częstotliwości impulsów pola elektrycznego. *Y. enterocolitica* była wysoce odporna na działanie pola elektrycznego o częstotliwości impulsów 28 MHz. Potraktowanie mięsa polem elektrycznym o częstotliwości impulsów 280 MHz skutkowało inaktywacją *Y. enterocolitica* obecną w mięsie wołowym przechowywanym w temperaturze -20 ^{0}C przez 30 dni. Potraktowanie mięsa polem elektrycznym o częstotliwości impulsów 2800 MHz okazało się wysoce skuteczne w eliminacji *Y. enterocolitica* zawartej w mięsie przechowywanym zarówno w temperaturze +4 ^{0}C jak i -20 ^{0}C.

1. INTRODUCTION

Pulsed electric field (PEF) is considered to be an alternative method for thermal methods of food preservation. The overall objective is to destroy both pathogenic microorganisms as well as saprophytic forms of microorganisms which constitute microbiological contamination of food products, while simultaneously maintaining the physico-chemical and organoleptic characteristics of foods [1]. Foodborne pathogens constitute a huge risk for human health and life. Good hygienic practice can decrease a level of microbiological contamination. However, the most important pathogens require PEF treatment to be completely eliminated from minced meat. PEF treatment belongs to safe and effective methods of meat decontamination. Such treatment does not possess any negative effects on sensory and nutritional features of meat. PEF treatment can be successfully carried out when meat is in a frozen state. PEF method constitutes an effective way of enhancing the safety of meat.

The use of pulsed electric field is a highly effective method of ensuring food safety. Its effectiveness depends on the degree of resistance of the microorganism. One of pathogens contaminating food and posing a serious threat to human health and life is *Yersinia enterocolitica*. It is a psychrophilic bacterium which can be isolated from heat-processed meat and dairy products [2]. Resistance of *Y. enterocolitica* is still unrecognized. There are many factors that have a significant impact on the effectiveness of PEF against the microorganism. They include the parameters of the technological process, the physico-chemical characteristics of raw materials contaminated with *Y. enterocolitica* as well as the natural immunity and physiological status of the microorganism [3].

For this reason, it is difficult to explicitly determine the degree of resistance of *Y. enterocolitica* to PEF. Some authors agree on the linear relationship between the reduction in a number of viable cells of *Y. enterocolitica* expressed as $\log_{10}$ CFU/g and their treatment time with PEF [4, 5]. Others do not confirm such a correlation [6, 7]. The aim of this study was to evaluate the effectiveness of pulsed electric field (PEF) against *Y. enterocolitica* multiplied in the ground beef meat stored for 3 days at +4 ^{0}C and for 30 days at –20 ^{0}C.

2. MATERIALS AND METHODS

2.1 The experimental material and growth conditions

The examined strain was *Y. enterocolitica* ATCC 35669. The strain was kept on slants of Tryptic Soy Agar with 0.6% yeast extract addition. The bacterial suspension was prepared in such a way that 50 ml of tryptic-soy broth was inoculated with

the examined microorganism, and the incubation was performed at 30 ^{0}C for 30 h to obtain a viable cell concentration of 7.5 $\log_{10}$ CFU/1 ml of suspension (3.5 x 10^7 CFU/ml). 5 g of minced beef meat was inoculated with 1 ml of earlier prepared bacterial suspension. The number of *Y. enterocolitica* in 1 g of meat after inoculation was 6.7 $\log_{10}$ CFU/1 g (5.6 x 10^6 CFU/g). The prepared meat samples were subjected to pulsed electric field.

2.2 PEF equipment

The meat samples inoculated with *Y. enterocolitica* were placed in a chamber between two parallel electrodes. Distance between electrodes was 1 m. The circuit configuration produced the waveform pulses at various frequencies. They amounted to 28, 280 and 2800 MHz. The electric field strength was 300 V/m. The actual power of the electric field in the chamber was measured by a probe connected to an oscilloscope (Tektronix, TDS 220, Wilsonville, Oregan, USA). During each irradiation, the temperature was maintained at a level below 35 ^{0}C. The treatment time was 15 minutes.

3. RESULTS AND DISCUSSION

On the basis of microbiological tests of beef meat samples treated with the pulsed electric field the significant differences in a number of viable cells of *Y. enterocolitica* were found. The parameter having a major impact on survival of pathogenic microorganism in minced beef meat was the pulse frequency of the pulsed electric field.

The survival of *Y. enterocolitica* in minced meat was tested in the irradiated samples stored for 3 days at +4 ^{0}C and for 30 days at –20 ^{0}C.

Figure 1 presents the growth of *Y. enterocolitica* in tryptic-soy broth at 30 ^{0}C. A viable cell concentration of 7.5 $\log_{10}$ CFU/1 ml of suspension (3.5 x 10^7 CFU/ml) was achieved after 30 h of incubation at 30 ^{0}C.

Figure 2 presents a number of *Y. enterocolitica* in minced beef meat stored at +4 ^{0}C for 3 days. The initial number of *Y. enterocolitica* in minced beef meat before its PEF treatment amounted to 6.7 $\log_{10}$ CFU/1 g (5.6 x 10^6 CFU/g). After meat treatment with PEF at the pulse frequency of 28 MHz and storage at +4 ^{0}C for 3 days, a number of viable cells of *Y. enterocolitica* did not change. Treatment of meat samples with PEF at the pulse frequency of 280 MHz and storage at +4 ^{0}C for 3 days did not lead to a decrease in *Y. enterocolitica* cells. Only treatment of meat samples with PEF

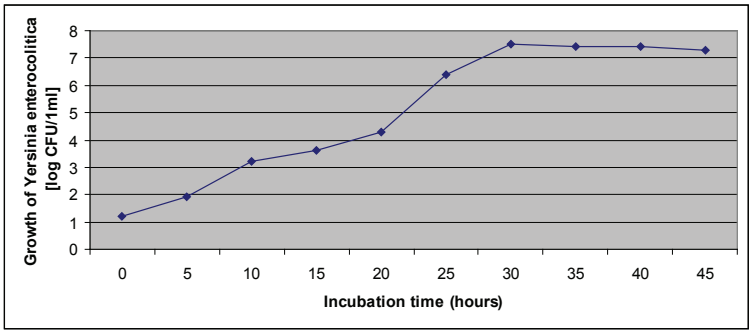

Figure 1 Growth of *Yersinia enterocolitica* in tryptic-soy broth at 30 ^{0}C
Rysunek 1 Wzrost *Yersinia enterocolitica* w podłożu tryptozowo-sojowym w temp. 30 ^{0}C

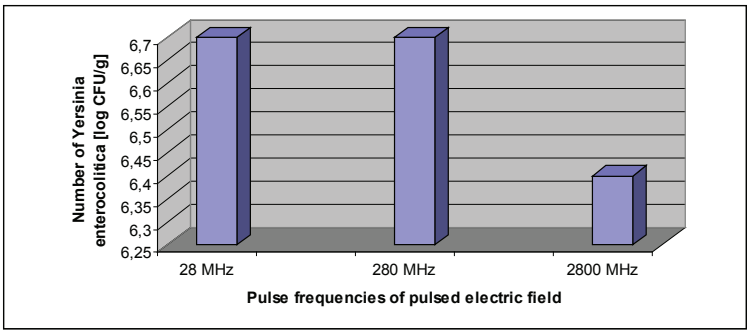

Figure 2 Number of *Yersinia enterocolitica* in minced beef meat stored at +4 ^{0}C for 3 days
Rysunek 2 Ilość komórek *Yersinia enterocolitica* w mielonym mięsie wołowym przechowywanym w temp. +4 ^{0}C przez 3 dni

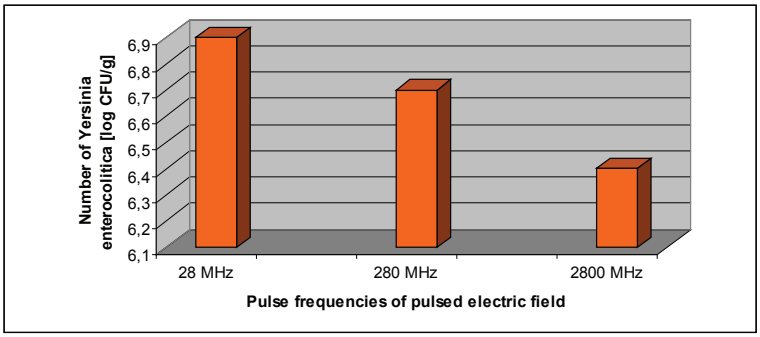

Figure 3 Number of *Yersinia enterocolitica* in minced beef meat stored at -20 ^{0}C for 30 days
Rysunek 3 Ilość komórek *Yersinia enterocolitica* w mielonym mięsie wołowym przechowywanym w temp. -20 ^{0}C przez 30 dni

at the pulse frequency of 2800 MHz and storage at +4 ^{0}C for 3 days caused a significant decrease in viable cells of *Y. enterocolitica* by 6.52 $\log_{10}$ CFU/1 g (3.3 x 10^6 CFU/g). A number of viable cells in meat amounted to 6.4 $\log_{10}$ CFU/1 g (2.3 x 10^6 CFU/g).

Figure 3 presents a number of *Y. enterocolitica* in minced beef meat stored at –20 ^{0}C for 30 days. The initial number of *Y. enterocolitica* in minced beef meat before its PEF treatment amounted to 6.7 $\log_{10}$ CFU/1 g (5.6 x 10^6 CFU/g). After its treatment with PEF at the pulse frequency of 28 MHz and storage at –20 ^{0}C for 30 days, a number of viable cells of *Y. enterocolitica* slightly increased to 6.9 $\log_{10}$ CFU/1 g (7.8 x 10^6 CFU/g). Treatment of meat samples with PEF at the pulse frequency of 280 MHz and storage at –20 ^{0}C for 30 days did not cause any change in a number of viable cells of *Y. enterocolitica.* Only treatment of meat samples with PEF at the pulse frequency of 2800 MHz and storage at –20 ^{0}C for 30 days caused a further slight increase in a number of viable cells of *Y. enterocolitica* by 1.8 $\log_{10}$ CFU/1 g. A number of viable cells in meat amounted to 6.4 $\log_{10}$ CFU/1 g (2.3 x 10^6 CFU/g).

The parameters which were a subject of research were the microbial growth phase, the temperature during growth (30 ^{0}C), the pulse frequencies of PEF, the treatment time and the storage of minced beef meat samples after PEF treatment. The above figures present the influence of the frequency of the pulses (28, 280 and 2800 MHz) on the survival of *Y. enterocolitica.* As it can be observed, the survival of the microorganism depends on the pulse frequencies of PEF. The pulse frequency at level of 2800 MHz occurred to be highly effective towards the inactivation of *Y. enterocolitica.*

The other researches investigated the influence of PEF on the survival of other pathogenic microorganisms such as *Escherichia coli* and *Listeria monocytogenes* [8]. PEF treatment causes many cell modification such as a cell volume decrease, a change of cell shape, a change in the cell wall composition. It is also known that the influence of PEF is strongly related to the microorganism being the subject of investigation. Each microorganism shows different resistance towards PEF treatment. There were many conducted studies to assess the temperature level at which a microorganism is the most sensitive towards PEF treatment. It was shown that most microorganisms are more sensitive towards PEF treatment when they are in the medium at lower temperature. This is connected with the higher membrane fluidity of cells which is caused by an increase in unsaturated fatty acids [9].

There are also other factors which influence the resistance of microorganism when it is treated with PEF. One of them is pH value of medium. However, it is difficult to reach a general conclusion to what extend the acidity of medium containing the given microorganism influences its resistance towards PEF treatment. It can generally be said that the microorganism shows lower resistance towards PEF when is in the medium at a significantly high acidity like meat. It was observed in case of *E. coli* and *L. monocytogenes* [10]. However, there were other microorganisms such as *S. enteritidis* and *S. senftenberg* which possessed a higher resistance in the medium at a relatively high acidity [11-13].

Another factor which should also be examined is the water activity which possesses the influence on the microbial resistance towards PEF [14-16]. Many studies confirmed that the microorganisms expressed a significantly lower resistance towards PEF when they were in the medium with a reduced water activity.

4. CONCLUSIONS

The results of the study presented in this paper prove that the pulsed electric field (PEF) is an efficient method in the inactivation of *Y. enterocolitica* in minced beef meat. The extent of the inactivation depends on a level of pulse frequencies. The treatment of meat with the electric field with the pulse frequencies of 28 MHz occurred to be ineffective towards *Y. enterocolitica.* The treatment of meat with the electric field with the pulse frequencies of 280 MHz occurred to be effective towards *Y. enterocolitica* contained in beef meat stored at -20 ^{0}C for 30 days. The treatment of meat with the electric field with the pulse frequencies of 2800 MHz occurred to be highly effective towards *Y. enterocolitica* contained in beef meat stored both at +4 ^{0}C and at -20 ^{0}C. Foodborne pathogens constitute a huge risk for human health and life and might be eliminated from food using PEF treatment. This method belongs to safe and effective methods of meat decontamination. PEF treatment can be successfully carried out when meat is in frozen state. PEF method constitutes an effective way of enhancing the safety of meat.

Acknowledgments
The cost of the research were covered by financial resour coming from grand No N N312 427639.

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