New methods for inactivation of *Alicyclobacillus acidoterrestris* spores in apple juice concentrate

Małgorzata Djas[1], Magdalena Bober[2], Marek Henczka[3]

[1] Warsaw University of Technology, The Faculty of Chemical and Process Engineering, e-mail: m.djas@ichip.pw.edu.pl.

[2] Warsaw University of Technology, The Faculty of Chemistry, e-mail: mbober@wp.pl.

[3] Warsaw University of Technology, The Faculty of Chemical and Process Engineering,
e-mail: m.henczka@ichip.pw.edu.pl

This paper is focused on new methods for inhibiting *Alicyclobacillus acidoterrestris* spores germination in apple juice concentrate. Four methods such as pulsed electric field (PEF), ultrasound (US), integrated method of PEF and US and microwaves (MW) have been investigated. The highest efficiency of spores inactivation was obtained after microwave treatment.

Keywords and phrases: *Alicyclobacillus acidoterrestris*, spores, inactivation, pulsed electric field (PEF), ultrasound (US), microwaves (MW).

Introduction

The presence of *Alicyclobacillus acidoterrestris*, and especially its spores, is currently a major concern of fruit and vegetable industry. These spore-forming bacteria cause flat-sour type spoilage in fruit juices and concentrates, attributed to the production of offensive-smelling metabolites. Undesirable changes of taste and smell of the contaminated product make it deficient and undrinkable, which results in the decrease of its market value.

The thermophilic and acidophilic characteristics of *Alicyclobacillus acidoterrestris* make it resistant to pasteurization [1]. Thermal processing can destroy vegetative forms, while it is practically impossible to eliminate thermally resistant spores. Membrane filtration is a commonly used method for elimination of microorganisms in industry. However, because of high viscosity of juice concentrates (268,06 mPa·s at 20°C for apple concentrate) application of this method is not efficient and consumes large amounts of energy [2]. For that reason, alternative and innovative methods for inactivation of *A. acidoterretris* spores have to be developed.

This paper is focused on four new methods for inhibition of *Alicyclobacillus acidoterrestris* spores germination in apple juice concentrate: pulsed electric field (PEF), ultrasound (US), combination of both methods (US→PEF, PEF→US) and microwaves (MW). PEF technology is based on high-voltage short pulses delivered to the product placed between two electrodes. Electroporation is believed to be the principle of this method. Application of high-intensity pulsed electric fields destabilizes microbial cell membrane and causes alterations in ion transport processes, which results in cell damage and death [3]. Ultrasound is able to disaggregate bacterial clusters and inactivate bacteria through mechanical, physical, and chemical effects of acoustic cavitation [4]. However, it has been shown that PEF and US methods used separately offer low and unsatisfactory inactivation degree of bacterial spores [5–7]. The influence of different process parameters as well as the order of applied methods on the elimination of bacteria was investigated [2]. Because inactivation mechanisms of US and PEF treatments are different, the present paper is focused on the possible additive and synergistic effects and the combination of the influence of ultrasound and electric current as an integrated method of inactivation of *A. acidoterrestris* spores. The identification of elementary mechanisms was enabled by the results of the undertaken research. Another technique presented in this paper is microwave processing. The actual mechanism of bacterial killing by microwaves still has not been resolved. In literature two conclusions can be found: the first one that microwave radiation itself induces a nonthermal effect on bacteria and the second one that there is heat generated by microwaves that plays a role in elimination of microorganisms [8, 9]. The aim of this study was to determine the efficiency of MW for *A. acidoterrestris* spores inactivation and possibility of magnifying the thermal effect by electromagnetic energy.

Materials and methods

Apple juice concentrate containing spores of *Alicyclobacillus acidoterrestris* was taken from fruit juice factory and stored at 4°C until processed. Concentrate samples were thoroughly mixed and then treated by ultrasounds, pulsed electric fields and microwaves according parameters summarized in the Table 1.

The US experiment was designed with constant power 330 W and treatment time 10 minutes. The PEF treatment was designed with different electric filed intensity and number of electric pulses as main parameters. The electric field intensity used were equal to 500 Vcm^{-1} and 2500 Vcm^{-1}, while the number of electric pulses were equal to 500 and 2000 with time pulse 2000 µs.

Besides US and PEF experiments conducted separately, application of both methods one after another was investigated (integrated method). The sample of concentrate was treated first with US and then with PEF and then in the reversed order, using the same parameters for each stage as shown in the Table 1.

Table 1. Treatment methods and process parameters.

Ultrasound		Pulsed electric field	
Power (W)	Time (min)	Electric field intensity (Vcm^{-1})	Number of pulses
330	10	500	500
		2500	2000

Microwaves						
Run	Effective power (W)	Irradiation time (s)	Energy (J)	Energy density (J/cm^3)	Initial temperature (°C)	Final temperature (°C)
1	40	270	10800	216	21	41
2	80	135	10800	216	21	44
3	120	90	10800	216	21	43
4	240	45	10800	216	21	46
5	400	27	10800	216	21	49
6	600	18	10800	144	21	40
7	120	240	28800	576	21	80
8	240	120	28800	576	21	85
9	400	72	28800	576	21	97
10	600	48	28800	384	21	89
11	800	36	28800	384	21	80

The microwave experiments consisted of two parts. First, samples were treated by microwaves at different times and power levels following the Table 1. In the second part, conventional heating at 100°C was compared with microwave heating (400 W) at the same temperature. Samples for testing were taken after reaching 100°C and after 30 s and 1 min of boiling.

After the treatments, samples were diluted with sterile deionized water. Heat shock at 80°C for 10 min was applied prior to testing in order to stimulate spores germination and to inactivate vegetative organisms. Samples were immediately cooled and filtered through sterile 0,45 µm membrane filters. Membranes were transferred onto a BAT agar surface. After incubating at 45°C for 5 days colonies were counted. The obtained results were expressed in CFU/ml of juice concentrate. *Alicyclobacillus acidoterretris* colonies at membrane filter are shown in Fig. 1.

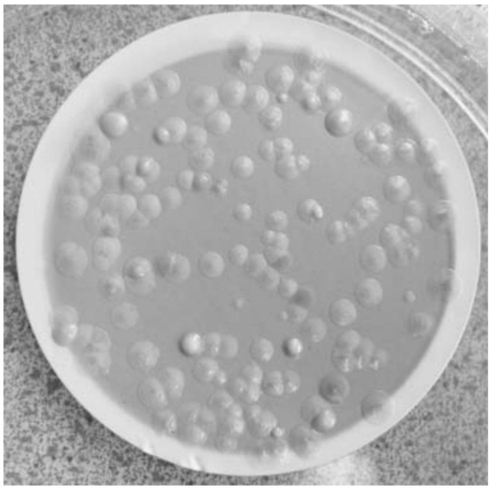

Fig. 1. *A. acidoterrestris* **colonies at membrane filter.**

Results and discussion

Efficiency of the method for inactivation of spores can be easily expressed by using dimensionless parameter S defined in the form of log-units:

$$S = \log \frac{N_0}{N} \tag{1}$$

where: N_0 — the initial spore concentration of not-treated samples [CFU/ml],
N — the spore number of processed samples [CFU/ml].

Increasing the parameter S shows the higher efficiency of the method. Negative numbers of parameter S show the effect achieved by breaking bacterial aggregates and the increase in the number of colonies.

US, PEF and integrated method (US→PEF, PEF→US) processing

The comparison of efficiencies of application US, PEF and integrated methods (US→PEF, PEF→US) on the inactivation of *A. acidoterrestris* spores in apple juice concentrate is shown in Fig. 2.

Analysis of the obtained experimental results allows to conclude that the combination of both methods (US→PEF, PEF→US) is an effective method of

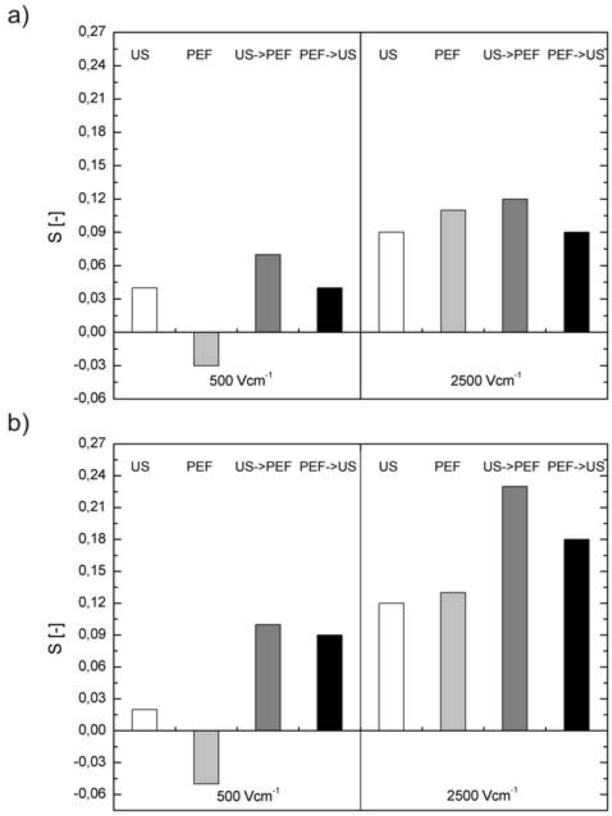

Fig. 2. Inactivation degree of *A. acidoterrestris* spores after a) 500, b) 2000 electric pulses.

inactivation of *A. acidoterrestris*. The highest efficiency of elimination of microorganisms was obtained, when a sample of concentrate was treated first with ultrasonic waves and then with electric current (US→PEF). High effectiveness of this inactivation method is a result of the combination of two different mechanisms of the influence of ultrasonic waves and pulses of electric field on bacterial spores. Two theories, explaining high effectiveness of this solution, have been formulated. According to the first theory, the dominant effect of sonication is based on destroying of cellular aggregates. Subsequently, the exposed bacterial cells are faced with destructive activity of electric pulses. According to the other theory, the damaged spores are transformed into vegetative forms during the ultrasonic disintegration process are then inactivated with the PEF method. The degree of inactivation obtained as a result of US→PEF method, irrespectively of the applied electric field intensity and of the number of electric pulses, was always higher in comparison to the degree of inactivation, resulting from the application of single elimination methods — US or PEF. The highest efficiency of *A. acidoterrestris* bacteria inactivation was obtained as a result of application of the combination of US→PEF methods for 2000 pulses and the highest electric field intensity with the value of 2500 Vcm^{-1}.

Increasing the electric field intensity from 500 to 2500 Vcm^{-1} increased the efficiency of inactivation of spores with US→PEF processing from 0,07 to 0,12 (at 500 electric pulses) and from 0,1 to 0,23 (at 2000 electric pulses), respectively. Increasing the number of pulses from 500 to 2000 (at electric field intensity 2500 Vcm^{-1}) resulted also in nearly doubling the reduction of spores number. Similar effect can be achieved for 2000 pulses by increasing the electric field strength from 500 to 2500 Vcm^{-1}. Negative numbers of parameter *S* (US treatment) show the effect achieved by breaking bacterial aggregates and the increase in the number of colonies.

Microwaves processing

Two approaches were used to study the microwave radiation effect on *A. acidoterrestris* spores. The first approach was to carry out the experiments at sublethal temperatures (<50°C) with constant energy consumption. The objective was maintenance of samples at low temperatures rather than strict temperature control. In the second approach irradiation time was extended and samples reached higher temperatures (80–100°C). Results of performed experiments are shown in Fig. 3.

At sublethal temperatures no significant inactivation occurred. Slight changes in the spore number were observed. This effect could be due to the presence of spores aggregates and also uneven distribution of spores on the filters which influence the precision of counting. Because of the high viscosity of apple juice concentrate, intensive mixing could have not be enough to obtain uniform distribution of spores.

On the other hand, high temperature conditions appeared to have significant effect on viability of spores. Efficiency parameter *S* increased from 0,24 to 0,41 when power of the MW processing increased from 120 to 400 W. MW-effect on the spores depended upon both the power and the treatment time. Despite constant energy consumption ([power]´[time] variable) in every run, short time of processing at higher power levels (600 and 800 W) resulted to be insufficient to obtain considerable efficiency.

The third experiment was conducted in order to find out if there is any additional lethality using MW irradiation as a mode of heating. Apple juice concentrate reached 100°C in 75±5 s at 400 W, while the necessary boiling time at gas burner was equal to70±5 s. This was because of much smaller sample volume used for conventional heating. The comparison of efficiency of both treatments is shown in Fig. 4. Inactivation was higher and obtained at shorter time for spores subjected to MW-heating. The most significant difference was observed at '0 s', when samples reached the boiling point (0,53-log and 0,15-log reduction respectively for MW and regular heating). In both cases increase of processing

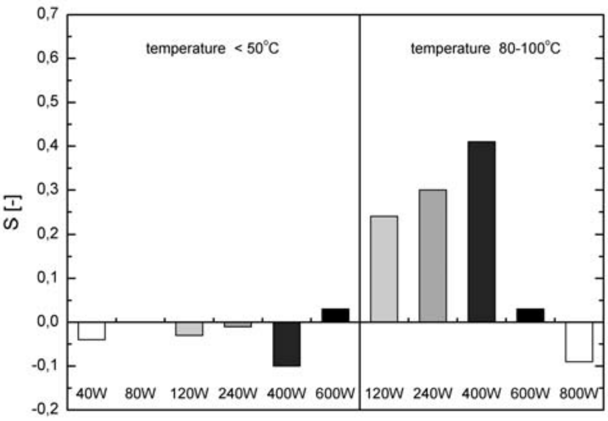

Fig. 3. Efficiency of microwave radiation at sublethal and high temperatures.

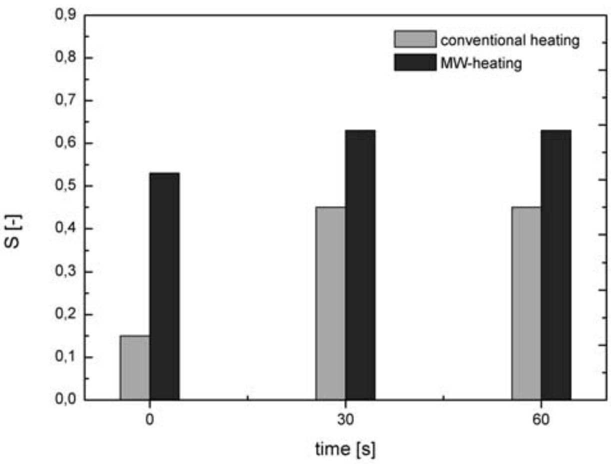

Fig. 4. Efficiency of microwave radiation and conventional heating after reaching 100°C, for 30 s and 60 s of boiling.

time from 30 s to 1 min did not cause any changes. Complete inactivation of spores could not have been observed with this procedure because of evaporation of samples.

Conclusions

The considerable efficiency of inactivation of *A. acidoterrestris* spores in apple juice concentrate was achieved with integrated method (US→PEF) and microwave processing. The highest reduction degree was observed as a result of microwave irradiation. These methods indicate the possibility of application on industrial scale.

The shortcoming of the US→PEF method is unfavourable increase of temperature of the concentrate during ultrasonic disintegration, which might cause the change of colour and taste and loss of nutritive properties of the concentrate. Application of this method on industrial scale would require the application of a cooling system, which would simultaneously increase the costs of the processing.

Analysis of the obtained results allows to assume that microwave energy is not able to destroy *A. acidoterrestris* spores without treatment of the heat. The results show that inactivation of spores is caused mainly by the thermal effect. However, higher efficiency of MW at comparable conditions with conventional heating may suggest the existence of synergistic interaction of microwaves and thermal heat. It indicates the possibility of using this method instead of thermal pasteurization due to the higher sporicidal activity. MW-processing is also assumed to require shorter time because of the strong penetrating effect of waves, which results in faster temperature rise. Nevertheless, further research on the influence of MW-heating on juice properties should be conducted.

References

[1] Chang, Su-Sen, and Dong-Hyun Kang. "*Alicyclobacillus* spp. In the Fruit Juice Industry: History, Chatacteristics, and Current Isolation/Detection Procedures." *Crit. Rev. Microbiol.* 30 (2004): 55–74.

[2] Henczka, Marek, and Małgorzata Djas. "Zintegrowana metoda dezintegracji mikroorganizmów w cieczach o podwyższonej lepkości." *Inż. Ap. Proc.* 1 (2010): 53–54.

[3] Gongora-Nieto, M.M., et al. "Food Processing by Pulsed Electric Fields: Treatment Delivery, Inactivation Level and Regulatory Aspects." *Lebensm.-Wiss. u.-Technol.* 35 (2002): 375–388.

[4] Joyce, E., et al. "The development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power and sonication time on cultured *Bacillus* species. *Ultrason. Sonochem.* 10 (2003): 315–318.

[5] Borthwick, Kathryn A.J., et al. "Improvement of Immunodetection of Bacterial Spore Antigen by Ultrasonic Cavitation." *Anal. Chem.* 77 (2005): 7242–7245.

[6] Grahl, T., and H. Märkl. "Killing of microorganisms by pulsed electric fields." *Appl. Microbiol. Biotechnol.* 45 (1996): 148–157.

[7] Cserhalmi, Zs., et al. "Inactivation of *Saccharomyces cerevisiae* and *Bacillus cereus* by pulsed electric fields technology." *Inn. Food Sci. & Emerging Technol.* 3 (2002): 41–45.

[8] Wu, Q. "Effect of high-power microwave on indicator bacteria for sterilization." *IEEE Trans. Biomed. Eng.* 43 (1996): 752–754.

[9] Yeo, C.B., et al. "Heat transfer analysis of Staphylococcus aureus on stainless steel with microwave radiation." *J. Appl. Microbiol.* 87 (1999): 396–401.