Praca oryginalna *Original paper*

Effect of concentrated microwave field on bacteria reduction and physical properties of egg white*)

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Summary

The aim of the study was to evaluate the optimal conditions to produce a significant reduction in bacteria population using a concentrated microwave field. The research with CMF was conducted in two different diluting agents: physiological solution and egg white. Using liquid egg white as one of the most sensitive materials it was important to find optimal conditions of pasteurization without destructive changes in egg white proteins' activity. Bacteria strains were isolated from the surface of egg shells and identified using an ATB System (Merck). They were compared with ATCC strains. Microwave impulses were generated in groups separated by well matched periods. One of the main findings of the investigation was that the destruction of microbes was not associated with an increase in temperature; the temperature of material subjected to microwave was not higher than 44°C.

Keywords: microwave, microwave field, microbial inactivation

The heat production of microwaves is mainly due to dipole excitation and ion migration. Friction energy is produced as a result of the orientation of the dipoles in the alternating electromagnetic field. The power absorption depends on various factors, e.g.: frequency of waves, temperature of processed sample, the magnitude of electric field in tissue, density or presence of dielectric components in examined liquids and tissues. Microwave is an efficient means to heat, dry and sterilize most of food products. Compared with conventional methods, microwave processing offers economic and significant amount of processing time and energy. Because of rapid heat transfer, nutrients, vitamins, taste, aroma and color of food contents are better preserved (2). Many examples of microwave applications in food technology have been reported in the literature, although these techniques have not been yet performed in food industry in all cases, e.g.: drying and freeze drying (production of pasta, fruit powder, vegetable concentrates and extracts, instant tee, coffee and dyes, final drying of potato chips also drying of mushrooms, fish protein, egg yolk paste, herbs) or baking of bread, pizza and cake, where microwaves heating is often combined with conventional process (13). This kind of processing kills corn pests, bacteria and limits fungal infections with confined thermal degradation of sensitive food components. Results are also observable on the destruction of aflatoxins in peanuts and trichinae in treated meat. Several theories had been proposed to explain microbial inactivation by microwave. One of them assumes, that the temperature level is the most important factor responsible for the lethal effects assuring irreversible heat-denaturation of enzymes, proteins and nucleic acids or other cellular constituents vital to cell metabolism or reproduction (8). This may be concluded from the results of some experiments with very dry agents, e.g.: bacterial spores (10) or vegetative cells and spores in dry soil (15), when no microbial reduction was observed without heating. In contrast to these observations, some authors obtained results confirming the concept of athermal effects, when the death of some microorganisms was noticed (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* or *Bacillus stearothermophilus* spores) (6, 16). Khalil and Villota (9) observed that 23 S ribonucleic acid (RNA) subunits of *Staphylococcus aureus* were damaged only in microwave-treated suspensions, compared to heat treatments. Other researches noticed in microbial cells some differences in enzymatic activity in a manner unlike, that observed in cells heated (4) and in the rate of

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phosphoanhydride bond hydrolysis in RNA (14). These differences were also cited as evidence for athermal effect. The mechanism of microwave effect has not known yet. The aim of the work was to evaluate the optimal conditions to produce a significant reduction in bacteria population using concentrated microwave field.

Material and methods

A new experimental CMF reactor, built and produced by Plazmatronika firm (Poland), mm is appropriated to lead organic synthesis, organic substances' selective preparations and sterilize various liquid food products. The test apparatus consists an oven magnetron, a treatment chamber with monomode adapter, a cooling device type RPC (reduced pressure cooler), temperature measurement devices and the computer to control operations. In experiment, CMF process involved the application of microwaves collected in various amount of packets contain various number of pulses, separated by well matched periods in range of parameters. Possibility of operations was expanded over amount of packets (1-10), amount of pulses in one packet (1-10), periods between packets (1-99 s), width of each impulse (1-9 × 10 ms), periods between pulses (1-99 × 100 ms) for samples of liquid white and physiological solution placed in stationary cell of treatment chamber's monomode adapter. The number of applied impulses and their packets were be regulated according to the need. Selected examples, grouped in A, B, C variants are presented in tab. 1.

Tab. 1. Selected examples, grouped in A, B, C variants

Variant	Amount of packets	Amount of pulses in one packet	Period between packets (s)	Width of each impulse (× 10 ms)	Period between pulses (× 100 ms)
A	10	10	1	9	1
B		10	7		
C		7	1		

Strains of *Escherichia coli*, *Acinetobacter lwoffii*, *Citrobacter freundii*, *Serratia liquefaciens*, *Pseudomonas aeruginosa*, *Staphylococcus gallinarum*, *Staphylococcus xylosus* and *Oligella* sp. were isolated from the surface of egg shells (laying hens line of Tetra SL) and identified using biochemical tests (ID 32 GN, ID 32 E, ID 32 STAPH, Merck) and computer analysis (ATB System, Merck). They were compared with ATCC strains of *Escherichia coli* 25922, *Staphylococcus aureus* 25923 and *Pseudomonas aeruginosa* 27853. The research with concentrated microwave field was conducted in two different diluting agents: phosphate-buffered 0.85% NaCl solution (1) and liquid egg white. Standardized inoculums were prepared by making a culture in nutritive broth (of colonies from 18-24 h culture grown on nutrient agar), collected by centrifugation and resuspended in NaCl solution (Sigma: 20 min./4000 r.p.m./temp. 4°C). After three washes using the same procedure, a suspension was diluted to obtain approximately 10^6 cfu/ml in samples with physiological solution and liquid egg white. The concentration of bacteria was recorded and adjusted with spectrometer at 560 nm, using 0.85% NaCl solution as a blank. The cell suspensions were treated by CMF and compared to cultures, which were not processed but were keeping at the same temperature, for the same time as each of the treated test samples. The inhibition growth by CMF was tested following the agar dilution procedure of the National Committee for Clinical Laboratory Standards (NCCLS: Approved Standard M 31-A, 1999) and the direct colony suspension method for using nutrient agar, fit for species of microorganisms. The plates were incubated at 37°C incubator and read after 24 h. In the absence of growth the sample was considered to indicate resistance to the CMF used.

Egg white, obtained from laying hens line of Tetra SL, was separated from yolk and shells and homogenized in Mixer B-400 (Büchi) with aseptic conditions retained. The functional properties of pasteurized liquid hen egg white: whipping ability (ΔV_f), foam stability (S_f), foam index (I_f) and percentage of gas in foam (G), were investigated to check the condition and structure-function relationships of white proteins treated with CMF with maintain process conditions, the same as for bacteria cells suspended in 0.85% physiological solution treated. To avoid the problem with gel-structure of egg white from new-laid eggs, all eggs were stored at 20°C for 21 days before test.

Results and discussion

About 100 samples were examined. Each sample placed in 20 ml cell, was controlled to note all temperature changes using thermocouple sensor. Selected examples, grouped in A, B, C variants, had constant value of packets' amount (10), width of each impulse (9 × 10 ms) and period between pulses (1 × 100 ms). The results of bacteria strains' reduction ratio (log cycle reduction „D"), depending on amount of microwaves in each packet and periods between packets applied are shown in figures 1-4. CMF treatment was conducted at ambient temperature. Initial temperature of samples was not higher than 10°C. In time of processing it

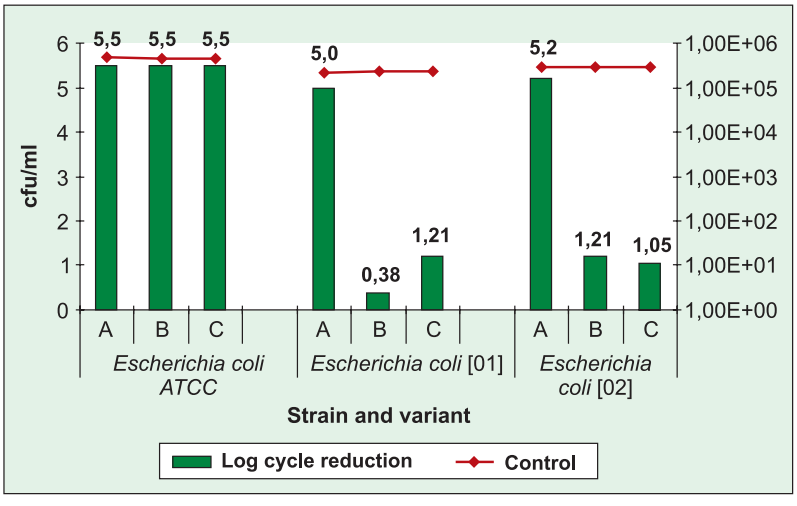

Fig. 1. Inactivation of *Escherichia coli* strains by CMF

increased and amount to 44°C/12 s of treatment time for A, 38°C/67 s for B and 39°C/12 s for C variant. The initial amount of microorganisms was within the range of 10^5 cells/cm^3, except *Pseudomonas aeruginosa* strains, when the concentration of cells was higher (10^6/cm^3). The process was effective in killing microorganisms, however, to achieve more than a 1-log cycle reduction, variant's A parameters were needed, depending on the bacteria. Except ATCC strains, the most significant reduction was noted in population of *Acinetobacter lwoffii* and *Oligella sp.* (about 2D). At the end of the process, the number of cells of *Pseudomonas aeruginosa F*, *Staphylococcus xylosus*, *Staphylococcus gallinarum*, *Escherichia coli* (02) was reduced in population by 1 log cycle. Less pulses (7) applied in packets could reduced population to 1 D (*Staphylococcus xylosus*, *Staphylococcus gallinarum*, *Staphylococcus aureus ATCC*, *Escherichia coli* (01), *Escherichia coli* (02) and *Serratia liquefaciens*). The most susceptible were both: *Acinetobacter lwoffii* and *Pseudomonas aeruginosa F* – variant C reduced bacteria below 3 D. A kind of diluent (physiological solution, egg white) considered for one of treated strains *Escherichia coli* (02) was not important for sensibility of bacteria. An effect of CMF on bacteria was comparable. However temperature of samples treated by CMF was not higher than 44°C depending on selected parameters, inconsiderable protein coagulation was noted in variances of liquid egg white when 10 packets of 10 pulses each one, separated 1 s period were applied. Evaluation of whipping properties of pasteurized white and results of the research are shown in tab. 2. Increase of foam volume was more efficient in samples treated by CMF when variant's A parameters were used compared to samples, which were not processed. The percentage of foam volume's increase for sample treated with 10 pulses classified in 10 packets was comparable to control. Also microwaves generated in variant C were even more suitable for quality of egg white. Because condition of white foam corresponds to the energy delivered by each pulse, stability of foam was satisfactory; only sample treated by less number of pulses (7 in each packet) was characterized by greater outflow volume. The lower value of foam index I_f was noted for samples treated with microwaves of variant C compared to control and to samples of variant A, which were more suitable than control one. Pulses applied in variants A and C were also very advisable for the percentage of gas in foam compared to samples, which were not processed.

Although the lethality of CMF treatment corresponds to the energy delivered by each pulse, variable regression in the group of selected variances did not show important

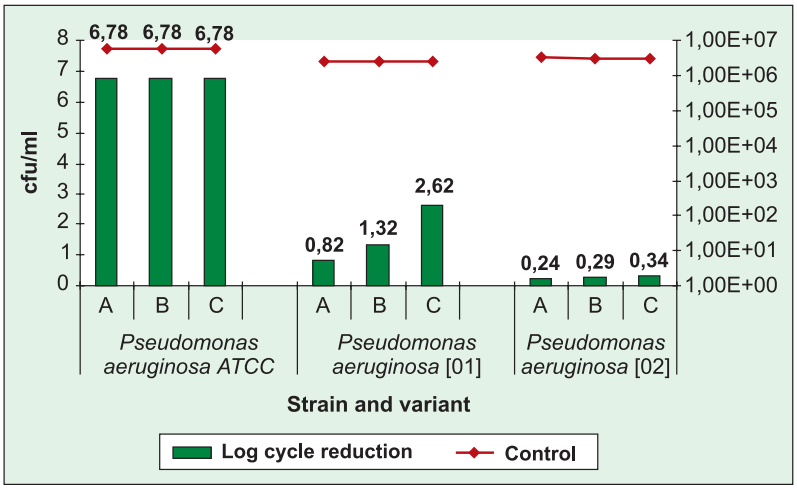

Fig. 2. Inactivation of *Pseudomonas aeruginosa* strains by CMF

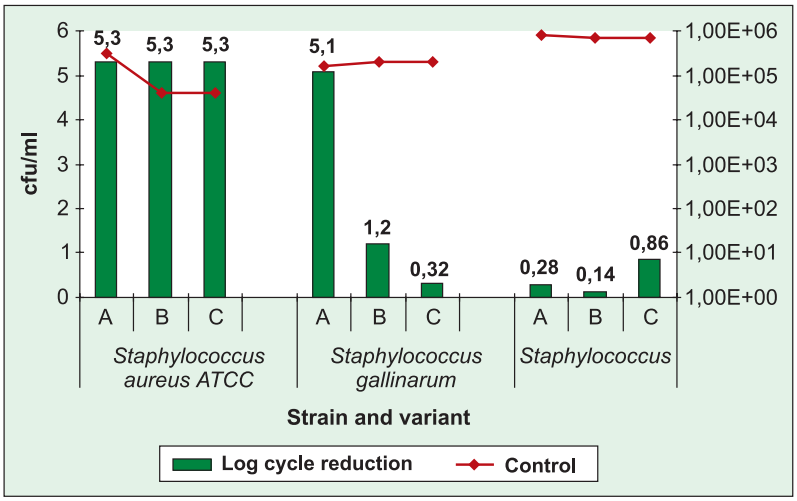

Fig. 3. Inactivation of *Staphylococcus aureus ATCC, S. gallinarum* and *S. xylosus* by CMF

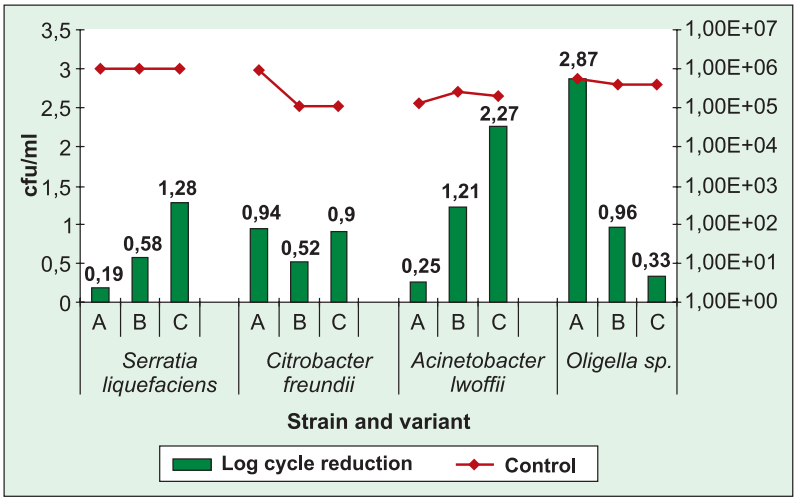

Fig. 4. Inactivation of *Serratia liquefaciens, Citrobacter freundii, Acinetobacter lwoffii* and *Oligella* sp. by CMF

interactions between number of pulses and intensity of their application. High reduction – from 1 to 5 D, to the initial amount of microorganisms was noted in variant A, when 10 packets with 10 pulses each one, separated 1 s period were used. It was a variant advisable for microbiological and technological analysis. For ATCC strains of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* – these parameters were sufficient to kill all bacteria cells in suspension. Strains isolated from the surface of eggs' shells were more resistant to lethality effect of microwave except cells of *Escherichia coli* (01) and (02). Longer period between packets relieved level of reduction. The results of the research indicate that the texture of foams, being described by the increase of its volume, stability, index and percentage of gas, were comparable with control one and in variant A were thereby more advisable for functional properties of pasteurized hen egg white. Increase of foam volume corresponds to the energy delivered by each pulse. Ovoalbumin, a predominant protein contributing to the functional properties of egg white, need some energy to reveal ability to rapidly adsorb at the air-water interface during whipping and undergo rapid conformational change and rearrangement at the interface (3). Improved functionality could be obtained without chemical or enzymatic modification. Novel protein structure could increase the usefulness of proteins in food applications (5, 7). Variant A with white treated by microwaves was characterized by the supreme stability and index. Index contained in value of 5-8 indicates rather average usability for food technology. Lower index of control and treated with CMF samples of white can be explain away the age of stored eggs. Continued investigating effect of CMF on quality high heat sensitive food susceptible to spoil (activity of microorganisms) is needed in spite of uncomfortable conditions connected with thermolability of egg products' components and foaming properties unwanted during CMF processing. The presence of bubbles leads to non-uniform treatment. It can be a reason of slight white's coagulation at sample although temperature noted after process was not higher than 44°C.

Tab. 2. Functional properties of hen egg white pasteurized by CMF

White treated by CMF									
Selected process conditions		Functional properties							
		Whipping ability (%)		Foam stability (%)		Foam index		Gas in foam (%)	
Pulses in packet	Period between packets	ΔV_f	Control sample	S_f	Control sample	I_f	Control sample	G	Control sample
10	1	642,44		61		3,91		83,30	
10	7	578,99	637,99	61	59	3,52	3,75	79,16	82,14
7	1	635,65		56		3,55		84,60	

Conclusions

Concentrated microwave field as pasteurization at reduced temperatures, on account of termolabile components of food like liquid white, has become a suitable method for food preservation. Hen egg white proteins have been extensively utilized as ingredients in food processing because of their unique functional properties, e.g. gelling and foaming, and in pharmaceutical industry as factors protecting the body against bacterial, viral or inflammatory diseases. Because of the high heat sensitivity of egg white proteins, egg products processed by traditional heat treatments exhibit operational problems due to protein coagulation in heat exchangers and the functional characteristics begin to be damaged at temperatures as low as 54°C. The results of the research indicate that it is possible to find optimal parameters to save sensitive liquid's components using non conventional pasteurization at temperatures not higher than 44°C.

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