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EFFECT OF MICROWAVE RADIATION ON MICROORGANISMS IN FISH MEALS*

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Abstract

This study was aimed at testing the effect of microwave radiation on survival of *E. coli*, *Salmonella* Enteritidis, *Enterococcus* spp. and *Clostridium* spores in two kinds of fish meals. The material used in the study consisted of samples of two kinds of fish meal – salmon and cod. In the experiment samples of both kinds of fish meals were inoculated with suspensions of tested bacteria and spores of *Clostridum sporogenes*. After inoculation the material was exposed to microwave radiation with a frequency of 2.45 GHz and microwave energy power of 0, 100, 180, 300, 450, 600 and 700 W, respectively, for 2.5 min for bacteria and 11 minutes for spores. Then the reisolated microorganisms were counted and theoretical lethal doses of radiation were determined. Among the studied vegetative forms of bacteria, the largest decreases in the numbers at the same radiation dose were observed in the rods of *E. coli*, whereas the smallest in enterococci. Spores of *Clostridium sporogenes* showed a considerably higher resistance to the effect of that factor. The power of dose resulting in the complete inactivation of the studied bacteria should be about 430 kJ×g^{-1}, and in the case of spores – 1 900 kJ×g^{-1}.

Key words: microwave radiation, inactivation of bacteria, spores, fish meals

Microwave radiation is a common method of food processing. Cooking, drying, blanching and thawing with microwave reduces time of the process and, if properly planned, enables avoiding the losses of nutritional quality of the product (Dong et al., 2011; Ghanem et al., 2012; Chandrasekaran et al., 2013).

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It was also proved that electromagnetic waves with a frequency from 300MHz to 300GHz, have a lethal impact on a number of pathogenic microorganisms, both those occurring only at the vegetative stage (*Salmonella* spp., *Listeria* spp., *Campylobacter* spp.) and those that are able to form spores highly resistant to unfavourable outside factors (*Clostridium* spp., *Bacillus* spp.) (Welt et al., 1994; Dańczuk and Łomotowski, 2008; Wu and Yao, 2010; Lu et al., 2011). Bactericidal effect of microwaves is used for medical equipment sterilization, hospital waste disposal or inactivation of pathogens in food (Park et al., 2006; Zieliński et al., 2007).

It is assumed that one of the mechanisms determining the inhibitory efficacy of microwave radiation towards microorganisms is certainly the so-called thermal effect. It results from the high temperature generated during this process, causing irreversible changes in the structure of proteins, enzymes and nucleic acids inside the microbial cells. Also cytoplasmic membranes and the cell wall undergo destruction, which in turn results in leakage from cells components necessary for their proper functioning. It is also proven that weakening of bacteria cell membrane results in increasing bactericidal effectiveness of some antibiotics (Nasri et al., 2013).

It is disputable, in turn, if there exists a non-thermal effect, specific only to microwave radiation, which is questioned by some researchers. It would be responsible for microorganisms elimination under conditions of temperatures not exceeding their point of thermal death (Woo et al., 2000; Buffo and Holley, 2006; Shamis et al., 2008).

Due to relatively low costs and the simplicity of this method, attempts at its application are made in many different fields of economy. In the present study, the effect of microwave radiation on the microorganism survival rate in two types of fish meals was tested. These products, which are a rich source of protein in animal feeds, can be also a habitat for bacteria of the genus *Salmonella* (Crump et al., 2002; Møretrø et al., 2003; Maciorowski et al., 2007). For this reason, among others, it is necessary to subject them to appropriate methods of processing, minimizing the epidemiological risk connected with their utilization. The effectiveness of microwave rays allows for the assumption that under optimized conditions they can be used for efficient hygienization of animal meals.

Material and methods

The material used in the study consisted of samples of two kinds of fish meal – salmon and cod. Cod meal contained on average 19.6% of total protein and 0.5% of crude fat. The content of Ca was 22–27%, and P 13–14%. Salmon meal contained on average 27.4% of crude protein and 1.8% of crude fat. The content of Ca was 16–25%, and P was 11–12%.

Production technology of these fish meals is the subject of a patent application at the Polish Patent Office (no. P-403123).

The submitted material was initially examined for presence of the total number of microorganisms, mould fungi, actinomycetes and rods of *Salmonella* spp., *E. coli*, enterococci and spores of sulfite reducing anaerobic bacteria.

In the experiment samples of both kinds of fish meals were inoculated with suspensions of *Salmonella* Enteritidis, *E. coli*, bacteria of the genus *Enterococcus* and spores of *Clostridium sporogenes* IW1306 (PKM Wrocław). The study was conducted in 3 repetitions for each microorganism, both in cod and salmon meal.

Bacterial suspensions were prepared in sterile Ringer's solution based on 24-hour cultures of the studied bacteria on the nutritional agar. The density of each suspension was estimated using a densitometer at 0.3 McF.

To obtain spores suspension, 0.5 ml of *Clostridium sporogenes* culture in BHI (Brain Heart Infusion) broth stored at 4°C was introduced to 10 ml of the liquid FTG medium and incubated under anaerobic conditions (Anaerobic System, Oxoid) for 24 hours at 37°C. Next 0.5 ml of culture in FTG medium was transferred to 10 ml of the Duncan-Strong medium and incubated under anaerobic conditions for 7 days at 37°C. After that period, the inoculated Duncan-Strong medium was centrifuged at a speed of 3 000 rpm for 15 minutes, washed twice and suspended in 50 ml sterile distilled water, obtaining a suspension with a density of 10^7 CFU×ml^{-1}.

Next 1 ml of individual bacterial suspensions and the spore suspension was added to samples of both types of fish meals with a weight of 7 g each, and then stirred to obtain a "dough" consistency. Inoculated material was dried for 45 min at 37°C.

From dried samples of both types analytical samples with a mass of 0.5 g were prepared, which were scattered in a thin layer on ceramic trays with an area of 6.25 cm^2. Then trays together with the material were exposed to microwave radiation with a frequency of 2.45 GHz and microwave energy power 0, 100, 180, 300, 450, 600 and 700 W, respectively, for 2.5 min for bacteria and 11 minutes for spores. Taking into consideration the above data, the power of microwave radiation dose (kJ×g^{-1}) was calculated according to the equation:

$$D = \frac{M \cdot t}{m} \tag{1}$$

where:

M – power of microwave energy (kW),

t – time of exposure (sec),

m – mass of a sample subjected to exposure (g).

The values of dose power, taking into account the effective radiation time, were presented in Table 1.

The number of all the studied bacteria was determined with the MPN (most probable number) method in a 3-tube set, making decimal dilutions of the radiated samples in appropriate media.

In the process of isolating rods of the genus *Salmonella* for initial multiplication (24 hours at 37°C) 1% buffered peptonic water was used. Selective multiplication was carried out using the liquid medium according to Rappaport (24 hours at 43°C). For the growth on a solid medium, the selective agar BPLS agar medium was used (24 hours at 37°C), on which *Salmonella* grew in the form of pale-pink colonies, dyeing the medium pink. Final identification consisted in the use of diagnostic sera according to the Kauffmann-White scheme.

Table 1. Microwave radiation dose (kJ×g^{-1})

Bacteria – 2.5 min.						
Microwave energy power (kW)	0.10	0.18	0.30	0.45	0.60	0.70
Effective radiation time (s)	24.3	39.1	64.3	94.7	124.6	150.0
Microwave radiation dose (kJ×g^{-1})	4.86	14.08	38.58	85.23	149.52	210.00
Spores – 11 min.						
Microwave energy power (kW)	0.10	0.18	0.30	0.45	0.60	0.70
Effective radiation time (s)	103.4	169.1	280.4	413.4	545.3	660.0
Microwave radiation dose (kJ×g^{-1})	20.68	60.88	168.24	372.06	654.36	924.00

To determine the number of *E. coli* rods, a series of decimal dilutions was made on MacConkey's broth. Inoculated broth was incubated at 43°C for 24 hours. The change of medium colour from purple to yellow and the presence of gas in the Durham tube were assumed as the positive result. After the incubation of broth, the material was cultured on selective solid media – ENDO agar. Plates with the culture were incubated at 43°C for 24 hours. Typical growth of *E. coli* rods on ENDO agar had the form of dark red colonies with green-gold fuchsine sheen. Final identification was performed using the API 20E test.

Enterococci determination was performed using the broth with glucose and azide (48 hours at 37°C) and agar with kanamycin, esculine and azide (48 hours at 37°C). Turbidity of the liquid medium with glucose and azide indicated the presence of enterococci in the sample. On the solid media with kanamycin, the growth of small colonies and dyeing the medium for a dark colour indicated the presence of enterococci. Final identification was carried out based on the serological Phadabac Strep Test.

To estimate the number of *Clostridium sporogenes* spores in the studied material, a series of 10-fold dilutions was prepared in tubes containing 9 ml of peptonic water. Then, to inactivate vegetative forms, the tubes were placed in water bath at 80°C for 15 minutes. After cooling the tubes with dilutions, the number of spores was determined based on the results of cultures made with the pour-plate method. For this purpose, 1 ml was taken 3 times from each dilution, introduced to sterile Petri dishes and poured with the DRCM medium solidified with agar. The cultures were incubated for 3 days at 37°C under anaerobic conditions. After the incubation, characteristic, black colonies of *Clostridium sporogenes* were counted on each plate.

Based on the results obtained, theoretical lethal doses, inactivation rate and doses causing a 90% reduction in the bacteria number were calculated. The theoretical lethal doses were calculated as the quotient of the intercept of the regression line equation (theoretical initial number of bacteria or spores at the beginning of process expressed in log MPN or log CFU and the slope of the regression line (elimination rate)). In order, the D_{90} doses were determined as the quotient of the number of bacteria or spores equal 1 log MPN or log CFU (90% of population) and the slope of the regression line.

The determined parameters were analysed statistically using the STATISTICA 10 PL (StatSoft®) software, which involved the estimation of significance of differences between them at the levels 0.05 and 0.01 based on the Bonferroni test.

Results

The results of initial investigations did not show the presence of chosen indicator bacteria, fungi or actinomycetes in the material samples. The total number of microorganisms did not exceed the value of 10^1 CFU×g^{-1}.

In the course of the conducted investigation, the sanitization of fish meal with the use of microwave radiation was proved to be effective. The results obtained made it possible to observe a gradual elimination of vegetative bacterial forms and the inactivation of *Clostridium sporogenes* spores along with an increase in a dose (Tables 2–5).

The initial number of the studied bacteria and spores remained at the level of 10^6–10^7 MPN×g^{-1} or CFU×g^{-1} (Tables 2–5).

Table 2. Changes in number of *Salmonella* Enteritidis depending on microwave dose

Dose (kJ×g^{-1})	Number of *Salmonella* Enteritidis (MPN×g^{-1})	
	type of fish meal	
	cod	salmon
Control sample	2.0×10^7	1.5×10^7
4.86	1.5×10^3	3.0×10^3
14.08	2.5×10^2	9.5×10^2
38.58	2.5×10^1	2.5×10^2
85.23	n.d.*	2.5×10^1
149.52	n.d.	n.d.
210.00	n.d.	n.d.

* – not detected.

Table 3. Changes in number of *E. coli* depending on microwave dose

Dose (kJ×g^{-1})	Number of *E. coli* (MPN×g^{-1})	
	type of fish meal	
	cod	salmon
Control sample	2.0×10^7	1.5×10^6
4.86	n.d.	4.5×10^2
14.08	n.d.	2.5×10^1
38.58	n.d.	n.d.
85.23	n.d.	n.d.
149.52	n.d.	n.d.
210.00	n.d.	n.d.

Table 4. Changes in number of *Enterococcus* spp. depending on microwave dose

Dose ($kJ \times g^{-1}$)	Number of *Enterococcus* spp. ($MPN \times g^{-1}$)	
	type of fish meal	
	cod	salmon
Control sample	2.5×10^6	4.5×10^6
4.86	2.0×10^5	2.5×10^5
14.08	2.5×10^4	2.5×10^5
38.58	2.5×10^3	2.5×10^4
85.23	2.5×10^3	4.5×10^3
149.52	2.5×10^3	2.5×10^3
210.00	2.5×10^2	2.5×10^3

Table 5. Changes in number of *Clostridium sporogenes* spores depending on microwave dose

Dose ($kJ \times g^{-1}$)	Number of *C. sporogenes* spores ($CFU \times g^{-1}$)	
	type of fish meal	
	cod	salmon
Control sample	5.00×10^6	5.83×10^6
20.68	3.63×10^6	4.20×10^6
60.88	3.57×10^6	4.03×10^6
168.24	2.83×10^6	2.10×10^6
372.06	1.03×10^6	5.96×10^5
654.36	2.50×10^5	1.43×10^3
924.00	4.70×10^2	1.11×10^3

The largest decreases in the number of the studied bacteria were observed in the case of E. coli, and the smallest for enterococci (Tables 2–4). Already a dose of 4.86 $kJ \times g^{-1}$ resulted in elimination of *E. coli* in the cod meal, and in the salmon meal the analogous effect was obtained at a dose of 38.58 $kJ \times g^{-1}$ (Table 3). The complete elimination of bacteria of the genus *Enterococcus*, in turn, was not observed, and at a dose of 210 $kJ \times g^{-1}$ their number fell to the levels of 2.5×10^2 $MPN \times g^{-1}$ and 2.5×10^3 $MPN \times g^{-1}$ in the cod and salmon meals, respectively (Table 4). In the case of *Salmonella* Enteritidis rods, the complete elimination was observed in the case of microwave radiation dose equal to 85.23 $kJ \times g^{-1}$ in the cod meal and 149.52 $kJ \times g^{-1}$ in the salmon meal (Table 2).

Spores of *Clostridium sporogenes* were not fully inactivated within the studied range of microwave radiation doses (Table 5). At the dose of 924 $kJ \times g^{-1}$ the number of active spores was 4.70×10^2 $CFU \times g^{-1}$ in the cod meal and 1.11×10^3 $CFU \times g^{-1}$ in the salmon meal (Table 5).

Based on regression line equations (Figures 2–5), the theoretical lethal doses and D_{90} doses were determined, as well as the elimination rate for the studied vegetative bacterial forms and *Clostridium sporogenes* spores.

Table 6. Statistical parameters describing the inactivation kinetics of selected microorganisms

Fish meal type	Bacteria	Theoretical lethal dose ($kJ \times g^{-1}$)	Elimination rate ($\log MPN \times (kJ \times g^{-1})^{-1}$)	D_{90} dose ($kJ \times g^{-1}$)
Cod	*S.* Enteritidis	76.92 A,a (±1.52)*	0.06 A,a (±0.005)	16.18 A,F,a (±1.32)
	E. coli	6.50 B,b (±0,05)	1.83 B,b (±0.02)	0.55 B,b (±0.01)
	Enterococcus spp.	373.02 C,c (±13.02)	0.01 A,c,e (±0.001)	72.99 C,c (±5.40)
		($kJ \times g^{-1}$)	($\log MPN \times (kJ \times g^{-1})^{-1}$)	($kJ \times g^{-1}$)
	C. sporogenes spores	1872.62 D,d (±59.46)	0.004 C,d (±0.0001)	270.27 D,d (±4.11)
Salmon	*S.* Enteritidis	136.86 E,e (±2.74)	0.03 A,a,c (±0.002)	29.59 A,e (±1.34)
	E. coli	36.24 F,f (±0.79)	0.13 A,f (±0.01)	7.72 B,F,f (±0.29)
	Enterococcus spp.	428.90 G,g (±8.14)	0.01 A,c,e (±0.001)	76.92 C,c (±3.02)
		($kJ \times g^{-1}$)]	($\log MPN \times (kJ \times g^{-1})^{-1}$)	($kJ \times g^{-1}$)
	C. sporogenes spores	1528.38 H,h (±14.79)	0.005 C,d (±0.0002)	222,22 E,g (±5.08)

A, B, C,… – highly significant difference ($P \leq 0.01$).
a, b, c,… – significant difference ($P \leq 0.05$).
* – standard deviation.

Theoretical lethal doses were highly significantly lower ($P \leq 0.01$) in the case of *E. coli* than for the other studied bacteria (Table 6). They amounted to 6.50 and 36.24 $kJ \times g^{-1}$, for the cod and salmon meals, respectively (Table 6). The observed difference resulting from the type of fish meal was highly significant ($P \leq 0.01$) (Table 6).

Theoretical lethal doses calculated for *Salmonella* Enteritidis were highly significantly different ($P \leq 0.01$) both in comparison with *E. coli*, and with enterococci (Table 6). They amounted to 76.92 $kJ \times g^{-1}$ in meal from cod and 136.86 $kJ \times g^{-1}$ in meal from salmon (Table 6). The difference connected with the type of meal was highly significant ($P \leq 0.01$) (Table 6).

Highly significantly higher ($P \leq 0.01$) theoretical lethal doses, as compared with the other bacteria, were found in the case of enterococci and they amounted to 373.02 $kJ \times g^{-1}$ in cod meal and 428.90 $kJ \times g^{-1}$ in salmon meal (Table 6). The difference connected with the meal type was highly significant ($P \leq 0.01$) (Table 6).

Theoretical lethal doses needed for the inactivation of *Clostridium sporogenes* spores were definitely higher ($P \leq 0.01$) as compared with vegetative forms of all the studied bacteria (Table 6). They amounted to as much as 1872.62 and 1528.38 $kJ \times g^{-1}$ in the meal from cod and salmon, respectively, whereas the difference resulting from the meal type was highly significant ($P \leq 0.01$) (Table 6).

The calculated inactivation rate of microorganisms of the *Enterococcus* spp. was the lowest of the vegetative bacteria forms and was equal to 0.01 log MPN× ×(kJ×g^{-1})$^{-1}$, both in the cod and salmon meals (Table 6). The highest inactivation rate, in turn, was observed in the case of *E. coli* rods and it was 1.83 log MPN× ×(kJ×g^{-1})$^{-1}$ in the meal from cod and 0.13 log MPN×(kJ×g^{-1})$^{-1}$ from salmon (Table 6). Statistically significant (P≤0.05) or highly significant (P≤0.01) differences in the elimination rate resulting from the genus of bacteria in the given type of meal were observed between *E. coli* and the other microorganisms, whereas those connected with the meal type were also recorded only in the case of *E. coli* rods (Table 6). Spores of *Clostridium sporogenes* underwent a highly significantly (P≤0.01) slower elimination in comparison with vegetative forms of bacteria, and a difference in the inactivation rate resulting from the type of fish meal was not statistically significant (P>0.05) (Table 6).

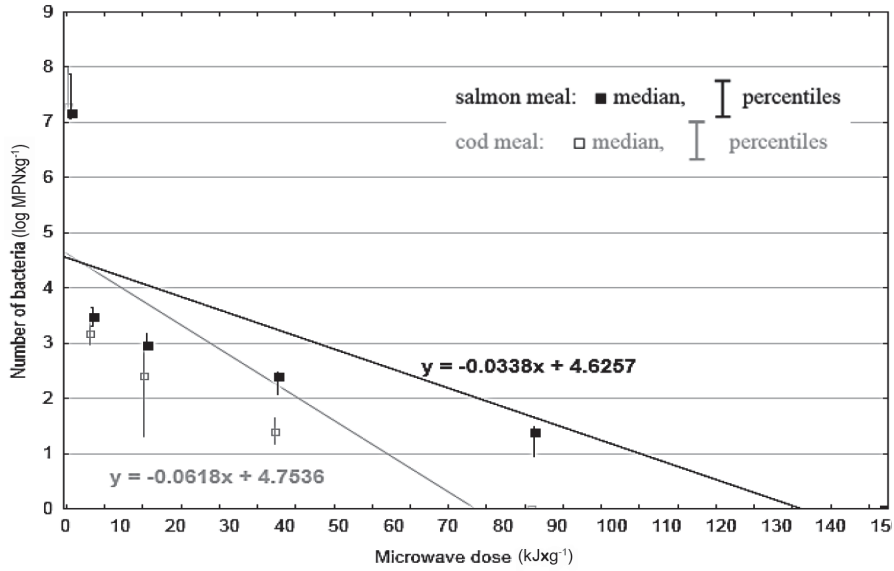

Figure 1. Regression lines describing the inactivation of *Salmonella* Enteritidis

Based on regression equations (Figures 1–4), microwave radiation doses causing a reduction in the number of the studied bacteria population by 90% (D_{90}) were calculated (Table 6). Reduction in the population number of vegetative forms of bacteria by 90% required the highest D_{90} dose (72.99–76.92 kJ×g^{-1}) in the case of enterococci, and the lowest (0.55–7.72 kJ×g^{-1}) for *E. coli* (Table 6). The differences shown between the studied microorganisms in the given type of meal were highly significant (P≤0.01) (Table 6). In turn differences resulting from the type of fish meal were statistically significant (P≤0.05) in the case of *Salmonella* Enteritidis and *E. coli* (Table 6).

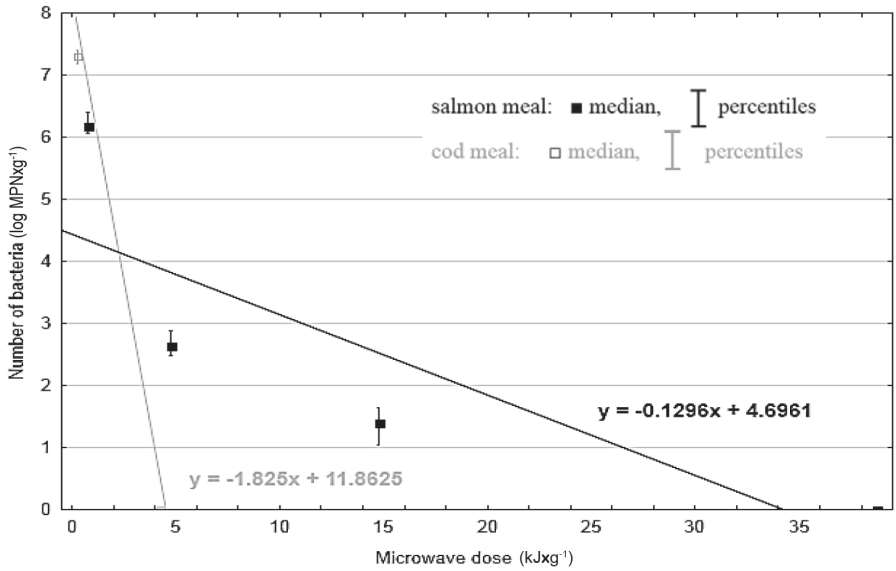

Figure 2. Regression lines describing the inactivation of *E. coli*

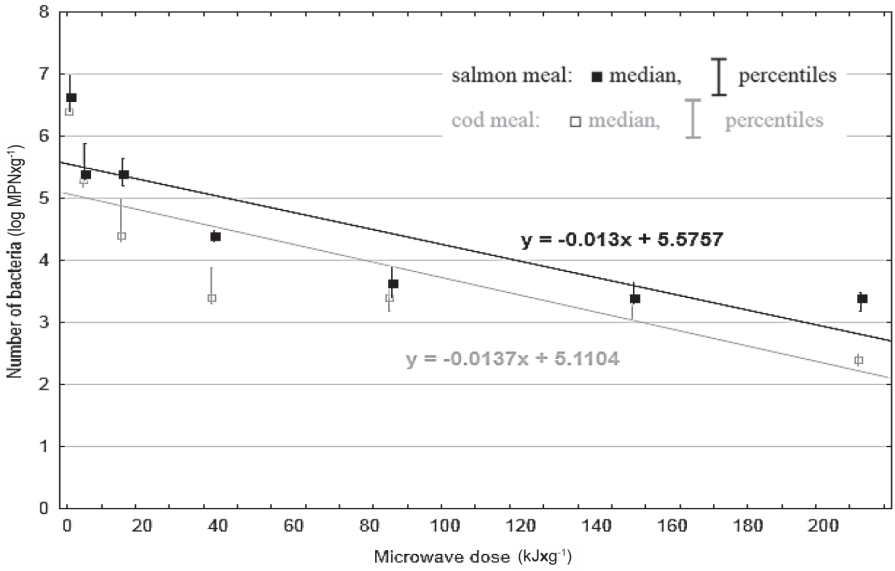

Figure 3. Regression lines describing the inactivation of *Enterococcus* spp.

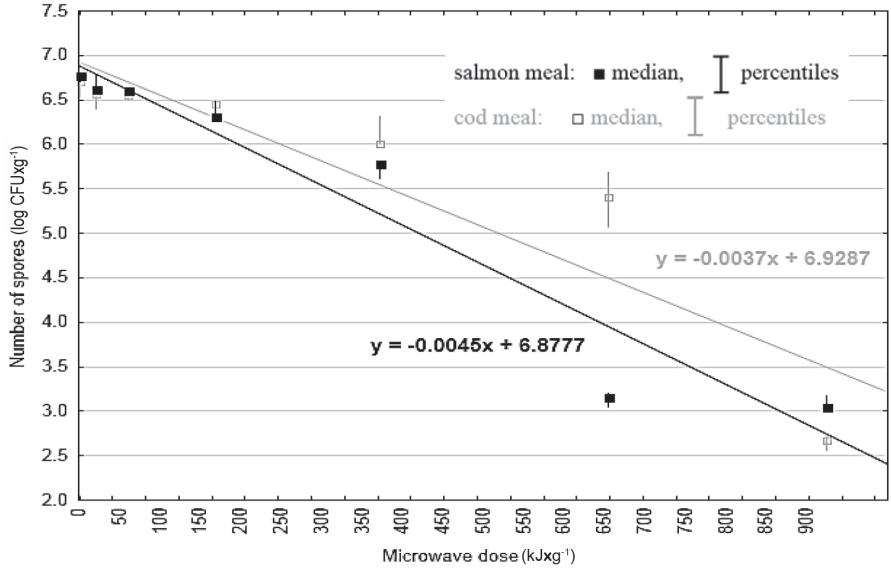

Figure 4. Regression lines describing the inactivation of *Clostridium sporogenes* spores

In the case of *Clostridium sporogenes* spores, D_{90} doses were highly significantly ($P \leq 0.01$) higher (222.22–270.27 kJ×g^{-1}) than those determined for vegetative forms of the studied bacteria, and the difference resulting from the type of fish meal was highly significant ($P \leq 0.01$) (Table 6).

The results obtained allowed the conclusion that for vegetative forms of the studied bacteria higher theoretical lethal doses and D_{90} doses of microwave radiation at a lower elimination rate were recorded in the meal from salmon than that from cod (Table 6). By contrast, a reverse tendency was shown for *Clostridium sporogenes* spores (Table 6).

Discussion

The effective use of microwave radiation for inactivation of microorganisms is dependent on many factors. The basic outside parameters determining the bactericidal effectiveness of microwaves include their power, frequency, the time of action and temperature (Shamis et al., 2008).

In the present study, the highest degree of reduction under the influence of microwave radiation was observed in the case of *E. coli*, which died in the cod meal already after the application of 4.86 kJ×g^{-1} (Table 3). The most resistant of vegetative forms turned out to be enterococci, isolated in both types of meals even after the application of a dose of 210 kJ×g^{-1} (Table 4). Also for other hygienization methods, *Salmonella* spp. was more resistant than *E. coli*. According to Nardi et al. (2011) the

UV dose needed for complete inactivation of *Salmonella* spp. reached 320 $J \times m^{-2}$ and, compared to *E. coli* – 110 $J \times m^{-2}$, appeared to be relatively high. Slightly different tendencies concerning differences in bacteria susceptibility to microwaves were observed by Dańczuk and Łomotowski (2008). The results of their study proved that a radiation dose necessary for the complete inactivation of *Salmonella* spp. in sewage is lower than that for the coliform bacteria. Both of them died after the application of a relatively low dose of radiation, not exceeding 12 $kJ \times g^{-1}$. According to Maktabi et al. (2011) reduction of *E. coli* viable counts after 15 s of microwave radiation ranged from 0.17 to 0.81 log $CFU \times ml^{-1}$ and was relatively low, compared to *Pseudomonas fragi* (2.00–3.27 log $CFU \times ml^{-1}$). The most effective antibacterial action against *E. coli* was achieved after combined treatment of laser, microwave and UV radiation. Their synergistic effect resulted in over 5 log reduction of *E. coli* counts (Maktabie et al., 2011).

In the study by Hong et al. (2004), a 90-second action of microwaves leading to generating a temperature of about 65°C resulted in the complete inactivation of coliform bacteria in sewage sludge. Obtaining a similar result using conventional heating required considerably longer time and a temperature of 100°C. Also Woo et al. (2000) reports that in the case of inactivation using microwaves a growth of temperature within the range from 50°C to 60°C is essential. Above this value, the effectiveness of the process of thermal reduction of *E. coli* and vegetative cells of *B. subtilis* was lower. De La Vega-Miranda et al. (2012) achieved 5 log cycles reduction of *Salmonella* Typhimurium in jalapeño pepper subjected to microwave treatment (25 s, 63°C, 950 W). Inactivation of *Salmonella* Typhimurium in the yolk of artificially infected eggs pasteurized with microwave radiation for 20 s, reached the value of about 1.2 log (Shenga et al., 2010).

A number of studies also confirm the possibility of obtaining the inhibitory effect of microwave radiation even below 45°C. Shamis et al. (2008), using a radiation source with the frequency of 18 GHz and the power of 16 W, obtained more than 60% degree of decontamination of raw meat surface inoculated with *E. coli* and *Staphylococcus aureus*. They also proved that at a high frequency of waves, repeating of irradiation allows for obtaining an increase in its bactericidal effectiveness.

The easily obtained thermal effect of microwave radiation makes it possible to predict its high effectiveness in inactivation of vegetative microbial forms. It is different in the case of spore forms, which sometimes are characterized by a very high thermoresistance, hindering their thermal destruction.

As expected, the results of the present study showed a considerably higher resistance of *Clostridium sporogenes* spores to the action of microwave radiation, in comparison with the other tested microorganisms. The dose equal to 210 $kJ \times g^{-1}$, which resulted in the complete inactivation of *Salmonella* Enteritidis and *E. coli* rods and a considerable reduction in the population of enterococci, did not exert a considerable effect on a change in the number of *Clostridium* spores (Table 5). The calculated theoretical radiation dose necessary to eliminate them was almost 1 900 $kJ \times g^{-1}$ in the cod meal and slightly higher than 1 500 $kJ \times g^{-1}$ in the salmon meal (Table 6).

Higher resistance of *Clostridium* spores to microwaves is also confirmed by Dańczuk and Łomotowski (2008). According to Kim et al. (2009), microwave radia-

tion leads to an increase in pressure inside *C. sporogenes* spores and their mechanical destruction. This process is accompanied by releasing DNA and Ca ions from the inside of spores. Leakage of proteins and DNA was also observed after irradiation of spores of *Bacillus licheniformis*. The presence of DNA in the bacterial cell suspension, both at the vegetative and spore stages, indicates damage of their cytoplasmic membranes (Woo et al., 2000). Such changes were not caused by conventional boiling and a lower power of radiation used by Kim et al. (2009).

Optimization of irradiation process parameters is not the absolute guarantee of hygienization efficacy of microwave radiation. In the case of food products, their chemical composition, volume, structure, pH and water content are also of great importance (Canumir et al., 2002; Valsechi et al., 2004). The low activity of water and high fat content contribute to an increase in the microorganism thermoresistance (Anaya et al., 2008).

Similar factors may determine the inhibitory effect of microwaves on microorganisms contained in the studied fish meals. The present study indicates the presence of highly significant differences in the survival of the studied microorganisms in different meal types. However, whereas enterococci, *Salmonella* Enteritidis and *E. coli* rods were isolated longer from the salmon meal irradiated with microwaves, *Clostridium* spores underwent a slower elimination in the cod meal (Table 6).

Studies on the possibility of using microwave radiation in the food industry prove its high effectiveness in reducing the number of pathogenic microorganisms, including also those of the genus *Salmonella*. Many authors, however, focus particular attention on problems connected with uneven distribution of the heat generated during the process, and appearance of the so-called hot and cold spots in irradiated food. This refers mostly to products with large sizes and irregular shapes, and is connected with the risk of some pathogenic microorganisms surviving (Lu et al., 2011; Pucciarelli and Benassi, 2005; Aziz et al., 2002).

A serious disadvantage related to using microwave for food disinfection is the possible deterioration of the radiated product. The undesired changes include chemical composition, nutritional quality and sensory properties of food. However, there are many data confirming relatively low, compared to conventional heat-treatment, effect of microwave processing on the quality of food (Chandrasekaran et al., 2013). Properly established and controlled parameters of the radiation may guarantee both high quality of the product and its microbiological safety (Tochampa et al., 2011).

The use of microwave radiation for hygienization of contaminated fish meal turned out to be a relatively effective method both towards vegetative and spore forms of bacteria, but species differences in resistance were observed. The power of dose resulting in the complete inactivation of the studied bacteria should be about 430 $kJ \times g^{-1}$ (but only 140 $kJ \times g^{-1}$ for *Salmonella* Enteritidis and *E. coli*), and in the case of spores – 1 900 $kJ \times g^{-1}$.

Homogeneous structure of meat and meat-and-bone meals allows for their even distribution, and then the control of thickness of the layer exposed to radiation. Together with the bactericidal effectiveness proved in the experiments, this can make an argument in favour of conducting further studies towards the use of microwaves as an effective method for sanitization of meat and fish meals.

References

Anaya I.I., Aguirrezabal A.A., Ventura M.M., Comellas L.L., Agut M.M. (2008). Survivability of *Salmonella* cells in popcorn after microwave oven and conventional cooking. Microbiol. Res., 163: 73–79.

Aziz N.H., Mahrous S.R., Youssef B.M. (2002). Effect of gamma-ray and microwave treatment on the shelf-life of beef products stored at 5°C. Food Control, 13: 437–444.

Buffo R.A., Holley R.A. (2006). Effects of food processing on disease agents. In: Food borne infections and intoxications, Riemann H.P., Cliver D.O. (eds). Elsevier Academic Press, UK, pp. 713–802.

Canumir J.A., Celis J.E., de Bruijn J., Vidal L.V. (2002). Pasteurisation of apple juice by using microwaves. Lebensm.-Wiss. Technol., 35: 389–392.

Chandrasekaran S., Ramanathan S., Tanmay Basak (2013). Microwave food processing – a review. Food Res. Int., 52: 243–261.

Crump J.A., Griffin P.M., Angulo F.J. (2002). Bacterial contamination of animal feed and its relationship to human foodborne illness. Clin. Infect. Dis., 35: 859–865.

Dańczuk M., Łomotowski J. (2008). Application of the microvare energy to the hygienization of sewage sludge (in Polish). Inż. Ochr. Środ., 11: 95–104.

De La Vega-Miranda B., Santiesteban-Lopez N.A., Lopez-Malo A., Sosa-Morales M. E. (2012). Inactivation of *Salmonella* Typhimurium in fresh vegetables using water-assisted microwave heating. Food Control, 26: 19–22.

Dong J., Ma X., Fu Z., Guo Y. (2011). Effects of microwave drying on the contents of functional constituents of *Eucommia ulmoides* flower tea. Ind. Crop. Prod., 34: 1102–1110.

Ghanem N., Mihoubi D., Kechaou N., Mihoubi N.B. (2012). Microwave dehydration of three citrus peel cultivars: Effect on water and oil retention capacities, color, shrinkage and total phenols content. Ind. Crop. Prod., 40: 167–177.

Hong S.M., Park J.K., Lee Y.O. (2004). Mechanisms of microwave irradiation involved in the destruction of fecal coliforms from biosolids, Water Res., 38: 1615–1625.

Kim S.Y., Shin S.J., Song C.H., Jo E.K., Kim H.J., Park J.K. (2009). Destruction of *Bacillus licheniformis* spores by microwave irradiation. J. Appl. Microbiol., 106: 877–885.

Lu Y., Turley A., Dong X., Wu C. (2011). Reduction of *Salmonella enterica* on grape tomatoes using microwave heating. Int. J. Food Microbiol., 145: 349–352.

Maciorowski K.G., Herrera P., Jones F.T., Pillai S.D., Ricke S.C. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. Anim. Feed Sci. Tech., 133: 109–136.

Maktabie S., Watson I., Parton R. (2011). Synergistic effect of UV, laser and microwave radiation or conventional heating on *E. coli* and on some spoilage and pathogenic bacteria. Innov. Food Sci. Emerg. Technol., 12: 129–134.

Møretrø T., Midtgaard E.S., Nesse L.L., Langsrud S. (2003). Susceptibility of Salmonella isolated from fish feed factories to disinfectants and air-drying at surfaces. Review Vet. Microbiol., 94: 207–217.

Nardi I.R., Nery V., Amorim A.K.B., Santos N.G., Chimenes F. (2011). Performances of SBR, chemical-DAF and UV disinfection for poultry slaughterhouse wastewater reclamation. Desalination, 269: 184–189.

Nasri K., Daghfous D., Landoulsi A. (2013). Effects of microwave (2.45 GHz) irradiation on some biological characters of *Salmonella* Typhimurium. C.R.Biol., 336: 194–202.

Park D.K., Bitton G., Melker R. (2006). Microbial inactivation by microwave radiation in the home environment. J. Environ. Health., 69: 17–24.

Pucciarelli A.B., Benassi F.O. (2005). Inactivation of *Salmonella* enteritidis on raw poultry using microwave heating. Braz. Arch. Biol. Technol., 48: 939–945.

Shamis Y., Taube A., Shramkov Y., Mitik-Dineva N., Vu B., Ivanova E.P. (2008). Development of a microwave treatment technique for bacterial decontamination of raw meat. Int. J. Food Eng., 4: 359–371.

Shenga E., Singh R.P., Yadav A.S. (2010). Effect of pasteurization of shell egg on its quality characteristics under ambient storage. J. Food Sci. Technol., 47: 420–425.

Tochampa W., Jittrepotch N., Kongbangkerd T., Kraboun K., Rojsuntornkitti K. (2011). The study of microwave heating time on chemical and microbiological properties and sensory evaluation in sweet fermented glutinous rice (Khao-Mark). Int. Food Res. J., 18: 239–248.

Valsechi A.O., Horii J., Angelis D. (2004). The effect of microwaves on microorganisms. Arquivos do Instituto Biológico, Sao Paulo, 71: 399–404.

Welt B.A., Tong C.H., Rossen J.L., Lund D.B. (1994). Effect of microwave radiation on inactivation of *Clostridium sporogenes* (PA 3679) spores. Appl. Environ. Microbiol., 60: 482–488.

Woo I.S., Rhee I.K., Park H.D. (2000). Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure. Appl. Environ. Microbiol., 66: 2243–2247.

Wu Y., Yao M. (2010). Inactivation of bacteria and fungus aerosols using microwave irradiation. J. Aerosol Sci., 41: 682–693.

Zieliński M., Ciesielski S., Cydzik-Kwiatkowska A., Turek J., Dębowski M., (2007). Influence of microwave radiation on bacterial activity and community structure in biofilm. Process Biochem., 42: 1250–1253.

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