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EFFECT OF MICROWAVE HEATING ON THE SURVIVABILITY OF *CAMPYLOBACTER* SPP. IN POULTRY NUGGETS

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The objective of this study was to assess the susceptibility of three bacterial strains of *Campylobacter*: *C. coli* ATCC 43478, *C. jejuni* ATCC 33291 and *C. jejuni* PZH, isolated from 1 month old infant with diarrhea, to microwave heating. Poultry nuggets were contaminated with the analysed bacterial strains and subsequently exposed to the treatment at three power levels of a microwave (at 2450 MHz): 340 W, 480 W and 760 W for 30 s, 60 s, 90 s, 120 s and 180 s. The experiments were performed in four series. The initial contamination of poultry nuggets was at 10^7 cfu/g. The results obtained showed that the application of power at 340 W and 480 W for 30 s caused a decrease in the number of bacteria of all analysed strains by 1 and 2 log cycles, respectively. The extension of the heating time up to 1 min reduced bacterial count by 3.5 and 4.5 log cycles, respectively. Upon the powers applied, the complete inactivation of the bacteria in poultry nuggets was reached after 90-s treatment. The treatment at 760 W reduced the number of bacteria by 4 log cycles after 30-s exposure. The complete elimination of *Campylobacter* spp. from the samples was obtained after 1-min treatment. No statistically significant differences were found in the survivability of the strains applied during exposure to microwaves.

INTRODUCTION

In the past few years, foodborne infections induced by *Campylobacter* spp. genus have been observed to be more frequent than those caused by *Salmonella* spp. or *E. coli.* Epidemiological studies have shown that *Campylobacter* spp. is currently the most frequently isolated human enteropathogen (approximately 400 mln cases a year throughout the world) and over the years the number of reported cases of infections with this bacteria have drastically increased [Jagusztyn–Krynicka & Brzuszkiewicz, 2003]. Among foods consumed, poultry and poultry products are considered as the major or one of the main sources of infections induced by *Campylobacter* spp. Simultaneously, a constant increase is being observed worldwide in the production and consumption of poultry products in different forms – from poultry carcasses, through culinary elements to processed products [Corry & Atabay, 2001; Wieczorek & Osek, 2005].

The economical, social and cultural transformations which proceeded at the turn of the century evoked alterations in life styles of a contemporary society. The observed increment in society's affluence with a simultaneous increase in professional activity, especially in the case of women, elicit endeavour to more comfortable life style. A growing number of people are dining out, mainly in restaurants, but also in bars (fast food) like: McDonalds, Kentucky Fried Chicken *etc.*, that use food produced with the industrial methods, often pre-treated one. Continuously, there is a growth in the demand for and interest in ready-to-eat and ready-to-heat or ready-to-cook food, *i.e.* the so-called "convenience food", among which poultry products are observed to prevail [Bartnikowska, 2001].

The thermal processing of such foods is normally performed by typical, conventional methods, such as: cooking, frying, stewing, baking, but microwave heating is becoming an increasingly popular alternative. The popularity of microwave ovens is noticed to grow successively. The number of their users is increasing as well. Microwave ovens have become common appliance in households as well as a regular piece of equipment in kitchens, bars and restaurants.

Taking into account the mentioned aspects, the aim of the research was to investigate the influence of microwave heating on the survivability of *Campylobacter* spp. in poultry nuggets.

MATERIAL AND METHODS

The study was conducted with three strains of *Campylobacter* spp.: *Campylobacter jejuni* ATCC 33291, *Campylobacter coli* ATCC 43478, obtained from the collection of the National Veterinary Research Institute in Puławy as well as *Campylobacter jejuni* – originating from a strain collection of the National Institute of Hygiene in Warsaw (PZH), isolated from one month old infant with diarrhoea (*C. jejuni* PZH). Poultry nuggets were contaminated with the mentioned bacteria. To this end, the initial inoculum was prepared

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each time, by washing colonies out of the surface of a dish with a sterile spatula and inserting 1 mL of a dilution fluid onto the dish' surface. From the resultant suspension, the inoculum was prepared with an optical density corresponding to "6" at the McFarland's scale using a densitometer (McFarland Densitometer SYNGEN DEN-1). So prepared suspension was administered by multiple injections with a sterile syringe to the nuggets interior (under the coat). For each experimental series, the coated poultry nuggets were purchased in a retail network in city of Olsztyn. The contaminated nuggets were heated in a microwave oven (MOULINEX Y 90 1100W/2450MHz). Each of the strains was exposed to microwave heating using three levels of power: 340 W, 480 W and 760 W for 30 s, 60 s, 90 s, 120 s and 180 s, and compared with the control sample not exposed to the microwave heating. Four series were made for each analysed strain of *Campylobacter*, power value and heating time.

After primary disintegration with a sterile scalpel, the nuggets (25 g) were transferred into a sterile bag, then 225 mL of liquid serial dilution was added and the sample was homogenized in a stomacher (STOMACHER Lab-Blender 400) for 1 min, thus obtaining the first dilution. Subsequently, further decimal dilutions were performed and inoculated from each dilution onto the surface of two parallel plates with mCCDA medium (modified charcoal cefoperazone deoxycholate agar). The plates were incubated at 41.5°C under microaerobic conditions for 44 h±4 h. After incubation, colonies of the *Campylobacter* spp. were counted as a weighted average in 1g, including two further dilutions, in accordance with guidelines of the Polish Standard [PN-EN ISO 7218:2007].

Immediately after heating in a microwave, the measurement of nuggets temperature was performed with an electronic thermometer (MT-1 INNOWA).

RESULTS

The results obtained were presented in Figures 1 to 4. The survivability of the three *Campylobacter* strains was shown as log of the number of bacteria per 1 g of poultry nuggets. All values were averaged due to the fact that no statistically significant differences were observed between 4 experimental series for each strain examined.

Figure 1 shows results obtained for the strain *C. jejuni* ATCC 33291. The microwave treatment at 340 W caused a complete reduction of bacteria number after 90 s, with average initial contamination at a level of 7.34 log cfu/g. The utilization of microwave heating for 30s decreased the number of bacteria of 1 log cycle to the level of 6.34 log cfu/g. After 60 s of microwave heating, the number of C. *jejuni* bacteria decreased by 3.5 log cycles, *i.e.* to 3.89 log cfu/g. Likewise, microwave heating for 90 s, 120 s and 180 s caused complete inactivation of the analysed strains.

In turn, 30-s microwave heating at 480 W with initial contamination of *C. jejuni* ATCC 33291 at a level of 7.34 log cfu/g diminished the contamination by 1.5 log cycle, that is to a level of 5.66 log cfu/g. The extension of microwave treatment by further 30 s reduced the bacterial count by the next 2.5 log cycles, *i.e.* to a level of 2.93 log cfu/g. The application of longer time of microwave heating at 480 W resulted in complete inactivation of the analysed strains.

During the application of microwave power of 760 W, 30-s heating led to a distinct decrease in the bacteria number to 3.58 log cfu/g, in comparison to the initial contamination of the control sample (7.34 log cfu/g). The complete inactivation of the analysed bacteria was observed as early as after 1 min of microwave heating.

Results obtained for the strain *C. jejuni* PZH are shown in Figure 2. The microwave heating at 340 W for 30 s diminished the initial contamination (7.25 log cfu/g) to a level of 6.20 log cfu/g. In turn, microwave heating for 1 min reduced the number of bacteria by 3.5 log cycle, *i.e.* to a level of 3.63 log cfu/g, whereas the application of longer time of the microwave treatment (90 s, 120 s and 180 s) led to the efficient inactivation of the strain from the samples.

In the case of applying the power of 480 W for microwave heating of *C. jejuni* PZH the initial contamination was 7.25 log cfu/g. After 30 s of microwave heating, the number of bacteria was observed to decrease by 2 log cycles, reaching a level of 5.15 log cfu/g, whereas 1-min exposure to microwaves diminished the contamination to 2.81 log cfu/g. Microwave heating for 90 s caused complete inactivation of *C. jejuni* PHZ in the samples.

The series in which the microwave power of 760 W was applied, the contamination level of the control samples was 7.3 log cfu/g. A reduction in bacterial count by 4 log cycles (to a level of 3.34 log cfu/g) was achieved after 30-s exposure to microwaves. The complete elimination of *C. jejuni* PHZ from the nuggets was observed as soon as after 1 min.

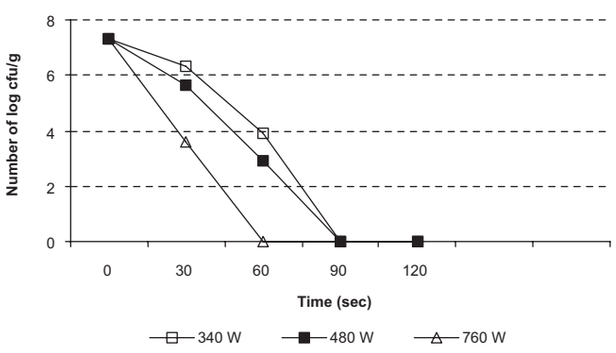

FIGURE 1. Survival of *Campylobacter jejuni* ATCC 33291 in poultry nuggets after microwave heating.

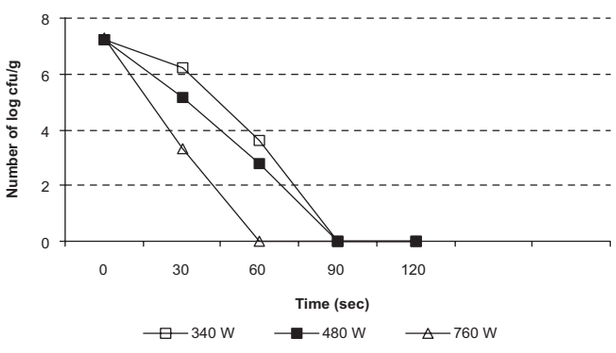

FIGURE 2. Survival of *Campylobacter jejuni* PZH in poultry nuggets after microwave heating.

Figure 3 presents results obtained for the strain *C. coli* ATCC 43478. Contamination level of control samples after microwave heating at 340 W was 7.328 log cfu/g. After 30-s exposure to heating, the reduction of contamination by 1 log cycle was achieved. The contamination was diminished by 3.5 log cycles after 1 min of microwave heating, whereas the complete bacterial inactivation was achieved after 90 s.

Contamination of the control samples with *C. coli* ATCC 43478, exposed to microwave heating (480 W) was at level of 7.25 log cfu/g. The reduction by 2 log cycles was observed after 30 s of microwave heating. Successive reduction by 2.5 log cycles was achieved after 60 s, when the contamination reached a level of 2.85 log cfu/g. Exposure for 90 s caused the complete inactivation of all *C. coli* in the nuggets.

The application of the microwave power of 760 W evoked the reduction of *C. coli* by nearly 4 log cycles after just 30 s. The extension of exposure time caused the complete elimination of bacteria.

Figure 4 shows the average maximum temperatures reached during the microwave heating. After 30 s of the treatment, the temperature inside the nuggets was 74.8°C, 84.3°C and 87.9°C for the power levels of 340 W, 480 W and 760 W, respectively. Further exposure for 1 min caused temperature increase up to 84.3°C, 89.6°C and 92,3°C, while after 90 s up to 86.2°C, 94.3°C and 95.7°C. The temperature measured inside the nuggets increased to 89.4°C, 96.5°C and 106.8°C after 2-min exposure, and up to 93.1°C, 103.7°C and 136.°C after another minute of microwave heating.

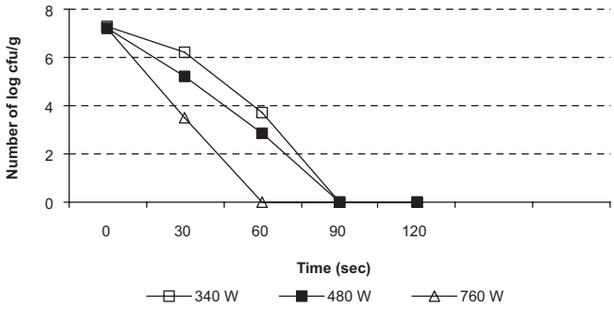

FIGURE 3. Survival of *Campylobacter coli* ATCC 43478 in poultry nuggets after microwave heating.

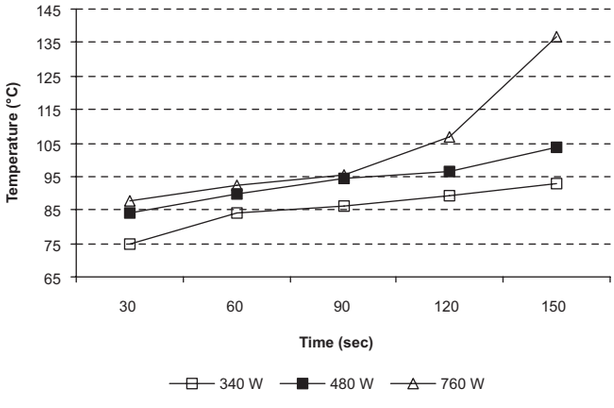

FIGURE 4. Temperature of poultry nuggets after microwave heating at different times.

DISCUSSION

Considering the own research and literature evidence, it appears that enteropathogens, including *Campylobacter* spp., show a high susceptibility to microwave heating. A number of authors point out the fact that the use of microwave ovens leads to a notable reduction of microbes in food.

Analyses of research results by Pucciarelli & Benassi [2005] indicates that microwave heating at 400 W caused a reduction in bacteria count by 1 log cycle after 30 s and by 3 log cycles after 60-s exposure in chicken nuggets contaminated with *S.* Enteritidis. The total elimination of *Salmonella* spp. from the product was achieved after 2 min of heating. Similar results have been obtained in our own research in the case of applying powers of 340 W and 480 W, when the reduction in bacteria count after 30-s exposure reached respectively 1 and 2 log cycles. The prolongation of the heating time up to 1 min diminished the bacteria count by 3.5 and 4.5 log cycles, respectively. The complete inactivation of the mentioned bacterial strains was achieved after 90 s of microwave heating at the powers applied. Upon the use of the power of 760W, the reduction by 4 log cycles was observed after 30 s, whereas the complete inactivation of *Campylobacter* was achieved after 1 min of microwave heating.

A research conducted by Abd El-Aal [1996] confirmed the high effectiveness of microwave heating, and thus the short duration of this process. On the basis of own study, this author demonstrated that the number of psychrotrophic bacteria in such products as: liver, hamburgers, poultry, meat, minced meat, sausage or fish fillets could be reduced by 4 to 6 log cycles as soon as within 30 s. Such a short time of microwave heating with the power of 800 W was also sufficient for the elimination of *E. coli* O157:H7 bacteria from portions of chicken with the initial contamination of 1.1×10^6 cfu/g [Apostolou *et al*., 2005]. What is more, according to Aziz *et al*. [2002] microwave heating with the power of 600 W for a short time applied to beef products contaminated with *Pseudomonas* and *C. perfringens* caused a significant reduction in bacterial count. In all cases (fresh beef, minced beef or beef hamburgers), the authors find the contamination of the analysed products to decrease by 1 log cycle after 20 s of heating. Notwithstanding, the microwave heating for 30 s enabled the reduction of bacterial contamination by 2 log cycles. The results obtained are consistent with those achieved by Cunningham [1980]. The author demonstrated that in raw poultry with the initial contamination at 10^4 cfu/cm^2 the application of microwave heating for 20 s caused the a reduction in the number of psychrotrophic bacteria by 1 log cycle. Simultaneously, 40 s of microwave heating reduced the bacterial count by 2 log cycles.

Uradziński *et al.* [1997] found that microwave treatment at 930 W for 8 min applied to poultry carcasses caused a reduction in the number of *C. jejuni* by 0-3 log cycles, depending on the strain analysed. The authors showed that 10-12 min of microwave heating enabled the complete elimination of *C. jejuni* from the samples. The temperature in poultry carcasses accounted for 52.5–97.5°C in the case of 8-min treatment, for 55.0–100°C in the case of 10-min treatment and for 70 – >100°C in the case of 12-min treatment. In a research by

Aleixo *et al*. [1985], the authors also found a substantial reduction in bacterial number in roasted turkeys exposed to microwave heating at 600 W. The number of *S.* Typhimurium bacteria in contaminated turkeys decreased after microwave heating to a level 9 to 45 cfu/g, with the initial contamination level reaching 6.0 x 10^8 cfu/g. The analysed turkeys (at 2.7 to 3.7 kg body weight) were treated for 55–87 min, with the maximum temperature of 76.6°C. In this study, the turkeys were also contaminated with *S. aureus*. In those turkeys (at 2.7–3.7 kg body weight) displaying the initial contamination at a level of 1.3 x 10^8, the 53.0-57.5 min exposure at the maximal temperature of 76.6°C reduced the bacterial number to a level of 279 to 720 cfu/g.

In the present study, the maximum temperatures in nuggets reached during the microwave heating exposure were determined as well. The exposure of the nuggets to microwaves for 1 min caused an increase in temperature to 84.3°C and 89.6°C, and after 2 min to 89.4°C and 96.5°C at the applied powers of 340W and 480W, respectively. However, lower temperatures were observed by Pucciarelli & Benassi [2005], who after 1 min of microwave heating (400 W) of poultry thighs observed the temperature within the range of 45-50°C, and after 2 min – 75°C. The application of a higher power of 800 W for 2 min caused a further temperature increase to 90°C, whereas in our own research the treatment at 760 W for 2 min increased the temperature to 136.7°C.

Different results about the efficiency of microwave heating in the reduction of bacteria number were obtained by Göksoy *et al*. [2000]. They showed that short exposure (30 s) of the contaminated chicken breast to microwave heat did not caused any changes in the number of bacteria. Results obtained in their study indicated that samples contamination with *E. coli* K12 and *C. jejuni* did not change after microwave heating, what is more, even a slight increase was observed in the bacterial count (up to 0.30 log cfu/g).

On the basis of literature data, as well as, our own results, it can be stated that effective reduction in bacterial number in food exposed to microwave heating, that assures health safety, depends – among other things – on the applied power of a microwave oven, time of heating, type of product, size of products to be heated and degree of their contamination with bacteria.

CONCLUSIONS

1. The microwave heating efficiently reduces the number of *Campylobacter* spp. in poultry nuggets.

2. The extent of reduction of *Campylobacter* spp. counts in nuggets was depended on the applied power, as well as, time of microwave heating.

3. The complete inactivation of *Campylobacter* spp. in the nuggets may be achieved upon 90-s exposure to microwave heating at the powers of 340 W and 480 W. Whereas, at the power of 760 W the complete elimination of bacteria was achieved after 1-min exposure.

4. None of the three analysed strains of *Campylobacter* spp. showed statistically significant differences in the survivability in poultry nuggets after microwave heating.

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