ation technology that employs ultra-high water pressure to destroy food-borne pathogens. HPP is independent of sample size and geometry, and can be applied with good retention of color, nutrients and flavor at ambient or moderate temperatures (Cheftel 1992, Martens and Knorr 1992, Lau and Turek 2007, Garriga and Aymerich 2009). HPP can be used to inactivate pathogens in raw materials, but also can be successfully used for food products that were previously heat-treated, and then were re-contaminated during slicing and packaging procedures, or through cross-contamination from raw meat to other food-items. Microbiological quality improvement was observed after the application of high pressure processing on fruits (Jacobo-Velázquez and Hernández-Brenes 2011, Argyri et al. 2014), oysters (San Marttn et. al 2002) and meat products (Garriga et al. 2004). Gogal et al. (2011) suggest that high hydrostatic pressure may be an effective treatment for norovirus-contaminated shellfish.

The aim of this study was to investigate the possibilities of C. jejuni elimination from the poultry meat by application of HPP. While the influence of high hydrostatic pressure on the survival of many food-borne pathogens is relatively well known, there is still a lack of comprehensive data on survival of C. jejuni. Exploring the possibility of C. jejuni inactivation in poultry meat is particularly important considering the constantly increasing consumption of poultry meat (Jayasena et al. 2013), as well the possibility of cross contamination, even after slaughter (Kruk et al. 2014). The results of this study will help to establish the parameters necessary to ensure the reduction of C. jejuni to the consumer safety level, and thus, reduce human exposure to contaminated poultry meat and its products. It will be also a source of information for inspection services, producers, and complement existing databases of technological parameters necessary to inactivate pathogens, where there is still little data on Campylobacter.

Materials and Methods

Test microorganisms

Bacterial suspension (inoculum) used in the study was a mixture of three Campylobacter jejuni strains: C. jejuni 34, C. jejuni 6 and C. jejuni 10/06. C. jejuni strains no. 34 and 6, obtained from the laboratory of the Department of Veterinary Protection of Public Health, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn (Poland), were isolated from chickens. C. jejuni strain no. 10/06, obtained from laboratory of the National Institute of Hygiene in Warsaw (Poland), has been isolated from clinical specimens from hospitalized children with diarrhoea. Strains used in the study had features typical of Campylobacter bacteria, i.e. characteristic rotary motion, no growth at 25 °C, positive reaction in tests for the presence of oxidase and catalase. All strains were maintained in brain hearth infusion broth containing 25% glycerol at … 80°C. Prior to the study bacteria were transferred onto plates of Karmali agar supplemented with modified Karmali selective supplement and were incubated for 48 h at 42°C under microaerophilic conditions. After three successive passages bacterial colonies taken from Karmali agar plates were suspended in nutrient broth supplemented with Preston campylobacter selective supplement containing 5% of laked horse blood. Suspensions obtained from three strains were mixed together in equal proportions to give a 15 ml mixture. The number of bacteria in the final suspension was determined by plating method and was about 10^8 cfu/ml.

Sample preparation

Animal-origin products (chicken breast) used in the study were purchased from commercial retail outlets in Warsaw. The percentage of fat and protein content and pH was determined (fat … 3.25%, protein … 21.5%, pH … 6.08). Prior to experiments, minced and then vacuum-sealed meat samples (10 g) were subjected to decontamination by gamma irradiation at a dose of 2 kGy in order to eliminate Campylobacter cells, the presence of which is highly probable in poultry meat and could affect the accuracy of the results. Until the start of experiments the samples were stored at -18°C. On the day of experiment bags were opened with sterile scissors and 0.2 ml of mixture (10^8 cfu/ml) of three C. jejuni strains was added to each bag containing 10 g of minced poultry meat. Since the high-pressure processed products require a sealed, flexible and quite durable packaging, after inoculation all samples were hermetically sealed in a polyamide … polyethylene packages.

High pressure treatment

The samples were exposed to high pressure treatment in the Institute of High Pressure Physics, Polish Academy of Sciences, Warsaw, Poland, where a lab-scale stand for food testing was constructed: high pressure piston-cylinder type food processor vessel with inner diameter of 110 mm equipped with internal heat exchanger and thermocouple located in the middle of the vessel. The working volume of

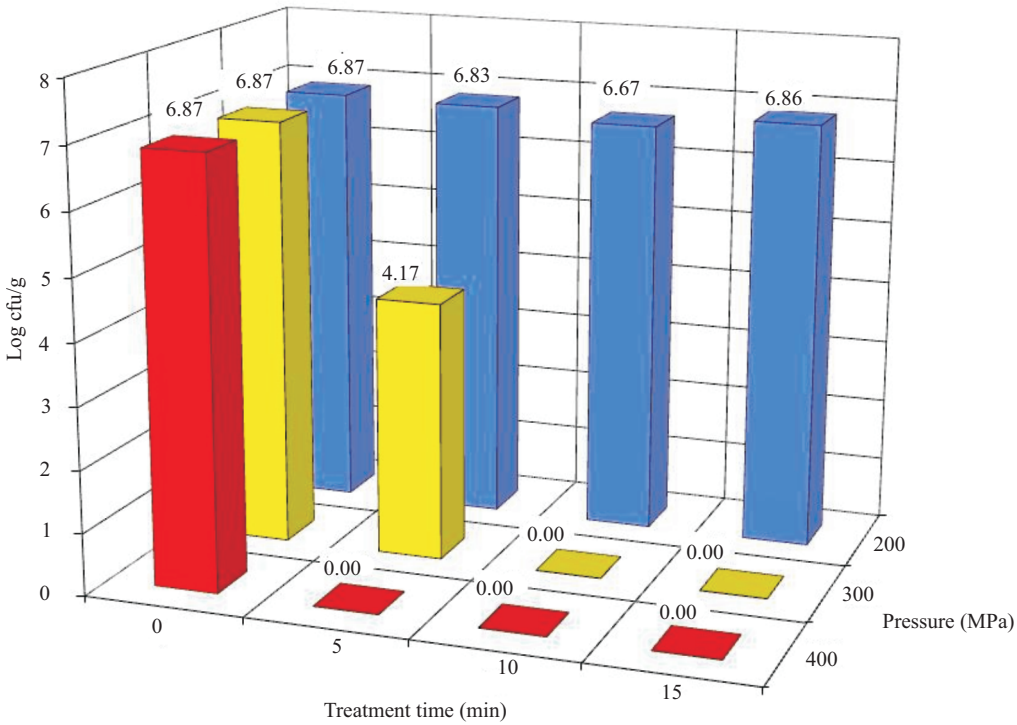

Fig. 1. Effect of high pressure treatment on survival of C. jejuni in poultry meat

Table 1. D-values and regression equation for estimating the reduction of C. jejuni in vacuum-packed poultry meat.

Pressure	D-value-1/b (min)	Regression equation	Correlation coefficient
200 MPa	NC	NC	NC
300 MPa	1.45	y = … 0.69 x + 7.11	-0.990
400 MPa	0.73	y = … 1.37 x + 6.87	-0.999

NC: Calculations were not conducted, no statistically important C. jejuni cells reduction were observed when subjected to 200 MPa for 5, 10 and 15 min.
Regression functions describe the correlation between the time application of pressure (x), and the number of surviving cells of C. jejuni (y). The strength of correlation between the parameters x and y describes the correlation coefficient.

sample holder was 1.5 l. The measurement of hydrostatic pressure inside of the chamber was done with the Pressure Gauge, strain gauge pressure transducer (type EBM 6045-700, KGT Kramer) and with the Bourdon type manometer, indicating the pressure under the piston rod. The pressure and temperature sensors were linked to the Computer Data Acquisition System (Memory Card MMC). This equipment allows for analysis of the variation of temperature in relation to pressure. A mixture of distilled water and propylene glycol (1:1) was used as a pressure-transmitting medium. The vessel was thermostated using the HUBER Compatible Control-Thermostat CC 245. The time needed to generate a pressure of 200, 300 and 400 MPa was 15-20 seconds. The time required to reduce the pressure to its initial value after the completion of the process was twice as long. Target high-temperature treatment was 4 °C, temperature variations depending on the pressure was 0-10°C. The samples were pressurized at 200, 300 and 400 MPa for 5, 10 and 15 min. Each experiment was carried out in duplicate and repeated in three independent runs. Unpressurized samples were used as a control.

Bacteriological analysis

In order to determine the number of C. jejuni cells that survived the HPP-treatment, quantitative bacteriological examination was carried out using the decimal dilutions method (McLandsborough 2005). The meat samples were homogenized for 2 minutes with a 9-fold amount of peptone water and decimal dilutions were made. From each dilution 1 ml was plated onto supplemented Karmali agar. Plates were incubated for 48 h at 42°C under microaerophilic conditions generated by using Gas Pak® envelopes. The bacterial counts were transformed into logarithms and

statistically analyzed. Statistical ANOVA analyses were carried out using the General Linear Models supplied through SPSS® 14.0 for Windows®. Mean values were compared using Tukey•s test.

Results

Effect of high hydrostatic pressure on the inactivation of C. jejuni cells in poultry meat is shown in Fig. 1. Pressure of 200 MPa, regardless of the time of its application, did not have a statistically significant effect on reducing the number of C. jejuni cells in poultry meat. Application of high pressure treatment with a value of 300 MPa for 5 min resulted in reduction of C. jejuni cells to a level of 4.17 log cfu/g. The prolonged application of 300 MPa pressure for a further 5 min resulted in reduction of C. jejuni cells below the detectable level. In samples treated with a pressure of 400 MPa after 5 min treatment were none of viable C. jejuni cells detected. On the basis of the results, the linear regression equation, D-values and 6D-values (Table 1) were calculated.

Discussion

Numerous studies have shown that high-pressure processing is a very attractive, alternative method of meats and fish preservation (Matser et al. 2000, Pierpaolo et al. 2000, Grochalska et al. 2001, Norton and Sun 2008, Bicche et al. 2009, Truong et al. 2014). As found in our previous studies high-pressure processing (300 MPa and 400 MPa) caused a significant C. jejuni reduction in cold-smoked salmon (Jackowska 2008).

C. jejuni was found to be resistant to pressure of 200 MPa when tested in poultry meat. Statistically significant effect of high pressure treatment on C. jejuni reduction was observed after applying a pressure of 300 MPa and 400 MPa. The effect was extremely strong when pressure of 400 MPa was applying … 5 min treatment resulted in reduction of C. jejuni cells below the detectable level. However, the same microbiological results were obtained when samples were subjected to pressure of 300 MPa for 10 or 15 min. This shows that the sensitivity of C. jejuni to the high pressure largely depends on the time of its application. The prolonged pressure of 300 MPa for another 5 minutes, resulted in reduction of C. jejuni cells by more than 4 log units. Table 1 shows the D-values of C. jejuni in poultry meat subjected to high pressure. For reduction C. jejuni by 6 log units (6D-values), considered as sufficient for consumer protection, the application of 300 MPa for 8.73 min, or 400 MPa for 4.37 min is needed. There was no significantly important reduction after applying 200 MPa pressure, therefore 6D-value for such pressure couldn•t be calculated. The results confirm that high hydrostatic pressure is an effective method to ensure the safety of poultry meat. C. jejuni was found to be quite pressure-sensitive. Most vegetative cells of other pathogens essential for food safety are readily killed in the range of 500-700 MPa (Farkas 2001). However, the results obtained by other authors are not unequivocal. Uradzi ski et al. (2008), applied 5, 15, 30 and 60-min pressurisation at 300 MPa to C. jejuni in poultry meat and showed that total reduction was achieved after 60-min pressurization. It is commonly reported, that Gram-negative pathogens are more pressure-sensitive than Gram-positive ones (López-Caballero et al. 1999). C. jejuni as a Gram-negative bacterium appears to be one of the most pressure-sensitive. In presented study the D-value for C. jejuni in poultry meat subjected to 300 MPa were 1.45 min, when other authors obtained higher D-values for other pathogens subjected to the same pressure range. One of the most pressure-resistant bacteria is Staphylococcusthat can survive treatment at 500 MPa for more than 60 min (Prokopov and Tanchev 2007). Gudbjornsdottir et al. (2010) studied the possibility of inactivation of L. innocua in cold-smoked salmon using a hydrostatic pressure of 400-900 MPa for a very short time (seconds). These authors demonstrated that for such short treatment time it is necessary to apply pressure with 700-900 MPa. Lori at al. (2007) applied the combination of pressure of 450 MPa and temperature 15ºC for 30 seconds which resulted in a reduction in C. jejuni by more than 6 log units in RTE poultry products. Such a short treatment time is possible by using the latest high-technology, and is dictated by the need to minimize the organoleptic changes in the pressurized product.

Sensitivity of microorganisms depends largely on the environment in which they are subjected to pressure. This regularity also applies to C. jejuni, which in food systems has greater resistance to high pressure than in other environments. Solomon and Hoover (2004) studied the effects of high pressure treatment on survival of C. jejuni in Bolton broth, phosphate buffer, UHT milk (whole and skim), soya milk, and chicken puree. It was found that high hydrostatic pressure with a value of 300-325 MPa applied for 10 min reduced the number of viable C. jejuni to below the detection limit when treated in broth. An additional 50-75 MPa was required to achieve similar levels of reduction when treated in food systems. The present study showed similar findings. Exposure of poultry meat to a pressure of 400 MPa for 10 min resulted in reduction of C. jejuni to below the detec-

tion limit. Similar range of reduction of C. jejuni in smoked salmon as a result of HPP was observed in our previous studies which were conducted in exactly the same conditions (Jackowska 2008). The D-value for C. jejuni in vacuum-packed smoked salmon subjected to 200 MPa and 300 MPa were 10.71 and 2.85 min, respectively. However, the application of 300 MPa pressure regardless of the time of its applications did not result in reduction of C. jejuni to below the detection limit … the cells showed a basic trend of decreasing the number along with increasing pressure value and treatment time. Different degrees of bacterial inactivation may be due to differences in the chemical composition of the pressurized products, especially the differences in the percentage of fat (poultry … 3.25%, salmon … 13.51%).

Nowadays, many researchers focus on the possibility of food-borne virus inactivation by the application of HPP technology (Kingsley et al. 2007, Grove et al. 2009, Tang et al. 2010, Terio et al. 2010, Gogal et al. 2011, Kingsley 2013), which is especially important taking into consideration the possibility of simultaneous elimination of C. jejuni cells. According to presented study, current standard parameters (275-300 MPa for several minutes) applied to shellfish as a Vibrio control (Kingsley 2013) might be not efficient for C. jejuni reduction to a level considered as sufficient for consumer protection.

Food manufacturers using high pressure technology for food preservation, stress that profitability is largely dependent on the duration of treatment. For economic reasons, therefore, it is preferable to use higher pressure for a shorter time. The results indicate that, from the hygienic point of view, this would be beneficial, since the application of higher pressure for a shorter time gives better microbiological effects. Increasing pressure of 100 MPa allows to reduce twice the time needed for viable C. jejuni cells reduction in poultry meat to a level sufficient to ensure consumer safety. This work has shown that for reducing C. jejuni in poultry meat by 6 log units (6D-values) the application of 300 MPa for 8.7 min or 400 MPa for 4.38 min is needed. However, further studies are needed to determine whether treatment in the pressure range 700-900 MPa for a very short period of time (seconds) will ensure consumer safety as regards the possibility of food poisoning caused by C. jejuni.

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