Acta Biochimica Polonica

Vol. 52 No. 3/2005, 721–724

on-line at: www.actabp.pl

High pressure processing for food safety*

M. Fonberg-Broczek[1]✉, B. Windyga[2], J. Szczawiński[3], M. Szczawińska[3], D. Pietrzak[4] and G. Prestamo[5]

[1]Laboratory of Biological Materials, Institute of High Pressure Physics, PAS, Warszawa, Poland; [2]Department of Food Research, National Institute of Hygiene, Warszawa; [3]Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, and [4]Department of Food Technology, Warsaw Agricultural University, Warszawa, Poland; [5]Instituto del Frio (CSIC), Ciudad Universitaria, Madrid, Spain;
✉e-mail: mfonberg@unipress.waw.pl

Received: 15 March, 2005; revised: 19 July, 2005; accepted: 28 July, 2005
available on-line: 15 September, 2005

Food preservation using high pressure is a promising technique in food industry as it offers numerous opportunities for developing new foods with extended shelf-life, high nutritional value and excellent organoleptic characteristics. High pressure is an alternative to thermal processing. The resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration, and type of microorganism. Generally, Gram-positive bacteria are more resistant to pressure than Gram-negative bacteria, moulds and yeasts; the most resistant are bacterial spores. The nature of the food is also important, as it may contain substances which protect the microorganism from high pressure. This article presents results of our studies involving the effect of high pressure on survival of some pathogenic bacteria — *Listeria monocytogenes, Aeromonas hydrophila* and *Enterococcus hirae* — in artificially contaminated cooked ham, ripening hard cheese and fruit juices. The results indicate that in samples of investigated foods the number of these microorganisms decreased proportionally to the pressure used and the duration of treatment, and the effect of these two factors was statistically significant (level of probability, $P \leq 0.001$). *Enterococcus hirae* is much more resistant to high pressure treatment than *L. monocytogenes* and *A. hydrophila*. Mathematical methods were applied, for accurate prediction of the effects of high pressure on microorganisms. The usefulness of high pressure treatment for inactivation of microorganisms and shelf-life extention of meat products was also evaluated. The results obtained show that high pressure treatment extends the shelf-life of cooked pork ham and raw smoked pork loin up to 8 weeks, ensuring good micro-biological and sensory quality of the products.

Keywords: high pressure processing, food safety, *Listeria monocytogenes, Aeromonas hydrophila, Enterococcus hirae*

Food products are an excellent environment for growth of pathogenic microorganisms, which may cause food-borne diseases. Quality and shelf-life of food products depend greatly on the properties of microorganisms contaminating the food. Despite the introduction of food standards obligatory in EU countries, epidemiologists believe that 75% of food-borne diseases are caused by bacteria (CAC, 2003). For this reason, the control of microorganisms is an important aspect of food quality and safety.

Many methods of food preservation are used for ensuring microbiological safety, among which high pressure processing (HPP) seems a very promising technique for food industry, as it offers numerous opportunities for developing new shelf life stable foods with extended shelf-life, high nutritional value and excellent organoleptic characteristics – minimally processed but safe for consumers (Fonberg-Broczek *et al*., 1999).

High pressure is an alternative to thermal processing. The resistance of microorganisms to

*Paper was presented at the International Review Conference on Biotechnology, Vienna, Austria, November 2004.

Abbrevations: CAC, Codex Alimentarius Commission; HPP, high pressure processing; ISO, International Standards Organisation; NCTC, The National Collection of Type Cultures, (located in the Centre for Infections, London, U.K.); PIW, Państwowy Instytut Weterynaryjny (National Veterinary Institute); PIS, Państwowa Inspekcja Sanitarna (State Sanitary Inspection); TSA, Tripticase soy agar; TSB, Tripticase soy broth; UHP, ultra-high pressure.

pressure varies considerably depending on the pressure range applied, temperature and treatment duration, and type of microorganism (Fonberg-Broczek *et al.*, 1999). As a result of technical progress and government support, the first high pressure processed food products appeared in Japan in the early 1990s. In Europe, high pressure processing (HPP) of foods was rather at the stage of research or pilot production in the last decade. EU legislation included HPP foods in the "novel food" category. EC Novel Food regulation (EC 258/97) has introduced a statutory pre-market approval system for novel foods across the whole of the European Union.

Recently, rapid progress of HPP toward commercial exploitation has been achieved, but still the process requires close collaboration between researchers, food and equipment manufacturers, as well as proper financial support.

In Poland, research on the application of high pressure for food preservation was initiated in 1992 by the High Pressure Research Center (now Institute of High Pressure Physics) of the Polish Academy of Sciences in collaboration with others Institutes. The research programmes, sponsored by the State Committee for Scientific Research and by EU funds, were devoted to high pressure processing of fruit products, fruit and vegetable juices, milk and meat products.

This paper presents some of our results concerning the effect of high pressure on the survival of *Listeria monocytogenes, Aeromonas hydrophila* and *Enterococcus hirae* in artificially contaminated food products. This microorganisms could be a source of food-borne diseases.

The usefulness of high pressure treatment for inactivation of microorganisms and extention of shelf life of two types of cooked pork ham and raw smoked pork loin is also discussed.

MATERIALS AND METHODS

Test organisms. The *Listeria monocytogenes* NCTC 11994 strain was obtained from the Spanish Type Culture Collection.

A mixture of five strains of *Listeria monocytogenes,* the PIW 1488 strain of *Enterococcus hirae* and mixture of three strains of *Aeromonas hydrophila* (PIW N. 98, PIS N. 98, IS N. 95) were obtained from the Polish National Veterinary Institute.

Sample preparation. *L. monocytogenes* were cultured on TBS at 30°C for 24 h. A loopful of the culture was streaked on Petri dishes containing TSA and after incubation at 30°C for 24 h a single colony was taken from each dish and inoculated on TSA slants and incubated at 30°C for 24–48 h. This pure culture of *L. monocytogenes* was washed using 1.5 ml tryptone salt diluent yielding bacterial population of 10^{10}. The cultures were used to contaminate sterile apple (pH 3) juice to yield bacterial density of 10^7–10^{10}/ml. Eppendorf tubes containing 1.5 ml of pure culture or contaminated apple juice, were introduced into flexible tubes filled with water, then sealed and exposed to the hydrostatic pressure of 100, 200, 300 and 400 MPa. The number of microorganisms was determined by plate count before and after treatment by plating 0.1 ml of the samples in nutrient agar (TSA). All samples were replicated 3 times.

Cured, sliced, pasteurised pork ham was divided into 10-g portions. Individual portions, made of two adjoining slices of ham were packed in PA/PE bags. The samples were inoculated with a mixture of five strains of *L. monocytogenes* (incubated in BHI at 37°C for 24 h) obtained from the Polish National Veterinary Institute. After inoculation all the samples were vacuum packed and exposed to high pressure treatment of 100, 200, 300 and 400 MPa.

Samples of ripened hard cheese (Gouda type) were placed in commercially used polyamide-polyethylene bags, inoculated with a) *E. hirae* or b) *A. hydrophila* (inoculum about 10^7/g) and sealed under vacuum. The samples were exposed to high hydrostatic pressure of a) 300, 400 and 500 MPa or b) 100, 200 and 400 MPa for 5, 10 and 15 min.

The numbers of surviving *E. hirae* and Aerobic Plate Counts (APC) were determined using Slanetz and Bartley agar (BTL) and nutrient agar prepared according to Polish Standards (PN-93-86034-04). The plates were incubated at 37°C for 48 h (Slanetz & Bartley agar) or at 30°C for 72 h (nutrient agar).

The numbers of *A. hydrophila* and Aerobic Plate Counts (APC) were determined using Ryan agar (Oxoid) and nutrient agar prepared according to Polish Standards. The plates were incubated at 30°C for 72 h.

The experiments were repeated 4 times.

The high pressure treatment was performed in Institute of High Pressure Physics, where a special stand for food testing was built (Fonberg-Broczek *et al.*, 1999). Samples were exposed to 100, 200, 300, 400, 500 MPa for 5, 10, 15 and 30 min.

The numbers of microorganisms were determined using standard ISO methods.

Statistical calculation. The numbers of surviving microorganisms were determined. The bacterial counts were transformed into logarithms, and D-values (the time required at a given pressure to reduce a bacterial population by 90%, or by one order of magnitude) were calculated using linear regression method ($y = a + bx$), where y — number of bacteria (log scale), x — time of treatment, a — initial number of bacteria, b — linear regression coefficient, and $D = -1/b$ (Szczawiński *et al.*, 1998). The statistical package "Statgraphics" was used for calculations.

RESULTS AND DISCUSSION

Listeria monocytogenes is a psychrophilic microorganism which grows at low temperature and wide a range of pH and is a causative agent of food related-listeriosis. This microorganism, which is generally associated with dairy products, has been detected in a wide variety of foods, such as raw meat, cheese, different pasteurised products, and even in vegetable produce (Prestamo *et al.*, 1999; Szczawiński *et al.*, 1997). Despite the decreasing number of cases of listeriosis reported in recent years, *L. monocytogenes* is still considered a pathogen posing serious threat to public health.

Aeromonas hydrophila belongs to pathogenic bacteria capable of rapid growth at 5°C. Inadequate cooking or lack of thermal treatment, low level of salt and prolonged storage under refrigeration are main factors which favour growth of aeromonas in foods. In the last decade ready-to-eat food products have often been implicated in food-borne outbreaks (Szczawiński *et al.*, 2001). Therefore, *A. hydrophila* is considered as one of the "emerging pathogens". The aim of the study was to define the usefulness of high pressure treatment for inactivation of *A. hydrophila* in sliced, vacuum-packaged hard cheese.

The count of *Enterococcus* spp. in milk products is a significant indicator of their hygienic quality and may be used to identify poor manufacturing practices (Szczawiński *et al.*, 2003). Enterococci cause some problems in milk industry, because they are capable of growth at temperatures from 7°C to 45°C and belong to the most thermoresistant vegetative bacteria, which may survive usual milk pasteurisation temperatures. They are also salt-tolerant and may grow in the presence of 6.5% sodium chloride. In ripened cheeses they induce undesirable organoleptic changes as embittering and improper consistency. Excessive growth of Enterococci can be the reason for the high content of histamine and tyramine in ripened cheeses (Fonberg-Broczek & Sawilska-Rautenstrauch, 1995).

This paper describes the results of our studies involving HPP:

— inactivation of *L. monocytogenes*, *A. hydrophila*, *E. hirae* in artificially contaminated ham, cheese and fruit juices, all containing bacteria at above 10^6 microorganisms per gram,

— the influence of high pressure treatment on microbial quality and shelf life prolongation of pork ham and loin.

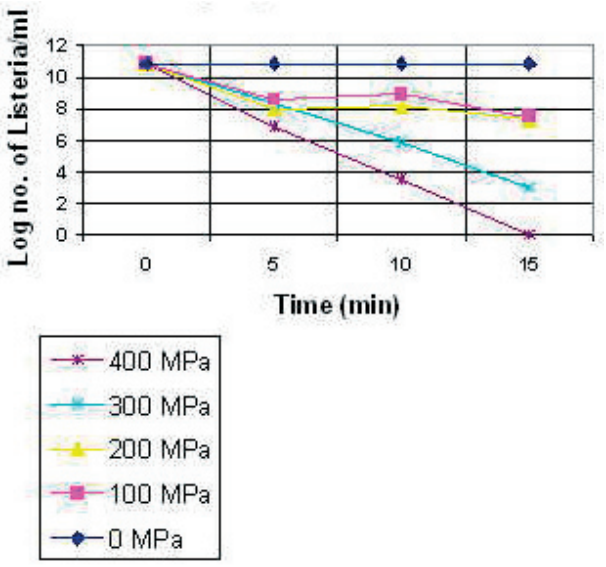

Figure 1. effect of pressure and treatment time on survival of *Listeria monocytogenes* (pure culture).

The obtained results indicate (Fig. 1 and Table 1) that in samples of pure culture of *L. monocytogenes*, as well as in hard cheese, meat products and fruit juice the number of this bacteria decreased proportionally to the increase in high pressure values and time of treatment and the effects of these two factors were statistically significant ($P \leq 0.001$).

Analysis of the data given in Table 1 (Szczawiński *et al.*, 1997; 2003) indicates that the Gram (+) *E. hirae* is much more resistant to ultra-high pressure treatment than the Gram (+) *L. monocytogenes* and the Gram (–) *A. hydrophila.* The results given in Table 1 allow one to calculate that for reduction of *E. hirae* in hard cheese by 6 log units application of 500 MPa for 96.18 min is needed (6D-value, the time required at a given pressure to reduce a bacterial population by 99.9999% or by 6 logarithmic cycles, which is usually considered as sufficient for consumer protection). In this case application of HPP

Table 1. D-values-time required for decimal reduction (minutes) of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Enterococcus hirae* at given pressure

UHP (MPa)	*L. monocytogenes* (ham)	*L. monocytogenes* (apple juice)	*A. hydrophila* (cheese)	*E. hirae* (cheese)
100	31.80	11.54	32.05	–
200	28.30	6.37	12.97	–
300	5.80	2.60	2.43	33.67
400	2.40	1.56	–	17.83
500	–	–	–	16.03

treatment at a temperature of 50–60°C could be useful (Kołakowski *et al*., 1994). *A. hydrophila* seems to be much more sensitive to HPP treatment than the other pathogenic bacteria tested in previous studies (Szczawiński *et al*., 2001).

Due to the growing popularity of the consumption of freshly squeezed fruit and vegetable juices (which may, however, be stored for up to several days), the preservation of these products through the use of high pressure processing seems very promising. This method conserves the sensory characteristics of a raw product (e.g., flavour, taste) whilst at the same time ensuring its micro-biological safety (Prestamo *et al*., 1999; Knorr, 1999; Cano *et al*., 1999).

The usefulness of high pressure treatment for inactivation of microorganisms and shelf-life extention of two types of cooked pork ham and raw smoked pork loin, produced according to two formulas that differed in the percentage of sodium nitrite (0.01% and 0.015%) and sodium chloride (1.5% and 2.5%), expressed as the percentage of the finished products, was studied (Grochalska *et al*., 2001; Karłowski *et al*., 2002).

The samples were exposed to the high hydrostatic pressure treatment of 500 MPa and 600 MPa. Microbiological, physicochemical and sensory investigations were performed after 24 h and 4, 6 and 8 weeks of storage at of 4–6°C. A significant decrease of microbiological parameters studied, such as total bacteria count, psychrophylic bacteria and enterococci was observed.

The results show that high pressure treatment extended the shelf-life of cooked pork ham and raw smoked pork loin up to 8 weeks. The products presented good microbiological and sensory quality (Pietrzak & Mroczek, 2003).

High pressure inactivation of pathogenic and spoliage microorganisms in meat requires further investigations, because of different response of microorganisms in different meat products. The effect of this technique depends on the pressure applied, time of exposure and temperature.

Acknowledgements

This publication and Conference participation was supported by EU Contract G1MA-CT-2002-04055, and by the Institute of High Pressure Physics, Polish Academy of Sciences — SPUB — PRENABIO

REFERENCES

CAC Codex Alimentarius Commission (2003) Report of the Thirty-Fifth Session of the Codex Committee on Food Hygiene, Alinorm 03/13A.

Cano PM, Hernandez A, De Ancos B (1999) Combined high-pressure/temperature treatments for quality improvement of fruit-derived products. In *Processing Foods. Quality Optimisation and Process Assessment* Oliveira FAR, Oliveira JC, eds, pp 301–313. CRC Press LLC.

Fonberg-Broczek M, Sawilska-Rautenstrauch D (1995) Zawartość histaminy i tyraminy w serach dojrzewających pobranych z obrotu. *Roczniki PZH* **46**: 244–246 (in Polish).

Fonberg-Broczek M, Arabas J, Kostrzewa E, Reps A, Szczawiński J, Szczepek J, Windyga B, Porowski S (1999) High pressure treatment of fruit, meat, and cheese products: equipment, methods and results. In *Processing Foods. Quality Optimisation and Process Assessment* Oliveira FAR, Oliveira JC, eds, pp 281–300. CRC Press LLC.

Grochalska D, Barabasz A, Windyga B, Ścieżyńska H, Mroczek J, Fonberg-Broczek M, Porowski S (2001) Wpływ metody UHP na cechy jakościowe i trwałość szynki wieprzowej. *Medycyna Wet* **57:** 924–928 (in Polish).

Karłowski K, Windyga B, Fonberg-Broczek M, Ścieżyńska H, Grochowska A, Górecka K, Mroczek J, Grochalska D, Barabasz A, Arabas J, Szczepek J, Porowski S (2002) Effects of high pressure treatment on the microbiological quality, texture and colour of vacuum packed pork meat products. *High Pressure Res* **22:** 725–732.

Kołakowski P, Reps A, Babuchowski A (1994) Przydatność wysokich ciśnień w utrwalaniu i przetwarzaniu żywności. *Przemysł Spożywczy* **48:** 108–111 (in Polish).

Knorr D (1999) Process assessment of high-pressure processing of foods: an overview. In *Processing Foods. Quality Optimisation and Process Assessment* Oliveira FAR, Oliveira JC, eds, pp 249–269. CRP Press LLC

Pietrzak D, Mroczek J (2003) Wpływ wysokich ciśnień na jakość i trwałość gotowanych wędzonek wieprzowych. *Postępy Techniki Przetwórstwa Spożywczego* **1:** 4–8 (in Polish).

Prestamo G, Sanz PD, Fonberg-Broczek M, Arroyo G (1999) High pressure response of fruit jams contaminated with *Listeria monocytogenes. Lett Appl Microbiol* **28:** 313–316.

Szczawiński J, Szczawińska M, Stańczak B, Fonberg-Broczek M, Arabas J, Szczepek J (1997) Effect of high pressure on survival of *Listeria monocytogenes* in ripened, sliced cheeses at ambient temperature. In *High Pressure Research in the Biosciences and Biotechnology.* Heremans K, ed, pp 295–298. Leuven University Press, Leuven, Belgium.

Szczawiński J, Pęconek J, Szczawińska M, Porowski S, Fonberg-Broczek M, Arabas J (1998) High pressure inactivation of *Listeria monocytogenes* in meat products. In: *Shelf life prediction for improved safety and quality of foods. Copernicus Project CIPA-CT94-0120.* Copi-Print Library Building University College, pp 181–186. Dublin.

Szczawiński J, Stańczak B, Pęconek J (2001) Possibility of inactivation of *Aeromonas hydrophila* in ripened, slices cheese by ultra high pressure. *Proceedings of lectures and posters*. "Hygiena Alimentorum " **22:** pp. 155–157.

Szczawiński J, Stańczak B, Pęconek J (2003) Survival of *Enterococcus hirae* in ripened cheese subjected to ultra high pressure. *Pol J Vet Sci* **6**: 267–269.