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The Influence of Moderate Pressure and Subzero Temperature on the Shelf Life of Minced Cod, Salmon, Pork and Beef Meat

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Summary

The effect of moderate pressure at subzero temperature on natural microflora of minced cod, salmon, pork and beef meat was studied. Pressure of 193 MPa at –20 °C caused the reduction of total bacterial count in pork and beef meat by 1.1 and 0.6 log cycles, respectively, and by about 1.5 log cycles in fish meat. Under these conditions the psychrophilic and psychrotrophic bacteria were below the detection limit (<10 CFU/g of sample) in pork and beef meat, while in cod and salmon meat they were reduced only by 1.3 and 2.0 log cycles, respectively. In all tested samples of meat treated with the pressure of 193 MPa at –20 °C, the number of coliforms was below 10 CFU/g. Under these conditions a significant reduction in the number of coagulase-positive *Staphylococcus* was also observed. During storage of samples at 4 °C after pressurization at 193 MPa and –20 °C, the inhibition of growth of all tested groups of bacteria was observed. Moderate pressure at subzero temperature does not ensure complete inactivation of bacteria; however, it allows the improvement of microbiological quality and extension of shelf life of food, which depends on the level of bacterial contamination of the initial raw material.

Key words: moderate pressure, subzero temperature, inactivation of microorganisms, microflora of minced pork, beef and fish meat

Introduction

High hydrostatic pressure is one of the most interesting methods for extending the shelf life and improvement of food safety. The doses of pressure that can be used depend strongly on the kind of preserved food. For preservation of some food products, *e.g.* fruit or vegetable juices, high doses of pressure can be used without changes in their sensory quality. However, in the case of meat preservation, it should be taken into account that high pressure treatment induces changes in the components and properties of meat. Some of these changes lead to the improvement of sensory quality of raw material and meat products, while others lead to their deterioration, as was reviewed by Cheftel and Culioli (*1*). Pressure treatment can induce changes in protein structure and texture (*2–5*). Pressurization of meat can also lead to changes in colour and lipid oxidation (*6*). In order to limit these undesirable reactions in meat, moderate pressure should be applied. Such pressure can also be beneficial for improvement of some functional properties of meat such as gelling strength of myofibrillar proteins, which is important for producing different restructured products based on surimi and fish mince products. Economical reasons also support the use of moderate pressure. It is obvious that this level of pressure is unable to achieve a substantial inactivation of microorganisms, however, it should allow the extension of shelf life of meat and meat products (*7–12*). One of the possibilities for achieving the increase in the inactivation of bacteria

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might be the use of moderate pressure in combination with subzero temperature. As has been shown in the model system, under these conditions bacterial cells are more sensitive to pressure than at ambient and low above zero temperatures (*13*,*14*). There is not much data in the literature about the effect of pressure at temperatures below 0 °C on natural microflora of meat. Some literature data show that pressure-assisted freezing (PAF) and pressure-assisted thawing (PAT) are very effective in inactivation of microorganisms (*15*).

The aim of this study is to determine the viability of natural microflora and shelf life of minced cod, salmon, pork and beef meat treated with pressures of 60 and 193 MPa at subzero temperatures. Our previous data show that the pressurization at subzero temperature does not increase the hardness of raw or cooked meat of fish and slaughter animals in comparison with the untreated samples (*16*). Therefore, such influence of pressure on the texture of meat does not limit the application of this technique for food preservation.

Materials and Methods

Preparation of food samples

Raw meat of cod, salmon, pork and beef was purchased from a local market and stored at 4 °C before use. Samples of meat and skinned fish fillets were minced in a mechanical grinder model 986.86 Zelmer (Zelmer S.A., Rzeszów, Poland), vacuum packed in polyethylene bags and pressurized.

The pressure was generated in a natural way as proposed by Hayakawa *et al.* (*17*), without using an oil pressure pump. The method is based on the phenomenon of generating pressure in response to the increasing volume of forming ice I_h in a sealed vessel filled with water and kept at subzero temperatures. Moreover, according to Bridgman (*18*), high pressure reduces the freezing and melting points of water to a minimum of –22 °C at 207.5 MPa. Therefore, above this temperature, the sample placed in a sealed vessel is affected by the pressure in unfrozen state. The equipment used to generate pressure during the experiments was designed and constructed by Edward Dunajski at the Department of Food Chemistry, Technology and Biotechnology, Chemical Faculty, Gdansk University of Technology, Gdansk, Poland. The details of the procedure were previously described by Malinowska-Pańczyk *et al.* (*19*).

Microbiological analysis

Microbiological enumerations were performed directly after the pressure treatments and after appropriate time of storage at 4 °C. Samples of meat (10 g) were transferred to a stomacher bag and homogenized in a stomacher (Masticator Basic Panoramic, IUL Instruments, Barcelona, Spain) with 90 mL of 0.1 % peptone water (1:9, by mass per volume) for 1 min. Successive decimal dilutions were also prepared in 0.1 % peptone water. Appropriate serial dilutions were then plated onto plate count agar (PCA, Merck KGaA, Darmstadt, Germany), and incubated for 48 h at 30 °C (total bacterial count or TBC) or for 10 days at 4 °C (psychrophiles and psychrotrophs). The number of coliforms was estimated using violet red bile dextrose agar (VRBD Agar, Merck KGaA). The most probable number (MPN) of coagulase-positive *Staphylococcus* was determined according to EN ISO 6888- 3:2003 (*20*). The media were purchased from Merck KGaA.

The results in the tables are average values from three replications±standard deviation. The differences between treatments were evaluated statistically by analysis of variance (one-way procedure) using the program Statgraphics, StatPoint Technologies, Inc., Warrenton, VA, USA.

Results and Discussion

Effect of pressure on microflora of pork and beef meat

Total bacterial count (TBC) in pork and beef meat amounted to 4.3 log CFU/g (colony-forming units per gram). Pressure of 60 MPa at –5 °C did not change the number of bacteria present in meat samples. Increase of pressure to 193 MPa (at –20 °C) caused reduction of TBC in pork and beef meat by 1.1 and 0.6 log cycles, respectively (Tables 1 and 2). At ambient temperature, the pressure at or below 200 MPa only insignificantly affected the TBC of beef meat (*8*).

The data presented in Tables 1 and 2 show that psychrophilic and psychrotrophic bacteria of meat are very sensitive to pressure, much more than bacteria growing at 30 °C, which include in a large part mesophiles. The number of psychrophilic and psychrotrophic bacteria were below 10 CFU/g of pork and beef meat (with their initial population amounting to 4 log CFU/g) pressurized at 193 MPa and –20 °C. Similar level of inactivation of psychrophiles or psychrotrophs in beef and pork meat was obtained at 20–25 °C after pressure treatment with 300 MPa (*8*,*21*). According to Yuste *et al.* (*22*), higher pressure sensitivity of psychrophilic than mesophilic bacteria is caused by the loss of their ability to grow at refrigeration temperatures.

During storage of pork and beef meat at 4 °C after pressurization at 193 MPa and –20 °C a delay in the bacterial growth was observed (Tables 1 and 2). After 6 days of storage the TBC in pressurized beef and pork meat was 3.3 and 1.5 log cycles lower than in the controls (unpressurized and stored meat). The psychrophilic and psychrotrophic bacteria in pork mince were below 10 CFU/g of sample for up to two days of storage at 4 °C. After 6 days, their number increased to 2.9 log CFU/g, but in the control samples it was 4.7 log cycles higher (Table 1). Ananth *et al.* (*21*) showed that in pork meat pressurized at higher pressure (414 MPa, 13 min, 25 °C) the growth of psychrotrophic microflora was stopped for up to 7 days of storage at 4 °C. In the case of pressure-treated (at 193 MPa and –20 °C) minced beef, the number of psychrophilic and psychrotrophic bacteria after 6 days was by about 0.9 log cycles higher than in the initial samples but 3.3 log cycles lower than in the unpressurized sample stored under the same conditions (Table 2).

The number of coliforms in the initial pork and beef meat amounted to about 250 CFU/g. Treatment of meat with pressure of 60 MPa at –5 °C did not affect the number of these bacteria, however, they were below detection limit (<10 CFU/g) after pressure treatment at 193 MPa

Table 1. The influence of pressure and temperature on natural microflora of minced pork meat after 2 and 6 days of storage at 4 °C. Control sample was not pressurized

Minced pork meat	*t*(storage)/day		
	0	2	6
	log(total bacterial count)/g		
Control sample	(4.30±0.04)[a]	(5.80±0.01)[a]	(7.4±0.7)[a]
Treated at 60 MPa and –5 °C	(4.10±0.01)[a]	(5.60±0.04)[a]	(8.2±0.1)[a]
Treated at 193 MPa and –20 °C	(3.20±0.09)[b]	(4.1±0.0)[b]	(5.90±0.01)[b]
	log(psychrophilic and psychrotrophic bacterial count)/g		
Control sample	(4.00±0.04)[a]	(5.80±0.06)[a]	(7.60±0.07)[a]
Treated at 60 MPa and –5 °C	(3.8±0.5)[a]	(5.60±0.04)[a]	(7.60±0.05)[a]
Treated at 193 MPa and –20 °C	n.d.	n.d.	(2.90±0.01)[b]
	log(total coliform bacterial count)/g		
Control sample	(2.50±0.05)[a]	(4.7±0.1)[a]	(6.90±0.05)[a]
Treated at 60 MPa and –5 °C	(2.6±0.2)[a]	(4.70±0.01)[a]	(6.7±0.1)[a]
Treated at 193 MPa and –20 °C	n.d.	n.d.	(2.60±0.02)[b]

n.d.=<10 CFU/g of sample
[a–b]for each bacterial count the values in a particular column followed by different letters differ significantly ($p<0.05$)

Table 2. The influence of pressure and temperature on natural microflora of minced beef meat after 2 and 6 days of storage at 4 °C. Control sample was not pressurized

Minced beef meat	*t*(storage)/day		
	0	2	6
	log(total bacterial count)/g		
Control sample	(4.3±0.1)[a]	(5.50±0.07)[a]	(8.2±0.1)[a]
Treated at 60 MPa and –5 °C	(4.20±0.02)[a]	(5.50±0.04)[a]	(8.30±0.02)[a]
Treated at 193 MPa and –20 °C	(3.70±0.01)[b]	(3.40±0.03)[b]	(4.90±0.08)[b]
	log(psychrophilic and psychrotrophic bacterial count)/g		
Control sample	(4.00±0.05)[a]	(5.3±0.3)[a]	(8.2±0.2)[a]
Treated at 60 MPa and –5 °C	(3.8±0.2)[a]	(5.6±0.1)[a]	(8.1±0.1)[a]
Treated at 193 MPa and –20 °C	n.d.	(2.60±0.02)[b]	(4.90±0.06)[b]
	log(total coliform bacterial count)/g		
Control sample	(2.4±0.2)[a]	(5.10±0.08)[a]	(7.60±0.04)[a]
Treated at 60 MPa and –5 °C	(2.30±0.03)[a]	(5.10±0.06)[a]	(7.6±0.1)[a]
Treated at 193 MPa and –20 °C	n.d.	n.d.	(3.50±0.09)[b]

n.d.=<10 CFU/g of sample
[a–b]for each bacterial count the values in a particular column followed by different letters differ significantly ($p<0.05$)

and –20 °C. During storage of meat for two days at 4 °C, they were still unable to grow, but after 6 days their number amounted to 2.6 and 3.5 log CFU/g (Tables 1 and 2). Similarly, Carlez *et al.* (*8*) also showed that this group of bacteria was not able to grow immediately after pressurization of minced beef meat at 450 MPa and 20 °C. Then, after 2 days of storage they started growing and on the ninth day their number amounted to about 2 log CFU/g (*8*). Under high pressure conditions bacterial cells can undergo sublethal injury and cannot be detected on selective media such as violet red bile lactose (VRBL) and VRBD agar that were used in our or work of Carlez *et al.* (*8*) to enumerate coliforms. Such phenomenon was also observed with temperature- or pressure-stressed *Escherichia coli* strains, which lost ability to grow on selective medium with bile salts (*23,24*). During storage, cells can repair their damaged metabolism and then they are able to grow. The time required to repair the injury depends on the sensitivity of the strain and the dose of applied pressure (*23*).

Effect of pressure on microflora of fish meat

The TBC in minced cod and salmon meat was relatively high; it amounted to 6.3 and 5.0 log CFU/g, respectively. Similarly as in pork and beef meat, the natural microflora of fish meat was not sensitive to the pressure

of 60 MPa at –5 °C. After treatment at 193 MPa and –20 °C, the TBC in minced fish meat decreased by about 1.5 log cycles. High reduction of total microflora in cod and salmon meat was reported by Schubring *et al.* (*15*) when pressure of 200 MPa was used in the PAT process. Bacteria were not detected in such thawed meat, whereas the one that was thawed using conventional method contained above 10^3–10^4 CFU/g. The high level of bacterial inactivation when using PAT can result from sublethal damage of bacterial cells caused by the freezing process. It is known that this kind of damage caused by different factors makes cells sensitive to pressure treatment. As a rule, the inactivation of bacteria in pressure-treated fish in the temperature range of 5–25 °C is lower than that obtained in our work. For example, the number of bacteria decreased by about 1 log cycle in salmon meat pressurized at 200 MPa and 5 °C (*25*). In the case of minced albacore tuna meat, the number of bacteria did not change even after pressurization at 310 MPa at 10 °C (*26*). Higher reduction of TBC, by 2 and 3.6 log cycles, in pressurized Atlantic salmon meat at 150 and 300 MPa, respectively, at room temperature was reported by Yagiz *et al.* (*12*).

As shown in Tables 3 and 4, psychrophilic and psychrotrophic bacteria that contaminated fish meat were more resistant to pressure treatment at 193 MPa and –20 °C

Table 3. The influence of pressure and temperature on natural microflora of minced cod meat after 2 and 6 days of storage at 4 °C. Control sample was not pressurized

Minced cod meat	*t*(storage)/day		
	0	2	6
	log(total bacterial count)/g		
Control sample	(6.30±0.03)[a]	(7.3±0.1)[a]	(9.50±0.07)[a]
Treated at 60 MPa and –5 °C	(6.30±0.08)[a]	(7.6±0.2)[a]	(9.50±0.05)[a]
Treated at 193 MPa and –20 °C	(5.00±0.01)[b]	(5.9±0.1)[b]	(8.5±0.1)[b]
	log(psychrophilic and psychrotrophic bacterial count)/g		
Control sample	(6.30±0.01)[a]	(8.10±0.03)[a]	(9.80±0.01)[a]
Treated at 60 MPa and –5 °C	(6.6±0.5)[a]	(8.20±0.01)[a]	(9.2±0.6)[a,b]
Treated at 193 MPa and –20 °C	(4.9±0.1)[b]	(5.80±0.01)[b]	(8.70±0.02)[b]
	log(total coliform bacterial count)/g		
Control sample	(3.60±0.05)[a]	(3.90±0.01)[a]	(8.00±0.04)[a]
Treated at 60 MPa and –5 °C	(3.70±0.08)[a]	(4.00±0.02)[a]	(5.80±0.6)[b]
Treated at 193 MPa and –20 °C	n.d.	n.d.	n.d.

n.d.=<10 CFU/g of sample
[a–b]for each bacterial count the values in a particular column followed by different letters differ significantly ($p<0.05$)

Table 4. The influence of pressure and temperature on natural microflora of minced salmon meat after 2 and 6 days of storage at 4 °C. Control sample was not pressurized

Minced salmon meat	*t*(storage)/day		
	0	2	6
	log(total bacterial count)/g		
Control sample	(5.0±0.2)[a]	(6.40±0.01)[a]	(7.50±0.07)[a]
Treated at 60 MPa and –5 °C	(4.80±0.02)[a]	(6.50±0.02)[a]	(7.40±0.08)[a]
Treated at 193 MPa and –20 °C	(3.4±0.1)[b]	(4.50±0.01)[b]	(6.7±0.1)[b]
	log(psychrophilic and psychrotrophic bacterial count)/g		
Control sample	(4.50±0.07)[a]	(5.90±0.04)[a]	(7.4±0.1)[a]
Treated at 60 MPa and –5 °C	(4.0±0.6)[a]	(5.90±0.04)[a]	(7.9±0.3)[a]
Treated at 193 MPa and –20 °C	(2.50±0.05)[b]	(3.70±0.07)[b]	(6.00±0.08)[b]
	log(total coliform bacterial count)/g		
Control sample	(3.3±0.4)[a]	(3.6±0.1)[a]	(6.90±0.05)[a]
Treated at 60 MPa and –5 °C	(3.0±0.1)[a]	(4.0±0.2)[a]	(6.20±0.08)[b]
Treated at 193 MPa and –20 °C	n.d.	n.d.	n.d.

n.d.=<10 CFU/g of sample
[a–b]for each bacterial count the values in a particular column followed by different letters differ significantly ($p<0.05$)

than those in the meat of slaughter animals. Pressurization caused reduction of their number by 1.3 and 2.0 log cycles in cod and salmon meat, respectively, and in pork and beef meat by 4.0 log cycles. These data were statistically significant ($p>0.05$). The differences in the resistance of psychrophilic and psychrotrophic bacteria in the meat of fish and slaughter animals may be a result of variation in the quantity and quality of microflora and their adaptation to low temperature and elevated pressure.

During storage of fish meat at refrigeration conditions, the TBC and number of psychrophilic and psychrotrophic bacteria increased, but after 2 days their numbers were still lower in pressurized samples than in control samples at day 0 of storage. After 6 days, TBC and psychrofiles and psychrotrophes in unpressurized salmon meat amounted to about 7.5 CFU/g, while their number was lower in meat pressurized at 193 MPa and –20 °C by 0.8 (mesophiles) and 1.4 (psychrophiles and psychrotrophes) log cycles. In unpressurized cod meat, the number of both groups of bacteria exceeded 10^9 CFU/g after 6 days of storage and was higher than in salmon meat, which resulted from higher microbial contamination of cod than salmon meat. After that time, in pressurized cod meat the number of mesophilic and psychrophilic and psychrotrophic bacteria was by 1 log cycle lower than in stored unpressurized sample.

The results presented in Tables 3 and 4 show that pressurization at 193 MPa and –20 °C allows the extension of the shelf life of fish meat for at least two days. Extension of shelf life for two days was obtained with vacuum packed salmon after high pressure treatment of 150 MPa for 10 min at 5 °C (*25*) and for 7 days in sea bass fillets treated at 500 MPa (*9*).

Similarly to the case of pork and beef meat, pressure treatment at 193 MPa and –20 °C of cod and salmon meat inactivated coliforms to the level below 10 CFU/g of samples (Tables 3 and 4). Moreover, they were unable to grow during 6 days of storage at 4 °C.

Effect of pressure on coagulase-positive Staphylococcus

S. aureus bacteria are considered as the most pressure resistant among vegetative form of bacteria (*17,27*). In most research pressure sensitivity of *S. aureus* was determined with a pure culture suspended in buffers or added to food matrix. Little attention has been paid to indigenous coagulase-positive species.

It was shown that samples coming from different kinds of meat as well as from different batches of the same meat differ in levels of coagulase-positive *Staphylococcus.* For example, the MPN of these microorganisms per 1 g of pork meat from batches I, II and III amounted to 24, 4.3 and 1.5, respectively (Table 5). The highest MPN of coagulase-positive *Staphylococcus* of 46 per g was determined in cod meat from batch I, while in batches II and III it was much lower, below 1. On the other hand, all three batches of salmon meat were characterized by similar and low MPN. These differences in MPN of coagulase-positive *Staphylococcus* were most likely caused by various hygienic conditions maintained after catching the fish.

Table 5. The most probable number of coagulase-positive *Staphylococcus* in 1 g of minced pork, beef, cod and salmon meat

Sample	I	II	III
Pork meat			
control sample	24	4.3	1.5
treated sample	2.3	1.5	0.36
Beef meat			
control sample	15	24	4.3
treated sample	0.72	2.3	0.92
Cod meat			
control sample	46	0.94	<0.30
treated sample	3.5	<0.30	<0.30
Salmon meat			
control sample	0.36	0.36	<0.30
treated sample	<0.30	<0.30	<0.30

Treated samples: meat pressurized at 193 MPa and –20 °C
Control sample was not pressurized

The pressure of 193 MPa at –20 °C caused a decrease of the number of coagulase-positive *Staphylococcus* (Table 5). In the most contaminated samples of meat with coagulase-positive *Staphylococcus*, the MPN decreased from 24 to 2.3 (pork from batch I and beef from batch II) and from 46 to 3.5 (cod from batch I). In the remaining samples, the MPN after pressurization was below 1. These results show that the pressure of 193 MPa at –20 °C reduced the number of coagulase-positive *Staphylococcus* below the level of 10^2 per g, which is acceptable according to Council Directive 94/65/EC. The decrease in the number of coagulase-positive *Staphylococcus* in meat is unexpectedly relatively high, although Carlez *et al.* (*8*) have also shown that Baird-Parker agar flora was significantly inactivated after pressurizing at 400 MPa and 20 °C. In the model system in phosphate-buffered saline, the number of *S. aureus* strains (PCM2054, PCM2101 and ATCC 29213) decreased after pressurization at 193 MPa and –20 °C only by 0.5 log cycle (*28*). It is known that microorganisms are usually more resistant to pressure treatment in food systems than in the buffer (*1,27*). This result is a consequence of the protective effect that food components exert on microorganism cells (*29*). Differences in the inactivation level of indigenous *Staphylococcus* and cells in pure culture may be caused by three factors. Firstly, differences in pressure sensitivity of microorganisms can occur among strains belonging to the same species (*27,30–32*). Alpas *et al.* (*30*) showed that among 7 strains of *S. aureus* species, one underwent inactivation by about 7 log cycles after pressure treatment at 345 MPa and 20 °C, whereas number of cells in populations of other strains did not change under these conditions. Secondly, other species besides *S. aureus* also belong to coagulase-positive staphylococci, such as *Staphylococcus intermedius, Staphylococcus hyicus,* or *Staphylococcus schleiferi.* There is no information in the available literature regarding pressure sensitivity of these bacteria in a buffer or in a food system. Thirdly, the inactivation by pressure is higher when the number of cells is low. García-Graells *et al.* (*33*) revealed that inactivation by

high pressure proceeds in a cell-density-dependent manner. *Escherichia coli* was more sensitive to high pressure and antimicrobial compounds at lower (10^6 CFU/mL) than at higher (10^9 CFU/mL) cell density.

Conclusions

Moderate pressure at subzero temperature does not ensure complete inactivation of bacteria; however, it allows the improvement of microbiological quality and extension of shelf life of food which depends on the level of bacterial contamination of initial raw material. A positive aspect of this technique is a considerable inactivation and inhibition of the growth of psychrophilic microflora that mainly participates in the spoilage of food during refrigerated storage. Moreover, under these conditions, significant inactivation of coliforms and relatively high degree of inactivation of coagulase-positive *Staphylococcus*, natural contaminants of raw meat, occurs. The effect of high pressure on individual species and strains of this natural microflora is worth checking in the future research.

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