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### Chapter

## High Hydrostatic Pressure Treatment of Meat Products

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#### **Abstract**

High hydrostatic pressure (HHP) treatment has been described to improve the microbiological safety and shelf life of ready-to-eat (RTE) meat products, as a nonthermal decontamination technology in the meat industry, applied at pre- or post-packaging. The pathogen widely studied in this product is *Listeria* monocytogenes that reflects the concern of the food industry. In general, microorganism's lethality during HHP treatment depends on specific intrinsic factors of the microorganism; those factors are related to food and technological factors of treatment. In addition to processing parameters, intrinsic factors of the food matrix also exert an effect on bacteria inactivation during pressure treatment. It is known that low water activity (a<sub>w</sub>) protects microorganisms against the effects of pressure. Predictive modelling is an important tool of the novel microbial food safety management strategy that provides with accurate information to demonstrate and guarantee the safety and shelf life of the food products. The chapter describes the effect of parameters on the efficiency of this technology on meat products over pathogens, composition and the sensorial quality consequences. The predictive modelling tool is introduced for the optimisation of meat treatment.

**Keywords:** high hydrostatic pressure, high pressure processing, microbial inactivation, extension shelf life, predictive microbiology

#### 1. Introduction

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Today the food security situation is continually under review and questioned as a result of several food-borne outbreaks that occurred. The company, mainly responsible for the safety of its products, strives to achieve techniques and procedures that allow it to ensure all risks and at the same time extend commercial shelf life and all this without altering the sensory characteristics of optimum quality. Consumers demand insistently fresher, healthier, safer and more convenient food, with good tasting and without preservatives.

In the case of ready-to-eat products (RTE), the need is even more pressing since it is a product for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level of microorganisms.

In this sense, European legislation (Regulation EC 2073/2005, [1]) establishes clear limits of various pathogens, such as regulating the presence of *L. monocytogenes* in ready-to-eat products, *Escherichia coli* in fruits, vegetables and live bivalve molluscs or *Salmonella* in ready-to-eat foods containing raw egg.

The occurrence of food-borne outbreaks in Europe has a decreasing tendency. A total of 5079 food-borne (including waterborne) outbreaks were reported by the European Food Safety Authority (EFSA) [2]. This report describes *Salmonella* as the commonest detected agent, and the highest-risk agent/food pairs the *Salmonella* in eggs and meat and meat products, and the analysis of strong-evidence food-borne outbreaks is associated with animal origin food [2]. In the case of meat products with longer shelf life, bacteria have more time to grow if they have the conditions (such as cooked sausages, cooked sliced ham and fermented salami) [3].

All the facts mentioned above has made companies look for alternative techniques that guarantee the safety of their products, as is the case of HHP. HHP has become a reality in the food industry and has spread worldwide [4]. This technique achieves a microbial inactivation without using high temperatures, so they manage to keep the sensory characteristics of the product almost intact, providing a larger commercial shelf life [5–7]. This preservation technique consists of the application of isostatic pressures, transmitted to foods uniformly and instantaneously by airdriven pumps through a liquid, generally water [8].

One of the main advantages of high pressure processing (HPP) is that it reaches acceptable microbial inactivation in meat products, but the sensorial and nutritional characteristics remain with good quality [9, 10].

In the meat industry, the application of HPP has focused on products ready for consumption with the additional aim of extending commercial life. For example, several studies have described the behaviour of *L. monocytogenes* in ready-to-eat meat products treated by HHP at different points of processes: prepackaging (liquid food) [11] and post-packaging (all types of food) [5, 12–19], the latter application being the most used [20]. In general, pathogen lethality during HHP treatment depends on various processing parameters such as the pressure level and holding time, temperature and food matrix. The optimisation of these parameters of the treatment for the pathogens' inactivation has been reinforced by the use of predictive microbiology tool that has been applied on different meat products [5, 15, 21–26].

Different organisations and administrations have recognised the listericidal effect of HHP treatments on RTE foods [27–29]. The objectives of this study are the revision of the effect of all parameters on the efficiency of this technology on meat products over pathogens and the sensorial quality consequences. Also, the predictive modelling tool for the optimisation of the meat treatment will be introduced.

#### 2. High hydrostatic pressure treatment of meat

HHP was very well accepted since its beginning as an alternative to thermal inactivation treatments and as an in-package cold pasteurisation process [4]. In the last three decades, the number of companies with HHP facilities has increased considerably in the world, from just a few in 1990 to more than 200 units and with an increasing capacity [30].

The inactivation of bacteria effect of high pressure was demonstrated 100 years ago, although the industrial technology was built up at the end of the twentieth century [20]. The system consists in exerting high and uniform pressure on the food, for enough time to achieve the desired effect. This is called adiabatic heat and occurs instantaneously with pressure increase and as the pressure is uniform over the product [20]. HHP is probably the most developed nonthermal technology commercially in the world market, mainly applied for sliced meat products, fruit jellies and jams, fruit juices, dressings for salad, raw oysters, ham and guacamole, among others.

The packaged food is usually submerged in water inside a tank, and through this, high pressure is caused. HHP treatment will inactivate bacteria, yeast, moulds and

enzymes equivalent to thermal pasteurisation processed but preserving the taste, colour and nutritious value of the product [4, 6, 23, 30, 31]. The treatment can be prepackaging (liquid food) or post-packaging (all types of food), although the latter is most frequently used [20]. The meat industry tries to apply the shortest HHP treatment on production lines as they can, currently from 3 to 6 min maximum [9, 32]; although many potential HPP applications would require long treatment times to ensure an adequate inactivation level of pathogens and spoilage microorganisms, pressure treatments alone would not be sufficient to guarantee food safety [33].

The application of HHP technology follows two basic principles: Le Chatelier principle and isostatic rule (Pascal principle). The first principle postulates that pressure accelerates reactions (phase change, changes in the molecular configuration, chemical reactions) that involve volume reductions and vice versa and inhibits reactions that occur with increases in volume. Since the medium used to transmit the pressure is usually water (incompressible fluid), the isostatic rule principle is verified in the HHP application, stating that "the increase in pressure applied to the surface of an incompressible fluid, contained in an undeformable container, is transmitted with the same value to each of its parts". The applied pressure is transmitted in an isostatic (uniform) and almost instantaneous way to all points of the food, regardless of its composition, size and shape. This prevents deformation of the product, despite being subjected to such high pressures, and makes it very homogeneous and does not have over-treated areas. When food is treated in its packaging, it must be flexible and deformable (it must tolerate volume reductions of up to 15%). The evacuation of gases from the interior is especially necessary to prevent their compression from reducing the pressurisation efficiency [34, 35].

The pressure range for a commercial purpose is usually from 100 to 600 Mpa [30], but this only can approach to a pasteurisation process but not commercial sterilisation where spores should be destroyed and more than 1000 Mpa should be applied for the sterilisation [30]. The new commercial unit implemented has increased in capacity and pressure, reducing the time to a few minutes, which helps manufacturers to reduce costs. The consumer has demonstrated a high level of acceptance of products treated with HHP because of the minimal changes in sensory and safety characteristics they perceive.

Two types of HHP treatment can be distinguished: the classical or also named single-pulsed HHP or the multi-pulsed high hydrostatic pressure (mpHHP). Difference between both is the number of compressions done. In the single HPP treatment, a compression hold for a certain time is followed by decompression to atmospheric pressure, while in the mpHHP more than one compression is applied with its respective decompression phase. It was reported that the mpHHP treatment, with few exceptions, is more effective than the classical or single-pulsed HHP treatment for inactivation of microorganisms in fruit juice, dairy products, liquid whole egg, meat products and seafood [4]. The reports of applying mpHHP on meat products describe better inactivation rates of *E. coli* O157:H7 and *Salmonella* Enteritidis in ground beef and chicken fillets, respectively, than the classic HHP [4, 36, 37]. Moreover, the mpHHP treatment could also be used to inactivate enzymes in foods and to increase the shelf life of foods [4].

The high pressure applied causes a temperature increase in the treated product, around 3°C per 100 MPa applied for water and 8–9°C for fat and oils and intermediated values for proteins and carbohydrates have been described [30, 38].

#### 2.1 Effect of HHP on food components

The effectiveness of HHP on meat products constituents depends on different factors as initial microbial, pH and ionic strength [39].

The pressure affects properties of water contained in food such as density, viscosity, dipole moment, dielectric constant, and surface tension and thermal properties such as freezing and melting point and consequently will exert its effect on enzymes, chemical reactions and microorganism [40, 41]. For example, high pressures reduce the freezing point of water to  $-22^{\circ}$ C at a pressure of 207.5 MPa because it prevents the increase in ice volume [40].

Whether the fat is affected or not by the treatment is very important because it has a significant impact on the sensory characteristics and it will depend on the intensity of treatment. It has been described that 450 MPa is applied during 154 s in dry fermented sausage; the total fatty acids and the stability of the fatty ones were not affected [42].

The denaturation that the proteins of the food undergo by the treatment of high pressures will depend on the level of pressure exerted, the pH and the temperature. Irreversible changes that have been described include the dissociation of oligomeric proteins into their subunit, aggregation or gelation of protein or changes in the conformation of the active site of enzymes. Reversible changes are observed when the pressures are between 100 and 300 MPa [30]. Proteins and sugars have been described as protective agents for bacteria in these treatments [5, 43–45].

#### 2.2 Effect of HHP on the sensory quality of food

The effect of HHP on the sensory quality of food depends on the conditions, pressures and time, but physical properties of the food play an important role in its sensory quality. The colour of meat is critical because it is the main criterion that consumers will evaluate before making purchases.

The significant change of the texture and visual appearance, colour in the raw meat, depends on the intensities of pressure, observing significant changes at HPP at 600 MPa, but not at lower as 175 MPa [46, 47]. Nevertheless, on cases of cured meat products, changes on colour mainly depended on water content and water activity [48].

In case of salted chicken meat, it has been described that, in general, the use of HHP treatment improved the texture of cooked meat and colour of raw meat, and it is proposed as a processing alternative to reduce NaCl content [49]. Siddig et al. [50] in other study concluded that the colour of chicken was slightly affected by treatment, but pH, moisture content and the oxidation of lipids were not substantially changed.

Pressure treatment of meat can promote oxidation reactions, and it is crucial to control the balance between pro- and anti-oxidants to prevent this phenomenon because it will affect the colour. Lipid oxidation has been extensively investigated in meat because it can react with proteins, leading to organoleptic modifications and the loss of nutritional value. In the case of meat, the oxidation is one of the most important mechanisms of the degradation of meat, which can be initiated endogenously via metallic ions, especially hemic iron, or via exogenous reactive oxygen species. This process will result in changes in the organoleptic properties of the meat, as degradations in colour, aroma and flavour. These effects will be related to the type of meat, the treatment used and the methods used to evaluate the reactions with the oxidation of lipids and proteins. The pressure above 400 MPa seems to be critical for the initiation of lipid oxidation [7].

#### 2.3 Effect of HHP on microorganisms

The effect of inactivation of HP on microorganisms in foods will depend on specific intrinsic factors of the microorganism, those related to food and technological factors of treatment [40, 51].

Among the intrinsic factors of microorganisms that will affect inactivation would be the number, species, strain and their physiological state [40, 51, 52]. Even the size of the microorganism has been described as influential [53]. In the different phases of physiological state, the cell and the membrane vary, and it has been observed that in the logarithmic phase of growth, it is more sensitive to the treatment of HHP and, in the stationary phases, it is more resistant [53].

The spores are even more resistant, and heat needs to be applied at the same time to inactivate them [34, 54]. For example, the spores of yeast and moulds had been reported to be inactivated by pressures of 600 MPa [9] although some species have been described as more resistant, as the ascospores of *Byssochlamys nivea* [40].

The factors related to food that affects the efficiency of treatment would influence variables such as pH, a<sub>w</sub>, salt concentration and the general composition of the food [40, 51, 55].

The treatment gains effectiveness by lowering the pH of the food [52] or adding antimicrobials [34]. In a study by Alfaia et al. [42] carried out in chorizo, it describes a significant increase in pH by increasing the intensity of the treatment, which was also found in other products such as raw sausage batter, fresh chicken breast fillets and raw poultry sausages [26, 56]. At high pressure, there is increased ionisation and redistribution of ions that can be the origin of the pH increase and also the release of imidazolium groups by histidine [57]. Alfaia et al. [42] verified that the HPP resulted in a significant increase (p < 0.001) of the pH of chorizo compared to the control samples and in a significant decrease of the  $a_{\rm w}$  (p < 0.01). The increase in pH was also reported on raw sausage batter, fresh chicken breast fillets and raw poultry sausages.

It has been observed that the decrease of  $a_w$  decreases the effectiveness of lethality of bacteria [4, 19], probably related to the stabilisation of protein, especially enzymes, which suffers less pressure [58]. It has been demonstrated that lyophilised L. monocytogenes treated with HPP was not inactivated [59]. On the other hand, it is also described that low  $a_w$  will inhibit the recovery of cells and potential growth during storage of the product treated by HPP [15, 60], that is to say that two antagonistic effects that could compensate each other.

Synergistic effects of HHP treatment with the addition of sodium lactate on the inactivation of *L. monocytogenes* in cooked chicken have been described [11].

Also, the fat content has been described as a parameter that affects the effectiveness of microorganism inactivation, having in general a protective effect of bacteria [5, 15, 19, 25]. High fat concentration decreases the inactivation of bacteria [15, 25], but it is also related to the pressure exerted; the higher the pressure of 650 MPa, the more is the protection [5, 18].

HPP and the addition of essential oils have similar effects on microbial structures, and thus they may act synergistically on the inactivation of microorganisms. Therefore, the combination of HPP with EOs is a promising alternative to expand the HPP food industry [61, 62].

The concentration of other components has been described affecting inactivation of bacteria as vitamins and amino acids [43], proteins [63], sucrose [64] and minerals such as calcium or magnesium [65].

Not only food component can affect the efficacy of HPP but also the food structure. Several authors have described it as an essential factor of variability on the resistance of microorganisms by comparing inactivation on food matrix and culture media where the food displays a protective effect against HHP [10, 66, 67].

Among the technological or process factors, the pressure exerted, the treatment time, the depressurisation rate, the temperature and the come-up time (CUT) required to reach the desired pressure should be mentioned [40]. If the CUT is prolonged, it is as if a pretreatment is performed, and the temperature is

fundamental, it seems that values of 45–50°C increase the inactivation of pathogens and yeasts [54].

It has been described in various publications that this treatment of HHP, 20–180 Mpa, can produce populations with sublethal damage [30, 68–70]. It is very important to take into account if the treatment carried out in food can produce this type of population since it would produce an estimate of economic life and erroneous security by being able to survive and revive over time even if it is in low concentrations.

The inactivation of *L. monocytogenes* in different meat products has been studied by several authors [60, 71], which reported that pressure treatments of up to 300 MPa are insufficient to inactivate it.

In fermented products such as chorizo, it has been described that the application of HHP can contribute to lowering the altering microbiota, without adverse effects on fermentative bacteria with a treatment of 400 MPa/154 s [42].

The mechanism of action of HHP on microorganisms has been described by various authors that causes damage to the cell membrane [30, 51, 72] and induces morphological changes in the microorganism [73].

The cell membrane is damaged and therefore causes irreversible damage and cell death. It produces crystallisation of the acyl chains of the phospholipid bilayer that leads to bud formation, intracellular material leakage and membrane rupture [30].

Proteins at pressures greater than 100 MPa hydrophobic interactions tend to increase in volume and will cause protein denaturation. In the case of enzymes, it generates conformation changes and, therefore, cell damage and death [34, 74].

There is also inactivation of enzymes related to DNA replication and transcription [34, 74].

### 2.4 Predictive modelling applied to meat products treated by HPP

Although the effectiveness of HHP application has been recognised by various authors to reduce the levels of various pathogens to acceptable levels in several foods, it is important to take into account the fact that the treatment can be sublethal and only cause lesions in subpopulations of microbial cells. These cells can recover from this type of lesions and grow during the period of storage of the product or before its consumption, reaching levels above the levels allowed by current legislation. Based on this, many authors evaluated and modelled the behaviour of *L. monocytogenes* during and after the treatment of APH in meat products, that is, throughout their useful life [15, 25, 75–77]. These models are essential tools for decision-making in the industry in terms of meeting microbiological criteria. In addition to the predictive models described, there are models in the literature that describe the probability of inactivation/recovery, or also called survival/death (logistic) interface models, of *L. monocytogenes* in meat products or culture media.

Predictive models of inactivation developed in culture media, once validated in specific food matrices such as chorizo, can be applied in the meat industry. Examples of these models would be those developed for *L. monocytogenes* and *L. innocua* (as a surrogated for safety purpose) in meat products [5, 19, 22, 24–26].

In **Table 1**, several types of predictive models that consider treatment inactivation and/or growth on storage phase of meat product can be observed.

Bover-Cid et al. [22] developed and validated a polynomial model of the inactivation of *L. monocytogenes* induced by HPP on dry-cured ham (Eq. (1)), as a function of the technological parameters: pressure intensities (347–852 MPa), pressure holding time (2.3–15.75 min) and fluid temperature (7.6–24.4°C). Pressure and time were the most critical factors influencing microbial inactivation, and the little effect was observed applying pressures below 450 MPa. The increase in holding time for

Reference	Meat product	Equation
Inactivation model during treatment		
Bover-Cid et al. [5]	Cured ham	$log\left( \text{N/}_{N_0} \right) = 38.653 - 34.29 \cdot a_w - 0.0237 \cdot P - 0.00349 \cdot F^2 + 0.000334 \cdot P \cdot F$
Growth model after treatment		
Hereu et al. [14]	Cooked ham	$log\left(N\right) = \ log \ \frac{10^{9.09}}{1 + \left(\frac{10^{9.09}}{N_0} - 1\right) \cdot exp\left(-(0.023 \cdot (T + 1.80))^2 \cdot \left(t - \left(\frac{\left(6.30 \cdot 23.85 T^2\right) \cdot ln\left(2\right)}{\left(0.023 \cdot (T + 1.80)\right)^2}\right)\right)\right)}$
Probability of recovery during and after	treatment	
Valdramidis et al. [18]	Uncured meat	$\begin{aligned} Logit(Pr) &= 62.08 - 1.83 \cdot 10^{-1} \cdot P + 1.38 \cdot 10^{-4} \cdot P^2 - 0.18 \cdot 10^{-3} \cdot P \cdot t_s - 4.25 \cdot 10^{-3} \cdot P \cdot a_W \end{aligned}$
Koseki and Yanamoto [78]	Saline solution	$Logit(Pr) = 12.9973 - 0.0775 \cdot P - 9.1909 \cdot log(t) + 2.3331 \cdot pH + 1.6674 \cdot IC$

N and  $N_0$  represent the final and initial concentrations of the pathogen, respectively. P = applied pressure; t = t reatment time;  $t_s = s$  torage time; F = t content;  $a_w = t$  water activity; T = t storage temperature; T = t initial concentration of the pathogen, T = t probability.

Table 1. Predictive models obtained during/after the process of inactivation of L. monocytogenes by HHP on meat products.

longer than 10 min and the temperature tested did not lead to a significant increase in inactivation of the pathogen.

$$\log (N/N_0) = -380.3164 + 292.5942 \cdot P_{log} - 56.1268 \cdot P_{log}^2 + 1.4090 \cdot t + 0.0133 \cdot t^2 - 0.6423 \cdot P_{log} \cdot t$$
(1)

Bover-Cid et al. [5] used the response surface methodology (RSM) (**Table 1**) to evaluate the effect of  $a_w$  and fat content in the inactivation of *L. monocytogenes* by HPP in dry-cured ham. Besides these two intrinsic factors, the pressure intensity (347–600 MPa, during 5 min) was also considered as an independent variable for model development. According to the best fitting polynomial equation, all the three factors evaluated influenced on HHP inactivation, reaching inactivation levels from 0.92 to 6.82 logs.

Hereu et al. [25] obtained inactivation curves of L. monocytogenes on sliced RTE cooked meat products, ham (Eq. (2)) and mortadella (Eq. (3)) (which differ mainly on fat concentration), during HPP at pressures from 300 to 800 MPa. Their results suggested that the fat content of mortadella would have a protective effect on L. monocytogenes to pressure, in comparison with cooked ham. The log-linear with tail primary model was adequate to describe the inactivation kinetics at different holding times, which means that a first-order kinetics was applicable to describe the inactivation before a tailing effect appeared that suggests the presence of a more resistant subpopulation of cells. Secondary model was also performed to establish the relationship between the primary kinetic parameters,  $\log K_{max}$  and  $\log N_{res}$ , and pressure treatments. Combining the equations resulted from the primary and secondary modelling approaches; the inactivation of L. monocytogenes could be estimated as a function of pressure and holding time

$$\log N/N_0 = \log \left[ \left( 10^{\log N_0} - 10^{8.0832 - 0.0121 \cdot P} \right) \cdot e^{-\left( 10^{-2.9869 + 0.0069 \cdot P} \cdot t \right)} + 10^{8.0832 - 0.0121 \cdot P} \right] - \log \left( N_0 \right) \text{(cooked ham)}$$

$$(2)$$

$$\log N/N_0 = \log \left[ \left( 10^{\log N_0} - 10^{8.6636 - 0.0125 \cdot P} \right) \cdot e^{-\left( 10^{-3.6586 + 0.0079 \cdot P} \cdot t \right)} + 10^{8.6636 - 0.0125 \cdot P} \right] - \log \left( N_0 \right) \text{(mortadella)}$$

$$(3)$$

Hereu et al. [14] built up another model for the estimation of growth of L. *monocytogenes* in sliced cooked meat products (cooked ham and mortadella) after pressurisation but includes other factors as two different inoculum levels ( $10^7$  or  $10^4$  cfu/g), two physiological states of cells (freeze-stressed or cold-adapted) and different storage temperatures (4, 8, and 12°C). The logistic model with delay (primary model) was fitted to data to estimate the lag phase ( $\lambda$ ) and the maximum specific growth rate ( $\mu_{max}$ ). Secondary modelling was performed, using the Ratkowsky square root model (**Table 1**) and the relative lag time (RLT) concept. They observed that the time to achieve a 2-log cfu/g concentration of L. *monocytogenes* was similar for both physiological states. Freeze-stressed cells were more resistant to pressures and showed more extended lag phase during storage than the cold-adapted bacteria.

Based on logistic regression (**Table 1**), [18] concluded that the recovery of L. *monocytogenes* in a simulated cured meat after HPP treatments is influenced by the pressure applied, the storage time and the synergistic effect of pressure and  $a_w$ . The effect of salt reduction on the recovery of L. *monocytogenes* following HPP in meat

systems was assessed. A protective effect was remarked at low a<sub>w</sub> values which led to low inactivation levels both immediately and during storage.

Koseki and Yanamoto [78] developed a probability model (a simple linear logistic regression model,  $R^2$  = 0.9213, **Table 1**) of recovery of *L. monocytogenes* on sliced cooked ham during and after HHP treatment, with a storage of 10°C during 70 days. Authors defined "recovery" as the detection of >10² cfu/g bacteria, and the ham score was "1" as when there was a recovery of cells and "0" when not. The treatment applied to 500 MPa for 10 min allowed the reduction of *L. monocytogenes* of 5 logs cfu/g, reaching below the detectable level (10 cfu/g). However, they described a gradual increase of bacterial count during storage that at the end of the experiment, reached 7–8 log cfu/g. This model does not only calculate the appropriate process condition of HPP treatment but also provides information for the estimation of risk of the recovery of *L. monocytogenes* during storage of the product.

Mussa et al. [79] obtained kinetic data on *L. monocytogenes* inactivation by HPP on pork chop samples. The variables studied were pressure intensities (200–400 MPa) and duration of pressure treatments (0–90 min). Interestingly, this is one of the few studies in which the pressure inactivation kinetics was analysed assuming a first-order kinetic process (Eq. (4)):

$$\log\left(N_{N_0}\right) = -kt\tag{4}$$

where N refers to the number of viable cells in samples after pressure treatments;  $N_0$  is the number of viable cells just before pressures achieved the intensities set in the experimental design; t is the time in minutes; and k is the reaction rate constant (min<sup>-1</sup>).

The D value, which is the treatment time at any given pressure required to produce one decimal reduction, was calculated as the inverse of the slope (Eq. (5)):

$$D = -\left(\frac{1}{slope}\right) \tag{5}$$

Two secondary models were assessed to describe the pressure dependence as a function of kinetic parameters (k and decimal reduction time D): Arrhenius-type and the pressure death time (PDT) models. Both models described well the kinetic parameters (R > 0.96). Higher lethal effects were observed when higher pressures were applied, with an increase in K values and a decrease in D values as pressure levels increased. The holding time also had a significant effect on inactivation.

The pressure ZHP (the pressure range between which the decimal reduction time changes by a factor of 10) was calculated as the negative of the inverse of the slope of the curve of log D values versus pressure as follows (Eq. (6)):

$$ZHP = -\left(\frac{1}{slope}\right) \tag{6}$$

Results indicated that to achieve a 5 log cfu/g reduction of L. monocytogenes levels, approximately 7.5 min of pressure holding time, when pressure is set to be 400 MPa (D value = 1.49 min), would be necessary. At the same conditions of this study, Mussa et al. [79] obtained a D value = 3.52 min on pork, which makes clear that, besides the technological parameters, the type and composition of food influence on the destruction kinetics of L. monocytogenes by HPP.

Oliveira et al. [80] evaluated the effect of HPP (600 MPa/180 s at 25°C) in combination with the application of natural phenolic bioactive carvacrol (at 200 ppm) to reduce *Listeria innocua* levels in a low-sodium sliced vacuum-packed turkey breast ham during 60 days of storage at 4°C. The initial contamination of

slices with L. innocua was  $\sim 10^6$  cfu/g of slice. The primary model of Baranyi and Roberts, fitted to data obtained during the storage period, showed a significant extension of shelf life of low-sodium vacuum-packed turkey breast ham, with the reduction of maximum population density and the increase in lag phase duration. L. innocua has been used as a surrogate of L. innocytogenes for processing plant safety purposes, as it has similar physiological and metabolic characteristics to those of pathogenic species [81].

The effect of HPP treatments and potassium lactate on inactivation of L. *monocytogenes* was evaluated by Lerasle [26] considering the variables pressure intensities (200–500), holding time (2–14 min) and potassium lactate concentrations of 0 or 1.8% w/w. The Weibull model was fitted to the inactivation data ( $log\ N$  versus time) obtained at the different pressure holding times evaluated. The secondary model was a linear regression that defines log b as a function of the pressure intensity and explanatory factors (Eq. (7)). Considering that the lactate concentration effect was not significant (ANOVA, p > 0.05), the secondary model was:

$$\log b = \log b * -\frac{P}{Z_p} + \varepsilon; \tag{7}$$

where  $Z_p$  might be interpreted as the pressure required to reduce b by 10-fold and  $logb^*$  is the y-intercept and  $\varepsilon$  the model error. The estimated values for the parameters are represented below (Eq. (8)):

$$\log b = 143 - \frac{P}{3.1} + \varepsilon \tag{8}$$

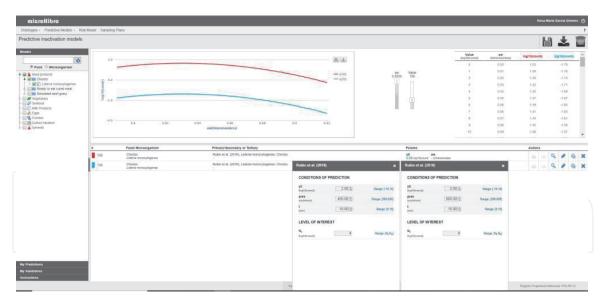
Combining primary and secondary models makes it possible to recalculate the log reduction obtained at various times and pressures intensities.

These models developed by Lerasle et al. [26] were subsequently applied in a multi-criteria framework combining safety, hygiene and sensorial quality to investigate the possibility of extending the shelf life of a ready-to-cook poultry product, using the HPP technology [82]. Models developed for *Salmonella* and *E. coli* were also considered in the framework in which the maximum allowed contamination level of *L. monocytogenes* was set to be 100 cfu/g (according to the microbiological criteria of the foodstuffs defined by the Commission Regulation (EC) No 2073/2005) [1]. The approach is a decision support tool for shelf life determination.

Also the significant inactivation effect (P < 0.001) of HHP (540 MPa/270 s) on *Enterobacteriaceae*, *E. coli* and *Pseudomonas*, coagulase-negative *Staphylococcus* (CNS) and LAB of natural casings and condiments used in the processing of cured meat sausage using response surface methodology was described by Fraqueza et al. [83]. Treated casings turned slightly whiter, but their resistance (FT) to breakage (i.e. casings structural integrity) was not affected.

Recently, Guillou and Membré [52] have carried out a hierarchical model based on a study of metadata of the determining factors in inactivation by the treatment of high pressures in different microorganisms and substrates, concluding that those more relevant factors studied were the species, the strain and the pH and that the most resistant species was *Staphylococcus* and the most sensitive *Salmonella*.

Novel approaches have been described as the potential use of Listex<sup>TM</sup> P100 in sausage "Alheira" combined with high hydrostatic pressure, applying Weibull model [84] and concluding that at mild HHP treatment, phage P100 remained active and seemed to present potential to be added in nonthermal inactivation of L. *monocytogenes*.



**Figure 1.**Screenshot of the microHibro web application of a predictive model of HHP treatment of chorizo.

High pressure processing and biopreservation can contribute to food safety by inactivation of bacterial contaminants. However, these treatments are inefficient against bacterial endospores such as *Bacillus* and *Clostridium* species. Moreover, HPP can induce spore germination [85]. In [85], it is reported that *Lactococcus lactis* strain CH-CH15 was able to regrow after HPP treatments, thus an excellent option to be preservative against *Bacillus* and *Clostridium* strains during chilled storage. The inactivation model used was fitted by using a reparametrized Weibull model, whereas growth curves of lactic acid bacteria were modelled with a logistic model.

Predictive microbiology modelling easy-to-use software has been developed to allow users involved in food safety management to use a tool to asses them and help them for decision-making. Several applications have been developed, but just a few had incorporated the prediction of HHP treatment. One of it is the "HP3", available online (www.hp3.cat) elaborated by the Institute of Agrifood Research and Technology (Spain), and another is microHibro (www.microhibro.com), built up by the University of Córdoba (Spain) (**Figure 1**).

For further information, there are several reviews as [4, 10, 23, 30, 31, 78].

#### 2.5 Other applications of HPP on meat products

Although the main application of HHP is enzymatic and microbial inactivation to extend commercial life and inactivate pathogens, other possible applications such as obtaining different types of fish, meat, egg and milk gels have been described. Likewise, this technology accelerates the diffusion of solutes in various foods, the solubilisation of gases and the extraction processes. The possibility of using high pressures to keep food at temperatures below 0°C in a liquid state (at 207.5 MPa, the water remains liquid at temperatures of -22°C) or to induce freezing (supercooling) and ultra-fast defrosting constitutes a promising new field of study and application in the food industry [34, 40]. Also applying low pressures, 100–150 MPa have been employed to tenderised pre-rigour meat of rabbit, chicken, pork and beef. Higher pressures, 250 MPa, has been applied, for example, before smoking to treat roast beef and bacon, to inactivate microflora of minced meat or to treat foie gras to extend shelf life [40, 41].

#### 3. Conclusion

High hydrostatic pressure treatment has been described to improve the microbiological safety and shelf life of ready-to-eat meat products, as a nonthermal decontamination technology in the meat industry, applied at pre- or postpackaging. There are a variety of factors that influence the treatment effect that should be taken into account when applied to food. The pathogen widely studied in this product is *L. monocytogenes* that reflects the concern of the food industry. The predictive modelling is an important tool of the novel microbial food safety management strategy that provides with accurate information to demonstrate and guarantee the safety and shelf life of the food products and also helps to the optimisation of the meat treatment.

#### Acknowledgements

We greatly acknowledged the Spanish government and European ERDF funding for supporting the RTA-2013-00055-C02-02 project, providing material and especially human resources, making possible the continuation of risk assessment and management activities at national and European level by our research group AGR170 "HIBRO".



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High Hydrostatic Pressure Treatment of Meat Products DOI: http://dx.doi.org/10.5772/intechopen.90858

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