NEW MILD TECHNOLOGIES IN MEAT PROCESSING: HIGH PRESSURE AS A MODEL TECHNOLOGY

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Abstract
As a consequence of market globalisation, the production and manufacture of meat products is at a stage of innovative dynamics. Consumers demand high quality and convenient meat products, with natural flavour and taste, and very much appreciate the fresh appearance of minimally processed food. To harmonize or to blend all these demands without compromising safety, it is necessary to implement new preservation technologies in the meat industry and in the food industry in general. High hydrostatic pressure (HHP) represents an attractive non-thermal process for meat products to avoid post-processing contamination. When combined with antimicrobials, like bacteriocins, the death rate may be increased because of sublethal injuries to living cells. HPP is a powerful tool to control risks associated with *Salmonella* spp. and *Listeria monocytogenes* in raw or marinated meats. The HPP treatment could extend the shelf life of the marinated beef loin by controlling the growth of both spoilage and pathogenic bacteria. As a general conclusion it can be stated that from both a physico-chemical and microbiological point of view, cooked pork ham, dry cured pork ham and marinated beef loin, vacuum-packed and high pressure treated at 600 MPa for 10 minutes at 30ºC, are substantially equivalent to the same untreated products.

Key words: preservation, meat products, high pressure, emerging technologies

1. Introduction

As a consequence of market globalisation, the production and manufacture of meat products is at a stage of innovative dynamics. In order to keep or to reinforce their leading position, meat and food companies need to take into consideration the evolution of the purchasing and consumption habits of consumers, as well as the perception and definitively the trends of the consumers' demands.
These consumers' demands are continuously changing, but some of the main parameters or axes are consolidating. Consumers demand high quality and convenient meat products, with natural flavour and taste, and very much appreciate the fresh appearance of minimally processed food. Besides, they require safe and natural products without additives such as preservatives and humectants.
To harmonize or to blend all these demands without compromising the safety of the products, it is necessary to implement new preservation technologies in the meat industry and in the food industry in general. Moreover, sliced vacuum packaged meat products and marinated vacuum packaged meats are the meat market segment with the greatest rate of increase in recent years. Those products are particularly liable to cross-contamination just before packaging; therefore, the need for implementing new preservation technologies in these products is of great importance.

2. Mild preservation techniques with possible applications in the meat industry

In the last century, several alternative or complementary preservation technologies to classical processing were developed. A good example is gamma irradiation, which is competely effective for food decontamination yet its consumer acceptability is low. For this reason, during the last decade different approaches have been studied and as a consequence there are several promising technologies currently being evaluated in small industrial production.
Many of these mild preservation technologies aim at energy saving and being environmentally friendly. All of them have the same goal of being mild for food but knocking out pathogenic and spoilage microorganisms. In this way, their use guarantees the natural appearance of the product. However, few mild technologies can be regarded as totally safe.
Several examples of these mild preservation techniques with good potentiality in the meat industry are: high pressure processing (HPP), controlled instantaneous decompression (DIC), oscillating magnetic fields (ohmic heating, dielectric heating, microwaves), high intensity pulsed light, X-rays and electron beams.
Nowadays, irradiation through electron beams and HHP offer real possibilities for their practical use in meat and derived products. In the same way, light pulses of high intensity can already be applied on the decontamination of surfaces or packaging material. Other alternative technologies like oscillating magnetic fields, or DIC are not ready for practical application; some new developments which are currently under research like acoustic waves, also need to be taken into account.
The existence of these technologies require an adaptation to the actual needs of each meat subsector as well as the generation of know-how. Besides, they offer a very wide field of experimentation through their combined use, as in the hurdle theory proposed by Leistner (1992).

2.1. High pressure processing (HPP)

HPP is a very promising technology for the preservation of sliced cooked and cured meat products, and it shows a big potential for the innovative development of new products with a relatively low energy consumption.
High pressure processing uses an isostatic pressure at room temperature and between 100-600 MPa. The pressure chamber is loaded and closed, degassed and the pressure is transmitted by the pumps through a liquid, generally water. The technology is based on the principle of Le Chatelier and the isostatic rule, so HPP is trasmitted in a uniform and instantaneous manner and the product, or its constituents, suffers volume changes under pressure. Precisely, high pressure accelerates reactions envolving a change of volume at molecular level and they are the key to understanding the biological effects on macromolecules and microorganisms.
In general, HPP at low or moderate temperature causes destruction of microbial vegetative cells and enzyme inactivation, without changing the organoleptic characteristics of the product and leaving the vitamins intact. However, the resistance of the microorganisms is very variable depending on the strain and the meat matrix to be treated. The efficacy of the treatment also depends on the achieved pressure, on the treatment temperature and on the exposure time. The HPP treatments can induce special effects on the products' texture and structure of a given food and accordingly can be used for the development of new products or to increase the functionality of some ingredients.
Therefore, the areas of experimentation with this technology for its industrial application in the meat sector comprise: establishment of the best treatment conditions in every product and commercial presentation. Combination of HPP with new packaging systems, natural antimicrobial substances, enzymatic cocktails, etc. Also promising are the development of new meat products based on cold gelification of starches, on non-thermal coagulation of proteins, on selective enzymatic inactivation, etc.

There are a range of pressure-treated food products already on the market. They include fruit preparations, fruit juices, rice cakes and raw squid in Japan, fruit juices, specially apple juice in France and Portugal and guacamole and oysters in the U.S.A.. Concerning meat products, so far there are two Spanish meat companies using HPP equipment daily (Esteban Espuña, S.A. and Campofrio Alimentación, S.A.). In the U.S.A., several meat companies have made this methodology available (e.g. Hormel Foods and Purdue Farms). Some equipment suppliers are: Flow International, ACB Pressure systems in France and Hyperbaric (Nicolás Correa group) in Spain.

2.2. Controlled instantaneous decompression (DIC)

DIC was developed at the Technological University of Compiègne (France) after studies carried out by Prof. Allaf in the 90's (University of La Rochelle, France).

This technique is applicable to products cut into pieces, in powder or pumpable, with the purpose of drying, texturizing, extracting biomolecules and for decontamination or sterilization. Energy consumption is reduced compared with classical techniques due to the short treatment times (from 5 sec to 1 min). It is a clean and environmentally friendly technology.

In fact, the DIC is a high temperature short time treatment (HTST). The food is placed at a temperature below 200ºC under a pressure below 20 bars for a few seconds. The pressure and temperature are obtained through steam, although it is also possible to use convection, conduction, microwaves etc. to obtain the desired temperature. The decompression is obtained through a very instantaneous communication of the treatment autoclave with a vessel of a much larger volume and reduced pressure, achieving consequently an instantaneous evaporation of part of the water from the product and a quick cooling, thus stopping the reactions of thermal degradation. The DIC treatments can induce important effects on the texture (alveocation) and in the functional properties of the product, conferring a good innovative tool for new products and new commercial presentations. The microorganisms are destroyed by thermal and mechanical effect.

2.3. Ohmic heating

Ohmic heating is an alternative thermal process generating internal heat in a solid product in a uniform way. It is an HTST process allowing the pasteurisation and sterilization of food. It is based on the passage of electricity through food with high resistance to conductivity. One of the main advantages of this technology is that it allows a continuous production without heat transfer. Besides, since it is a rapid treatment allowing a pasteurisation or a sterilization at lower cooking temperatures, the harm caused by the heat in the conventional treatments is minimized. Therefore, the nutrients are not destroyed and the organoleptic characteristics are maintained producing a higher feeling of freshness. It is a clean technology, environmentally friendly and with low energy costs. Its combination with an aseptic packaging system allows a high preservative effect on the treated products.

The main disadvantages of the ohmic system arise from the need to treat pumpable products and a significant energy cost. The density of intensity admissible recommended is of 4000 A/m^2. The maximum tension is limited by the costs of the wiring and the control equipment. In Europe, there are several pilot plants: in the United Kingdom, APV (www.apv.com) with a capacity of treatment of 750 Kg/h; in France, Centre Technique de Conservation des Produits Agricoles (CTCPA) and Institute des Sciences et Techniques de Valenciennes. Some equipment suppliers are: APV and CTCPA.

2.4. Dielectric treatment or radiofrequency

Dielectric heating is based on the fact that the oscillation of water molecules produces friction and consequently heat is generated. The oscillating is due to the change of cycles at a very high frequency. The word "dielectric" can be used in all the electromagnetic frequencies, including those of the infrared spectrum, but it is generally accepted that the term "dielectric" is developed at frequencies between 1 and 100 MHz, producing a wavelength of several meters.

The radiofrequency waves are generated through a device called a magnetron applicator. The generated electromagnetic wave is an energetic wave changing its energy content and width as it gets through a medium. It is this periodic oscillation of the wave polarity and its passing the zero that causes a tension on the ions, atoms and molecules, which is converted into heat and consequently the bigger the forces' field the bigger the global effect. It has to be taken into account that the dielectric frequencies are not ways of heating but of energy, being evident through their interaction with the materials; in some way the materials heat themselves. In this process, it is important to control the leaks of radiation to avoid interference with radiofrequencies and more importantly for human safety.

The main advantages of the dielectric heating in respect to the conventional systems are an increment in the heating speed, uniformity of heating with no gradients, a very precise control of the heating process, the ability to start and stop the heating process instantaneously while regulating the applied power; an improvement in the quality of the products susceptible to surface overheating in the traditional systems; changes in the functionality of certain food ingredients (protein denaturation, starch gelification etc.). Some available pilot plans are: AGIR-Agroalimentaire Innovation Research in Talence Cedex (France) and in the U.S.A. Biological Systems Engineering. Washington State University. The available equipment suppliers are: Petrie Technologies Ltd. and Radyn (Proctor Staryfield Inc.) both of them in the United Kingdom.

2.5. Microwave treatments

The technology based on microwaves relies on the same principle as the radiofrequency but it uses higher frequencies between 300 MHz and 300 GHz and thus wavelengths between 1 mm to 1 m. Even though the principle is the same, the generation methods of the microwave frequencies and the equipment needed are very different. This technology can be used in the thawing, heating, cooking, drying and frying of foods. In the thawing and/or heating there are significant advantages over the conventional methods as the shortening of the thawing time from hours to minutes, the reduction of the plant space devoted to thawing and the elimination of thawing chambers, an increase in the hygienic conditions and a decrease in the microbial load of the thawed products.

When microwaves are used as a cooking method, they allow the reduction of the temperature and time of treatment due to the decrease in the temperature gradient and consequently, a reduction in the cooking losses is achieved, at the same time retaining the initial characteristics of the product. Its use in the development of snacks, fried without oil, envisages a very interesting field in product development and innovation. The combined use of this technology with classic drying processes like air or vacuum drying, allows the reduction in processing time as well as in energy costs, making the already existing infrastructures in the purely conventional processes more profitable. The available equipment suppliers in Europe are: APV and Petrie Technologies Ltd. United Kingdom.

3. High pressure processing in the meat industry

High hydrostatic pressure processing is the main emergent preservation technology with more prospects nowadays for its application in the meat industry.

3.1. Effect of HPP on meat constituents

Meat is a very rich medium for growth of microorganisms, it is mainly constituted by water, protein (15-21%), fat (0.5-25%), oligonutrients and vitamins (especially rich in B group vitamins). From a physical point of view an increase in pressure has a physical effect on the molecules, for they get closer to each other; leading to phase transitions which are reversible after depressurization. This is what happens to water and lipids. From a chemical point of view, HPP is softer than a thermal treatment. The covalent bonds are not broken but the weak energy bonds like hydrogen bonds and the hydrophobic bonds can be irreversibly modified (Cheftel, 1995).

The pressure effects on water comprise mainly a decrease in the melting point under pressure and an increase in the ionisation leading to a decrease in pH under pressure. These variations are reversible at low pressures but they can contribute by modifying the characteristics of the products subjected to high pressure. Above 150 MPa there are colour changes similar to those in cooked meat. When pressure is higher than 400 MPa ferrous myoglobin becomes ferric and the globin protein is denatured. Calpastatin is inhibited from 200 MPa while calpains are degraded above 400 MPa.

At pressures lower than 200 MPa lysosomes break down, the autolytic activity increases and the meat tenderization is higher. Cathepsin H and aminopeptidases are inactivated from 200 MPa and cathepsin D is inactivated when pressure reaches 500 MPa (Montero & Gómez-Guillén, 2002). The vitamins and sugars in meat and meat products are not modified by HPP, however the polysaccharides can be modified. In general, gel formation is inhibited by HPP since the pressure can modify the transition temperature from sol to gel. Gelation can be induced by pressure and then the gels formed are softer and brighter. HPP brings about a reversible passage of lipids from liquid to solid state leading to gelation. When there is a mixture of lipids, HPP can lead to a separation of different phases resulting in the destruction of cell membranes. The primary structures of proteins are slightly sensitive to HPP, the modification of weak bonds can lead to protein denaturation or on the contrary to enzyme activation. The effects are variable depending on the protein types and the processing conditions.

3.2 Effect of HPP on meat microorganisms

The rate and the inactivation kinetics of the microorganisms under HPP depends on several parameters like: the type of microorganism, the level of pressure, the time of treatment, the temperature, pH, the water activity and the food composition. In general, the inactivation increases with pressure.

3.2.1. Sites of pressure damage on microorganisms

The inactivation of microorganisms by HPP is probably the result of a combination of factors. HPP does not inhibit or destroy a unique cellular site or a unique cell function, so cell death is due to multiple or to accumulated damage inside the cell (Simpson & Gilmour, 1997).

The cell membrane is the primary target of pressure damage, mainly through altering its permeability as a consequence of phospholipid cristallization. Other cellular functions sensitive to pressure include: ion exchange modifications, fatty acid composition, ribosome morphology, cell morphology, protein denaturation and inhibition of enzyme activity, destabilization of DNA replicative complex, vacuole formation, etc. The differences in membrane properties can be an important factor in determining the pressure or stress sensitivity of a given microorganism. In several microorganisms, it has been shown that the sublethal damage is initiated by membrane phase transitions affecting mainly transport proteins (Vogel, Molina-Guiterrez, Ulmer, Winter & Gänzle, 2001). This can be a general mechanism of cell death. In general, cell death increases with pressure but it does not follow a first order kinetics (logarithmic death rate), since there is a tailing off in inactivation (Kalchayanand, Sikes, Dunne & Ray, 1998a). In the case of non-linear survivor curves, cell inactivation is thought to be due to multiple events or cumulative damage to the cell. This is consistent with the generally accepted belief that high pressure affects a combination of microbial processes and does not inhibit or destroy just one specific cell site or function (Hoover, Metrick, Papineau, Farkas & Knorr, 1989).

3.2.2. Microbial resistance to HPP

Generally speaking, gram-positive microorganisms are more resistant to HPP than gram-negatives and so are spores. Cell morphology also has an effect on pressure, with bacilli being more sensitive to pressurization than cocci.

Cells subjected to stress other than pressure (e.g. sublethal heat, cold-shock) become more resistant to pressure. Exponentially growing cells are more sensitive to pressure than cells in the stationary phase. Stress is induced during the stationary phase of growth through starvation or acidification (Archer, 1996).

Temperature, as expected, plays an important role in microbial inactivation for HPP. At optimal growth temperatures, inactivation is less than at higher or lower temperatures of growth. Membrane fluidity can be more easily disrupted at temperatures above the optimal growth. It has also been observed that the membrane fatty acids of the barophylic microorganisms become more polyunsaturated when pressure of growth is increased (Smelt, 1998).

When cells have been cold-shocked before HPP, they become more resistant to pressure. This can be explained by the fact that the mechanisms allowing low-temperature growth involve maintenance of cellular membrane fluidity. Adaptation of membranes to low temperatures is accomplished by altering the branching and decreasing the length of the membrane fatty acids, thereby resulting in higher levels of survival after pressure treatment (Wemekamp-Kamphuis, Karatzas, Wouters, & Abee, 2002). The induced levels of cold shocked proteins (CSP) might protect a cell exposed to high pressure. This protection against pressure resulting from low temperature treatment could be important for processing technology (e.g. if cold storage of food products is combined with pressure treatments).

Differences in pressure sensitivity of pathogenic strains of several species (*Listeria monocytogenes*; *Staphylococcus aureus*; *Escherichia coli* and *Salmonella typhimurium*) have been reported by several authors (Simpson & Gilmour, 1997; Alpas, Kalchayanand, Bozoglu, Sikes, Dunne & Ray, 1999). In a meat model system two different strains of *E.coli*, despite being similarly sensitive to pressure, showed very different growth kinetics during storage after pressurization (Fig 1) (Garriga, Aymerich, Costa, Monfort & Hugas, 2002a). Phenotypic diversity between strains, as for example in membrane fatty acid composition, can be the cause of different inactivation rates to pressure. Such variation in strain sensitivity is an important consideration, specially when setting up processing regimes designed to inactivate a particular species of microorganism. Therefore, as many strains as possible would have to be screened for their resistance to pressure before it could be established with any degree of confidence that a particular treatment would be effective at eliminating, or reducing the numbers of a particular microbial species sufficiently.

3.2.3. Effect of food constituents on microbial survival after HPP

According to Archer (1996), in real food situations two effects always determine microbial safety and stability: the effect of the food constituents during treatment and the effect after treatment during the recovery of the microorganism.

The pressure-resistance of microorganisms can be affected by many intrinsic and environmental parameters, the nature of the suspending media being one of the most important. Results obtained in buffers or synthetic media cannot be directly extrapolated to real food situations. It is known, that complex, low-acidity food matrixes such as meat (Table 1) and particularly milk (García-Graells, Masschalck & Michiels, 1999), tend to protect bacteria against HPP inactivation compared to phosphate buffer.

The ability of bacteria to survive high pressures can be greatly increased when treated in nutritionally rich media containing substances like carbohydrates which may provide protection against damage (Hoover et al. 1989). The presence of carbohydrates and proteins as in food emulsions, increase the pressure resistance of some microorganisms (Simpson et al. 1997). After comparing the microbial inactivation in spiked meat products with different water activities (Table 2), it was observed that the viability loss of *Staph. aureus* and lactic acid bacteria in dry cured ham was the lowest, compared to marinated raw beef and cooked ham. In fact, by decreasing the water activity, the resistance of microorganisms to HPP is increased as was previously observed in synthetic media (Oxen & Knorr, 1993).

Bacteria are more sensitive to suboptimal pH after heat or pressure treatment. Thus, not only pH fall enhances inactivation during treatment, but inhibits outgrowth of cells injured sublethally by heat or pressure. Apart from pH effects, no specific effects of organic acids have been observed. This might be due to the fact that pressure favours ionisation and that organic acids are particularly inhibitory in the undissociated form. Sorbic acid acts as an organic acid, but it also interferes with the microbial membrane, being more active in combination with pressure (Mackey, Forestiere & Isaacs, 1995).

3.2.4. Synergistic effects of antimicrobial compounds and HPP

Different experiments have shown that HPP alone may not be a sufficiently safe process under all conditions, thus it may be necessary to use a hurdle type of approach by combining HPP with one or more other factors that act synergistically.

Several factors have been proved successful in this regard like low pH (Alpas, Kalchayanand, Bozoglu & Ray, 2000), mild temperature processing with antimicrobial peptides (García-Graells et al. 1999), lysozyme and the lactoperoxidase system in milk (García-Graells, Valckx & Michiels, 2000) as well as the use of antimicrobial peptides in vitro (Kalchayanand et al. 1998a, 1998b), and meat products (Garriga, Aymerich, Costa, Monfort & Hugas, 2000a).

Gram-negative bacteria such as *E.coli* or *Salmonella* are normally insensitive to bacteriocins of lactic acid bacteria as they lack specific receptors, but they can be sensytised to nisin or other bacteriocins when pressurized (Kalchayanand et al, 1994). This fact might be explained by the specific action of bacteriocins, as they interact with the cell membrane and possibly could penetrate into the inner cell membrane.

The behaviour of several foodborne bacteria inoculated in a meat model system with added bacteriocins (enterocins A and B, sakacin K, pediocin AcH or nisin) after pressurization (400 MPa, 10 min, 17ºC) and during chilled storage was investigated (Garriga et al. 2002a). Although *Staphylococcus* was the genus least sensitive to pressurization, the samples including nisin displayed lower and significantly different counts during the 4ºC storage than the rest of the treatments. A greater inactivation of *E.coli* (>6 $\log_{10}$) in the presence of nisin was recorded, the number of survivors remained unchanged during storage at 4ºC for 61 days (Fig. 2), suggesting that, after pressure, the injured survivors became sensitive to nisin, resulting in the loss of viability.

Nisin was also the bacteriocin capable of maintaining slime-producing lactic acid bacteria below the detection limit (<10^2 cfu g^{-1}). *L. monocytogenes* in treatments with sakacin, enterocins or pediocin was kept <10^2 cfu g^{-1} till the end of storage (61 days). *Salmonella* London and *S.* Schwarzengrund counts in every treatment were kept at the level obtained after pressurization, with no significant differences between bacteriocin treatments during the chilled storage. The results obtained highlight that the use of some bacteriocins increases the lethality of some pathogenic and spoilage bacteria subjected to moderate high pressure (400 MPa), allowing the extension of the shelf life of the product.

Masschalk, Van Houdt & Michiels (2001) describe two types of sensitisation of bacteria to antimicrobial compounds by high pressure in buffer systems. One type is transient sensitisation, whereby bacteria exhibit sensitivity to the antimicrobial only during the time they are being held under pressure, with resistance being restored to the level of unpressurized cells immediately upon relief. This is the case with lysozyme and nisin (Hauben, Wuytack, Soontjes & Michiels, 1996). The other type is persistent sensitisation, whereby the bacteria remain sensitive for at least several hours after pressure treatment, like the sensitisation for low pH and for the lactoperoxidase system (García-Graells, Hauben & Michiels, 1998) (García-Graells et al. 2000). This latter form of sensitisation involves small diffusible antimicrobial molecules that can penetrate the gram-negative outer membrane. With larger antimicrobial molecules such as bacteriocins no persistent sensitisation is observed, probably because they fail to penetrate the outer membrane of gram-negative bacteria after pressure treatment.

3.2.5. Effect of process conditions on HPP effectivity on microbial inactivation

Different pressurization equipment and different pressurizing media can give different results in microbial survival during storage. Moreover, different pressure-temperature-time profiles perceived during a real process at different locations in the HP vessel may result in a pronounced non uniform distribution of microbial inactivation. Contrary to classical heat pasteurization or sterilization process, the critical control point (coldest spot) is located near the wall of the HP vessel, because heat transfer occurs between the HP vessel wall and the liquid bulk, resulting in a decrease in the temperature of the product fraction near the wall. The effect of the come-up time (ramp rate), the depressurization rate and the control of the temperature of the process has not yet been fully investigated. A slow ramp rate might induce a stress response and hence make the process less effective and it is often thought that a fast depressurization rate might contribute to a fast inactivation rate.

After pressurizing sliced skin-packaged cooked ham at 600 MPa for 6 min at 31ºC in two different machines, the counts of lactic acid bacteria (LAB) during chilled conditions were evaluated (Fig 3). In the samples pressurized in equipment A, LAB counts were kept below the detection level (10^2 cfu g^{-1}) during the sampling time (120 days at 4ºC). The samples pressurized in equipment B, were kept below the same detection level for the first 30 days of storage, although by the end of the sampling time they reached 10^8 cfu g^{-1} In a similar experiment (Table 3) comparing the pressurization effect on artificially inoculated samples of dry cured ham with *Listeria monocytogenes*, in two different high pressure machines (A and C), it was observed that the same treatment in equipment A inactivated *L.monocytogenes* completely (absence in 10 g) during the storage time (120 days) while the treatment with equipment C resulted in the presence of the pathogen in all samples and sampling times.

The differences observed could not be attributed to the samples since they were manufactured at the same place and sampled with the same protocol, but to the equipment used, suggesting there is a need to establish validation protocols for high pressure equipment before experimentation trials.

4. Shelf life extension in meat products treated with HPP

4.1. Fresh products

The application of HPP to fresh meat products results in a cooked-like aspect, and sometimes the products may develop a rubbery consistency. Murano, Murano, Brennan, Shenoy & Moreira (1999) tested the usefulness of applying a mild heat treatment at 50ºC simultaneously with HPP in ground pork patties to lower the D values of *L.monocytogenes* obtained with only HPP. With a treatment of 414 MPa and 50ºC for 6 min they obtained a 10-$\log_{10}$ reduction in the most resistant strain of *L.monocytogenes*. Shelf life studies were also conducted, spoilage levels for control samples were reached after 5 days of storage at 4ºC and after 28 days for treated samples. Sensory evaluation of uninoculated grilled patties showed that panellists could not distinguish between those treated by heat and HPP and untreated controls. Thus, treatment by HPP in combination with mild heating can be used successfully to produce safer, long-lasting fresh pork without affecting quality.

Marinated beef loin, which is a raw uncooked meat product with high water activity, a low level of salt and without nitrite, harbours a mixed flora of spoilage and pathogenic microorganisms from the slaughterhouse cutting and trimming operations. Sliced, skin vacuum-packaged marinated beef loin was treated by HPP at 600 MPa for 6 min at 31ºC. Aerobic, psychrophylic and lactic acid bacteria counts showed at least a 4 $\log_{10}$ cycle reduction after treatment and remained below the detection limit ($<10^2$ cfu g^{-1}) during the chilling storage of 120 days, helping to prevent the sour taste and off-flavours while untreated samples reached 10^8 cfu g^{-1} after 30 days in the same conditions. *Enterobacteriaceae* were kept below 10 cfu g^{-1} during the whole storage period in HPP treated samples, while untreated samples reached 10^5 cfu g^{-1} after 30 days.

HPP is a powerful tool to control risks associated with *Salmonella* spp. and *Listeria monocytogenes* in raw or marinated meats (Table 4). Most of the untreated samples showed presence in 25 g from one or both of the pathogens, whereas all pressurized samples showed absence in 25 g (Garriga et al. 2002b). The HPP treatment could extend the shelf life of the marinated beef loin by controlling the growth of both spoilage and pathogenic bacteria.

4.2. Cooked Ham

Sliced vacuum-packaged cooked ham is a highly perishable product due to its composition, pH and water activity and the lack of a background flora competing with spoilage or pathogenic microorganisms. The physico-chemical and microbiological characteristics of cooked ham do not represent any hurdles to bacterial growth. Its shelf life depends on the hygienic characteristics of the final product after post-processing as well as to the packaging methods where cross-contamination is more likely to occur. The techniques used to reduce cross-contamination include good manufacturing practices, post-pasteurisation after packaging or even the use of "white rooms" at the slicing and packing stage.

Sliced, skin vacuum-packed cooked ham treated by HPP at 600 MPa for 6 min showed a significant delay in the growth of spoilage associated microorganisms compared to untreated samples, thus contributing to the maintenance of organoleptic freshness for at least 60 days after treatment (Garriga et al. 2002b). The HPP process helped to prevent any sour taste, off-flavours, ropiness and colour changes. Thus, HPP processing on cooked ham in the conditions mentioned above was an effective process to avoid the growth of yeasts and *Enterobacteriaceae*, with the potential to produce off-flavours and gas. Accordingly, it contributed to the shelf life extension in this highly perishable meat product.

4.3. Dry Cured ham

Dry cured-ham is a dry, bone-in, salted and dried, non-fermented meat product. Because of the low water activity and high salt content of this type of product, spoilage microorganisms are mainly gram-positive cocci and yeasts. They may be present on the surface of whole hams and reach the sliced product during final boning, slicing and packaging operations. Sliced, skin vacuum packed dry cured ham samples, treated by HPP at 600 MPa for 6 min, showed a significant reduction of at least two $\log_{10}$ cycles for spoilage associated microorganisms after treatment. The surviving microbiota was kept at low levels during the storage period; contributing to the preservation of the organoleptic freshness during shelf life (120 days) and helping to prevent off-flavours, sour taste and gas formation. *Enterobacteriaceae* and *Escherichia coli* were below the detection limit, both in HPP and untreated samples. *Listeria monocytogenes* was present (in 25 g) in one untreated sample, but absent in all HPP treated samples during the whole storage period. (Garriga et al. 2002b).

5. Impact of mild preservation techniques on packaging films

When using new preservation technologies involving the use of packaging techniques, it is very important to study the safety of the material, the possible formation of compounds that influence smell and taste of packed food and the mechanical and physical properties like strength and barrier properties. The HPP needs watertight and airtight packages than can withstand an elargement corresponding to the compressibility of the product. This deformation is of 15% at 600 MPa for a vacuum package.

Several studies carried out in Japan showed that monolayer or multilayer plastic films currently used in the agrofood industries are not modified in their barrier properties and migration rates after HPP from 400 to 600 MPa.

Berg, van Boxtel & Jongbload (2001) studied the effects of high intensity light (HIL), HPP and e-beam irradiation on two packaging materials: polyethylene (PE) and polyethylene terephtalate (PET). They concluded that the three preservation techniques can influence both PE and PET film properties. E-beam irradiation has the most pronounced effect on polymer materials. The migration from PE decreased but on the other hand several volatile compounds were formed. The strength of PE decreased after irradiation. PET is much less sensitive to irradiation. HIL and HHP have less effects on polymer materials like PE and PET.

Much is still unknown about the effects of the emergent preservation techniques on polymer materials. Further research is necessary to be able to guarantee the quality and safety of food packed in materials that have been treated with HIL, HHP or e-beam.

6. Regulation in the EU

The commercialisation of food products manufactured under high pressure have produced two different attitudes in regard to regulation either within the EU or outside the EU.

In countries outside the EU, there is currently no particular legislation for HPP treatment. In the U.S.A. the traditional health regulations are applied. In this country there are already products in the market treated by HPP like guacamole and oysters without any specific regulation.

In EU countries, the national regulations for new products have been replaced, in the application of the precautionary principle, by a community regulation for novel foods and ingredients (CE 258/97) in force since 1997. This legislation for "Novel Foods" establishes an evaluation and a license system compulsory for new foods and new processes. High pressure processed foods are novel foods since they fulfil two conditions: their history of human consumption has so far been negligible and secondly they have been produced by a new manufacturing process.

In July 2001, after the last meeting of the commission in charge of "novel foods" several decisions were taken for a simplification of the regulation. In this sense, it was admitted that if it is possible to show that the new product is substantially equivalent to a product already on the market, then the product can be treated at a national regulation level and will not need to comply with the "novel food" regulation. The dossier will have to be sent to the EU for information only.

7. Demonstration of the substantial equivalence of HPP meat products

After evaluating the proximate composition of marinated beef loin, cooked ham and dry cured ham pressurized at 600 MPa for 10 min at 30ºC compared to control non-pressurized samples (Table 5), small differences have been observed which could be more related with the variability of samples and raw materials than with the technological procedures. A slight decrease in phosphate content was detected in samples of HPP-treated dry cured ham, indicating a possible enhancement of phosphatase activity. The differences in chloride and phosphate contents ($p<0.001$) fell within the typical variability between samples in whole muscle meat products. As a general conclusion HPP did not show any influence in the proximate composition of cooked ham, dry cured ham and marinated beef loin.

Non significant differences were found in the nonproteic nitrogen fraction in the three meat products studied when HPP treated and compared to controls. In the same sense, no differences were observed in their aminoacid content (García-Regueiro, Sárraga, Hortós, Díaz, Valero & Rius, 2002). These results agree with a lack of protein breakdown due to HPP.

For the fatty acid composition and the cholesterol content in the three products studied no significant differences between samples were found, with the exception of ω6 acid. With this fatty acid, an increased stability was observed in pressurized marinated beef loin ($p<0.05$). According to the levels obtained in cholesterol oxides, less cholesterol oxidation was obtained in pressurized products. 7-ketocholesterol which was high in beef control samples was strongly reduced in beef subjected to HPP. However, it is necessary to study if HPP processing could have some influence on the recovery of cholesterol oxides by analytical methods.

The vitamin content did not present any significant differences between HPP treated and untreated samples, at least on the B group vitamins.

In general, no significant differences were found in the mineral composition of pressurized samples compared to control. The decrease of calcium content in HPP cooked ham (Table 6) is difficult to explain and more experiments should be carried out to verify if the solubility of some ions is modified by HPP. An increase in the iron content of HPP beef loin can be explained according to the results of Ledward (2001), who reported a release of iron from non-heme complexes at pressures higher than 400 MPa as well as from the heme proteins denaturation above 300 MPa. Such changes do not apparently occur in cured meats.

As a general conclusion it can be stated that from a physico-chemical point of view, cooked pork ham, dry cured pork ham and marinated beef loin, vacuum-packed and high pressure treated at 600 MPa for 10 minutes at 30ºC, are substantially equivalent to the same untreated products.

The effect on the bioavailability of nutrients was also assessed. The solubility of proteins in cold 1% SDS was higher in marinated meat HPP than in untreated samples, whereas no differences were found in dried cured ham or cooked ham. The proteins solubilized in this medium are representative of the cytoplasmatic fraction, excluding most of the myofibrillar proteins. The solubility of the myofibrillar protein fraction in a selective solvent (1M KCl) was markedly reduced by pressure treatment, but it is even more dramatically decreased by traditional cooking. Analysis by SDS-PAGE of different conditions of protein extraction, showed only minor differences confirming that pressure did not affect the primary structure of proteins. Nevertheless, precipitation by TCA after KCl extraction as well as solubilization by 6M urea and SDS-PAGE confirmed the lower major proteins' solubility in the pressurized materials except in dry cured ham.

From a microbiological point of view, substantial equivalence of HHP products was assessed and proved that the differences found were in the direction of better hygiene and safety aspects (see 4.).

8. Potential risks of high pressure processing

There are no published reports available on toxicity studies of HPP treated foods. It is well known that HPP can modify the activity of some enzymes and the structure of some proteins. Although covalent bonds are not affected, hydrogen bonds as well as hydrophobic and intermolecular interactions may be modified or destroyed.

From the above some concern on the potential risks of HPP may arise. It is necessary to compile data in order to clarify the role of HPP towards toxicity, allergenicity, loss of digestibility and the eating and nutritional quality of foods.

Allergenicity is a concern in the safety assessment of novel foods. The incidence of food allergies is constantly and rapidly increasing as is their severity and the number of foods involved. So far, insufficient results on HPP foods are available. In heat treated products, protein denaturation reduces the allergenicity of many foods but heat-denatured proteins can also present new antigenic sites. New studies on the putative allergenicity of HPP treated foods are envisaged. Could HPP processing become a technology to obtain hypoallergenic foods? Could it be possible that HPP foods create or unmask new immunoreactive structures? These are questions that need an answer.

The digestibility of HPP treated foods has been the subject of numerous studies. HPP induces dissociation of oligomeric proteins (Balny, 2001) with a potential increase in their digestibility. In fact, "in vitro" enzymatic digestibility is enhanced in heated or pressurized meat proteins (de Lamballerie-Anton, Delépine & Chapleau, 2001). Heating or a treatment of 200 MPa results in higher digestibility than a treatment of 500 MPa (both at 10ºC for 10 min).

Many reports on the digestibility of pressurized food components have been published. No differences in digestibility of starch, lupin and pea proteins were reported (Klepacka, Porzucek, Piecyk & Salanski, 1996; Raabe & Knorr, 1997) Other authors reported an increase in digestibility of bean proteins, bluefish products and beef rounds after pressurization (Ohmori, Shigehisa, Taji & Haya shi, 1992; Klepacka et al. 1996). Protein digestibility with no removal of the lipid fraction, and carried out by proteolysis, shows that pressure-treated hams have a better digestiblity than their unprocessed counterparts (Benomi, F. personal communication).

9. Prospects for the future

In the near future, the new non-thermal technologies will very likely replace current technologies. However this may cause confusion to the consumer. Does this mean that current technologies are not guaranteeing the safety of foods we are consuming every day?. New technologies can tackle the problem of new emergent pathogens which concern the consumers but they could also be very useful for the development of new products.

A representative survey (Baron et al. 1999) of consumer attitudes concerning HPP of foods was carried out among 300 adults aged 14 years and over in France, Germany and the United Kingdom in face-to-face computer assisted personal interviews. The variable to be predicted using the model was the willingness to buy products preserved by HPP. The acceptability values were 71% for France, 74% for Germany and 55% for the U.K. The average acceptability rate of 67%, was clearly above the threshold value of 60% (a pragmatic market research

threshold) which is extremely positive for such an emerging technology. The best predictor which optimises the classification result of potential buyers and non buyers in the three countries is mainly the hope for more personal advantage from this new technology.

Before the total implementation of the new preservation technologies, several issues need to be addressed such as: the mechanisms of microbial resistance and adaptation to these new technologies, the mechanisms of microbial and enzyme inactivation, the identification of the most resistant and relevant microorganisms in every food habitat, the role of bacterial stress, the robustness of the technologies, the increased safety versus current technologies and last but not least, the legislation needed to implement them.

In some years, there will be new technologies to be used: gamma irradiation, electron beams, microwave heating, ohmic heating, high pressure, pulsed electric field, submerged arcing, pulse lights on surfaces etc. Some of them have a high likelihood of being used in combination with other technologies.

The application in the real world of the new technologies are new challenges to the food technologists and food researchers. The need to convince consumers and stakeholders about the improvement these new technologies represent, is a must. To do so, it is very important to present convincing data, to identify stakeholders and to provide clear, objective and unbiased information including the potentially negative aspects and their limitations. It is very important to demonstrate that the technology is available or that there is existing potential to develop a given technology.

Pressure treatment is maybe, the most available emergent technology. However, it is still costly, mainly because of the initial capital expenditure, and this may limit its application. It is expected than these costs will go down as a consequence of further progress in technology, the acceptance of and resultant investment in the requisite equipment for HPP by an increasing number of manufacturers. As an example, the treatment cost of cooked ham is 0.1€ per Kg which is a cost quite affordable for the consumer.

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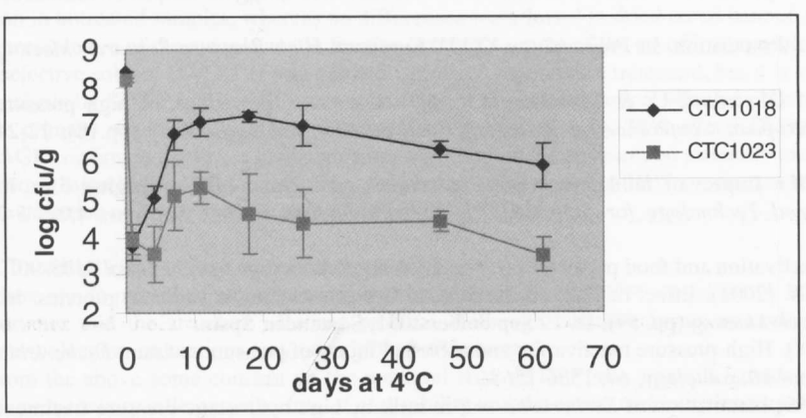
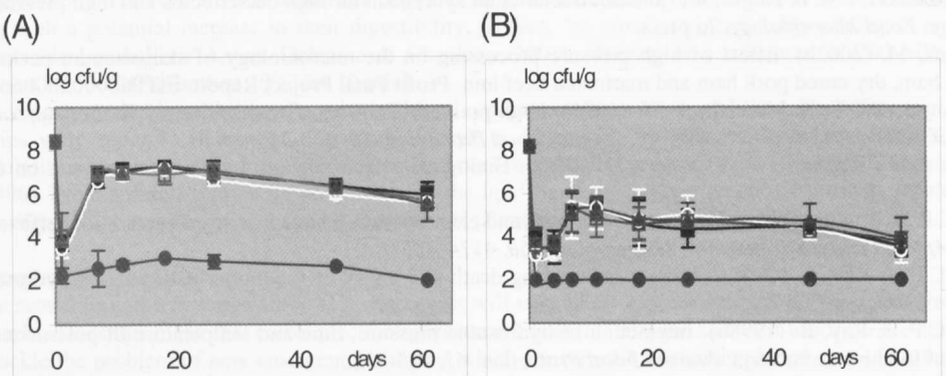
Fig 1 Behaviour of two different strains of *E.coli* (CTC1018 and CTC1023) in a meat model system after pressurization (400 MPa 10 min. 17ºC) during chilled storage. Values are the mean of triplicates.



(Garriga et al. 2002a)

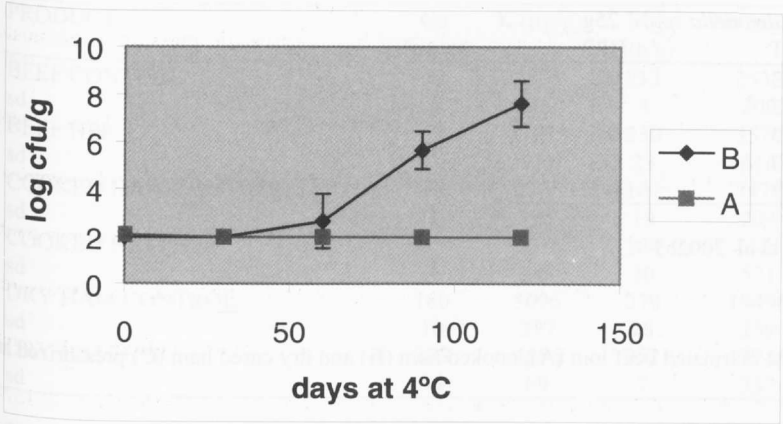
Fig 2 Behaviour of (A) *Escherichia coli* CTC1018 and (B) *E.coli* CTC1023 after HHP at 400 MPa for 10 min at 17ºC in a meat model system supplemented with bacteriocins during storage at 4ºC. Values are the mean of triplicates.



(Garriga et al. 2002a)

Treatments: ○ enterocins A and B, ▲ sakacin K, □ pediocin AcH, ● nisin A, ■ control
Values are means ± standard deviation.The minimum level of detection was 2 $\log_{10}$ cfu g^{-1}

Fig 3 Kinetics of growth of Lactic acid bacteria in cooked ham during chilled storage after pressurization (600 MPa, 31°C, 6 min) in two different pressurization machines (A and B). Values are the mean of triplicates.



(Garriga et al. unpublished)

Table 1 Bacterial decrease in log_{10} after pressurization at 500 MPa, 40°C for 10 min in phosphate buffer and in a spiked meat model consisting of cooked ham homogenized with water (3:1). Results are expressed in log_{10} cfu g^{-1}. Values are the mean of triplicates.

Strain	Phosphate buffer	Meat model
C. piscicola LMG2739	5.79	4.67
Ent. faecium CTC492	6.79	4.67
E.coli CTC1007	7.23	3.97
E.coli CTC1018	5.53	5.54
E.coli CTC1023	5.78	4.51
Lact. sakei CTC494	7.01	3.98
Lact. sakei CTC746	6.33	4.22
Lc. carnosum CTC747	6.33	3.91
L. innocua CTC1014	7.92	4.55
P. acidilactici F	5.60	3.84
Staph. carnosus LTH2102	4.75	1.29

(Garriga et al. unpublished)

Table 2 Comparison of microbial inactivation (*Staphylococcus aureus* and Lactic Acid Bacteria (LAB)) after pressurization (600 MPa, 6 min, 31°C) in spiked meat products with different water activities. Values are the mean of triplicates.

Bact. species[a]	Skin vacuum packed products[b]					
	Marinated beef		Cooked ham		Dry cured ham	
	Time 0	After HHP	Time 0	After HHP	Time 0	After HHP
Staph. aureus	3.62	0.95	3.70	2.58	2.74	2.19
Viability loss	**2.67**		**1.12**		**0.55**	
LAB	4.94	0.95	5.63	1.06	4.23	2.65
Viability loss	**3.99**		**4.57**		**1.58**	
A_w	0.985		0.978		0.890	

[a]Counts expressed as log_{10} cfu g^{-1}. 0.95 means the values were below the detection limit (10 cfu g^{-1}). [b]Triplicates for each product and sampling time. (Garriga et al. unpublished)

Table 3 Inactivation of *Listeria monocytogenes* (pool of three strains) after HHP in spiked sliced and skin vacuum-packed dry cured ham. Comparison of two different machines at 600 MPa, 31°C, 6 min. Values are the mean of triplicates.

Time	Equipment A	Equipment C
Before HHP	2,71[a]	2,75[a]
1 day after HHP	0/2[b]	3/3[b]
30 days after HHP	0/3[b]	3/3[b]
60 days after HHP	0/3[b]	3/3[b]
120 days after HHP	0/3[b]	2/3[b]

[a]log_{10} cfu g^{-1}. [b]Number of samples with presence of *L.monocytogenes* in 10 g/number of investigated samples (Garriga et al. unpublished)

Table 4 Investigation of presence of *Listeria monocytogenes* and *Salmonella spp.* in HPP treated marinated beef loin (600 MPa, 31°C, 6 min) compared to untreated (NT) during storage of samples at 4°C.

	L. monocytogenes / 25 g		*Salmonella spp.* / 25g	
Days	NT	HPP	NT	HPP
0	2/3[a]	0/3	3/3	0/3
30	2/3	0/3	2/3	0/3
60	3/3	0/3	2/3	0/3
90	1/3	0/3	0/3	0/3
120	1/3	0/3	2/3	0/3

[a] number of positive samples/ investigated samples (Garriga et al. 2002b)

Table 5 Proximate composition of pressurized meat products: marinated beef loin (A), cooked ham (B) and dry cured ham (C) pressurized at 600 MPa, 10 min 30°C.

(A)

	MARINATED BEEF LOIN			
	Control	SD	HPP	SD
Moisture (%)	74.11	0.60	73.78	0.65
Fat (%)	4.54	0.76	3.68	0.46
Protein (%)	20.64	0.83	21.43	0.50
Hydroxiproline (ppm)	677.0	316.7	558.6	130.3
NO_2 (ppm)	5.00	0.00	5.00	0.00
NO_3 (ppm)	9.67	2.31	15.67	4.04
Chloride (%)	0.74	0.03	0.83	0.09
Ash (%)	1.68	0.13	1.96	0.08
Carbohydrates (%)	0.71	0.04	0.65	0.06
Phosphate (ppm)	4786	411	3795	320
Ascorbate (ppm)	<10	0.00	<10	0.00
pH	5.44	0.01	5.80	0.03

(B)

	COOKED HAM			
	Control	SD	HPP	SD
Moisture (%)	75.20	0.24	74.02*	0.40
Fat (%)	2.63	0.38	2.97	0.89
Protein (%)	22.67	0.58	20.64	1.44
Hydroxiproline (ppm)	993.7	136.3	1043.3	56.52
NO_2 (ppm)	103.3	6.66	91.0	3.00
NO_3 (ppm)	38.33	3.06	38.0	3.61
Chloride (%)	2.06	0.04	1.80***	0.01
Ash (%)	3.16	0.05	3.18	0.09
Carbohydrates (%)	0.52	0.03	0.52	0.02
Phosphate (ppm)	4592	74	3061***	269
Ascorbate (ppm)	234	16	219	14
pH	6.42	0.02	6.52	0.04

(C)

	DRY CURED HAM			
	Control	SD	HPP	SD
Moisture (%)	50.64	0.28	50.17	1.03
Fat (%)	12.9	1.46	14.6	1.36
Protein (%)	30.56	0.70	29.88	0.50
Hydroxiproline (ppm)	2035.3	144.3	1873.0	18.08
NO_2 (ppm)	5.00	0.0	7.67**	0.58
NO_3 (ppm)	98.67	3.51	81.67*	12.7
Chloride (%)	3.76	0.10	4.63	0.14
Ash (%)	6.24	0.09	6.41	0.11
Carbohydrates (%)	0.19	0.02	0.22	0.04
Phosphate (ppm)	4590	360	3663	980
Ascorbate (ppm)	58	1	74	6
pH	5.48	0.44	6.11	0.03

* $p<0.5$ ** $p<0.01$ *** $p<0.001$; SD = standard deviation (García-Regueiro et al. 2002)

Table 6 Mineral composition of marinated beef loin (BL), cooked ham (CH) and dry cured ham (DH) pressurized at 600 MPa, 10 min 31ºC.

PRODUCT	Ca (µg/g)	K (µg/g)	Mg (µg/g)	Na (µg/g)	Cu (µg/g)	Fe (µg/g)	Zn (µg/g)
BEEF CONTROL	69	3374	213	2533	1.63	16.96	22.47
sd	2	96	3	200	0.08	0.77	1.39
BEEF HPP	69	3701*	230	3574	1.91*	20.37**	20.35
sd	19	111	23	616	0.11	0.29	6.03
COOKED HAM CONTROL	98	2765	167	7472	1.55	8.10	17.43
sd	3	195	19	209	0.05	0.72	0.63
COOKED HAM HPP	75**	2536	184	7321	1.37*	6.99	15.86
sd	4	283	20	571	0.05	0.61	1.14
DRY HAM CONTROL	180	5096	278	19496	1.93	11.55	25.26
sd	10	297	28	259	0.07	0.98	2.82
DRY HAM HPP	203	4656	252	19745	1.81	13.28	22.76
sd	35	69	7	337	0.06	1.28	1.92

* $p<0.5$; ** $p<0.01$; Analyses were done by ICP; sd = standard deviation. (García-Regueiro et al. 2002)

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