

**EFFECT OF HIGH PRESSURE PROCESSING ON THE MICROBIOLOGY OF SKIN-
VACUUM PACKAGED SLICED MEAT PRODUCTS: COOKED PORK HAM, DRY
CURED PORK HAM AND MARINATED BEEF LOIN**

FINAL REPORT

MONELLS, JANUARY 11th 2002

EFFECT OF HIGH PRESSURE PROCESSING ON THE MICROBIOLOGY OF SKIN-VACUUM PACKAGED SLICED MEAT PRODUCTS: COOKED PORK HAM, DRY CURED PORK HAM AND MARINATED BEEF LOIN

GROUP PRESENTATION AND REFERENCES

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The Food Microbiology and Biotechnology Unit of IRTA (Institute for Food and Agricultural Research and Technology) at Meat Technology Centre, Monells (Girona) Spain, will contribute to the project with its expertise in the field of microbiological analyses, isolation and characterization of several foodborne pathogens. The group had been working in several national funded projects dealing with the study and the application of lactic acid bacteria as starter or bioprotective cultures, as probiotics for animal safety and in the study of high pressure processing in combination with antimicrobials for the quality assurance of meat products. The group is also involved in an European Project Harmonization and Standardization of diagnostic Polymerase Chain Reaction (PCR) for detection of foodborne pathogens (FOOD-PCR). QLK1-CT-1999-00226. 5th Framework Program for the implementation of a Thematic Network. 2000-2002.

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INTRODUCTION

High hydrostatic pressure is being increasingly investigated in food processing. It causes microbial inactivation and therefore extends the shelf life and enhances the safety of food products. Yeasts, molds, and vegetative cells of bacteria can be inactivated by pressures in the range of 200 to 700 MPa.

Microorganisms are more or less sensitive to pressure depending on several factors such as type, strain and the phase or state of the cells. In general, Gram-positive organisms are usually more resistant than Gram-negative. High pressure processing modifies the permeability of the cell membrane, the ion exchange and causes changes in morphology and biochemical reactions, protein denaturations and inhibition of genetic mechanisms.

High pressure has been used successfully to extend the shelf life of high-acid foods such as refrigerated fruit juices, jellies and jams. There is now an increasing interest in the use of this technology to extend the shelf life of low-acid foods such as different types of meat products.

OBJECTIVE

Evaluation of sanitary risks and sensorial properties microbial indicators in sliced, skin-vacuum packaged meat products after High Pressure Processing.

Pressurisation was done at 600 MPa for 6 minutes.

Samples from treated and untreated products were kept at +4°C for 120 days.

The aim of this work was to compare pressurised products (HPP) with untreated products (NT).

METHODS

For the microbiological analyses 15-20 g of sample were homogenized (1/10) in Peptone 0.1%, NaCl 0.85% and serially diluted. An appropriate volume was poured in selective media. Incubation temperature and time are detailed below.

All analyses were carried out in triplicate at each sampling time.

The results were expressed as log CFU/g.

Listeria monocytogenes and *Salmonella spp.* were investigated in 25 g, *Campylobacter spp.* in 10 g. The results were expressed as presence or absence.

Sampling time:

Before HPP, after HPP, and during storage: at 30, 60, 90 and 120 days.

Culture media:

Aerobic total count: PCA at 30°C, 72 hours.

Psychrophiles aerobic total count: PCA at 8°, 7 days.

Lactic acid bacteria: MRS at 30°C, 72 h. in anaerobiosis.

Enterobacteriaceae: VRBG at 30°C, 24 h.

Yeasts and Fungi: SDA 2% at 25°C, 5 days.

Escherichia coli: Coli ID 37°C, 48 h.

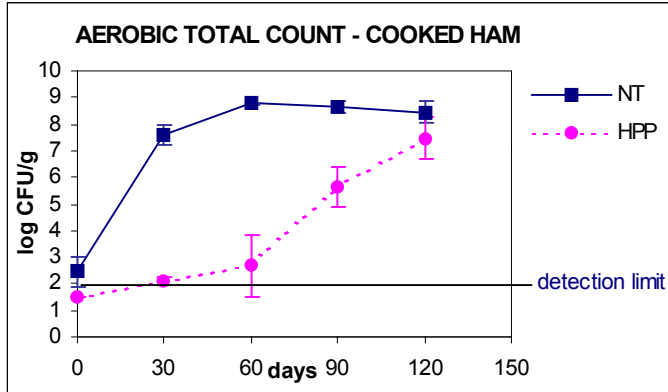
Staphylococcus aureus: Baird-Parker 37°C, 48 h.

Listeria monocytogenes: Pre-enrichment in UVM I at 30°C 24h, UVM II 30°C 24h., followed by selective isolation in Palcam 30°C 24 h. Suspected colonies confirmed by PCR.

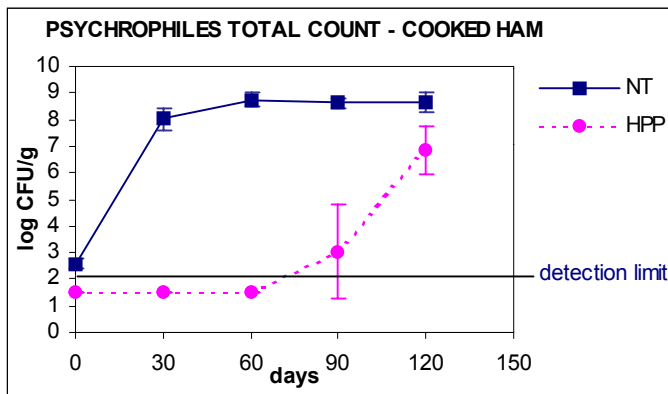
Salmonella spp. and **Campylobacter spp.** were investigated according to ISO 6579 and ISO 10272, respectively. Typical colonies were confirmed by API test.

RESULTS

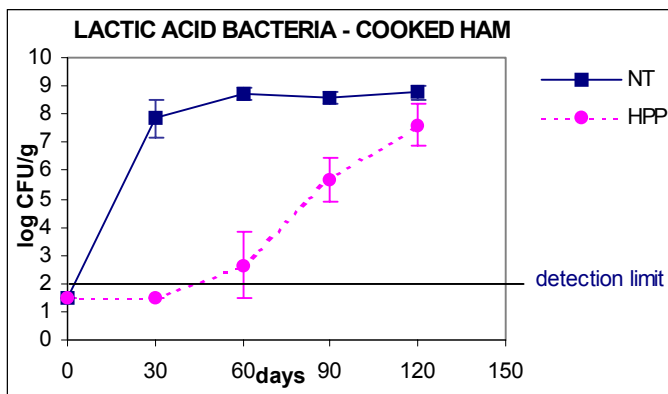
1) COOKED PORK HAM



HPP delayed growth, keeping low aerobic total counts: $< 10^4$ CFU/g at least for 60 days after treatment.

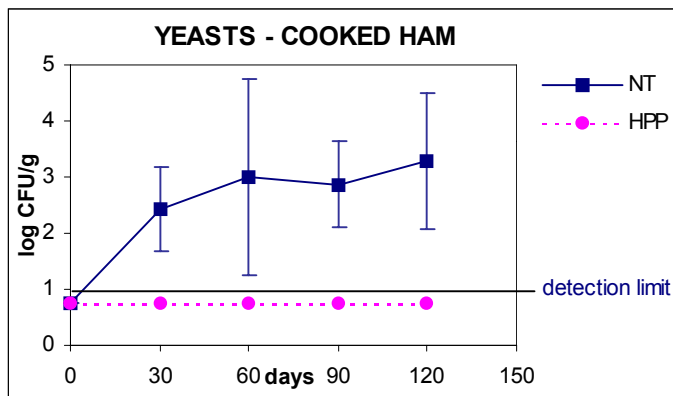


HPP delayed growth, keeping low psychrophiles total count: under the detection limit < 100 CFU/g, at least for 60 days after treatment.

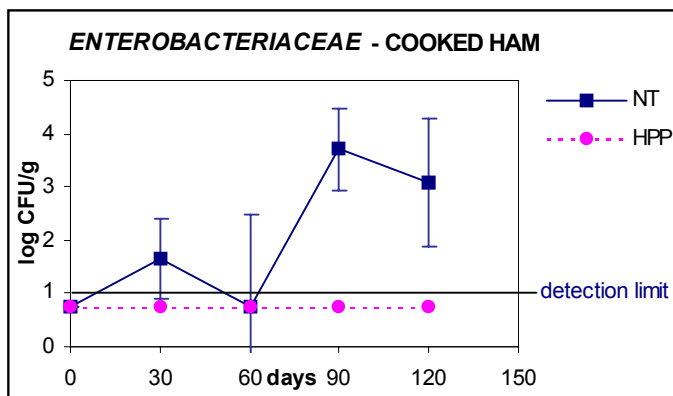


HPP delayed growth, keeping low LAB count: $< 10^4$ CFU/g at least for 60 days after treatment.

Lactic acid bacteria (LAB) were the main flora during the whole storage period.

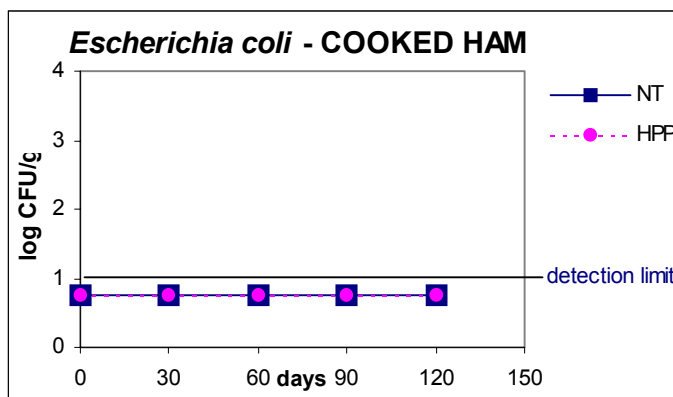


No growth of yeasts: HPP was very effective in preventing yeast growth by keeping the yeast count under the detection limit, < 10 CFU/g, during the whole storage period.

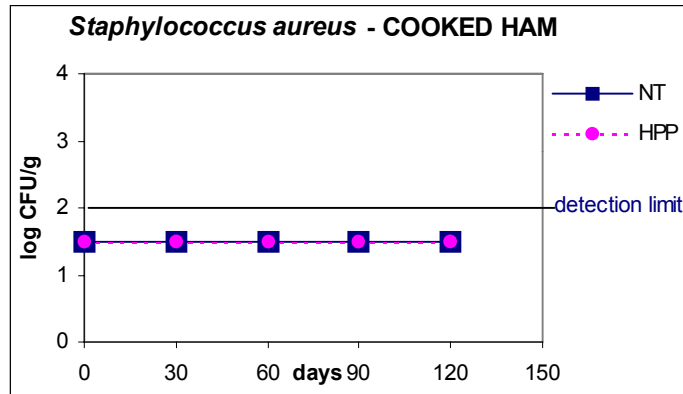


No growth of *Enterobacteriaceae*: HPP was very effective in preventing the *Enterobacteriaceae* growth by keeping the counts below the detection limit, < 10 CFU/g, during the whole storage period.

Enterobacteriaceae growth in non-treated samples showed a great variability during the storage period.



Escherichia coli was below the detection limit, < 10 CFU/g, during all the storage period, both in non-treated (NT) and pressurised (HPP) samples.



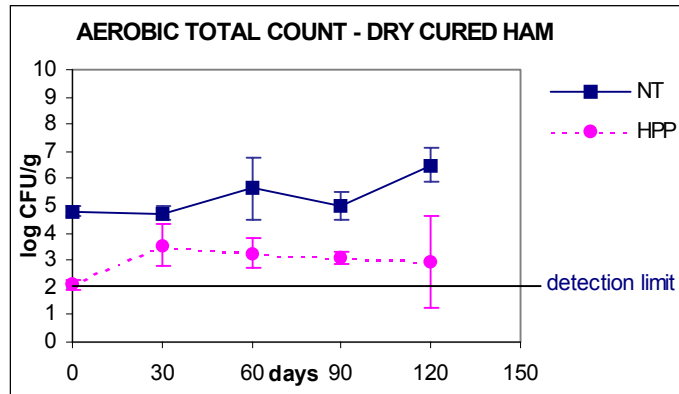
Staphylococcus aureus was below the detection limit, < 100 CFU/g, during all the storage period, both in non-treated (NT) and pressurised (HPP) samples.

Investigation of several pathogens (presence / Absence) in Cooked Pork Ham

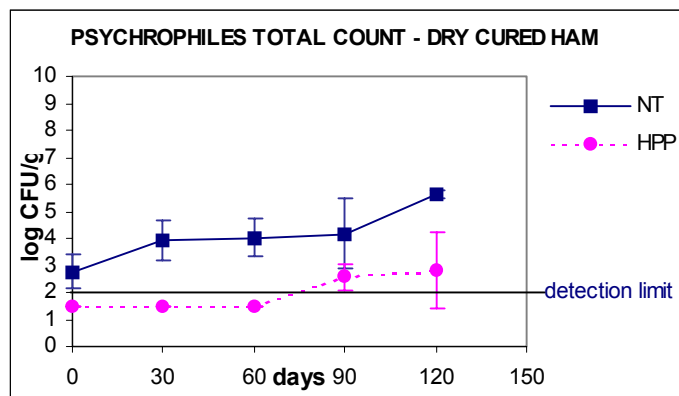
	<i>Listeria monocytogenes</i> / 25 g		<i>Campylobacter</i> spp. / 10 g		<i>Salmonella</i> spp. / 25g	
days	NT	HPP	NT	HPP	NT	HPP
0	0/3	0/3	0/3	0/3	0/3	0/3
30	0/3	0/3	0/3	0/3	0/3	0/3
60	0/3	0/3	0/3	0/3	0/3	0/3
90	0/3	0/3	0/3	0/3	0/3	0/3
120	0/3	0/3	0/3	0/3	0/3	0/3

Absence of *Listeria monocytogenes* in 25 g, *Campylobacter* spp. in 10 g, and *Salmonella* spp. in 25 g, in all samples (NT and HPP) of cooked ham tested, at any time during the storage.

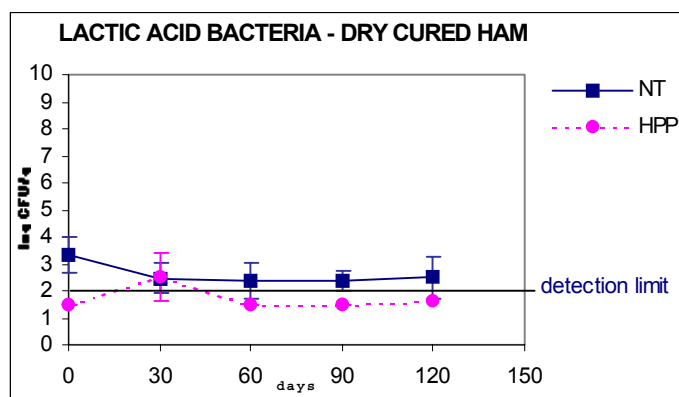
2) DRY CURED PORK HAM



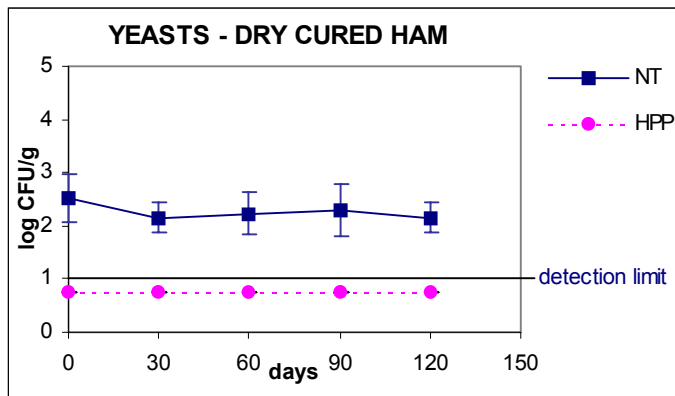
HPP reduced (more than 2 logs) the aerobic count (mainly salt tolerant Gram-positive cocci) in HPP samples, which keep lower count than NT samples during the storage period.



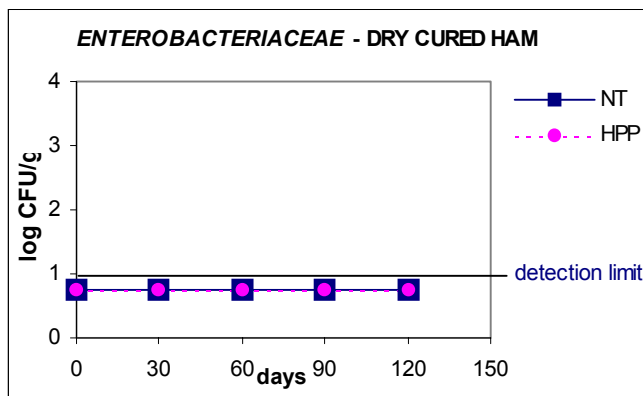
HPP reduced the psychrophiles total count under the detection limit < 100 CFU/g, at least for 60 days after treatment, keeping lower count than NT samples during the rest of the storage period.



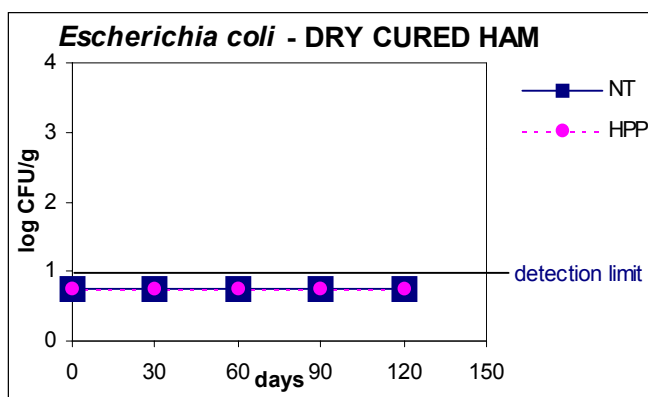
HPP reduced the lactic acid bacteria count under the detection limit < 100 CFU/g, in most HPP samples. Lactic acid bacteria were present only in very low count also in NT samples.



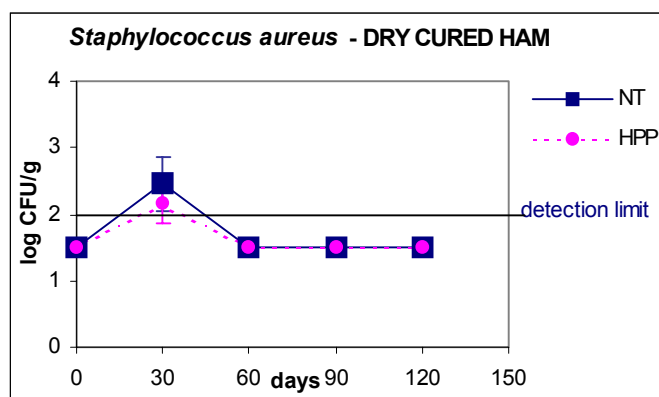
No growth of yeasts in HPP samples: HPP was very effective in preventing yeast growth by keeping yeasts count under the detection limit, < 10 CFU/g, during the whole storage period.



Enterobacteriaceae were below the detection limit, < 10 CFU/g, during all the storage period, both in non-treated (NT) and pressurised (HPP) samples



Escherichia coli was below the detection limit, < 10 CFU/g, during all the storage period, both in non-treated (NT) and pressurised (HPP) samples.



Staphylococcus aureus was below the detection limit, < 100 CFU/g, in most samples during all the storage period, with low count for both NT and HPP.

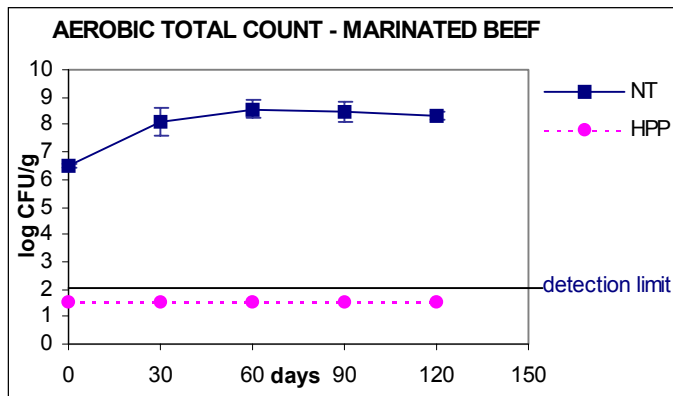
Investigation of several pathogens (Presence / Absence) in Dry Cured Pork Ham

days	<i>Listeria monocytogenes</i> / 25 g		<i>Campylobacter spp.</i> / 10 g		<i>Salmonella spp.</i> / 25g	
	NT	HPP	NT	HPP	NT	HPP
0	1/3	0/3	0/3	0/3	0/3	0/3
30	0/3	0/3	0/3	0/3	0/3	0/3
60	0/3	0/3	0/3	0/3	0/3	0/3
90	0/3	0/3	0/3	0/3	0/3	0/3
120	0/3	0/3	0/3	0/3	0/3	0/3

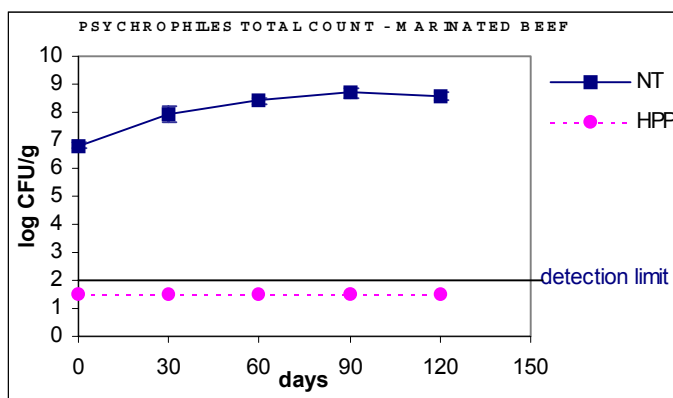
Absence of *Campylobacter spp.* in 10 g, and *Salmonella spp.* in 25 g, in all samples (NT and HPP) of dry cured ham tested, at any time during the storage time.

Presence of *Listeria monocytogenes* in 25 g, only in one untreated sample. **Absence** of *Listeria monocytogenes* in 25 g, in all HPP samples.

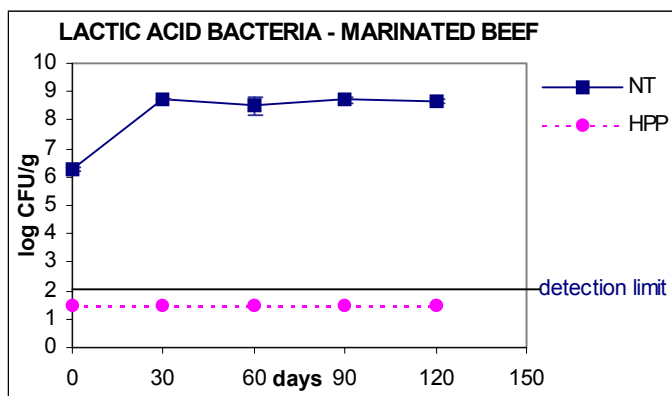
3) MARINATED BEEF LOIN



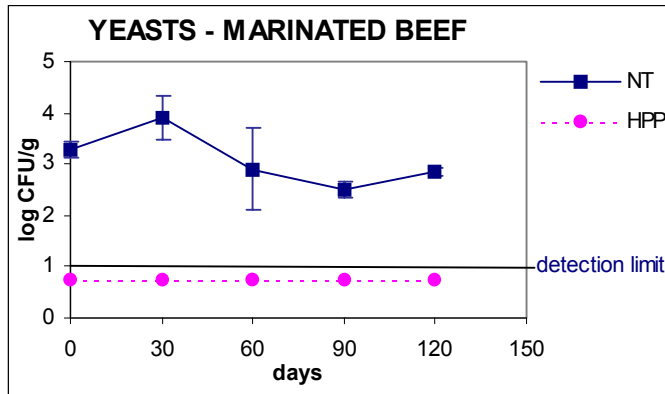
HPP reduced the aerobic total count after treatment by more than four log cycles, under the detection limit < 100 CFU/g in all HPP samples. Aerobic total count was below the detection limit in all HPP samples, during the whole storage period.



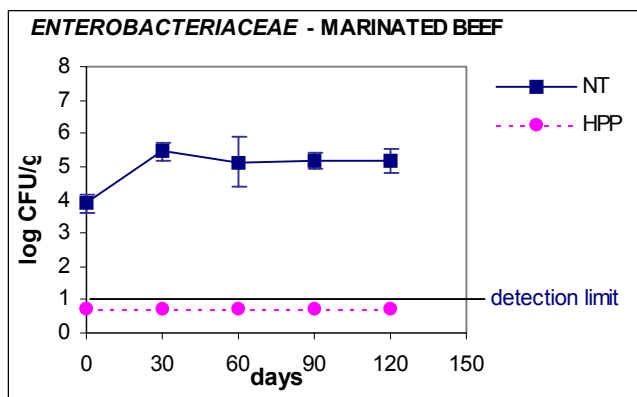
HPP reduced the psychrophiles total count after treatment by more than four log cycles, under the detection limit < 100 CFU/g in all HPP samples. Psychrophiles total count was below the detection limit in all HPP samples, during the whole storage period.



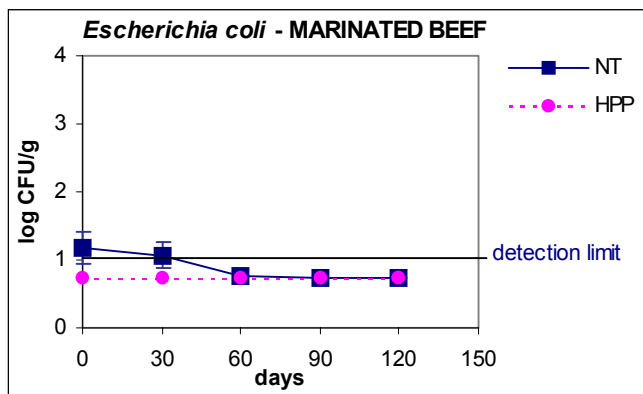
HPP reduced the lactic acid bacteria count after treatment by more than four log cycles, under the detection limit < 100 CFU/g in all HPP samples. Lactic acid bacteria count was below the detection limit in all HPP samples, during the whole storage period.



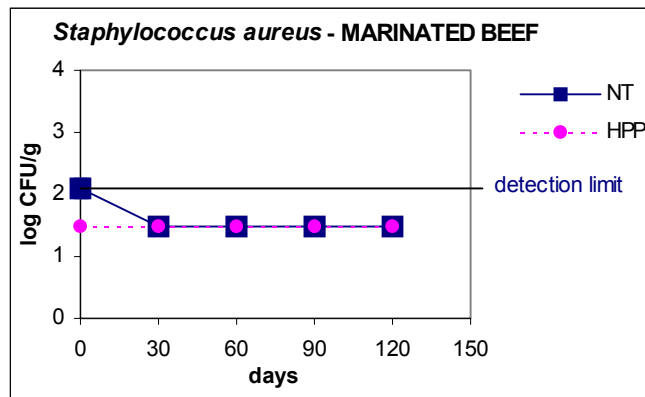
HPP reduced the yeast count after treatment by more than two log cycles, under the detection limit < 10 CFU/g in all HPP samples. Yeast count was below the detection limit in all HPP samples, during the whole storage period.



HPP reduced the *Enterobacteriaceae* count after treatment by three log cycles, under the detection limit <10 CFU/g in all HPP samples. *Enterobacteriaceae* count was below the detection limit in all HPP samples, during the whole storage period.



Escherichia coli was below the detection limit, < 10 CFU/g, during all the storage period in pressurised (HPP) samples. *Escherichia coli* were present only with very low count in untreated (NT) samples at the beginning of the storage period.



Staphylococcus aureus was below the detection limit, < 10 CFU/g, during all the storage period in pressurised (HPP) samples.

Staphylococcus aureus was present only with very low count in untreated (NT) samples at the beginning of the storage period.

Investigation of several pathogens (Presence / Absence) in Marinated Beef Loin

days	<i>Listeria monocytogenes</i> / 25 g		<i>Campylobacter</i> spp. / 10 g		<i>Salmonella</i> spp. / 25g	
	NT	HPP	NT	HPP	NT	HPP
0	2/3	0/3	0/3	0/3	3/3	0/3
30	2/3	0/3	0/3	0/3	2/3	0/3
60	3/3	0/3	0/3	0/3	2/3	0/3
90	1/3	0/3	0/3	0/3	0/3	0/3
120	1/3	0/3	0/3	0/3	2/3	0/3

Absence of *Campylobacter* spp. in 10 g. in all samples (NT and HPP) of Marinated beef tested, at any time during the storage time.

Absence of *Listeria monocytogenes* in 25 g, and *Salmonella* spp. in 25 g, in **all HPP samples** of marinated beef tested, at any time during the storage time.

Presence of *Listeria monocytogenes* in 25 g, in **nine out of fifteen untreated NT samples**.

Presence of *Salmonella* in 25 g, in **nine out of fifteen untreated NT samples**.

SUMMARY

Cooked ham

Microorganisms are present at very low levels in freshly sliced packaged cooked ham and they mainly come from cross-contamination during slicing and packaging.

Because of the high water activity of this product, spoilage microorganisms (lactic acid bacteria), grew quickly to 10^8 CFU/g in all the untreated samples in 30 days.

Sliced, skin vacuum packed Cooked Pork Ham samples, treated by High Pressure Processing at 600 MPa for 6 minutes, showed a significant delay in the growth of spoilage associated microorganisms compared to untreated samples, contributing to keep the organoleptic freshness for at least 60 days after treatment. The HPP process helped to prevent sour taste, off-flavours, ropiness and colour changes.

Yeasts and *Enterobacteriaceae* showed growth up to 3 log cycles during the storage period (120 days) in untreated samples, while in all HPP samples the number of survivors were kept under the detection limit during the whole storage period, meaning a significant improvement in the microbiological safety of HPP treated sliced cooked ham.

High Pressure Processing, in the conditions used in this assay, was an effective process to avoid growing of yeasts and *Enterobacteriaceae* with potential capacity to produce off-flavours and gas.

Escherichia coli and *Staphylococcus aureus* were below the detection limit, both in HPP and untreated samples.

Campylobacter, *Listeria monocytogenes* and *Salmonella spp.* showed absence in HPP and in untreated samples.

Dry cured ham

Dry Cured Pork Ham, is a raw, bone-in, dried, non-fermented meat product.

Because of the low water activity and high salt content of this product, spoilage microorganisms are mainly Gram-positive cocci and yeasts. They are present at the surface of the whole ham, and they reach the sliced product during final boning, slicing and packaging operations

Sliced, skin vacuum packed Dry Cured Pork Ham samples, treated by High Pressure Processing at 600 MPa for 6 minutes, showed a significant reduction of at least 2 log cycles after treatment for spoilage associated microorganisms, maintaining the survivors at low levels during the storage period, and contributing to preserve the organoleptic freshness during the whole storage period investigated (120 days), 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. *Staphylococcus aureus* was under the detection limit in most of HPP and untreated samples.

Campylobacter and *Salmonella spp.* showed absence in all the samples. *Listeria monocytogenes* was present (in 25 g.) in only one untreated sample, but absent in all HPP treated samples during the whole storage period investigated (120 days).

Marinated Beef Loin

Marinated beef loin, is a raw, uncooked meat product, with high water activity, low level of salt, without nitrite and with a mixed flora of spoilage microorganisms and pathogens from the slaughterhouse, cutting and trimming operations.

Sliced, skin vacuum packed Marinated Beef Loin, treated by High Pressure Processing at 600 MPa for 6 minutes, showed a very significant reduction of at least 4 log cycles after treatment for Aerobic, Psychrophiles and Lactic Acid Bacteria. In HPP samples the number of survivors remained unchanged and below the detection limit (< 100 CFU/g) during the whole storage period investigated (120 days), helping to prevent sour taste and off-flavours, while untreated samples reached 10^8 CFU/g after only 30 days of storage.

Yeasts in HPP samples showed a reduction of at least 2 log cycles and were kept under the detection limit (< 10 CFU / g), in all HPP samples during the whole storage period investigated (120 days).

High Pressure Processing was very effective in reducing nearly 3 log cycles the *Enterobacteriaceae* counts, and in keeping them under the detection limit (< 10 CFU/g) during the whole storage period in all HPP samples. Untreated (NT) samples showed counts of 10^5 CFU/g.

In all HPP samples, *Escherichia coli* and *Staphylococcus aureus* were kept under the detection limit (< 10 CFU/g or < 100 , CFU/g) respectively, during the whole storage period.

Campylobacter spp. recorded absence in 10 g in all HPP and untreated samples.

High Pressure Processing is a powerful tool to control risks associated with *Salmonella spp.* and *Listeria monocytogenes* in raw or marinated meats as shown from these data:

Nine out of fifteen untreated samples showed presence of *Listeria monocytogenes* in 25 g.

Nine out of fifteen untreated samples showed presence of *Salmonella spp.* in 25 g.

Fifteen out of fifteen HPP samples showed **absence** both of *Listeria monocytogenes* and *Salmonella spp.* in 25 g. at any time during the whole storage period (120 days).

Most of the untreated samples (NT) showed presence in 25 g for one of for both pathogens, whereas all pressurized samples (HPP) showed absence in 25 g.

CONCLUSIONS

High Pressure Processing at 600 MPa for 6 minutes is an efficient method to delay the growth of spoilage microorganisms in all the sliced skin packaged meat products investigated.

High Pressure Processing at 600 MPa for 6 minutes reduces significantly the safety risks associated to *Salmonella* and *Listeria monocytogenes* in all the sliced skin packaged meat products investigated.

