Application of novel processing techniques developed for the inactivation and inhibition of food-borne pathogens

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PathogenCombat for safe food

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Food processing techniques

Food can be altered:

Mechanically	→	Strokes, injuries
Physically	→	Temperature and humidity fluctuations
Chemically	→	Browning, oxidation
Biologically	→	Enzymes Microorganisms Insects and rodents

Food processing techniques

Objective:

Obtain safety product + increase the shelf life

Different processing techniques based:

- Elimination
- Avoid re-contamination
- Inhibition
- Inactivation

Food processing techniques

Elimination

Remove the microorganisms and their enzymes from food

Few application, only in liquid

- ✓CENTRIFUGAL
- ✓SETTLING
- ✓FILTRATION

Food processing techniques

Avoid re-contamination

Avoid contamination after application of different conservation method

- ✓**Packaging**
- ✓**Aseptic processing**
- ✓**Hygienic storage**

Also avoid or reduce mechanics, physics and chemicals alterations

Food processing techniques

Inhibition

Prevent microorganisms' development.
Distancing some factors from the optimal value for the growth of microorganisms

Temperature

COOLING
FREEZING

VERY COMMON

a_w DEHYDRATATION
LYOPHILIZATION
ADITION OF SOLUTE

pH ACIDIFICATION (Direct/Fermentation)

Redox potential

PACKAGING (Vacuum/Modified atmospheres)

Inhibitory substances, etc

Food processing techniques

Inactivation

permanent inactivation of all or some of the microorganisms

- ✓**High temperatures** — **VERY COMMON**
- ✓**Ionizing radiation**
- ✓**High hydrostatic pressure**
- ✓**Electric pulses**
- ✓**Intense light pulses**
- ✓**Magnetic fields**
- ✓**Protective cultures, etc**

Novel processing techniques

Food-borne outbreaks

Food-borne Outbreaks

Salmonella spp.
Campylobacter spp.
L. monocytogenes
STEC
St. aureus
Cl. botulinum
Cl. perfringens
.....

Consumers demand product
safety
good quality
healthy
fresh appearance

New technologies are needed

Novel mild and hurdle processing techniques

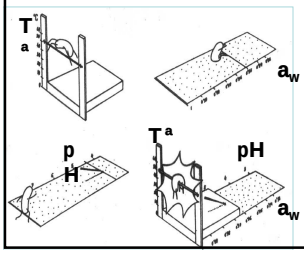
Novel processing techniques

Conventional processing techniques

Application of only one technique, with **drastic conditions**, which **modified intensively** the food

Novel processing techniques: mild techniques & hurdle technology

Application of two or more mild preservation techniques in combination, **softer**, and without **modified intensively** the food



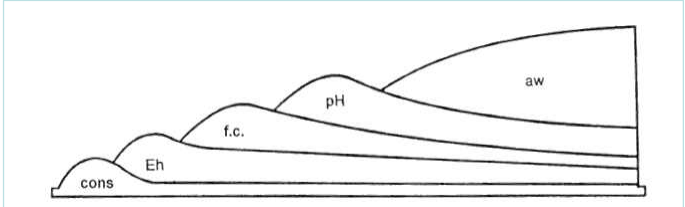
- ✓Obtain healthy and nutritious food
- ✓Less artificial additives
- ✓Preserve sensorial characteristic

Leistner, 1994

Novel processing techniques

Application in traditional product without know it

Fermented/Cured products

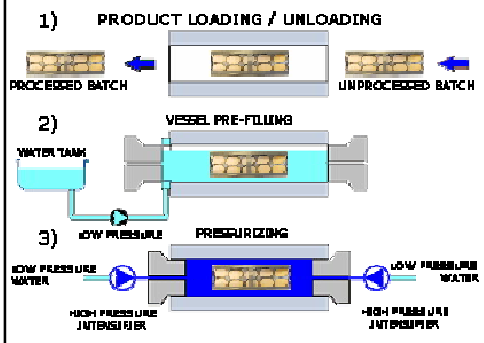


Novel processing techniques

Studies developed in Burgos University

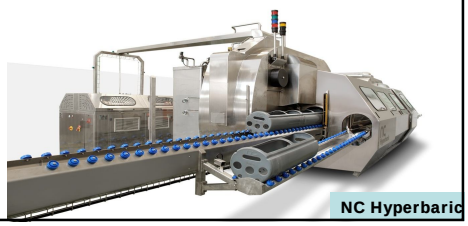
- **High Hydrostatic Pressure**
- **Modified Atmospheres packaging**
- **Protective Cultures**
- Surface pasteurization

High Hydrostatic pressure : HHP



High Pressure Processing (HPP) is a pasteurization method by which products, in its final package, are introduced into a vessel and subjected to a high level of isostatic pressure, (between 300 – 600 Mpa), transmitted by water.

This technology started to be developed at an investigation level in the 90's, and in the last years it began to be successfully implemented in food industries worldwide.



NC Hyperbaric

High Hydrostatic pressure : HHP

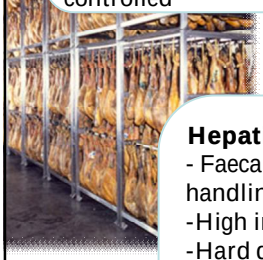
Applied to:

Dry cured meat: hams and loins
Fresh meat: suckling lamb “Lechazo de Castilla y León”
Cook meat product: blood pudding “ Morcilla de Burgos”

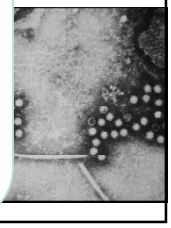
HHP in dry cured meat

Dry-cured meat
-Very popular and traditional
-Very stable and safe **ready-to-eat** food product, but there is a risk of **bacterial growth**, which have to be controlled

L. monocytogenes
Ubiquitous bacteria, that grow at refrigeration temperatures, in presence of 10%NaCl and nitrites
Susceptible groups: Pregnant women; neonates and elderly



Hepatitis E virus (HEV) and Noroviruses
- Faecal-oral transmission route (Incorrect food handling)
-High infective virus
-Hard detection:
  -Lack of efficient methods (in food)
  -Viruses do not spoil food
-It can survive at freezing conditions
-MAP technology does not provide protection
-Heating and chlorination are effective treatments



HHP in dry cured meat

Experiment 1: Challenge test using a cocktail of different L. monocytogenes strains in vacuum packaged dry-cured ham

AIM

To evaluate the efficacy of high pressure processing to inactivate *Listeria monocytogenes* in inoculated dry-cured ham

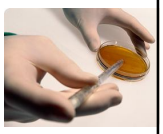
HOW DID WE DO?

Listeria monocytogenes
CECT 932
CECT 934
CECT 4032
CECT 5366

About 10^5 cfu/ml

Sliced dry-cured ham
Around 10^4 cfu/g

High Pressure Processing
500 MPa & 600 MPa
3min at 10 – 12 °C



Microbial analyses
At 24 h; 10, 50 & 75 days

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Hyperbaric

HHP in dry cured meat

Experiment 1: Challenge test using a cocktail of different L. monocytogenes strains in vacuum packaged dry-cured ham

RESULTS

Counts (log cfu/g) and detection of *L. monocytogenes* along storage in HHP treated and non treated samples (nd. Not detected)

	Time(days)			
Sample	*0*	*10*	*50*	**75**
Control	nd	nd	nd	nd
Inoculated Non-HHP	3.70	3.06	3.00	2.60
HHP (500MPa)	2.44	<1	nd	nd
HHP (600MPa)	<1	<1	nd	nd

HHP did not effect on dry-cured meat colour

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Hyperbaric

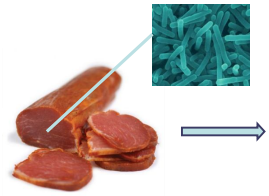
HHP in dry cured meat

Experiment 2: Natural contaminated dry-cured loins

AIM

To assess the efficacy of high pressure processing to inactivate native *Listeria monocytogenes* in naturally contaminated dry-cured loin

HOW DID WE DO?



Natural contaminated Iberian dry-cured loins

High Pressure Processing
600 MPa /10min (15 °C)

Microbial analysis
- Conventional methods
- Molecular Biology Methods

Instrumental sensory analysis
- Colour
- Odour profile

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HHP in dry cured meat

Experiment 2: Natural contaminated dry-cured loins

RESULTS

Detection and enumeration of *Listeria monocytogenes* by conventional and PCR techniques on naturally contaminated dry-cured loins

	Enumeration		Detection (in pressure-treated samples)	
	Conventional		Conventional	PCR
Samples	(untreated)	(600 MPa, 10 min)		esence
Loin A	3.11	<1	4/12	4/12
Loin B	1.85	N. D.	0/5	0/5
Loin C	3.36	<1	3/5	2/5
		Total	7/22	6/22
		%Total	31.8%	27.3%

SLIGHT CHANGES IN SENSORY ATTRIBUTES OF DRY-CURED LOINS, UNNOTICED BY PANELLISTS

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HHP in dry cured meat

Experiment 3: Challenge test using different surrogate viruses of Hepatitis E virus inoculated in dry cured hams

AIM

To evaluate the efficiency of the HHP technology to reduce or eliminate the presence of some viruses, in a real food matrix like dry-cured ham.

HOW DID WE DO?

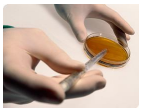
Hepatitis E virus are very infectious!!

- Wildtype strain
- ✓ **Three bacteriophages**
  - ATCC 15597-B1
  - ATCC 13706-B1
  - ATCC 13706™

Dry-cured ham
FCV 10^6 units/g
BP 10^6 & 10^8 units/g

High Pressure Processing

Pressure	Time
200 MPa	1 min
300 MPa	5 min
400 MPa	10 min
500 MPa	



Microbial analysis

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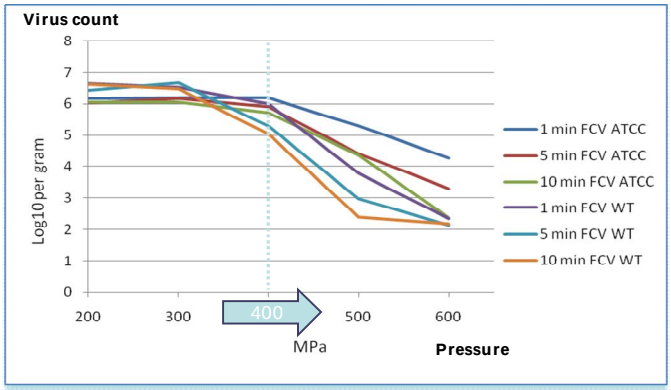
HHP in dry cured meat

Experiment 3: Challenge test using different surrogate viruses of Hepatitis E virus inoculated in dry cured hams

RESULTS

Survival of feline calicivirus (FCV) ATCC VR-2057 and wild type (WT) strains in pressure-treated dry-cured ham at different HHP conditions



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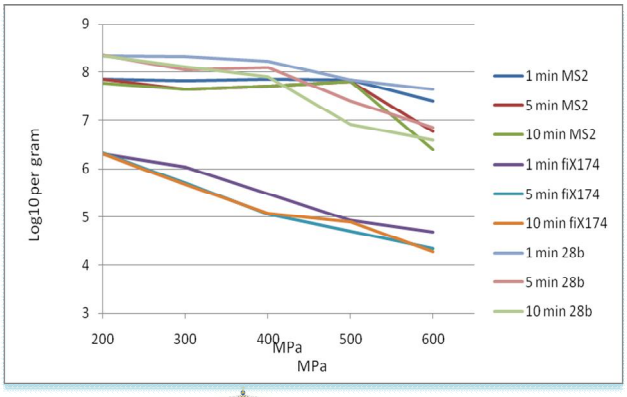
HHP in dry cured meat

Experiment 3: Challenge test using different surrogate viruses of Hepatitis E virus inoculated in dry cured hams

RESULTS

Survival of bacteriophages strains: MS2, ϕX174 and *Salmonella typhimurium* phage 28b in pressure-treated dry-cured ham at different HHP conditions



Reduction at 600 MPa during 10 min about 2 $\log_{10}$

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HHP in suckling lamb meat

Suckling lamb meat "Lechazo de Castilla y León"

- Fed exclusively with maternal milk and slaughter between 20-35 days
- High appreciable by consumers: mild flavor, tender and juicy texture.
- Characteristic pale-pink color and low fat
- Protected geographical indication (PGI)
- Short shelf-life (about 5 days)

***Escherichia coli* (STEC)**

- O157 serotype: High virulence and low infectious dose
- Vulnerable groups: Children and elderly
- EFSA (2007): "Ruminants are recognized as their natural reservoir of VTEC, in particular STEC O157"
- Wide range of temperature growing
- It survive freezing temperatures, and low pH
- Thermal inactivation and chemical disinfection and irradiation are effective treatments

HHP in suckling lamb meat

Experiment 4: Sensory test assessing pressure-treated suckling lamb meat at different high hydrostatic pressure conditions

AIM

To evaluate how do high pressure conditions affect suckling lamb meat appearance and select optimal conditions (pressure and pressure-time) that minimise meat colour change compared to fresh meat

HOW DID WE DO?

Vacuum-packaged suckling lamb meat

High Pressure Processing

Pressure	Time
150 MPa	2 min
225 MPa	6 min
300 MPa	10 min

Colour
Lightness (L*)
Redness (a*)
Yellowness (b*)

Sensory analysis
Meat colour
Fat colour
Brightness
Acceptability

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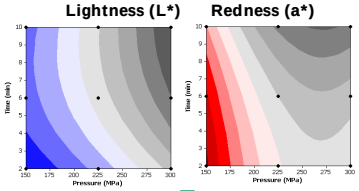
Hyperbaric

HHP in suckling lamb meat

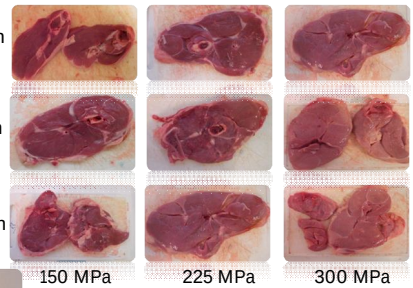
Experiment 4: Sensory test assessing pressure-treated suckling lamb meat at different high hydrostatic pressure conditions

Panellists noticed a difference in meat appearance at 300 MPa

Lightness (L*) **Redness (a*)**



Time



Maximum HPP conditions without noticeable colour changes
Pressure: 250 MPa
Holding time: 3 min

250 MPa , 3min

Pressure

Non pressure-treated sample

High pressure processing conditions **delayed one week** formation of unpleasant odours in lamb meat

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COLEAR

Hyperbaric

HHP in suckling lamb meat

Experiment 5: Challenge test using a cocktail of Escherichia coli strains in vacuum packaged suckling lamb meat

AIM

To evaluate the efficacy of a mild high pressure treatment to inactivate *Escherichia coli* in inoculated vacuum-packaged suckling lamb meat

HOW DID WE DO?

Escherichia coli
NCTC 12079 (O157:H7)
CECT 504
CECT 434

Around 10^5 cfu/ml

Vacuum-packaged suckling lamb meat steaks

High Pressure Processing
250 MPa / 3 min (From Exp. 4)
13 °C

Microbial analysis
At 24 h; 3, 8, 12, 15 & 19 days

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COLEAR

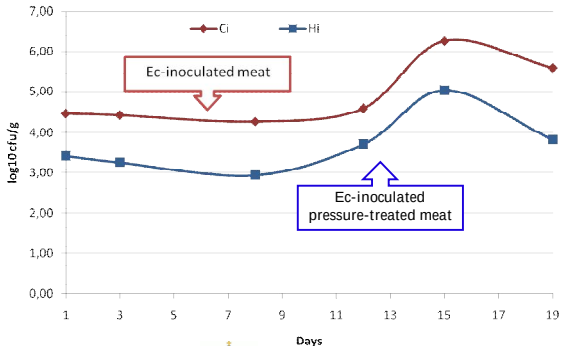
Hyperbaric

HHP in suckling lamb meat

Experiment 5: Challenge test using a cocktail of Escherichia coli strains in vacuum packaged suckling lamb meat

RESULTS

Escherichia coli (EC) **O157 strain** (NCTC 12079) count evolution (log10 cfu/g) in untreated (CN), **EC inoculated (Ci),** pressure-treated (HHP) and **EC-inoculated pressure-treated (Hi)** suckling lamb meat in refrigerated storage.



Reduction of <1 $\log_{10}$ cfu/g EC O157

Escherichia coli NCTC 12079 is baroresistant !

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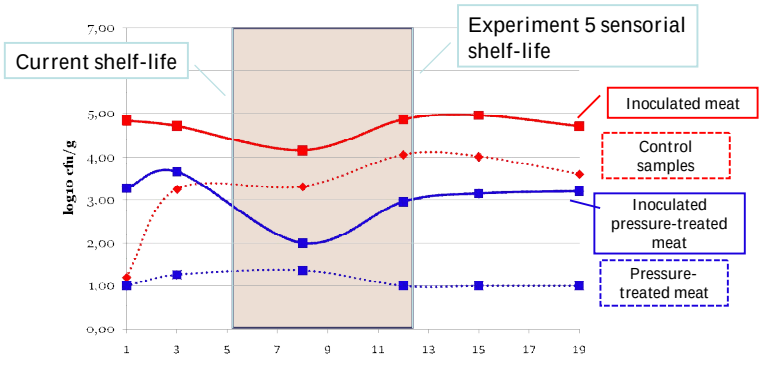
Hyperbaric

HHP in suckling lamb meat

Experiment 5: Challenge test using a cocktail of Escherichia coli strains in vacuum packaged suckling lamb meat

RESULTS

Escherichia coli (EC) **non-O157 strains** count evolution (log_{10} cfu/g) in untreated (CN), EC inoculated (Ci), pressure-treated (HHP) and EC-inoculated pressure-treated (Hi) suckling lamb meat in refrigerated storage



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HHP in blood sausages

"Morcilla de Burgos"
- Traditional and very appreciable meat cook product by Spanish consumers
-Ingredients: blood, rice, onion, fat, salt and spices

Vacuum packaging and cooling storage (4ºC): shelf-life 14 to 21 days

Spoilage microbial bacteria: *Weissella viridescens*, *W. confusa*, *Leuconostoc mesenteroides*, *L. carnosum*, *Lactobacillus fructosus* and *L. sakei*.

HHP in blood sausages

Experiment 6: study of vacuum blood sausages in HPP

AIM

To evaluate the efficacy of HHP in a cook traditional product (blood sausages)

HOW DID WE DO?

Blood sausages **Cook** **Vacuum-packaged**

High Pressure Processing

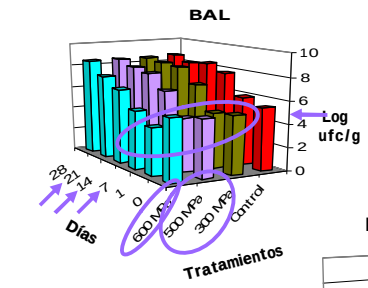
Pressure	Time
300 MPa	10 min
500 MPa	10 min
600 MPa	10 min

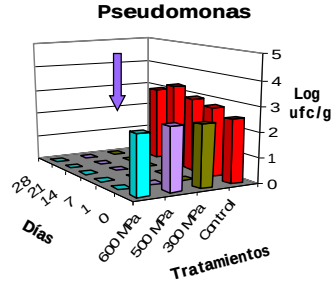
Microbial analysis
At 0,1,7,14 and 28 days

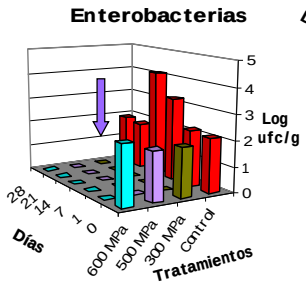
HHP in blood sausages

Experiment 6: study of vacuum blood sausages in HPP

RESULTS







Other experiments LAB 3 log ufc/g, shelf-life increase till 36 days

Diez et al. 2008

Modified atmosphere packaging

Modified atmosphere packaging (MAP):
Elimination of air in a package followed by injection of a gas or gas mixture, selected according to the properties of food, where packaging works as barrier and isolating (in greater or lesser degree) the inside environment of the outer atmosphere

-Dynamic atmosphere: product metabolism, absorption of gas by the product and permeability

-Object: increase the shelf life of food products, decreasing the growth of spoiled microorganisms and keeping the safety and quality

-Gases: O_2, CO_2, and N_2

Modified atmosphere packaging

Experiment 7: Study of suckling lamb meat under modified atmospheres

AIM

To evaluate the efficacy of different MAP to increase the shelf-life of suckling lamb meat

HOW DID WE DO?

suckling lamb meat steaks

Atmospheres:
15/30 (%O2/%CO2) (C)
70/30 (%O2/%CO2) (O)
15/85 (%O2/%CO2) (H)
Air (A)

Microbial analyses
(conventional, PCR & DGGE)
Physic-chemical analyses
Sensory analyses
At day 0, 4, 7, 11, 14 and 18

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Modified atmosphere packaging

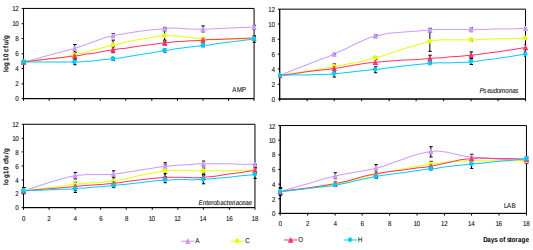
Experiment 7: Study of suckling lamb meat under modified atmospheres

RESULTS

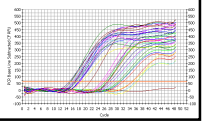
Physic-chemical and sensory analysis:

pH & aw constant
TBARS increase along the time
Color: L* constant
a* lower in H and higher in O
b* lower in C and higher in H
Sensory shelf-life: A day 4; C day 7; O day 11; **H day 14**

Microbiology analysis:
Conventional



PCR: STEC was present along all the study



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Modified atmosphere packaging

Experiment 7: Study of suckling lamb meat under modified atmospheres

RESULTS

DGGE:
- *Br. thermosphacta*, *C. divergens* and *L. sakei* appears all the time independently of the MAP
- CO2 is responsible for *Pseudomonas* and *Psychrobacter* disappearance
- Atmosphere with 85%CO2 there are less species

MAP with 85% CO_2 obtained the best results

Problem: exudation ⟶ 50-60%CO_2

Size (bp)	Closest relative	%Identity	Source[b]
164	*Carnobacterium maltaromaticum*	99	FJ656722
160	*Lactobacillus sakei*	93	FJ040202
154	*Carnobacterium divergens*	100	FJ656716
101	*Bacillus sp.*	96	DQ658919
162	*Staphylococcus equorum*	99	GQ169122
154	*Bacillus cereus*	100	GQ226038
167	*Enterobacter hormaechei*	99	FJ608246
162	*Psychrobacter sp.*	98	GQ169118
62	*Psychrobacter sp.*	100	AY700222
146	*Psychrobacter sp.*	99	AF513418
166	*Acinetobacter sp.*	100	EU977657
164	*Brochothrix thermosphacta*	99	AY543029
167	*Pseudomonas sp.*	99	FM161360
162	*Pseudomonas fluorescens*	99	AJ971392
165	*Pseudomonas sp.*	99	AM421976
101	*Pseudomonas sp.*	100	FN377722
125	*Ovis aries*	100	AM711877
166	*Jeotgalicoccus sp.*	97	GQ304892
147	*Escherichia sp.*	100	DQ857009

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Modified atmosphere packaging

Experiment 8: Challenge test using a cocktail of Escherichia coli strains in suckling lamb meat

AIM

To evaluate the efficacy of different modified atmospheres to inactivate *Escherichia coli* in inoculated suckling lamb meat

HOW DID WE DO?

Escherichia coli
NCTC 12079 (O157:H7)
CECT 504
CECT 434

Around 10^5 cfu/ml

suckling lamb meat steaks

Atmospheres:
15/60 (%O2/%CO2) (A)
15/30 (%O2/%CO2) (B)
Vacuum (V)
Air (A)

Microbial analysis
At days 1,4, 7, 10,14, 17

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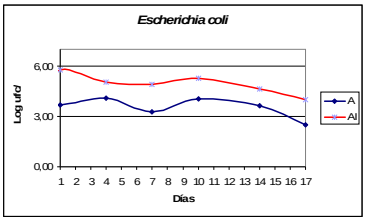
Modified atmosphere packaging

Experiment 8: Challenge test using a cocktail of Escherichia coli strains or L. monocytogenes strains in suckling lamb meat

RESULTS



- All MAP improved microbiological shelf-life of suckling meat lamb, delaying the growth of mesophilic aerobic bacteria (shelf-life from 7 to 11 days)
- MAP avoid *E.coli* growth, throughout chilled storage. No inhibit
- MAP treatment kept sensory characteristics of suckling lamb meat such as colour and odour
- According to microbiological and sensory results, the best performance atmosphere was High CO2-content MAP

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Protective Cultures

Lactic acid bacteria (**LAB**) are common use as protective cultures

During their growth released to the environment metabolic products as lactic acid, acetic acid, hydrogen peroxide or bacteriocins, which have antimicrobial properties

Bacteriocins are peptides or peptide complexes, synthesized ribosomal, with bactericidal or bacteriostatic activity against species, usually closely related to the production strain

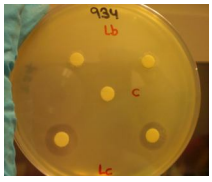
Bacteriocin-producing microorganisms used in food are:*Carnobacterium, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* y *Propionibacterium*

Protective Cultures

Antimicrobial activity

PCD101 (*Lactobacillus pentosus*) (0/8) *L. monocytogenes* strains (4 CECT & 4 isolated from food samples

PCK18 (*Leuconostoc pseudomesenteroides*) (6/8) *L. monocytogenes* strains (4 CECT & 4 isolated from food samples)



Protective Cultures

Experiment 9: Challenge test using a cocktail of L. monocytogenes strains in suckling lamb meat under modified atmospheres

AIM

To evaluate the efficacy of *L. pseudomesenteroides* against *L. monocytogenes* in inoculated suckling lamb meat under modified atmospheres

HOW DID WE DO?

L. monocytogenes
CECT 5366,
CECT 934,
CECT 4032
LTA0022

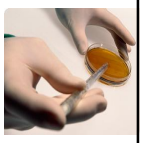
Around
10^4 cfu/ml

suckling lamb meat steaks

Atmospheres:
15/60 (%O2/%CO2) (A)
15/30 (%O2/%CO2) (B)
Vacuum (V)
Air (A)

10^4 cfu/ml
10^7cfu/ml

Leuconostoc pseudomesenteroides PCK 18



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COLEAR

PROBIOTICAL
MOFIN ALCE GROUP

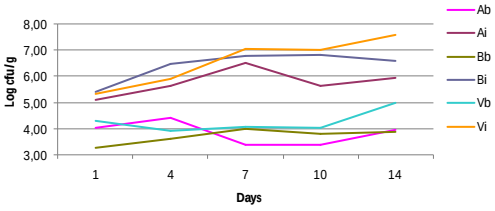
Microbial analyses (At day 0, 1, 4, 7, 10, 14 and 18)

Protective Cultures

Experiment 9: Challenge test using a cocktail of L. monocytogenes strains in suckling lamb meat under modified atmospheres

RESULTS

Listeria monocytogenes



PCK18 log 7 cfu/g

- **Reduce 2-3 log** *L. monocytogenes* in lamb meat

-Atmosphere with high CO_2 keep the lowest load of *L. monocytogenes*

- Protective culture-inoculated samples causes odour problems

PCK18 log 4 cfu/g

- Protective culture-inoculated samples did not modified the sensory characteristics
- *L. monocytogenes* inoculated in lamb meat was not reduced
- Atmosphere with high CO_2 keep the lowest load of *L. monocytogenes*

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Surface pasteurization

Experiment 10: Challenge test using a cocktail of L. monocytogenes strains in dry cured ham

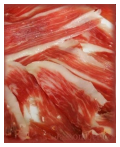
AIM

To evaluate the efficacy of surface pasteurization against *L. monocytogenes* in dry cured ham

HOW DID WE DO?

L. monocytogenes

Around 10^7 cfu/ml



Dry cured ham
Vacuum packaging

Surface Pasteurization
80ºC 2 min hot water immersion.
100º C 2 min hot water immersion.

Microbial analysis
At days 1,4, 7, 10,14, 17

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Surface pasteurization

Experiment 10: Challenge test using a cocktail of L. monocytogenes strains in dry cured ham

RESULTS

1) **80°C: 2 log reduction**. Sensory parameters affected, but acceptable.
2) **100°C: 7 log reduction**, sensory parameters were affected. Unacceptable.

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THANK YOU FOR YOUR ATTENTION