STAZIONE SPERIMENTALE PER L'INDUSTRIA DELLE CONSERVE ALIMENTARI

Validazione processo produttivo per *Listeria* e *Salmonella*

Silvana Barbuti

Dipartimento Microbiologia

Tel. 0521 795 267

Mail: silvana.barbuti@ssica.it

Guida Pratica

per la sicurezza microbiologica in impianti produttori di Prosciutto di Parma che esportano verso Paesi terzi a «tolleranza zero»

NORMATIVE DI RIFERIMENTO - IGIENE NELLA PRODUZIONE

Reg. 852/2004 - Sull'igiene dei prodotti alimentari

Reg. 853/2004 - Norme specifiche in materia di igiene per gli alimenti di origine animale

- ☐ Obblighi generali
- ☐ Requisiti generali e specifici in materia di igiene
- ☐ Analisi dei pericoli e punti critici di controllo (HACCP)
- ☐ Manuali di corretta prassi operativa

Reg. 2073/2005
Criteri microbiologici per gli alimenti

- ☐ Criteri di sicurezza (safety) alimentare
- ☐ Criteri di igiene di processo

9 CFR 301, 303 *et al*. 2001
Performance Standards for the Production of Certain Meat and Poultry Products

- ☐ RTE Meat and Poultry Products
- ☐ Performance Standards and HACCP
- ☐ The Proposed Performance Standards
  - ☐ A. Lethality
  - ☐ B. Stabilization
- ☐ Listeria monocytogenes
  - ☐ A. Proposed Requirements for Controlling L. monocytogenes
  - ☐ B. Shelf-life and Labeling

HACCP

Concetto fondamentale dell'HACCP: eliminare o ridurre a livelli accettabili i pericoli che possono essere insiti nella produzione di un certo alimento

Validazione: ottenere evidenze che una misura di controllo o una combinazione di misure è implementata correttamente ed è in grado di controllare il pericolo

HACCP Principles

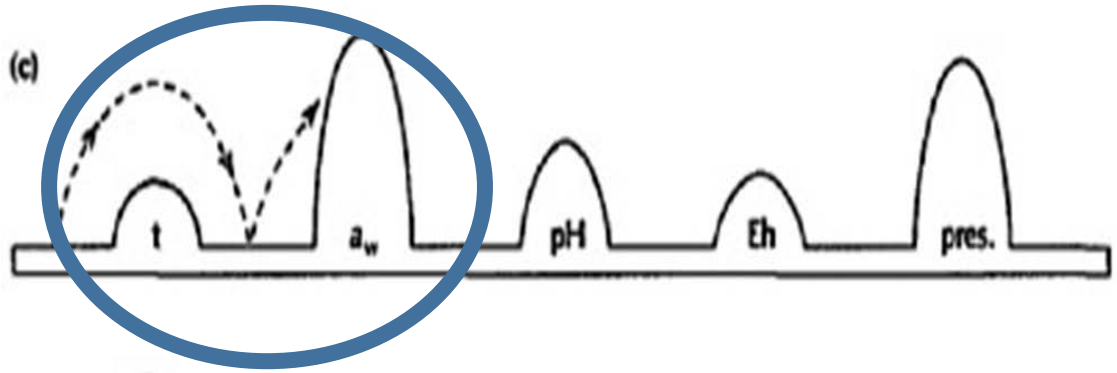
1 Hazard Analysis
2 Critical Control Points
3 Critical Limits
4 Monitoring
5 Corrective Actions
6 Verification, including Validation
7 Record-keeping

Il processo produttivo è in grado di garantire l'eliminazione di batteri patogeni

«step di letalità» ≈ «**ostacoli multipli»** con valutazione di una "letalità effettiva" dell'intero processo produttivo

I fattori che contribuiscono alla sicurezza/stabilità dei prodotti carnei stagionati sono:

- attività dell'acqua (aw)
- pH
- tempo/ temperature/ umidità relativa
- sale
- microbiota



Validazione di processo

Codex Alimentarius 2008 - 9 CFR 417.4

I processi possono essere validati attraverso l'uso di differenti approcci che comprendono:

- ☐ La letteratura scientifica
- ☐ L'uso dei modelli predittivi
- ☐ Studi di Microbial challenge test

Non tutti questi approcci devono essere utilizzati ma spesso alcuni approcci possono essere combinati per fornire sufficienti evidenze.

Listeria in prosciutto crudo

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Listeria monocytogenes prevalence, contamination levels and strains characterization throughout the Parma ham processing chain

Vincenza Annunziata Prencipe [a,*], Valentina Rizzi [a], Vicdalia Acciari [a], Luigi Iannetti [a], Armando Giovannini [a], Andrea Serraino [b], Davide Calderone [c], Andrea Rossi [d], Daniela Morelli [a], Lucio Marino [a], Giacomo Migliorati [a], Vincenzo Caporale [a]

[a] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, 64100 Teramo, Italy
[b] Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, Facoltà di Medicina Veterinaria, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia, Bologna, Italy
[c] Consorzio del Prosciutto di Parma, Via Marco dell'Arpa 8/b, 43100 Parma, Italy
[d] Centro Ricerche Produzioni Animali – C.R.P.A. S.p.A., C.so Garibaldi, 42 42121 Reggio Emilia, Italy

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Processing chain

ABSTRACT

In order to estimate prevalence, levels and patterns of *Listeria monocytogenes* contamination, a total of 774 swine carcasses were traced along the Parma ham production chain. Analyses were conducted on isolates originated from the same carcass, collected at different stages during processing, resulting in 0.2% (faeces at intestine removal from carcasses), 3.0% (swabbing of carcasses), 12.5% (fresh hams) and 2.0% prevalence (dry-cured hams). The highest contamination levels of *L. monocytogenes* were reached in fresh hams after cutting and were followed by a marked decrease during the subsequent processing stages. All the 132 isolates were characterized by serotyping and Pulsed-Field Gel Electrophoresis (PFGE). Transfer of *L. monocytogenes* between different stages of the processing chain was not reported, whereas processing itself has proved to be an important cause of contamination. The sole isolate of fecal origin belonged to a pulsotype that was uncommon to any of those recovered in carcasses, fresh hams and dry-cured hams, indicating that contaminations from farms does not significantly affect Parma ham production. For the majority of the strains isolated from the same production plants, PFGE profiles were highly similar. In several cases, the same pulsotypes were recurrently detected, over time, in carcasses and fresh ham samples sharing the same processing environment. A_w levels were also measured, showing that drying of the ham surface was able to induce a considerable decrease of the contamination levels, although unable to ultimately remove *L. monocytogenes*.

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1. Introduction

Listeria monocytogenes is a Gram-positive bacterium that causes severe food poisoning in humans. Severe listeriosis is mainly associated with groups of people considered most at risk, such as the elderly, pregnant women, immunocompromised persons (European Food Safety Authority, 2007) and may result in 16% mortality rate. (CDC, 2009).

Control of *L. monocytogenes* contamination is particularly difficult, since the pathogen can survive and grow in adverse conditions including refrigeration temperatures (2°-4 °C), a pH range of 4.0–5.0 and high saline concentrations (a_W 0.90–0.92; 11.5% NaCl) (Gandhi & Chikindas, 2007; Nolan, Chamblin, & Troller, 1992; Parish & Higgins, 1989). Ready-to-eat (RTE) products represent a major source of foodborne illness since they are not subjected to treatments able to reduce *L. monocytogenes* loads before consumption. Several outbreaks have been associated with the consumption of RTE food such as raw milk products, soft cheese, smoked fish products, sliced meat products and delicatessen (European Food Safety Authority, 2007).

Among RTE products, Parma ham is at risk of contamination from *L. monocytogenes* due to its physical and chemical features, the processing equipment and the environment used in the various processing phases (Grisenti et al., 2004). Parma ham represents a substantial source of income for the Italian pork industry, being exported worldwide. So far, very few studies focused on the dry-cured ham production process (Barbuti, Ghisi, & Campanini, 1989; Giovannini et al., 2007). Also, previous Authors have documented the occurrence of *L. monocytogenes* in dry-cured ham following experimental inoculation (Grisenti et al., 2004; Portocarrero, Newman, & Mikel, 2002; Reynolds, Harrison, Rose-Morrow, &

* Corresponding author. Tel.: +39 0861 3321x461; fax: +39 0861 332251.
E-mail address: v.prencipe@izs.it (V.A. Prencipe).

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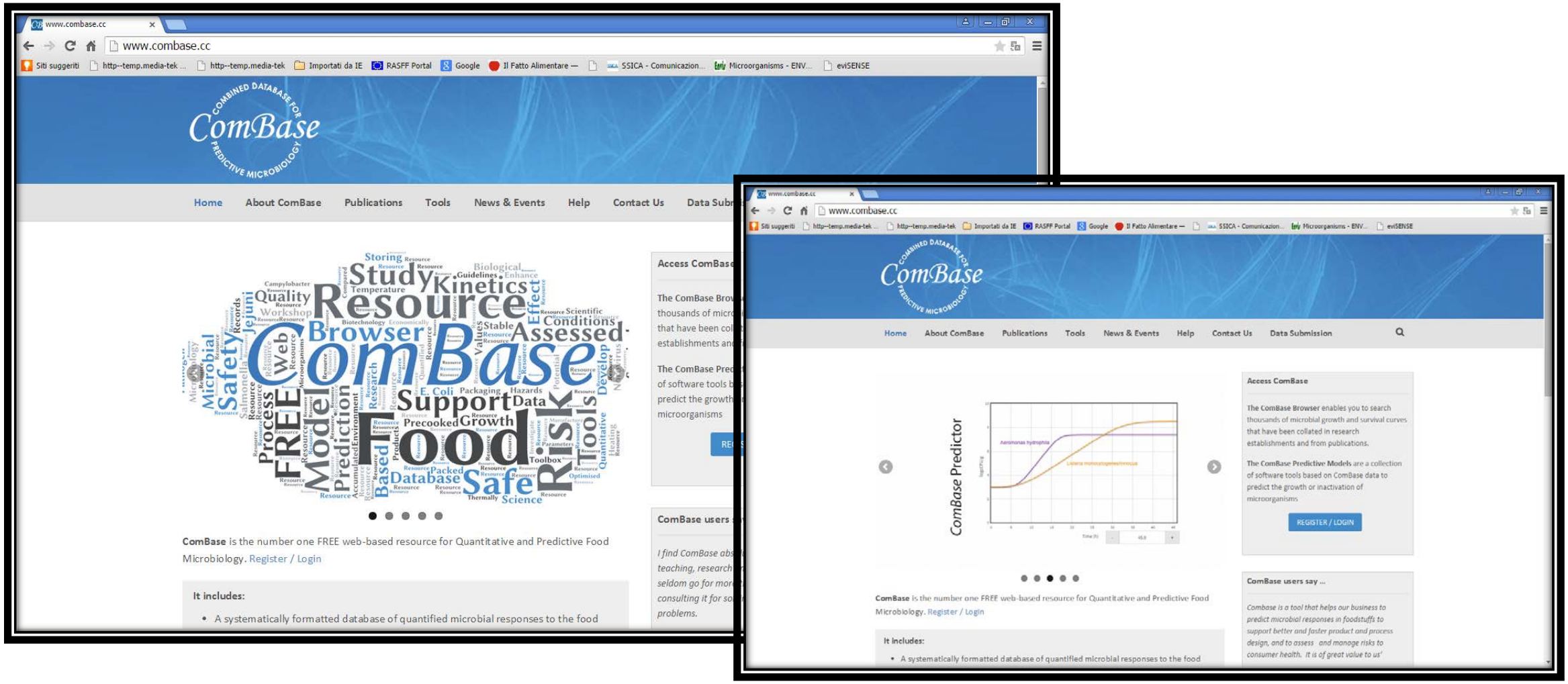
1. Contaminazione superficiale
2. Basso livello di rilevamento
3. Esclusa la possibile crescita durante il processo produttivo
4. Ricontaminazione attraverso l'ambiente di lavorazione

Software di modellazione

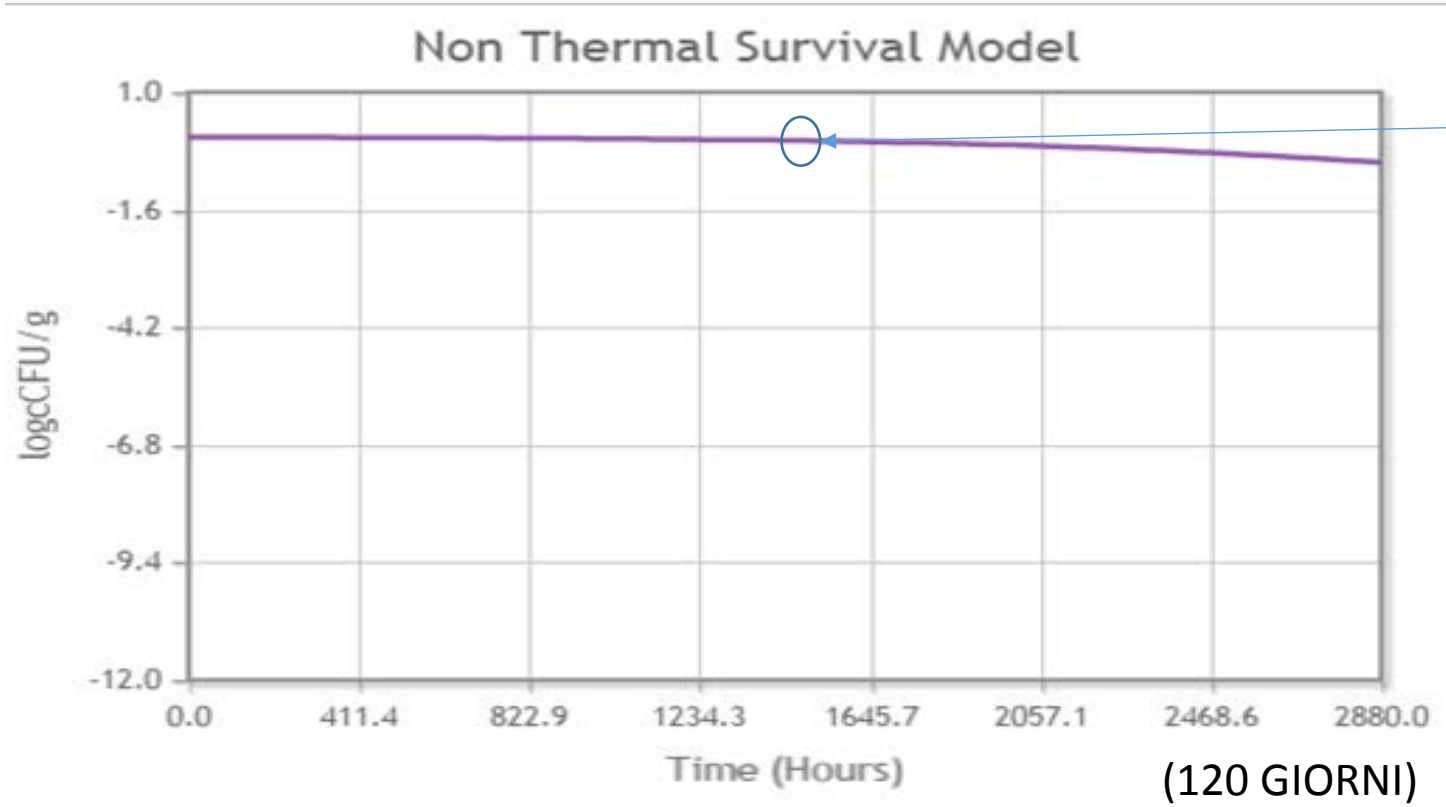


Dati registrati durante il processo produttivo

Fasi di lavorazione	Prelievi superficiali°			Parametri tecnologici	
	A_w	NaCl %	Umidità %	Giorni	T°C
Fine sale	0,885*			25	5
Fine riposo	0,906	4	38,5	65 - 100	5
Lavaggio					
Asciugamento	0,884	3,74	34,7	5 -7	16 - 23
Prestagionatura	0,874	3,13	29,8	90	16
Sugnatura					
Stagionatura 1	0,909	3,38	36,1	150	17
Stagionatura 2	0,911	3,34	40,7		17 -18

° *I dati sono stati estrapolati da:* *N. Simoncini et al. / Food Microbiology 24 (2007) 577–584*

Fasi “fredde” salagione e riposo - *Listeria*



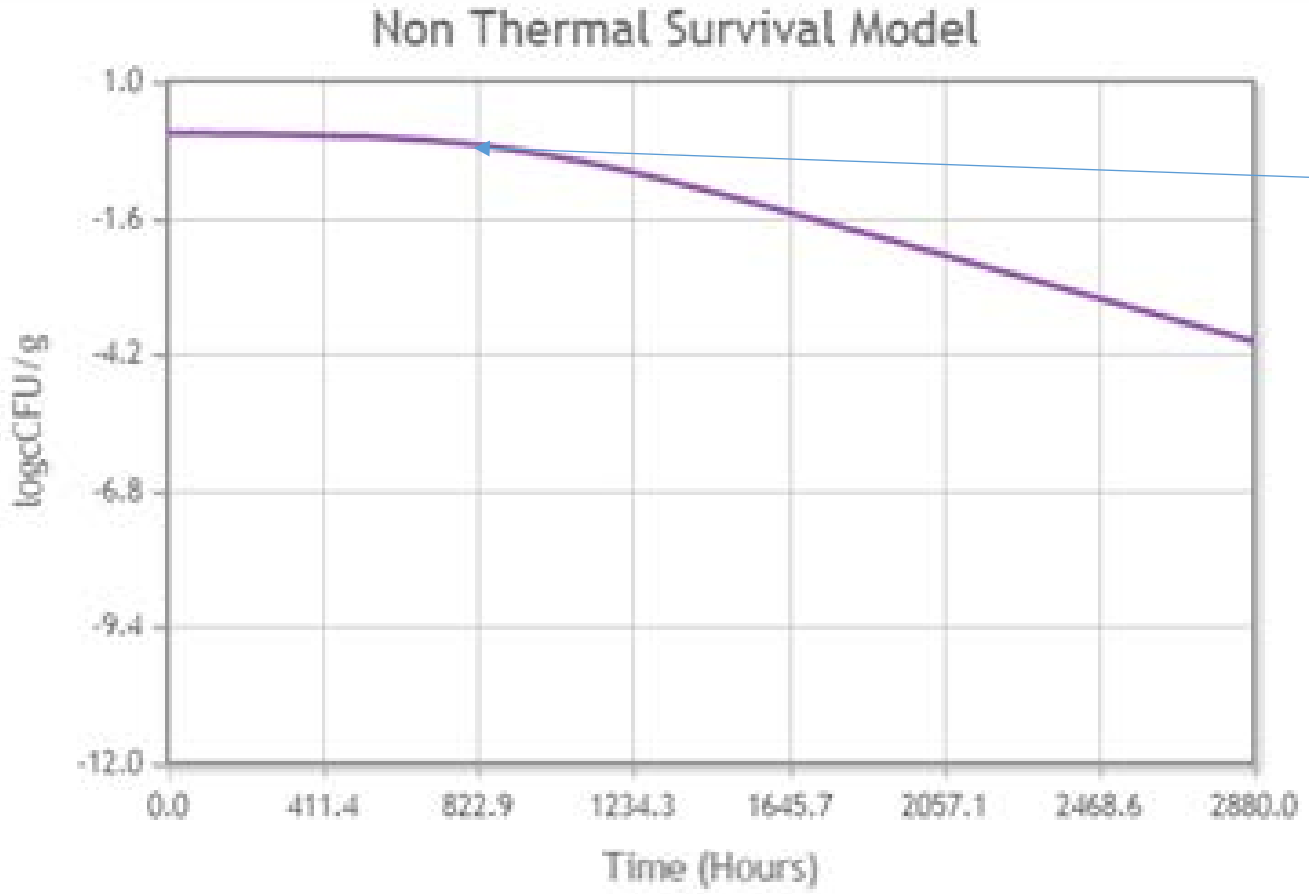
66 gg

(120 GIORNI)

— Listeria monocytogenes/innocua

Initial Level	Phys. state	Temp (C)	pH	Aw	Max.rate (log.conc/h)	D-value (Hours)
0	1.2e-2	5	6	0.91	**-0.001**	**1239.261**

Fasi “fredde” salagione e riposo - *Salmonella*

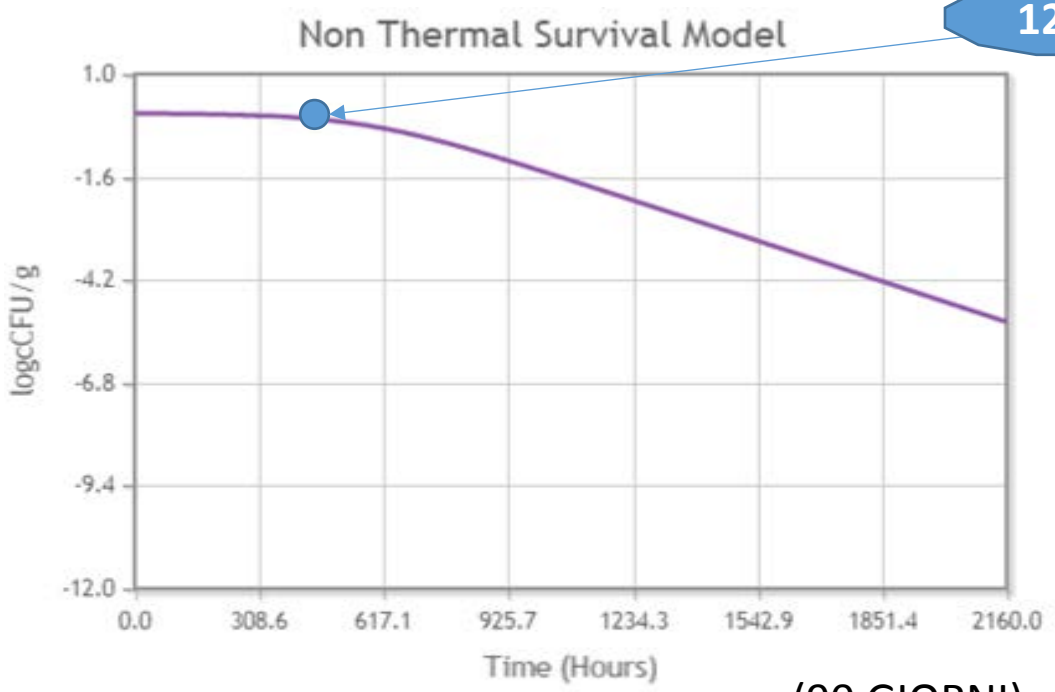


34 gg

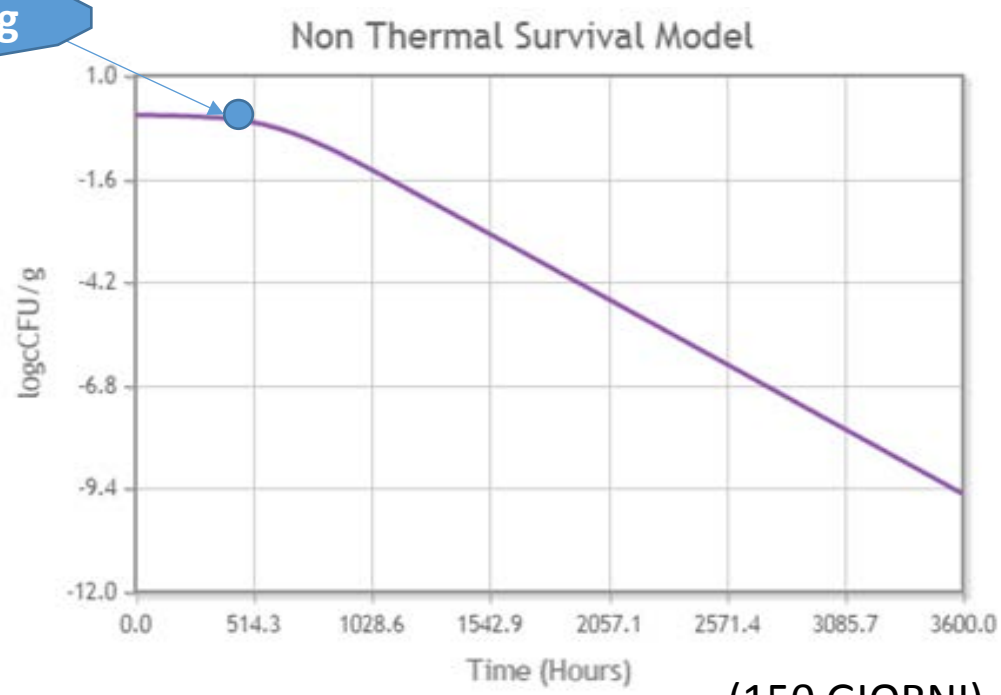
Initial Level	Phys. state	Temp (C)	pH	Aw	Max.rate (log.conc/h)	D-value (Hours)
0	1.5e-2	5	6	0.91	-0.002	497.728

Fasi “calde” prestagionatura e stagionatura – *Listeria*

12 gg



(90 GIORNI)



(150 GIORNI)

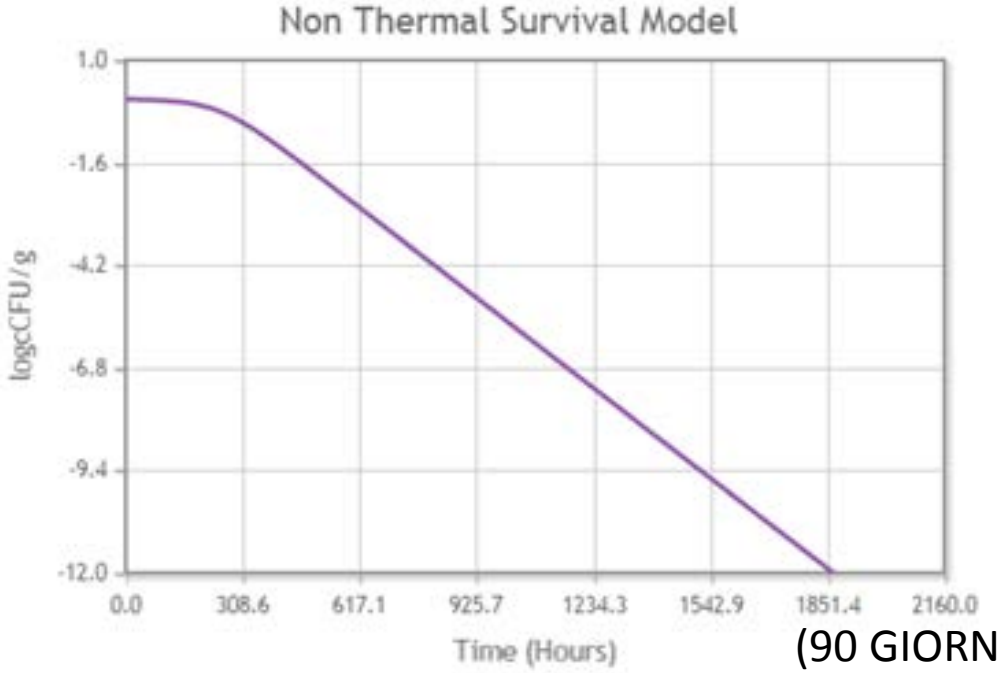
— Listeria monocytogenes/innocua

Initial Level	Phys. state	Temp (C)	pH	Aw	Max.rate (log.conc/h)	D-value (Hours)
0	1.2e-[illegible]	16	6	0.885	-0.003	301.353

— Listeria monocytogenes/innocua

Initial Level	Phys. state	Temp (C)	pH	Aw	Max.rate (log.conc/h)	D-value (Hours)
0	1.[illegible]e-2	17	6	0.91	-0.003	314.847

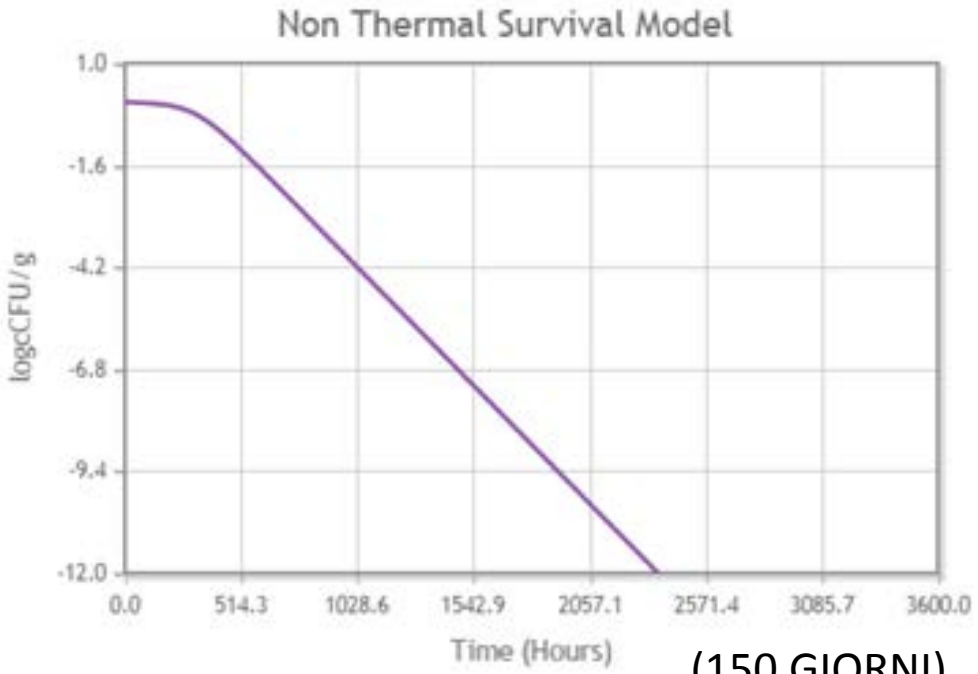
Fasi “calde” prestagionatura e stagionatura - *Salmonella*



(90 GIORNI)

— Salmonella spp

Initial Level	Phys. state	Temp (C)	pH	Aw	Max.rate (log.conc/h)	D-value (Hours)
0	1.5e-2	16	6	0.885	-0.007	135.152



(150 GIORNI)

— Salmonella spp

Initial Level	Phys. state	Temp (C)	pH	Aw	Max.rate (log.conc/h)	D-value (Hours)
0	1.5e-2	17	6	0.91	-0.006	170.928

Microbial challenge test di processo

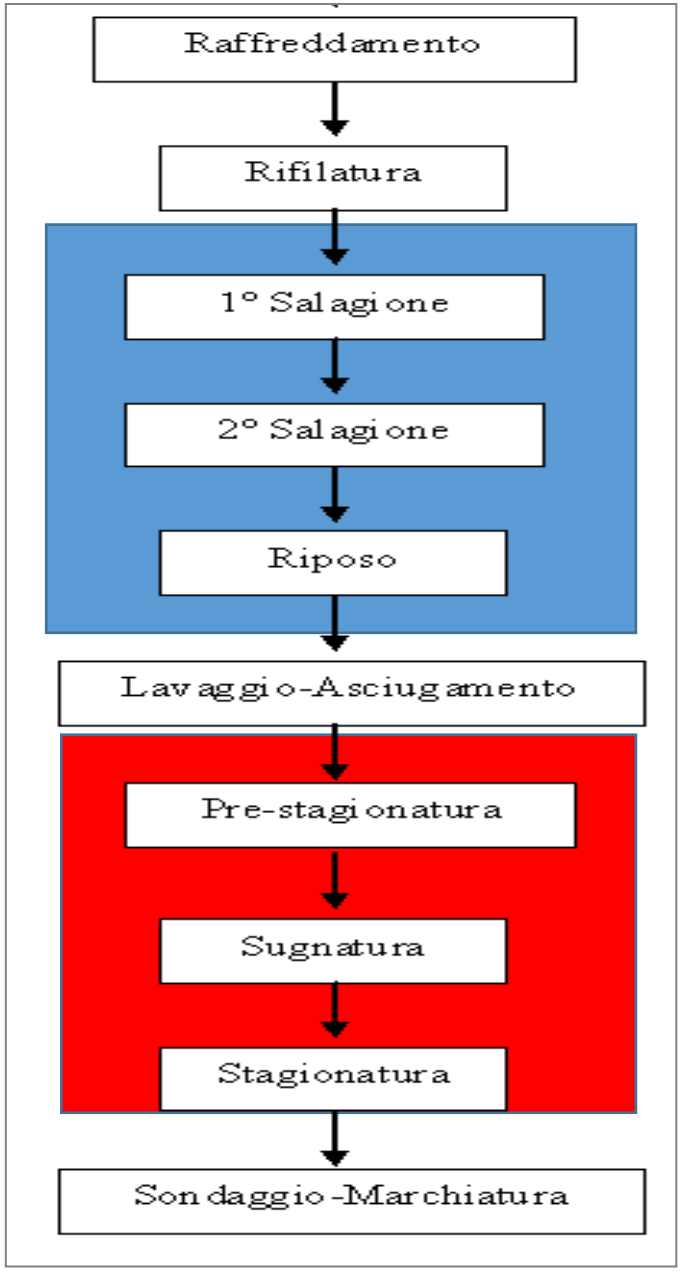
Consiste in una simulazione effettuata in laboratorio di ciò che accade ad un prodotto durante la produzione, la distribuzione e la manipolazione

MCT di processo - comporta l'inoculo, in condizioni ambientali controllate, di un numero rilevante del microrganismo oggetto dello studio nella materia prima e la valutazione di cosa succede al microrganismo durante il processo produttivo

CROSS-CONTAMINAZIONI DURANTE LA PRODUZIONE

ARRIVO COSCE FRESCHE		
STOCCAGGIO COSCE FRESCHE	SALATURA E RIPASSO	DISSALATURA
		
TOELETTATURA	SUGNATURA	CONTROLLO QUALITA' E MARCHIATURA
		
SPEDIZIONE		

MCT DI PROCESSO



LISTERIA	SALMONELLA
10^6UFC/G 10^4 UFC/G (2 D)	10^6UFC/G 10^5 UFC/G (1D)
10 UFC/G (5D)	100 UFC/G (4D)

LISTERIA	SALMONELLA
10^5UFC/G	10^5UFC/G
ASSENZA IN 25 G (> 5D)	ASSENZA IN 25 G (> 5D)

MCT - PROCESSO PRODUTTIVO

ICoMST 2009
55th International Congress of Meat Science and Technology

PE6.10 Validation of the Manufacturing Process of Italian Dry-Cured Ham (Prosciutto) for the Inactivation of Listeria Monocytogenes and Salmonella spp. 361.00

Silvana Barbuti (1) silvana.barbuti@ssica.it, Maria Silvia Grisenti(1), Maria Angela Frustoli (1), Giovanni Parolari (1)
(1)S.S.I.C.A.

Abstract— In order to validate the manufacturing process of Italian typical dry-cured ham, two independent microbial challenge tests (MCT) were performed. The hams were obtained from heavy pigs then processed according to guidelines for Italian typical dried hams. A mixture containing Listeria monocytogenes and Salmonella spp. were inoculated (5-6 log CFU g-1) onto the surface (N. 3 leg areas) of either raw hams (MCT1) or hams at mid ripening time (MCT2) and microbiological analyses were made at several stages of processing. Results from both MCTs show a 4-log reduction for both pathogens over a time span of 4 months (MCT1) and 6 months (MCT2), respectively. The log reduction did not differ (P>0.05) between the three inoculation areas, whose aw values were always <0.92 at the last sampling time. It is concluded that processing hams according to given guidelines is effective to achieve pathogen-free Italian-type dried hams.

S. Barbuti is with Stazione Sperimentale Industria Conserve Alimentari, 43121 Parma, ITALY. (corresponding author to provide phone: +39-0521 795267; fax: +39 0521 795218; e-mail: silvana.barbuti@ssica.it).

M.S. Grisenti is with Stazione Sperimentale Industria Conserve Alimentari,(e-mail: silvia.grisenti@ssica.it).

M.A. Frustoli is with Stazione Sperimentale Industria Conserve Alimentari,(e-mail: angela.frustoli@ssica.it)

G. Parolari is with Stazione Sperimentale Industria Conserve Alimentari,(e-mail: giovanni.parolari@ssica.it).

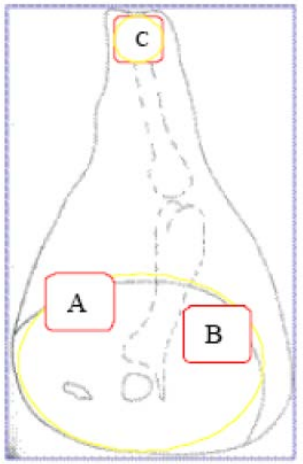
Index Terms— Processing validation, Italian dry cured ham, Listeria, Salmonella, microbial challenge test.

I. INTRODUCTION

ITALIAN typical dry-cured hams have been traditionally manufactured according to given procedures, such as those established by the Parma Ham Consortium Regulation (DPR, 1978). Basic manufacturing steps include salting, resting, washing, drying, maturing and ageing [3], with the first two stages being accomplished under refrigeration (0-4°C), while the subsequent phases require greater temperatures (15-20°C). Because of the long curing time (>12 months since salting) and consequent reduction of aw values, the finished hams are generally regarded as ready-to-eat safe meat products posing no microbial foodborne risk. Apart from investigations performed on other types of hams [4, 5], no systematic work has been done on Italian dried hams to demonstrate the ability of the manufacturing process to inactivate such major pathogens as Salmonella and Listeria monocytogens which are potential contaminants of raw meat or at successive phases of processing. Sea salt, the only additive allowed in manufacturing, is added to the legs by a salting machine and partly manually by sprinkling the salt onto specified leg portions such as the aitch and the femur head bone. Moreover, at six months of processing (end of maturing), the ham surface is manually covered with a spreadable mince made up of ground pork fat and salt (ham fattening) in order to prevent excessive surface drying. Being manually performed, these two steps (salting and fattening) may be regarded as potential sources of microbial contamination, in addition to the contamination of the meat itself. Accordingly, a comprehensive microbial challenge test of traditionally-made dried hams should take into account two independent stages of manufacturing such as salting and fattening, with the former reflecting contamination from meat + salt, and the latter from fat application. Therefore we designed two independent MCTs aimed at validating the manufacturing process of Italian typical dry cured hams as possibly affected by microbial contamination at the salting and fattening steps respectively.

II. MATERIALS AND METHODS

A. Processing parameters Two MCTs were performed, with the hams prepared or processed as described below. 1st MCT. N. 62 pork hind legs from a local slaughterhouse were selected and manufactured in compliance with requirements



A FASI PREDDE
- 5D Listeria
- 4D Salmonella

B FASI CALDE
- >5D Listeria
- >5D Salmonella

C INTERO PROCESSO PRODUTTIVO
- >5D PATOGENI

SOPRAVVIVENZA DI LISTERIA DURANTE LA FASE FINALE DI STAGIONATURA

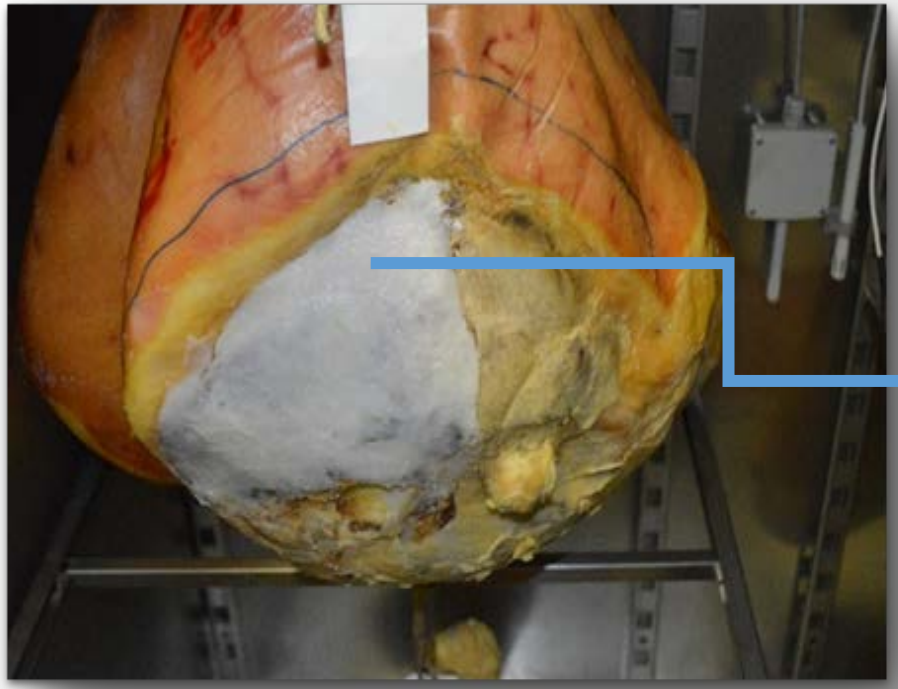
DISEGNO SPERIMENTALE

RIMOZIONE DI PARTE DELLA SUGNA

INOCULO CON LISTERIA

STAGIONATURA

RISULTATI

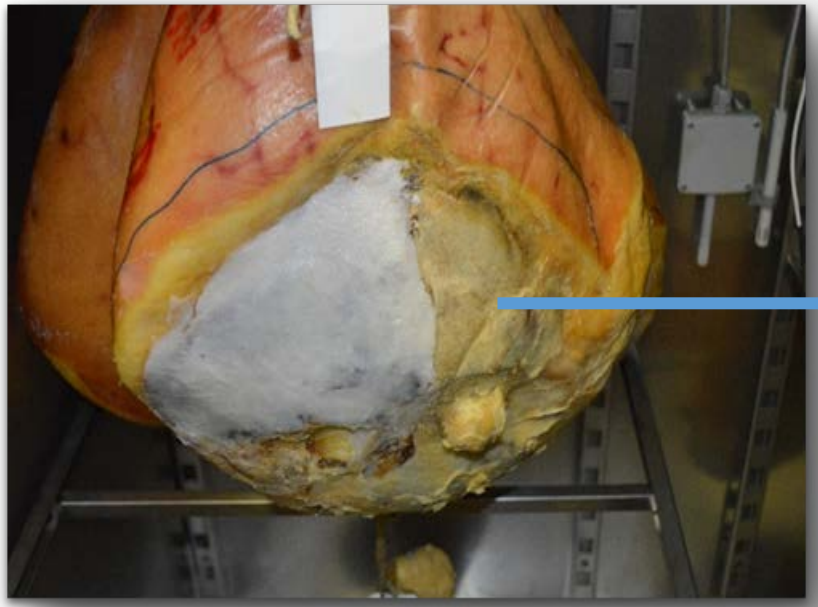


CONTAMINAZIONE SOTTO LA SUGNA

AZIENDA	T=0	T=12	T=30	T=90
A	presente (<5 ufc/g)	presente (<5 ufc/g)	assente	assente
B	presente (<5 ufc/g)	presente (<5 ufc/g)	assente	assente
C	presente (<5 ufc/g)	presente (<5 ufc/g)	assente	assente

RISULTATI

CONTAMINAZIONE SOPRA LA SUGNA



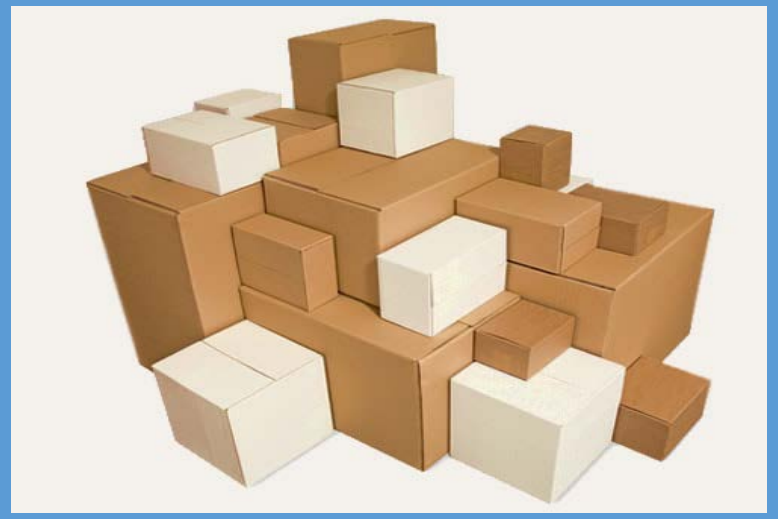
AZIENDA	T=0	T=30	T=90 (media di cinque campioni)
A	10 ufc/g	assente	assente
		assente	
		assente	
B	10 ufc/g	assente	assente
		assente	
		assente	
C	70 ufc/g	5 ufc/g	assente
		10 ufc/g	
		assente	

RISULTATI

CONTAMINAZIONE SOPRA LA COTENNA E SUL GRASSO

PORZIONE	AZIENDA	T=0	T=30	T=90
COTENNA	A	10 ufc/g	assente	assente
			assente	
			assente	
	B	80 ufc/g	assente	assente
			assente	
			assente	
	C	30 ufc/g	**PRESENTE (<5 ufc/g)**	assente
			assente	
			assente	
GRASSO	A	60 ufc/g	assente	assente
			assente	
			assente	
	B	50 ufc/g	assente	assente
			assente	
			assente	
	C	20 ufc/g	**PRESENTE (<5 ufc/g)**	assente
			assente	
			assente	

CROSS-CONTAMINAZIONI DOPO LA PRODUZIONE



Considerazioni

Validazione del processo produttivo

- La letteratura scientifica
- L'uso dei modelli predittivi
- Studi di Microbial challenge test

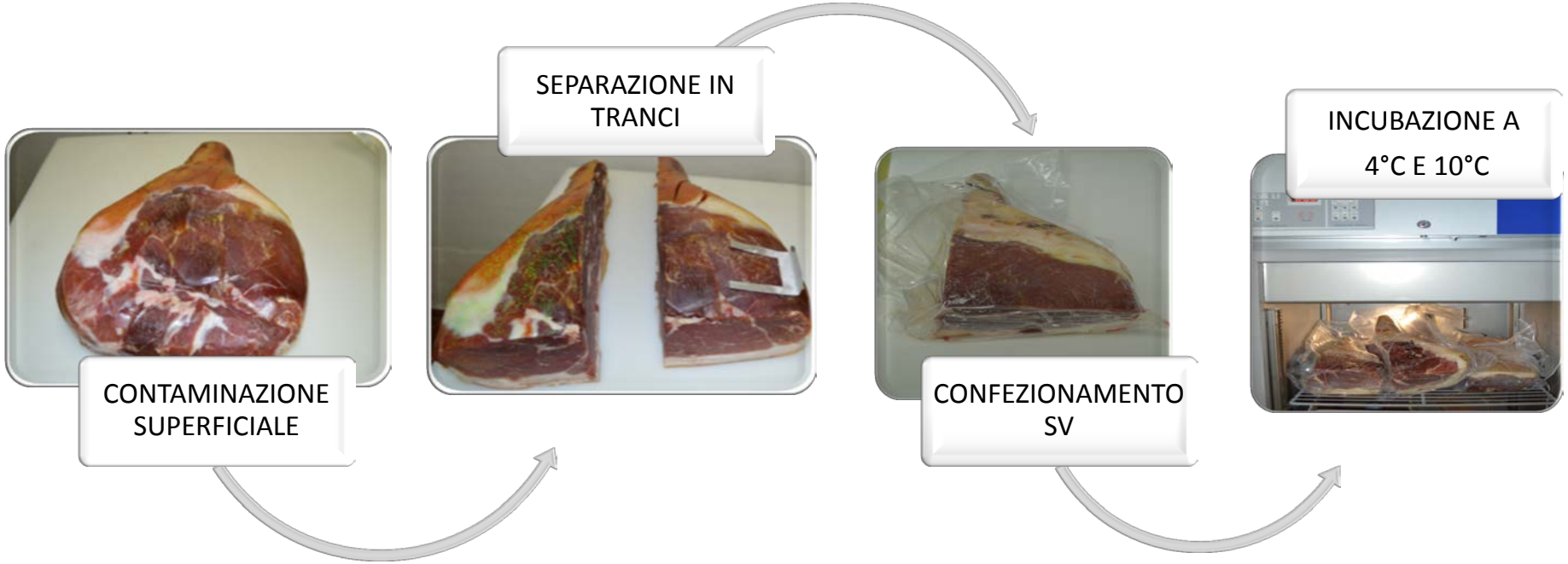
Il processo produttivo è in grado di garantire l'eliminazione di batteri patogeni

Tutti ???

- Salmonella
- Listeria monocytogenes

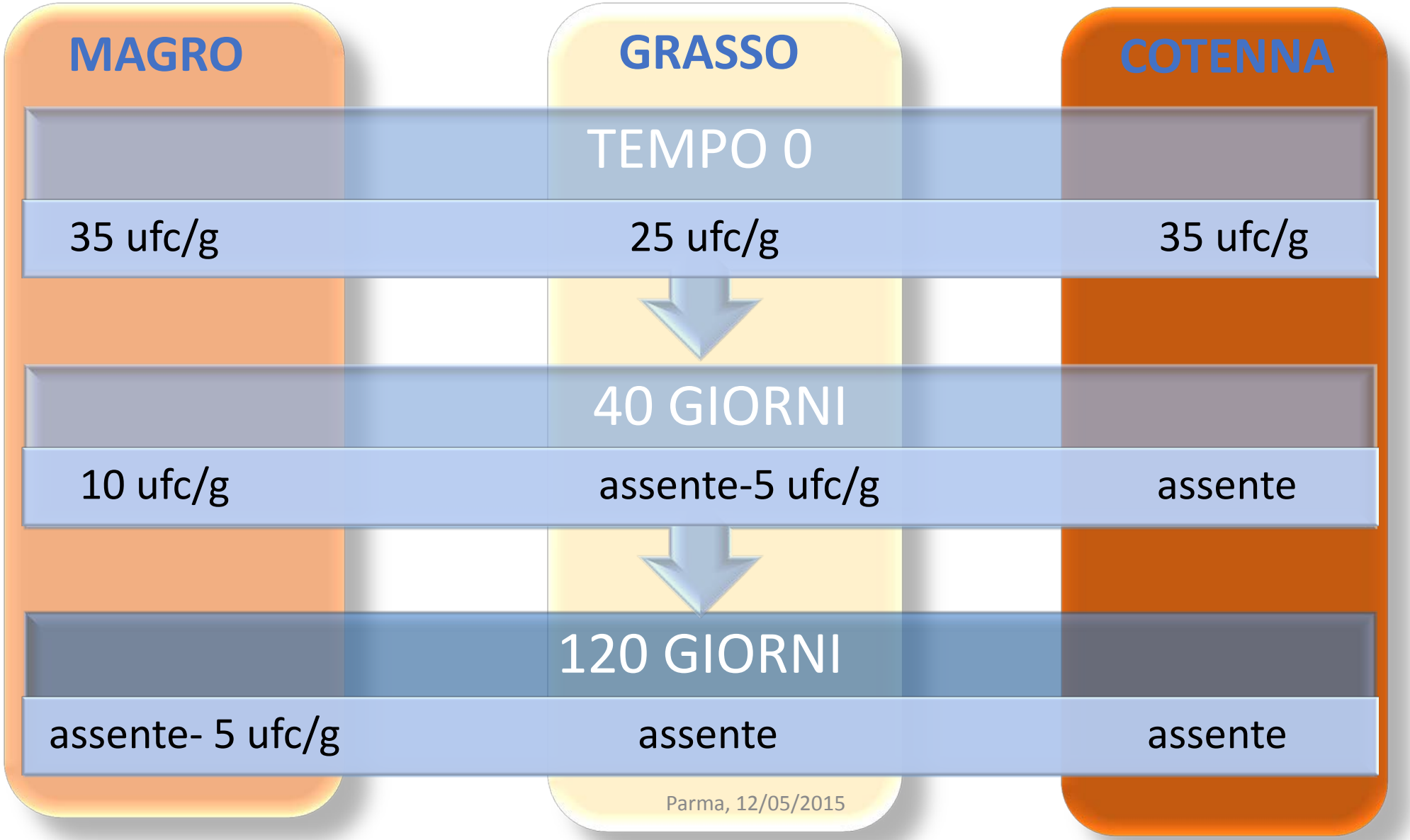
SOPRAVVIVENZA DI LISTERIA IN TRANCI SOTTOVUOTO

DISEGNO SPERIMENTALE



SHELF-LIFE A 4°C

SHELF-LIFE A 10°C



CONTROLLO DI LISTERIA IN FASE POST LETALE

FSIS Compliance Guideline:
Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Meat and Poultry Products

January 2014

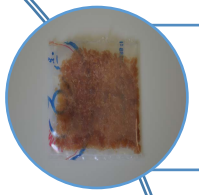
TRATTAMENTI POST LETALI

- Trattamento con le alte pressioni
- Trattamento di pastorizzazione con acqua calda/vapore
- Radiazione ultraviolette (UV)
- Tecnologia raggi infrarossi
- Radiazioni ionizzanti
- Ultrasuoni
- Radiofrequenze

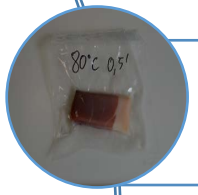
AGENTI ANTIMICROBICI

- Batteriofagi
- Ozono
- Acido lattico
- Acido acetico
- Sodio o potassio lattato
- Sodio diacetato

TRATTAMENTI DI PASTORIZZAZIONE CON ACQUA CALDA



TERMORESISTENZA SU STRATO SOTTILE

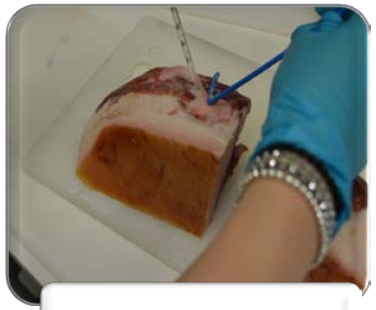


TRATTAMENTO DI PICCOLI TRANCI

TRATTAMENTO DI TRANCI

TRATTAMENTO DI MATTONELLE NON CONFEZIONATE

PROVE DI IMMERSIONE DI TRANCI IN ACQUA- 90°C/1’



INOCULO

CONFEZIONAMENTO E TRATTAMENTO

ASPETTO DOPO TRATTAMENTO

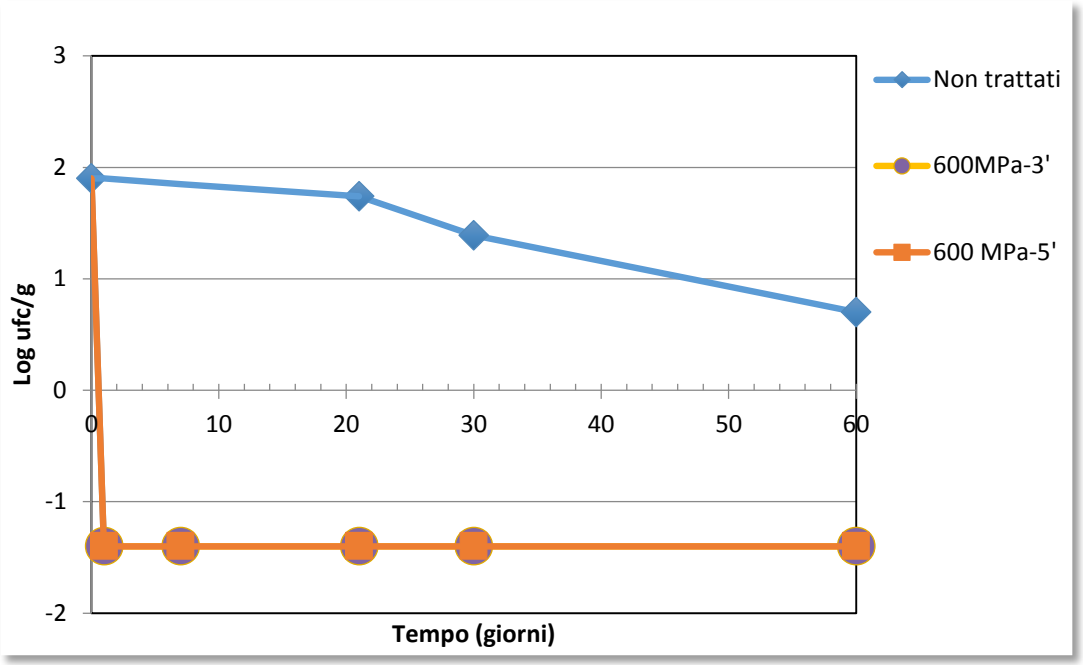
DOPO 7 GG A 4°C

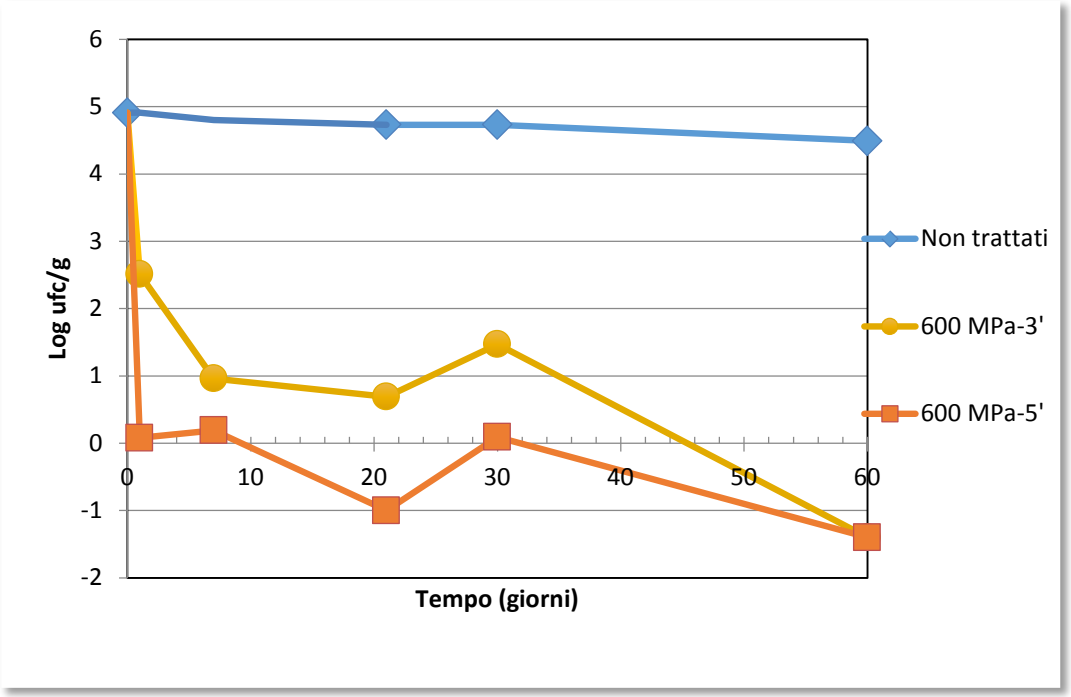
PRELIEVO PER ANALISI

RISULTATI

	MAGRO	GRASSO	COTENNA
Livello contaminazione iniziale	3,74±0,14 Log ufc/g	2,37±0,46 Log ufc/g	2,26±0,29 Log ufc/g
Contaminazione media dopo trattamento	1,14±0,48 Log ufc/g	< 10 ufc/g	< 10 ufc/g
Numero di riduzioni decimali	D 2,60±0,51	D > 1,67	D > 1,56

APPLICAZIONE DELLE HPP IN PROSCIUTTO CRUDO INOCULATO CON LISTERIA MONOCYTOGENES



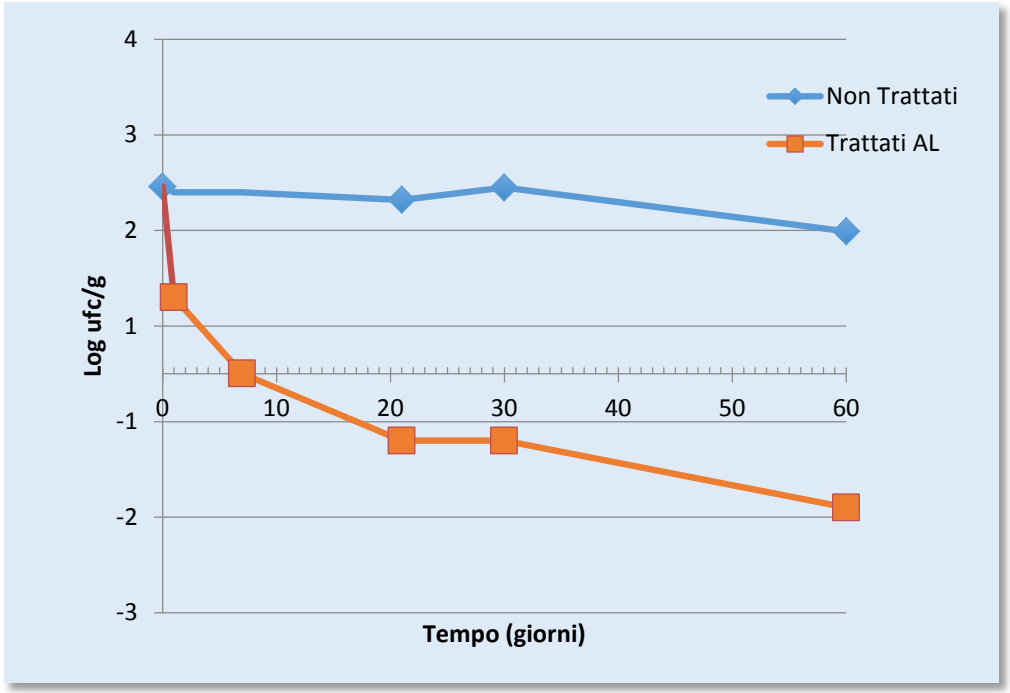


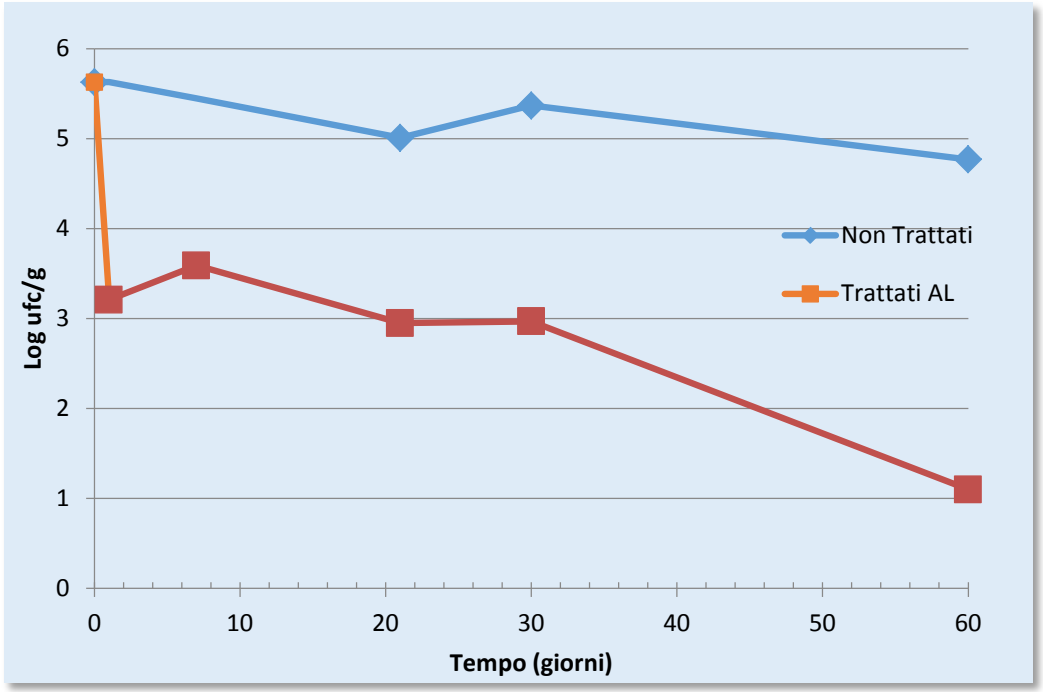
Ind. Conserve 78, 441, 2003
Ind. Conserve 79, 37, 2004
Meat Science 62, 367, 2002

ACIDO LATTICO IN PROSCIUTTO CRUDO INOCULATO CON LISTERIA

Listeria in prosciutto crudo

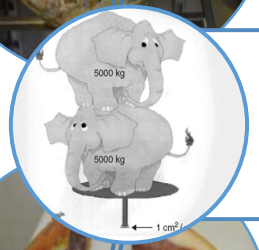
- 10^2 ufc/g: 1 riduzione logaritmica
- 10^5 ufc/g: 2,4 riduzioni logaritmiche





CONCLUSIONI

90°C/1’ D>1



HPP FINO A 5 D

ACIDO LATTICO FINO A 2,4 D