Universitat de Girona

FOOD SAFETY IN FAST DRYING (QDS PROCESS®) OF DRY-CURED MEAT PRODUCTS: HIGH PRESSURE AND NaCl-FREE PROCESSING IMPLEMENTATION

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PhD Programme in Technology

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El Dr. Josep Comaposada i Beringues, director del Subprograma de noves tecnologies de procés a la indústria alimentària (Programa de tecnologia alimentària) i la Dra. Anna Jofré i Fradera, investigadora del Programa de seguretat alimentària de l'IRTA,

CERTIFY:

That they have supervised the present PhD thesis entitled "Food safety in fast drying (QDS process®) of dry-cured meat products: High pressure and NaCl-free processing implementation" and presented by Katharina Stollewerk for obtaining the Ph.D. degree.

CERTIFIQUEN:

Que aquest treball titulat "Food safety in fast drying (QDS process®) of dry-cured meat products: High pressure and NaCl-free processing implementation" i presentat per Katharina Stollewerk per a l'obtenció del títol de doctora ha estat realizat sota la nostra direcció.

Dr. Josep Comaposada Beringues **Dra. Anna Jofré Fradera**

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"Todo parece imposible – hasta que se hace"

(Neslon Mandela)

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Published Works

Stollewerk, K., Jofré, A., Comaposada, J., Ferrini, G., Garriga, M. 2011. Ensuring food safety by an innovative fermented sausage manufacturing system. Food Control 22, 1984-1991.

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Position in Food Science & Technology Category: 11/126 (1st quartile)

Stollewerk, K., Jofré, A., Comaposada, J., Arnau, J., Garriga, M. 2012. The effect of NaCl-free processing and high pressure on the fate of *Listeria monocytogenes* and *Salmonella* on sliced smoked dry-cured ham. Meat Science 90, 472-477.

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Stollewerk, K., Jofré, A., Comaposada, J., Arnau, J., Garriga, M. 2012. NaCl-free processing, acidification, smoking and high pressure: effects on growth of *L. monocytogenes* and *Salmonella* in QDS processed® dry-cured ham. Meat Science, submitted for publication: 15th of February, 2012.

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Abbreviations

AESAN	Agencia Española de Seguridad Alimentaria y Nutrición
AFNOR	Association Française de Normalisation
ANICE	Asociación Nacional de Industrias de la Carne de España
approx.	approximately
ATP	Adenosine triphosphate
a_w	Water activity
BHA	Butylhydroxyanisole
°C	Degree Celcius
ca.	Circa
Ca	Calcium
CAC	Codex Alimentarius Commission
$CaCl_2$	Calcium chloride
CFU	Colony forming units
Cl	Chloride
CO_2	Carbon dioxide
CRL/AFSSA	Community Reference Laboratory/Agence Française de Sécurité Sanitaire des Aliments
DNA	Desoxyribonucleic acid
EFSA	European Food Safety Authority
e.g.	For example
EU	European Union
FSIS	Food Safety and Inspection Service
FDA	Food and Drug Administration
g	Gram
GCC+	Gram-positive catalase-positive cocci
GDL	Gluconodeltalactone
h	Hour
HACCP	Hygiene and critical control points
HEPA	High-efficiency absolute filter
Hg	Mercury
HHS	U.S. Department of Human and Health Services
HP	High pressure
ICMSF	International Commission on Microbial Specifications for Foods
i.e.	That is
IFT	Institute of Food Technologists
K	Potassium
KCl	Potassium chloride
kg	Kilogram
LAB	Lactic acid bacteria
log	Logarithm
MAFF	Ministry of Agriculture, Fisheries and Food
MAP	Modified atmopshere packaging
MAPA	Ministerio de Agricultura, Pesca y Alimentación
$MgCl_2$	Magnesium chloride
min	Minute
mm	Millimeter
Mn	Manganese
MPa	Megapascal
µm	Micrometer
n	Number of tested samples
Na	Sodium
NaCl	Sodium chloride
NACMCF	National Advisory Commitee On Microbiological Criteria For Foods
NAOS	Nutrition, Physical Activity and Obesity Prevention
N°	Number
PCR	Polymerase chain reaction
pI	Isoelectric point
PSE	Pale soft exudative
psi	Pounds per square inch
QDS	Quick Dry Slice
RASFF	Rapid alert system for food and feed
RH	Relative humidity
RTE	Ready-to-Eat
Tgase	Transglutaminase
ufc	Unidad formadora de colonias
U.S.	United States
vs.	Versus
WHO	World Health Organisation
% w/w	Weight in weight percentage

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Summary

Dry-cured meats play an important part in many diets, providing valuable proteic and fat nutrients and interesting flavours. These products, due to the intermediate water activity and acid pH values achieved through the fermentation, drying and ripening period, extend considerably the shelf-life of perishable raw meat. NaCl, which is added during processing, additionally exerts an important preservative effect. Nevertheless, to respond to the fast changing demands of today's consumers, industry is interested in new technologies, such as the QDS process®, which permits the shortening of the drying phase and therefore involves an economical advantage over the traditional method. Another way of innovation takes into account public health protection campaigns against illnesses linked to nutrition, and consists in the complete substitution of the NaCl-content in the production of dry-cured meat products by KCl, potassium lactate and sugars ("NaCl-free processing"). However, although both technologies are feasible from a technological point of view, their food safety impact must be clarified. The application of a high pressure treatment could be useful to improve the food safety of reformulated products and/or products of which the traditional production process has been modified.

With the objective to study food safety aspects of innovative meat technologies and combinations among them, QDS, NaCl-free processing and high pressure were integrated in the production of two types of dry-cured meat products. Dry fermented sausages (chorizo) were produced at acid (4.8) and low acid (5.2) pH and hurdles such as acidification and smoking were introduced in the production of dry-cured hams. All products were challenged with low levels (< 2 log CFU/g) of *Listeria monocytogenes* and *Salmonella*, whereas inoculation was performed in the meat batter of chorizos and directly on the surface of dry-cured ham slices. Pressurisation (600 MPa, 5 min, 13ºC) was applied as an in-package-cold pasteurisation. The fate of the pathogenic microorganisms in addition to technological microbiota and physicochemical parameters were investigated throughout the production and/or the storage period under refrigeration.

Neither the QDS technology nor NaCl-free processing affected the particular hostile environment of chorizo or dry-cured ham and pathogenic microorganisms did not grow in any of the products. However, the fate of both pathogens was affected by NaCl-free processing, acidification, smoking and pressurisation. In all types of chorizo except the low acid traditionally dried and NaCl-free processed one, pathogens were eliminated during production (acidification and drying). Accordingly, the application of a high pressure treatment would only be necessary to assure food safety of chorizos with less hurdles. In dry-cured ham, the combination of acidification and smoking was the most inhibitory and by means of pressurisation both pathogens were eliminated from all types of dry-cured ham throughout refrigerated storage.

Thus, the combination of QDS and NaCl-free processing could be useful for the fast development of safe and healthy sliced dry-cured meat products. Depending on the product, however, a high pressure treatment may be required, of which the effectiveness must be specifically evaluated, as in the case of products with a complete reduction of the NaCl content, where the bactericidal effect of pressurisation can decrease.

Resumen

Los productos crudo-curados están presentes en muchas dietas, proporcionando proteínas, grasas y flavores interesantes. Estos productos, debido a sus valores de pH ácido y una actividad de agua intermedia alcanzados durante la fermentación, la maduración y el secado, tienen una vida útil considerablemente más larga que la de la carne fresca. El NaCl, que se añade durante el procesado, ejerce también un importante efecto conservante. Además, para poder responder rápidamente a las demandas de los consumidores, la industria está interesada en la aplicación de nuevas tecnologías como el procesado QDS®, que permite acortar la fase de secado, lo que posibilita el desarrollo de nuevos productos y supone una ventaja económica frente al secado tradicional. Otra vía de innovación, el "procesado sin NaCl añadido", tiene en cuenta los objetivos propuestos en la estrategia NAOS (Estrategia para la Nutrición, Actividad Física y Prevención de la Obesidad) para prevenir enfermedades nutricionales y consiste en la sustitución completa del NaCl, usado durante la manufacturación de los productos crudo-curados, por KCl, lactato potásico y azúcares. Sin embargo, aunque ambas tecnologías son viables desde un punto de vista tecnológico, su impacto en la seguridad alimentaria de los productos finales tiene que ser evaluado. La aplicación de altas presiones puede mejorar la seguridad alimentaria de productos reformulados y/o de productos donde el proceso tradicional de producción ha sido modificado.

Con el objetivo de valorar el impacto que tienen sobre la seguridad alimentaria tecnologías innovadoras en la industria cárnica y combinaciones entre ellas, el procesado QDS®, el "procesado sin NaCl añadido" y las altas presiones se integraron en la producción de dos tipos de productos crudo-curados: embutido fermentado (chorizo ácido, pH 4.8, y poco ácido, pH 5.2) y jamón curado (con y sin acidificación y/o ahumado). Los productos se inocularon con niveles bajos (<2 log ufc/g) de los patógenos *Listeria monocytogenes* y *Salmonella* en la masa cárnica, en el caso de los chorizos, y en la superficie de las lonchas, en el caso del jamón curado. Las lonchas envasadas al vacío se sometieron a un tratamiento de alta presión de 600MPa, 5 min y 13ºC. La evolución de los patógenos, así como de la microbiota tecnológica y los parámetros físico-químicos se investigó a lo largo de la producción y/o período de almacenaje en refrigeración.

Ni el procesado QDS® ni el "procesado sin NaCl añadido" afectaron el ambiente inhóspito del chorizo y el jamón curado, el cual impidió el crecimiento de los patógenos. Sin embargo, la evolución de ambos patógenos se vio afectada por el "procesado sin NaCl añadido", la acidificación, el ahumado y la presurización. En todos los tipos de chorizo, excepto el poco ácido secado de forma tradicional y el "procesado si NaCl añadido", los patógenos fueron eliminados durante la producción (acidificación y secado). Así, la aplicación de las altas presiones sólo fue necesaria para asegurar la seguridad alimentaria en el caso del chorizo con menos obstáculos para el crecimiento microbiano. En jamón curado, la combinación de acidificación y ahumado fue la más inhibitoria tanto en productos estándar como "procesados sin NaCl añadido" y con el tratamiento de alta presión se logro ausencia de *L. monocytogenes* y *Salmonella* en todos los tipos de jamón curado durante el almacenaje en refrigeración.

Consecuentemente, el procesado QDS y el "procesado sin NaCl añadido" son útiles para el rápido desarrollo de productos cárnicos crudo-curados loncheados sanos y seguros. Sin embargo, dependiendo del tipo de producto podría ser necesaria la aplicación de un tratamiento de alta presión cuya eficiencia tendrá que ser valorada específicamente, ya que en el caso de productos elaborados sin la adición de NaCl, el efecto bactericida del tratamiento puede disminuir.

Resum

Els productes crus-curats són presents en moltes dietes, proporcionant proteïnes, greixos i flavors interessants. Aquests productes, degut als valors de pH àcid i una activitat d'aigua intermitja assolits després de la fermentació, la maduració i l'assecat, tenen una vida útil considerablement més llarga que la de la carn fresca. El NaCl, que s'afegeix durant el processat, exerceix també un important efecte conservador. A més, per poder respondre ràpidament a les demandes dels consumidors, la industria està interessada en l'aplicació de noves tecnologies com el processat QDS®, que permet escurçar la fase d'assecat facilitant així el desenvolupament de nous productes i suposant una avantatge econòmica enfront l'assecat tradicional. Una altra via d'innovació, el "processat sense NaCl afegit", té en compte els objectiu proposats a l'estratègia NAOS (Estrategia para la Nutrición, Actividad Física y Prevención de la Obesidad) i consisteix en la substitució total de l'NaCl per KCl, lactat potàssic i sucres. De totes maneres, encara que les dues tecnologies siguin viables des del punt de vista tecnològic, el seu impacte en la seguretat alimentaria dels productes resultants cal que sigui avaluat. L'aplicació d'altes pressions pot millorar la seguretat alimentaria de productes reformulats i/o productes on el procés tradicional ha estat modificat.

Amb l'objectiu de valorar l'impacte que tenen sobre la seguretat alimentaria tecnologies innovadores en la indústria càrnia i combinacions entre elles, el processat QDS®, el "processat sense NaCl afegit" i les altes pressions hidrostàtiques es van integrar en la producció de dos tipus de productes crus-curats: embotit fermentat (xoriço àcid, pH 4.8, i poc àcid, pH 5.2) i pernil curat (amb i sense acidificació i/o fumat). Els productes es van inocular amb nivells baixos (<2 log ufc/g) dels patògens *Listeria monocytogenes* i *Salmonella* a la massa càrnia, en el cas dels xoriços i a la superfície de les llenques, en el cas del pernil curat. Les llenques envasades al buit es van sotmetre a un tractament d'alta pressió de 600 MPa, 5 min y 13ºC. L'evolució dels patògens, així com de la microbiota tecnològica i els paràmetres físico-quimics es va investigar al llarg de la producció i període d'emmagatzematge en refrigeració.

Ni el processat QDS ni el "processat sense NaCl afegit" van afectar l'ambient inhòspit del xoriço i el pernil curat, el qual va impedir el creixement dels patògens. Malgrat això, l'evolució d'ambós patògens es va veure afectada pel "processat sense NaCl afegit", l'acidificació, el fumat i la pressurització. En tots els tipus de xoriço, excepte el poc àcid assecat de forma tradicional i el "processat sense NaCl afegit", els patògens van ser eliminats durant la producció (acidificació i assecat). Així, l'aplicació de les altes pressions només va ser necessaria per assegurar la seguretat alimentaria en el cas del xoriço amb menys obstacles pel creixement microbià. En pernil curat, la combinació d'acidificació i fumat va ser la més inhibidora tant en producte estàndard com en "processat sense NaCl afegit" i amb l'aplicació d'alta presió es va aconseguir absència de *L. monocytogenes* i *Salmonella* en tots els tipus de pernil durant el període d'emmagatzematge en refrigeració.

Conseqüentment, el processat QDS i el "processat sense NaCl afegit" són útils pel ràpid desenvolupament de productes carnis crus-curats llescats sans i saludables. Malgrat això, depenent del tipus de producte pot ser necessària l'aplicació d'un tractament d'alta pressió l'eficàcia del qual s'haurà de valorar específicament, ja que en el cas de productes elaborats sense l'addició de NaCl, l'efecte bactericida del tractament pot disminuir.

I. JUSTIFICATION

Dry-cured meats are generally considered as shelf-stable products (Barbuti & Parolari, 2002; Reynolds, Harrison, Rose-Morrow & Lyon, 2001). Their food safety is based on a number of hurdles, which in combination do not allow the growth of pathogenic microorganisms of concern for meat products, such as *Listeria monocytogenes* and *Salmonella*. The most important preservative factors assuring food safety in dry-cured meat products are the intermediate water activity (a_w), which is achieved during long ripening times. Salt (NaCl), which is added during processing, contributes to reduce the a_w and exerts an important preservative effect.

Recent innovations performed in this field include “the QDS drying technology” to speed up production by shortening the drying phase and the “NaCl-free processing”, a new strategy where no NaCl is added during production.

The technological advantages of the QDS process®

The fast adaption to new consumption trends raises much commercial interest in the fast production of dry-cured meat products. For the sliced product market, the QDS process® technology presents a large number of advantages compared to the traditional drying technology for raw cured products: It allows for much better control of the production process and of the product quality, which stands for yield improvement and waste reduction. The energy consumption of the high speed process is notably lower and the methodology offers great flexibility in production planning. The shorter process allows a just-in time workflow, which would reduce the financing of the stock. From the point of view of investment, this new technology requires much less space than the traditional system. Additionally, it permits the development of new formats other than the traditional round shape and also new products in line with the lifestyle tendencies of today’s consumers, who demand RTE products in small formats (Comaposada et al., 2010).

The need for reducing salt from the diet

Sodium is an essential ingredient, which is added in human diet mostly in the form of sodium chloride (NaCl), which is the common salt. In earlier times, it was viewed as a food preservative that enhanced human health by killing or limiting growth of food-borne pathogens and spoilage organisms. However, in recent decades, with increasing consumption of many different processed foods containing high levels of sodium, the perception of dietary salt has evolved to a point where it is now considered to be a potential health threat (Doyle & Glass, 2010). In Europe, the average daily sodium intake was reported to lie at about 3-5 g, this corresponds to 8-11 g NaCl (EFSA, 2005) and to levels which twice exceed recommended doses. Sodium occurs naturally in fresh beef, pork, and poultry meats at relatively low levels, ranging from 50 to 70 mg sodium per 100 g (Verma & Banerjee, 2012). Dry-cured meat products however, are heavy salt contributors to the diet owing their salt content (5.5% in dry-cured ham, 4.6% in “salchichón extra” and 3.9% in “chorizo extra”, AESAN, 2011) to traditional production procedures using high amounts of NaCl. Eating habits estimates suggested that dry-cured meat products contribute with 26.2% to the common salt intake and represent herewith the second largest group after cereals and cereal products (AESAN, 2011).

Strategies to protect public health and the socio-economic impact of reducing NaCl from food

The World Health Organisation (WHO) estimated that globally 62% of cerebrovascular disease and 49% of ischaemic heart disease were attributable to elevated blood pressure (systolic > 115 mm Hg) and that heart diseases are the leading cause of death for persons over 60 years of age and the second cause of death for persons aged 15 – 59 years (WHO, 2007). It should also be noted that the metabolic syndrome can enhance blood pressure response to sodium so that sufferers are more salt sensitive than those without the syndrome (Chen et al., 2009; Hoffmann & Cubeddu, 2007) and that adverse cardiovascular

events can occur more frequently in patients with sodium-sensitive hypertension (Morimoto et al., 1997).

With the aim to preventively protect population from illnesses linked to nutrition, public health politics have elaborated programmes on national (NAOS) and international (WHO) levels. To reduce the risks associated with cardiovascular disease, population-wide salt reduction strategies have been stated to be the most cost-effective (WHO, 2007). In relation to reducing the salt intake, studies were performed reporting the economic and social benefits and the improvement of life quality for the population (Beaglehole, Ebrahim, Reddy, Voute & Leeder, 2007; Dall et al., 2009; Palar & Sturm, 2009). Bibbins-Domingo et al. (2009) recently reported that a 3 g/day-reduction in salt intake (about 1.2 g of sodium) would result in 6% fewer cases of new heart disease, 8% fewer heart attacks and 3% fewer deaths. However, considering the high contribution (70-75%) of manufactured goods to total household dietary salt intake (AESAN, 2011), it gets apparent that it is not possible to meet the recommended target levels of 5 g/day of NaCl (2 g/day sodium) by simply reducing the amount of discretionary salt added to food by consumers (Stringer & Pin, 2005). Therefore, NAOS and WHO strategies not only encourage consumer awareness towards healthier food, but also involve industry with the aim to develop and promote products which contribute to a healthier choice, among them, products with a reduced content of NaCl.

In this context Arnau, Comaposada, Serra, Bernardo & Lagares (2011) recently submitted a patent application that deals with the complete exclusion of NaCl from the production process of dry-cured meat products. As part of this patent application, authors proposed to combine “NaCl-free processing” with the QDS technology. According to the European Regulation (EC) Nº 1924/2006 on nutrition and health claims made on foods, a product can be designated “sodium-free or salt-free” when it contains no more than 0.005 g of sodium, or the equivalent value for salt, per 100 g. Although in the submitted procedure no NaCl was added during the production process, the designation “sodium-free” cannot be used, due to the amout of NaCl naturally present in meat. Thus, the term “NaCl-free processing” was

chosen for describing the production process of dry-cured meat products without the use of NaCl.

The fundamental question of this PhD thesis

From a socio-economic point of view, the application of both technologies is useful and can contribute to the fast production of healthy dry-cured meat products. From a food safety point of view, however, changes in traditional processing and/or product reformulations could have a significant impact on the originally safe character of dry-cured meat products (Figure 1.), which up to date has not been evaluated.

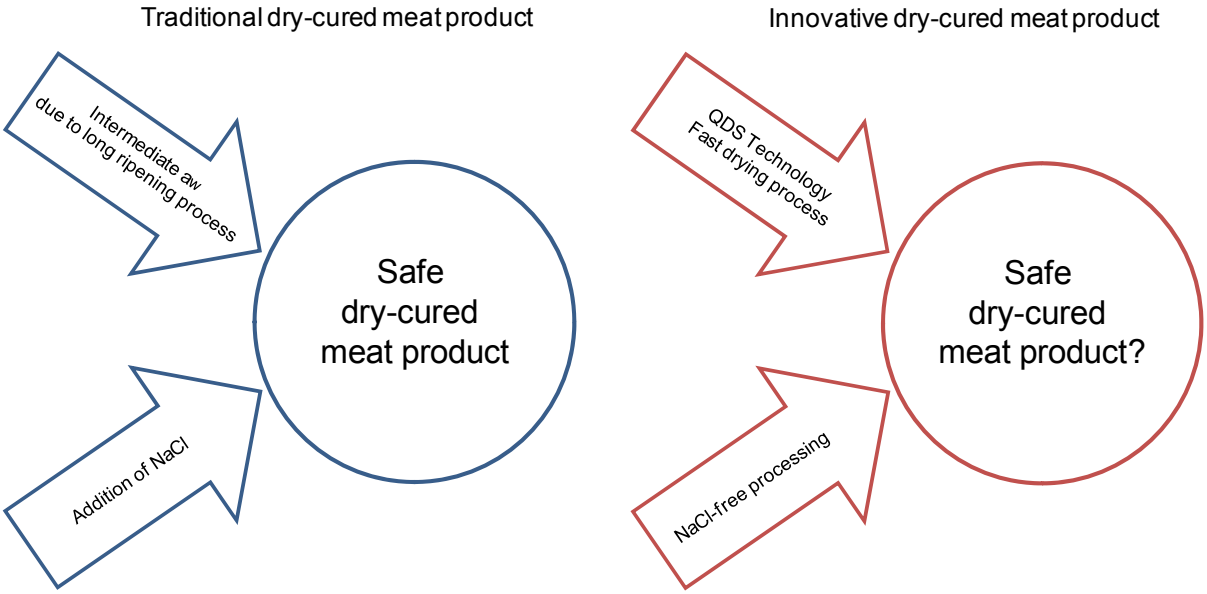

Figure 1. The fundamental question of this PhD thesis

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II. INTRODUCTION

1. Dry-cured meat products

Dry-cured meat products such as sliced dry-cured ham or dry fermented sausages can be categorized as ready-to-eat (RTE) foods, which are intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern (European Commission, 2005). RTE dry-cured meat products such as chorizo and dry-cured ham belong to the product classes “chopped or comminuted fermented meats” and “whole piece meat products” respectively, according to Campbell-Platt, (1995).

1.1. Chopped or comminuted fermented meats

All types of fermented sausages belong to this group. They are usually made out of ground meat, most often pork or beef (initial pH 5.4-6.0), fat, salt, curing agents (nitrate and nitrite), carbohydrates, spices and additives (Campbell-Platt, 1995; Fernández, Ordóñez, Bruna, Herranz & de la Hoz, 2000; Ordóñez & de la Hoz, 2001). The mixture is stuffed into casings, of which artificial ones are rather used for products to be sliced because they show higher water permeability and resistance, constant diameter and are easier to remove before slicing than natural ones (Arnau, Serra, Comaposada, Gou & Garriga, 2007). The fresh sausages are fermented, optionally smoked and dried to the target water content at controlled temperature and relative humidity (RH). The official classification of fermented sausages varies from country to country (Adams, 1986; Zeuthen, 1995) and may be based on the final moisture content (moist: 50-60%, semi-dried: 35-50% and dried: 20-35%, Campbell-Platt, 1995). From a microbiological point of view, fermented sausages are best subdivided on the basis of a_w and surface treatment (Table 1).

Table 1. Classification of fermented sausages (modified from Lücke, 1998)

Category	Ripening times	Final a_w	Application of smoke	Examples
Dry, mould ripened	>4 weeks	<0.9	No	Genuine Italian salami, French *saucisson sec*
Dry, mould ripened	>4 weeks	<0.9	Yes (during fermentation)	Genuine Hungarian salami
Dry, no mould growth	>4 weeks	<0.9	Yes or No	German *Dauerwurst*
Semi-dry, mould ripened	<4 weeks	0.9-0.95	No	Various French and Spanish raw sausages
Semi-dry, mould ripened or not	>4 weeks	0.9-0.94	No	Spanish dry fermented sausages
Semi-dry, no mould growth	<4 weeks (usually 10-20 days)	0.9-0.95	Yes (with exceptions)	Most fermented sausages in Germany, The Netherlands, Scandinavia, USA, etc.
Undried, spreadable	<2 weeks	0.94-0.96	Yes or No	German *Streichmettwurst*

Additional criteria for classification include the casing diameter, the degree of comminution of ingredients, the animal species used for obtaining the raw material, the fat content and type of tissue used, as well as spices, seasonings and other non-meat ingredients used (Lücke, 1998; Ordóñez et al., 2001). In Europe, dry fermented sausages have the longest tradition. In Spain, about 50 varieties are catalogued although many more are produced in craft industry (MAPA, 1997). The most characteristic Spanish products are “chorizo” and “salchichón”, of which the main difference consists in the paprika added to chorizo.

During fermentation the pH decreases through glycolysis by lactic acid bacteria (LAB). According to the achieved pH level, dry fermented sausages can be classified in “acid” and “low acid”. Low acid dry fermented sausages with a pH of ≥ 5.3 (Aymerich et al., 2006; Ordóñez et al., 2001) are typical Mediterranean products, associated with a long maturation period. Acid dry fermented sausages (final pH 4.6-5.0) are commonly produced in Central and Northern Europe at medium (20-24ºC) and in the United States at high (*ca*. 37ºC) fermentation temperatures (Jessen, 1995; Ordóñez et al., 2001). These variations are related to the differences of used starter cultures: while in North America typically *Pediococcus acidilactici* is used, which has its growth optimum at *ca*. 40°C, in Europe, mixed

cultures consisting of LAB (*Lactobacillus* or *Pediococcus*) and gram-positive, catalase positive cocci (GCC+, *Staphylococcus* and *Kocuria*) are applied (Jessen, 1995; Lücke, 2000). Acid dry fermented sausages usually contain carbohydrates (glucose, 1-8 g/kg to achieve a pH < 5.2; (Garriga & Aymerich, 2007) as substrates for LAB to facilitate fermentation and consequently the pH decrease. Traditionally, LAB fermentation was due to endogenous microbiota or performed by "back-slopping" (Campbell-Platt, 1995). Nowadays however, in meat industry, where big volume batches are produced to guarantee safety and standardize product properties (starter cultures represent important organoleptic contributions, (Lücke, 1998), fermentation is mostly achieved through starter culture application (Garriga et al., 2007; Zeuthen, 1995). Alternative to LAB fermentation, the pH decrease can also be produced by the addition of chemical additives, such as gluconodeltalactone (GDL) or encapsulated acids (e.g. lactic and citric acids). GDL hydrolyzes to gluconic acid while encapsulated acids comprise a mechanism for slow and targeted acid release by employing coating materials that take time to dissolve or break down (Gibbs, Kermasha, Alli & Mulligan, 1999).

During the whole ripening (including fermentation) and drying, flavour, colour and texture, characteristic for dry fermented sausages are formed due to several enzymatic (endogenous and microbial enzymes) and chemical reactions, including lipid oxidation, Maillard reactions and Strecker degradations (Garriga et al., 2007; Lücke, 1998; Ordóñez, Hierro, Bruna & Hoz, 1999; Toldrá, 1998).

1.2. Fermented whole piece meat products

To this group belongs dry-cured ham, which is usually made from the hind leg of pork (pH_{24} between 5.8 and 6.4; Weber, 2003). The production process is based on a salting-curing step where curing salts and optionally other additives (e.g. ascorbate and carbohydrates) are absorbed, followed by a resting period at a temperature below 5ºC until a_w decreases below 0.96 to prevent growth of undesirable microorganisms (Leistner, 1985; Weber, 2003). During

the drying period, temperature is gradually raised as a_w decreases to accelerate the drying process and the development of the typical aged flavour (Arnau et al., 2007).

Dry-cured hams are principally produced in Europe and North America with some production in South America, Asia, Oceania and Africa (Campbell-Platt, 1995). Depending on the geographical region of manufacturing, different procedures have been established, which discern basically (i) in the preparation of the used meat (entire hams with femur bone *vs*. boned hams *vs*. restructured meat chunks), (ii) in the salting method (dry salting *vs*. brine immersion), (iii) in smoke application or not and (iv) in the duration of the ageing period (Flores, 1997). These technological differences together with the raw material characteristics determine the quality of the finished product (Arnau, Guerrero, Gou & Monfort, 2001).

Within Europe, in Mediterranean countries (mainly Spain, France and Italy) the traditional hams are more frequently prepared out of hams containing femur bone (bone-in hams), which are dry salted, non-smoked and submitted to an ageing period from six months to two years. Products elaborated in Central Europe (Germany, Austria, Switzerland) are rather made out of boned hams or meat chunks) and manufacturing is characterized by brine immersion and vacuum tumbling, subsequent drying and/or smoking and ageing for 3 to 12 months (Arnau et al., 2007). While dry salting achieves a better osmotic dehydration, brine immersion provides less consumption of salt. Smoking involves the typical smoked colour and flavour and has antibacterial and fungicide properties (Flores, 1997). Therefore smoking inhibits growth of surface bacteria and moulds, to which products are more susceptible in the cold damp climates found in Central Europe. A comparison of the most common European products regarding their differences in manufacturing is represented in Table 2.

Table 2. Some types of dry-cured ham (modified from Campbell-Platt, 1995)

Type	Area of Production	Raw material	Salting	Smoking	Drying and maturation
Jambon d'Ardenne	Belgium	Bone-in ham	Dry salting or Brine curing, (no brine injection)	Yes	> 4 months
Jambon de Bayonne	France	Bone-in ham	Dry salting	No	9-10 months
Iberico	Spain	Bone-in ham	Dry salting	No	18-24 months
Prosciutto di Parma	Parma, Italy	Bone-in ham	Dry salting	No	12-18 months
Prosciutto di San Daniele	Italy	Bone-in ham	Dry salting	No	> 12 months
Schwarzwälder	Germany	Boned ham	Brine curing	Yes	3 months
Serrano	Spain	Bone-in ham	Dry salting	No	> 7 months
Westphalian	Germany	Boned ham	Dry salting and brine curing	Yes	> 6 months

Regarding dry-cured meat products, although found in most parts of the world, Europe is the major producer and consumer of these products (Campbell-Platt, 1995). In Spain in 2009, 1,251 tonnes of meat products were produced, of which 20% were dry-cured hams and 15% dry fermented sausages (ANICE, 2012). Evaluation of Spanish meat consumption evolution between 2004 and 2008 showed that among cured products, ham was keeping a leading position and together with “chorizo” made up to 60% of the total consumption of cured products (European Commission, 2011).

2. Food Safety of dry-cured meat products

Dry-cured meat products are generally regarded as shelf-stable and safe meat products and they have rarely been implicated in food poisoning (Barbuti & Parolari, 2002; Reynolds, Harrison, Rose-Morrow & Lyon, 2001). This fact is due to the presence of particular preservative factors called “microbiological hurdles” that are applied or develop during manufacturing and protect the food product against undesired microorganism growth (Leistner, 2007). In dry-cured meat products, the following factors assure microbiological stability:

- The decrease of a_w, which is caused by solutes (salt, carbohydrates, etc.) and dehydration throughout production and subsequent drying. It is the only factor of

increasing importance along ripening of a dry-cured meat product due to progressive desiccation to a $a_w < 0.9$ (Jofré, Aymerich & Garriga, 2009a).

- The pH decrease produced by acidification (due to endogenous LAB, applied starter cultures or chemical substances) plays a more crucial role in dry fermented sausages than in dry-cured ham. Accordingly, low acid dry fermented sausages, due to the absence of the acidity hurdle, are more at risk than acid products and may require alternative preservative factors to achieve an equal food safety standard (Jofré et al., 2009a). In this regard, it is worth mentioning that LAB also act as competitive microbiota and therefore represent an additional hurdle (Lücke, 1998). In whole piece meat products fermentation is not typical because carbohydrates are rarely added and LAB are not the predominant microorganisms (Arnau et al., 2007). In the same sense, Reynolds et al. (2001) reported the pH changes observable in dry-cured ham to be more subtle than those found in dry fermented sausages. Although pH in German raw hams indeed decreases slightly during the first two weeks to values of normally 5.7-5.9, this decrease is not comparable to that happening in dry fermented sausages (Weber, 2003). From a technological point of view, acidification of dry-cured meat products improves cohesiveness of the meat mixture when the pH decreases below the isoelectric point of myosin (pI 5.4) (Hamm, 1986).

- NaCl, nitrate and nitrite, the curing salts, contribute to a large extent to both food safety and quality. Salt, in addition to decrease a_w, has an important bactericidal effect (explained below). The microbial enzyme nitrate reductase reduces nitrate to nitrite, which acts as an antioxidant and prevents or retards microbial growth, apart from its colour stabilizing and flavour effect (Honikel, 2007).

- Lactate is a compound that can be found in food naturally formed during processing or added as an ingredient (Ray, 2004). Its acid form is GRAS listed and is widely

used as food additive for preservation purposes, due to its antimicrobial character that is debited to its ability to acidify the cytoplasm of the cell and its a_w lowering effect (Shelef, 1994).

- Smoking (mainly used in Central European meat processing) has antibacterial and fungicide properties which can be attributed to formaldehyde and phenolic compounds (Girard, 1988; Toth & Potthast, 1984).

2.1. Pathogenic microorganisms in dry-cured meat products

Raw meat is highly perishable due to its pH near 7, its a_w > 0.97 and its highly nutritive nature, representing optimum conditions for the growth of most bacteria. After some time of refrigerated storage in air, microflora of fresh meat largely consists of gram-negative, oxidase-positive rods, particularly psychrotrophic pseudomonads and psychrotrophic *Enterobacteriaceae*, while gram-positive organisms including LAB usually occur only in small numbers (Gill, 1982; Lücke, 1998). High levels of hygiene during meat processing are crucial as long as raw material contamination, for example through gastrointestinal tract, feet, hides, or skins of slaughtered animals, is still the primary source of contamination (Garriga et al., 2007). The contaminating microbiota includes technologically important microorganisms but also spoilage and pathogenic bacteria (Garriga et al., 2007), of which *L. monocytogenes* and *Salmonella* are the most commonly linked to food-borne illness outbreaks derived from RTE food products (Moore, 2004).

Instruments and surfaces in processing plants and human handling (infected personnel or healthy carriers) can further easily contribute to cross-contaminations (Garriga et al., 2007; Jaroni, Ravishankar & Juneja, 2011). With the objective to assess the efficiency of hygienic practices, Talon et al. (2007) showed in a survey of microbial ecosystems of environments in 54 processing units of fermented sausages that sporadic contamination with pathogens was recorded and that *Salmonella* was detected at 4.8% and *L. monocytogenes* at 6.7% of the

equipment samples. In this context it has to be stressed that the ability of *L. monocytogenes* to form biofilms represents a particular threat for food processors, because when a biofilm is formed, it is subsequently very difficult to eradicate from food processing environments (Jaroni et al., 2011). *Salmonella* contamination of meat was similarly reported to be often provoked by cross-contamination *via* ambient and contaminated equipment (ICMSF, 1996).

For marketing, convenience and quality reasons, nowadays dry-cured meats are vacuum- or MAP (modified atmosphere) packaged and stored, distributed and displayed at refrigeration temperatures. These conditions positively affect the product appearance and shelf-life (Gounadaki, Skandamis, Drosinos & Nychas, 2007). Additionally, vacuum-packaging has been described to prevent the growth of aerobic microorganisms (Ahn & Byungrok, 2007). However, at the same time, vacuum-packaging prevents the further reduction of a_w that suffer whole piece dry-cured products during storage and which represents a significant additional hurdle for the inactivation of possible pathogens in the product (Jofré et al., 2009a). In this sense, growth of *L. monocytogenes* and *Salmonella* was outlined to be a possible hazard in vacuum-packed foods (MAFF, 1991).

A high level of protection of public health is one of the fundamental objectives of food law, therefore, general food safety requirements are laid down in the European Commission Regulation (EC) N° 2073/2005 on microbiological criteria for foodstuffs, according to which non-complying food must not be placed on the market. For *Salmonella*, the food safety criteria demands for “Meat products intended to be eaten raw, excluding products where the manufacturing process or the composition of the product will eliminate the salmonella risk” absence in 25 g (n=5) for products placed on the market during their shelf-life. For *L. monocytogenes*, the food safety criteria demands for “RTE foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes” that 0 out of 5 samples result <100 CFU/g for products placed on the market during their shelf-life. In comparison, in the United States, the more stringent zero tolerance policy is applied, according to which *L. monocytogenes* must be absent in 25 g of the

product. The European 2073/2005 as well as the U.S. Food Safety and Inspection Service (FSIS, 2002) regulations consider RTE-products as stable and not supporting *L. monocytogenes* growth, if they comply with at least one of the requirements listed in Table 3. Other food products can also belong to this category, however, they must be **subjected to scientific justification** (European Commission, 2005).

Table 3. Conditions validated to prevent growth of *L. monocytogenes* in RTE-products

Conditions	Regulation
pH ≤ 4.4	EC 2073/2005
a_w ≤ 0.92	EC 2073/2005
pH ≤ 5.0 and a_w ≤ 0.94	EC 2073/2005
Shelf life < 5 days	EC 2073/2005
pH < 4.5	FSIS 2002
pH < 5.0 + refrigeration	FSIS 2002
a_w < 0.9	FSIS 2002
a_w < 0.92 + refrigeration	FSIS 2002
pH < 5.5 and a_w < 0.95	FSIS 2002
Presence of antimicrobial agent validated to inhibit *L. monocytogenes* growth (e.g. lactate)	FSIS 2002
Product that is held below 0°C and labeled „keep frozen“	FSIS 2002
Product that has recieved a post-lethality treatment validated to be lethal against *L. monocytogenes*	FSIS 2002

These harmonised criteria should form an integral part of the implementation of HACCP (hygiene and critical control points)-based procedures and other hygiene control measures and were set to prevent differing interpretations. In spite of existing microbiological criteria, in 2010, 181 human deaths due to listeriosis and 62 deaths due to non-typhoidal salmonellosis were estimated in the European Union (EFSA, 2012).

2.2. *L. monocytogenes*

Bacteria of the genus *Listeria* are gram-positive, facultatively anaerobic, non-spore-forming and motile by means of flagella. They are rod shaped, measuring 0.5 µm in diameter and 1 to 2 µm in length. Six species have been detected of which *L. monocytogenes* is the most infectious but only the heamolytic strains of *L. monocytogenes* are pathogenic. From an

epidemiological point of view, the serotypes 1/2a, 1/2b and 4b are the most important ones out of 13 identified (Rocourt & Buchrieser, 2007).

Disease caused by *L. monocytogenes* is not frequent, but can be severe, with a high mortality rate in populations at risk. Listeriosis in humans is accompanied by mild flu-like symptoms such as headache, chills, and fever, along with gastrointestinal symptoms like nausea, vomiting and diarrhea. Immunocompromised people, infants, pregnant women and elderly people are more at risk for contracting the disease and meningitis, abortion, and prenatal septicaemia are some of the primary manifestations, which in serious cases can be fatal (Food and Drug Administration (FDA), 2001). The dose of infection of *L. monocytogenes* is not well known, apparently it is higher than 100 cells, but it can depend on strain and host factors such as age, health and exposure to certain foods (NACMCF, 1991). The incubation period is extremely long and lasts from 3 to 70 days (Forsythe, 2010). Especially if untreated, mortality may exceed 25% in predisposed groups (Farber & Harwig, 1996). In the years 2009 and 2010, according to the EFSA, 1,645 and 1,601 confirmed listeriosis cases in humans were recorded, respectively, among which the fatality rate was *ca*. 17% (EFSA, 2011; EFSA, 2012).

Listeria can grow and survive in between wide pH (4.39 - 9.4) and temperature ranges (-0.4 to 45ºC) and at relatively low a_w levels (> 0.92) in broth, when other parameters are at optimum (ICMSF, 1996). The pathogen was furthermore described to be able to grow in the presence of nitrite (Campbell-Platt, 1995) and in up to 12% NaCl (% w/w, Stringer & Pin, 2005). *L. monocytogenes* is psychrotrophic and able to grow in food under various conditions: its growth limit in food with a neutral pH and with high content of nutrients stabilizes at 0ºC (Walker, Archer & Banks, 1990). Depending on ambient conditions, the growth limiting a_w value vary and has been described to lie at 0.93 in meat products (ICMSF, 1996).

In nature, *L. monocytogenes* is ubiquitously distributed. It has been isolated from different ambient including soil, water, diverse animal and vegetal sources, feed and water residues.

Moreover, *L. monocytogenes* has been found in at least 37 mammalian species, both domestic and feral, as well as in at least 17 species of birds and possibly of fish and shellfish; it is furthermore plausible that 1-10% of humans may be intestinal carriers of *L. monocytogenes* (Forsythe, 2010). It can also be found in a wide range of food, raw or processed, where it can survive and multiply fast during storage. Foods implicated in outbreaks include milk, butter, cheese, RTE meat products, surimi, smoked mussels and trout, and vegetables (ICMSF, 2001). *L. monocytogenes* is one of the microorganisms of most concern in food, because it causes only little or no deterioration to the product which is supporting its growth (ICMSF, 2001).

2.3. *Salmonella*

Salmonella belongs to the *Enterobacteriaceae* family and is gram-negative, facultatively anaerobic, non-spore-forming and rod shaped, and motile forms have peritrichous flagella. They can ferment glucose while producing acid and sometimes gas (ICMSF, 1996). There are more than 2,600 serovars of *Salmonella.* In the EU, *S*. Enteritidis and *S*. Typhimurium are the serovars most frequently associated with human illness (EFSA, 2012). Human *S*. Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat, while *S*. Typhimurium cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat.

Salmonella can cause gastroenteritis, enteric fever and sepsis. The infection dose can vary from 20 to 10^6 cells depending on the serotype, food, and host vulnerability (age and health state) (Forsythe, 2010). Very low infection doses have been observed (< 100 cells) in water and fatty foods or foods with buffer capacities (ICMSF, 1996). The incubation period lies between 16-72 hours and the illness can take from 2 to 7 days.

The pathogen can multiply in a wide range of temperature (5.2-46.2ºC) and pH (3.8-9.5). *Salmonella* growth is significantly affected by the a_w value that promote growth optimally at *ca.* 0.99 and inhibit growth below 0.94 in broth. However, due to its high desiccation

tolerance, *Salmonella* was reported to be able to survive for a year or more in foods with low a_w such as chocolate, black pepper, peanut butter and gelatine (ICMSF, 1996).

Salmonella lives in the intestinal tract of human and animals as either pathogen or commensal and its distribution in nature is ubiquitous. Many foods, mainly of animal origin or contaminated with residual water, have been identified as vehicles of transmission of this pathogen to humans. According to the rapid alert system for food and feed (RASFF, 2010). *Salmonella*-caused food poisonings are amongst the most frequently reported. In 2010, a total of 99.020 confirmed cases of human salmonellosis were reported in the EU (EFSA, 2012). RTE-products such as salad vegetables, leafy greens, meat, poultry, seafood, dairy, eggs, and tree nuts, along with herbs, spices and dried seeds, have all been found to be contaminated with *Salmonella* (Jaroni et al., 2011). In Europe in 2012, *Salmonella* in foodstuffs was mainly detected in meat and products thereof (EFSA, 2012).

2.4. Incidence of *L. monocytogenes* and *Salmonella* in dry-cured meat products

Despite containing microbiological hurdles, various studies performed on dry-cured meat products have shown the survival of *L. monocytogenes* and *Salmonella* during manufacturing and/or subsequent ripening (Barbuti et al., 2002; Encinas, Sanz, García-López & Otero, 1999; Glass, Doyle, 1989; Hajmeer, Basheer & Cliver, 2006; Ihnot, Roering, Wierzba, Faith & Luchansky, 1998; Johnson, Doyle & Cassens, 1990; Nightingale, Thippareddi, Phebus, Marsden & Nutsch, 2006; Reynolds et al., 2001; Varabioff, 1992). In 2010, according to the EFSA, 0.5% and 0.6% of the analysed RTE products of pig meat did not comply with the microbiological criteria EC2073/2005 for *L. monocytogenes* and *Salmonella,* respectively (for *Salmonella*: n=11,675; for *L. monocytogenes*: n=22,158; EFSA, 2012).

Growth limits of pathogens can be tested and physicochemical parameters can be set at specific values for general purposes, but microorganisms may respond different in food products due to the complex interactions among physicochemical parameters and matrix composition (Brocklehurst, 2004) and the possible protective effects of some food components. Hence, to estimate and predict pathogenic microorganism behaviour in food products, especially when their processing includes new technologies or compositional changes, the careful investigation of hurdle effects in challenge test studies with the target food product and the microorganism of concern is indispensible and must precede commercialization.

3. Shortening the production of dry-cured meat products

Dry-cured hams and dry fermented sausages are traditional products prepared since the earliest civilizations to preserve meat and are still produced in large quantities due to their appreciated and typical flavour characteristics. However, their elaboration process is very time consuming, due to the long lasting drying-ripening times depending on the product, which take from a few weeks in small calibre fermented sausages up to years in Iberian hams obtained from Iberian pigs fed and fattened with acorns (Arnau et al., 2007). Therefore, processors of ripened food products have been searching for methods to speed up manufacturing to make the production of ripened food more flexible. In the 1970s, first attempts to accelerate the maturation of ripened foods were carried out in cheese, with the purpose of enhancing the lipid and protein breakdown by different strategies (Fernández et al., 2000). Most of the approaches to cheese ripening enhancement were related to elevated ripening temperature, enzyme addition to milk or curd, addition of slurries containing cheese flavour components or addition of modified or non-modified cheese related microorganisms (El-Soda, Madakor & Tong, 1999).

For dry fermented sausages, the objective of the first assays for accelerating the production was to remove as much water as possible prior to fermentation. Lu & Townsend (1973) shortened the drying period by incorporating freeze-dried meat into the meat block of a dry sausage formulation. With the incorporation of pale soft exudative (PSE) meat in the meat block (meat with reduced water holding capacity) Townsend (1980) succeeded in reducing the drying time of fermented sausages for *ca*. 40 to 50%. Chin, Keeton & Lacey (1996) investigated the shortening of the drying period to increase the efficiency of pepperoni production by the application of vacuum during drying, which may accelerate water evaporation from the surface by increasing internal capillary flow. As a result, authors reported a reduction in drying time of *ca*. 30% without noticeable quality defects. Another technique to shorten the drying time of fermented sausages comprises the reduction of the

product calibre (Arnau et al., 2007). In this way, the distance for water to reach the product surface is reduced. Soy protein isolates were described to stimulate LAB starter growth and speed up the fermentation process (Hagen, Naes, & Holck, 2000). Similarly Mn^{2+}, which can be found in some spices, accelerates the pH drop and stimulates lactobacilli growth, however, the magnitude and perseverance of the stimulating effect produced by Mn^{2+} differs with the type of LAB starter (Hagen et al., 2000; Vandendriessche, Vandekerckhove & Demeyer, 1980; Zaika & Kissinger, 1984). To eliminate the 12–48 h fermentation period, chemical acidification was proposed as an alternative to LAB fermentation (Barbut, 2005).

In order to speed up the process for dry-cured hams, several production techniques based on facilitating cure penetration and weight loss have been proposed, including boning and skinning pork legs prior to cure application, trimming of subcutaneous and intermuscular fat, blade tenderization and tumbling (Kemp, Abidoye & Langlois, 1980; Kemp & Fox, 1985; Marriott, Graham & Claus, 1992; Marriott, Graham, Shaffer & Phelps, 1987; Montgomery, Kemp & Fox, 1976; Ockerman & Organisciak, 1978). By using vacuum impregnation techniques in salting processes of meat, faster salting kinetics could be obtained with a more even salt distribution in the product and with increased process yields (Chiralt et al., 2001). The brine thawing/salting operation was introduced as a method in which frozen hams can be processed directly: Instead of using fresh pile salted ham, or thawing the ham in a cold chamber and proceeding to pile salting as usually done, thawing and salting are performed simultaneously (Barat, Grau, Montero, Chiralt & Fito, 1997). The brine thawing/salting operation was reported to involve changes in the different steps that constitute the whole process: salting, post-salting and maturation (Barat, Grau, Pagán-Moreno & Fito, 2004). Authors demonstrated that the use of brine thawing/salting with saturated brine in fresh and thawed hams allowed 58% and 61% time reductions, respectively, in reaching a NaCl concentration similar to the one obtained in the traditional pile salting method (Barat, Grau, Ibañez & Fito, 2005).

To accelerate production, Jessen (1995) highlighted the application of starter cultures to salt brines of dry-cured hams, which stabilize and improve colour and flavour. In boned hams the salting process can be accelerated by trimming the products of skin and subcutaneous fat and salting them directly combined with the curing mixture in a tumbler under vacuum. Once the curing mixture has been absorbed, the pieces could be treated with transglutaminase (TGase; EC 2.3.2.13), an enzyme that has the property to form crosslinks between protein molecules (DeJong & Koppelman, 2002; Kuraishi et al., 1997; Motoki & Seguro, 1998). Hams are then stuffed into casings and kept at 5ºC for a period longer than 2 h (Arnau et al., 2007). For the drying of restructured hams, water-permeable plastic bags have been proposed to minimize handling, to improve hygiene and binding, to start drying earlier and to prevent crusting, mould growth and mite infestation (Serra, Gou, Fulladosa, Costa & Arnau, 2007). By increasing the temperature and reducing the RH of air, the drying of the dry-cured ham process can also be speeded up (Arnau et al., 2007).

3.1. The Quick Dry Slice (QDS) process® – a fast drying technology

In terms of time, the drying period in the production of dry-cured meat products is the limiting step, requiring much energy and therefore contributing in a large extent to the total costs of the manufacturing process. A fast drying method would hence not only facilitate the reduction of drying, but also bring along a reduction in capital and labour. At the same time, the profit margin and the product competitiveness would increase, due to the possibility of rapid adaptation to marketing trends and production of small quantities. Some safety concerns, such as mould growth, lipid oxidation and mite infestation, which could happen during long traditional drying periods, are additionally reduced.

To meet all these requirements, the Quick-Dry-Slice (QDS) process® has been patented for sliced products (Comaposada, Arnau, Gou & Monfort, 2004). Dry-cured meat products are fermented to the desired pH in the case of dry fermented sausages, or prepared according

to manufacturing protocols in the case of dry-cured hams, and then frozen, sliced and dried in a continuous system with the application of convective air (Metalquimia S.A., Girona, Spain). Figure 2 represents a schematic flow-diagram comparing QDS and traditional processing for dry fermented sausages and dry-cured ham. With the QDS system, the traditional drying process could be drastically reduced, to approx. 45 min, depending on slice diameter and thickness.

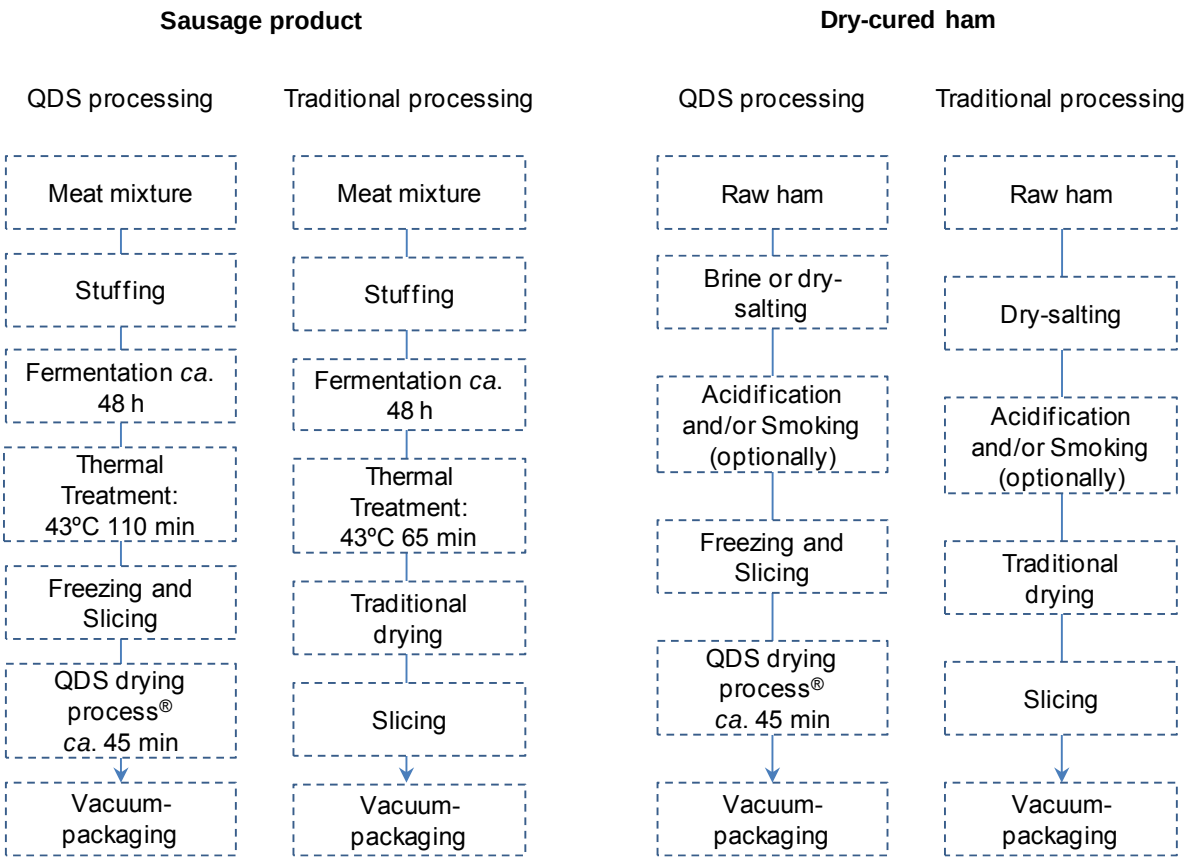

Figure 2. Schematic representation of the production of QDS products and comparison with traditional processing

3.1.1 The QDS process® pre-prototype

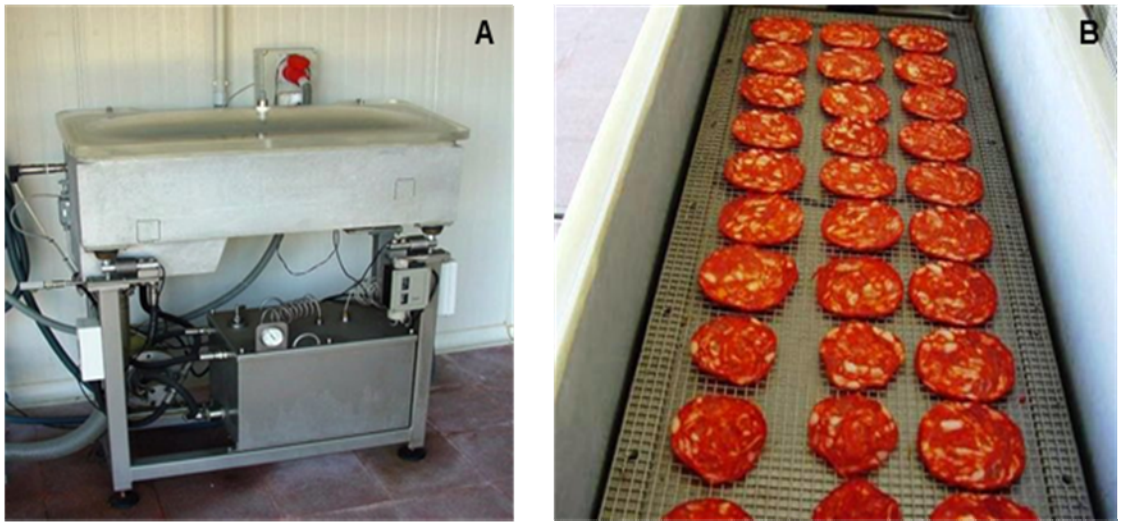

Figure 3. A: QDS process® pre-prototype equipment; B: tray loaded with dry fermented sausage slices

The first QDS equipment was constructed out of a hermetically sealable tank (Figure 3 A), in which only one metallic tray fit. In this pre-prototype the vacuum drying could be performed. The trays were manually loaded, with product slices (Figure 3 B), weighed and placed.

3.1.2 The QDS process® prototype equipment

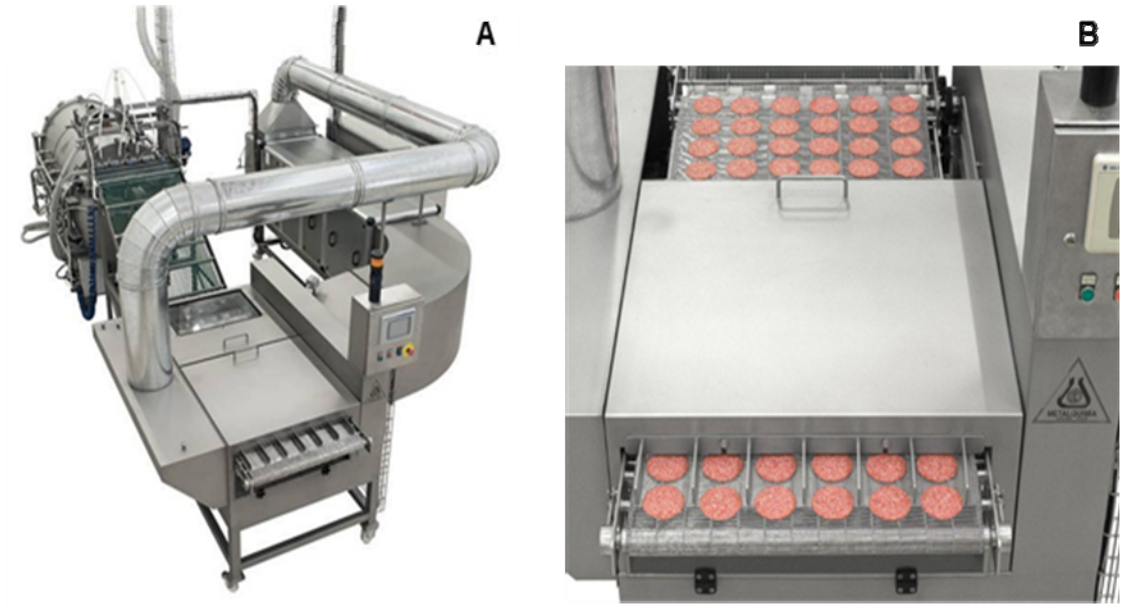

Figure 4. A: QDS process® prototype equipment; B: convective drying zone loaded with dry fermented sausage slices

Pictures kindly provided by Metallquimia S.A., text adapted from Comaposada et al. (2010).

The equipment was designed by the company Metalquimia S.A. (Figure 4 A) for performing QDS processing in a continuous system. It consists of a zone for loading frozen slices, a tempering and forced-air drying zone and a vacuum-drying zone to eliminate the water from the slices that is most difficult to extract. Slices are deposited on stainless steel conveyor belts (Figure 4 B) so that water can drain from the slices during the forced-convection drying phase and the vacuum-drying phase. The tempering-drying air used in the forced-convection phase is conditioned by using a high-efficiency absolute filter (HEPA) to minimize contamination of the air contacting the product. Additionally and in order to regulate the tempering-drying speed, control is exercised on temperature, RH and velocity of the air to which the product slices are exposed. The vacuum-drying phase is mainly regulated by the working pressure and the heating temperature. The different process sections are connected

by means of conveyor belts and mechanisms (gantry robots) for loading and unloading the slices. The entire process is regulated with a Programmable Logic Controller that also allows for monitoring and recording control parameters.

In the development of QDS processing, research on the prototype equipment revealed that it was possible to reduce or eliminate the vacuum drying stage for most products, replacing it with a longer convective drying time. The obtained products showed equivalence to those produced with the original QDS process®, while the production was more simple and required less energy. So, the industrial line for the QDS process® was constructed, using a convective drying.

3.1.3 The QDS process® industrial line

Here is a scheme of a QDS process® line with an average production capacity of 400 kg/h.

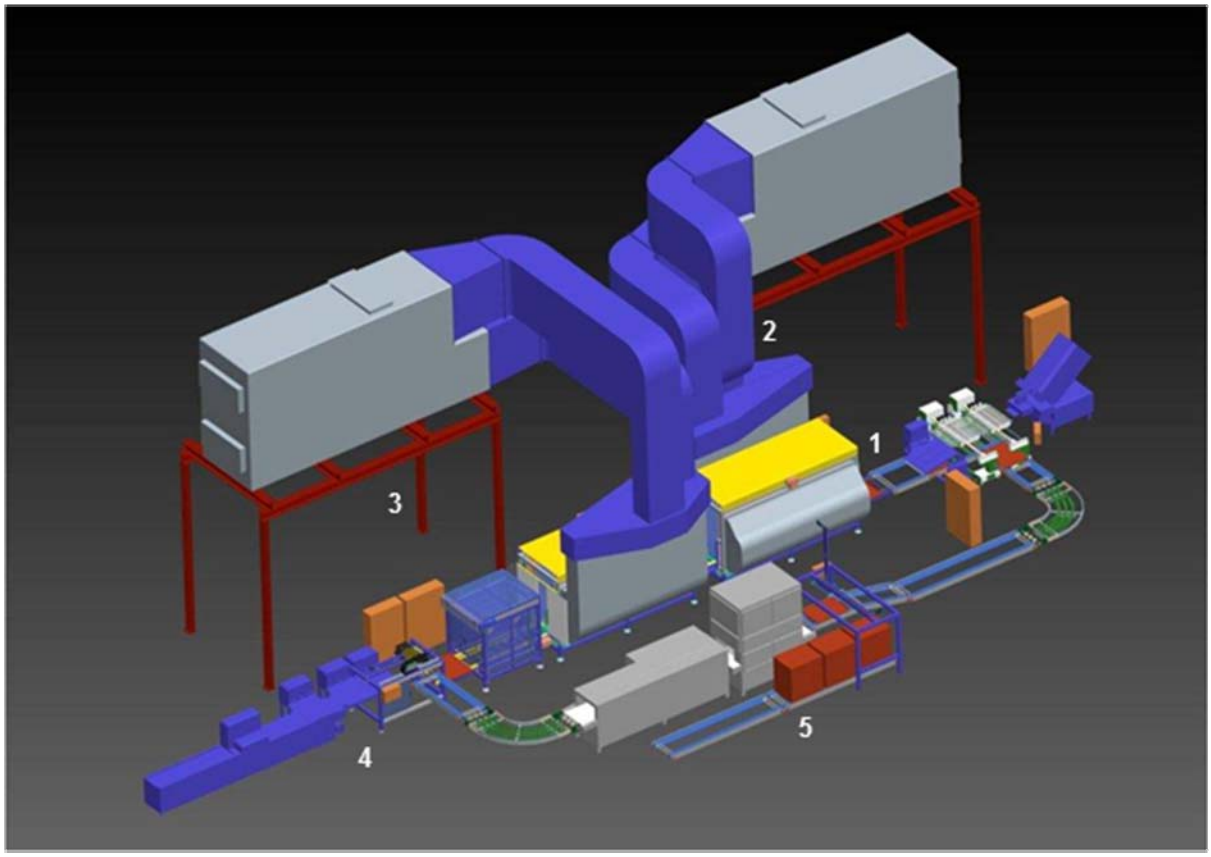

Figure 5. QDS process® industrial line

The product is sliced in the slicing zone (1) and distributed on perforated plastic trays, which transport the slices to the convective drying zone (2). Slices are dried by means of a lateral flow of air at controlled temperature and humidity. Due to the short processing time required for drying with QDS, higher processing temperatures can be tolerated without altering the sensory characteristics of the product (Comaposada et al., 2010). These temperatures, usually between 20 and 30ºC, would allow, in temperate climate zones, the use of air conditioners that automatically regulate and re-circulate the mixture of outer air ("free-cooling") instead of using refrigeration equipments. After passed through the QDS process® drying tunnels and with the aim to adapt the temperature of the slices to packaging

conditions, trays pass through the accumulator-cooler zone (3). After final weighing and definite weight loss calculation, slices are supplied to the packaging zone (4) where they are placed in thermoformer forms. The washing zone (5) consists of a sanitation and drying tunnel and is used for the cleaning of the trays.

3.1.4 Effects of QDS drying on product characteristics

Some differences in organoleptic properties were noticed between QDS and traditionally processed dry fermented sausages, especially less acid flavour was recorded in QDS dried products (Comaposada et al., 2008). This observation was probably due to differences in pH evolution: whereas pH normally changes during traditional drying (Leistner, 1995), values maintained during QDS drying (Comaposada et al., 2008) due to the no formation of an acid gradient between the outer and central part of the slice and the even removal of volatile acids during drying (Arnau et al., 2007). Colour was more intense in the QDS processed than in traditionally dried products and some sensitive colorants (e.g. Ponceau 4R) did not fade during the process (Comaposada et al., 2008).

At the food safety level, a preliminary study suggested that dry fermented sausages manufactured with the QDS system did not show important differences when compared to a traditionally dried fermented product (Arnau et al., 2007). However, the effect of QDS processing on the food safety of different types of dry-cured meat products containing different hurdles has not yet been evaluated.

4. The design of NaCl-free processing

Salting is one of the oldest techniques of preservation and allowed the extension of nutritional benefits of meat from times of plenty to times of scarcity during many years (Stringer et al., 2005). The development of other preservation techniques such as refrigeration and better packaging, transport and storage, however, reduced the need of high salt levels to maintain product integrity. Nevertheless, the exclusion of NaCl from the

production of meat products is a challenging task, because salt contributes to functional and organoleptic product characteristics and exerts a preservative effect.

4.1. The effect of NaCl on functional and organoleptic product characteristics

In meat, salt alters the osmotic equilibrium and increases the water holding capacity: while the inclusion of Cl^- in the meat matrix provokes the loss of the myofibrilar structure, Na^+ cations are pulled close to the filament surfaces creating an uneven distribution of ions in the water phase. This establishes an osmosis-like force within the filament lattice pulling water molecules into the system (Offer & Knight, 1988). The contribution of NaCl to protein solubilisation permits the binding of the product and therefore the achievement of the desired texture (Desmond, 2006).

In this sense, one of the particular problems associated with partial or complete NaCl reduction is related to changes in the binding and thus, in texture (Arnau, Comaposada, Serra, Bernardo & Lagares, 2011).

Further, the characteristic organoleptic properties of dry-cured meat products including texture and flavour are due to enzymatic processes (proteolysis, lipolysis and lipid oxidation), which generate peptides, free amino acids, free fatty acids and various volatile compounds during the whole production and ripening period (Molly et al., 1997; Toldrá, 2006; Toldrá, Flores & Sanz, 1997). It was suggested that a certain level of NaCl was important for the stabilisation and activation of muscle proteases and exerts a promoting effect on lipolytic activity (Costa Corredor, 2010). However, in dry-cured ham, the decrease of proteolytic activity throughout the ongoing salting process was related to the inhibiting effect of high salt concentration (Sárraga, Gil, Arnau & Monfort, 1989).

NaCl is the saltiest sodium ingredient (Doyle & Glass, 2010) and its flavour enhancing properties increasing the characteristic flavour of meat products can be related to its effect

on a_w (Matulis, McKeith & Brewer, 1994; Ruusunen, Särkkä-Tirkkonen & Puolanne, 1999). Due to a_w reduction, the concentration of other compounds in solution is increased, their volatility is enhanced and therefore their sensory perception. The intensity of the salty taste was demonstrated to be increased by glutamic acid and aspartic acid, which are formed during proteolysis, (Careri et al., 1993) but diminished by ingredients like sugars or special organic or inorganic salts used to reduce the sodium content in meat products (Boadas, Gou, Guarida & Arnau, 2000), which should be kept in mind when implementing product reformulations with the aim to reduce or exclude the NaCl amount. Another particular problem with low-salt meat products is that not only the perceived saltiness, but also the intensity of the characteristic flavour decreases (Ruusunen et al., 2005).

4.2. The effect of NaCl on microbiological stability

The preservative effect of NaCl is primarily due to its ability to lower a_w (Marsh, 1983; Sofos, 1983). Bacterial cells maintain the osmotic equilibrium with their surrounding media that means that an addition of ions, for example through NaCl, causes a water efflux through their semi-permeable membranes, which leads to shrinkage of the cytoplasmic volume. This efflux is also called plasmolysis (Csonka, 1989; Stringer et al., 2005). As cells must maintain a suitable level of cytoplasmic water for effective functioning of cell components, they try to maintain homeostasis by active accumulation of ions or uptake or synthesis of compatible solutes. The energy expended in these activities reduces and eventually prevents growth (Stringer et al., 2005). In this sense a sudden onset of plasmolysis was reported to cause inhibition of nutrient uptake and DNA replication and triggered an increase in the ATP levels of cells, which could lead to inhibition of macromolecular biosynthesis (Csonka, 1989). Salt concentrations between 3 and 7% were described to potentially inhibit enzymes important for glycolysis and the acid citric cycle (Krebs-cycle) (Csonka, 1989). However, early research showed that the preserving effect of NaCl involves more than dehydrating capacity. Magnesium sulphate was shown to have greater dehydrating effect on proteins than NaCl,

but was not as bacteriostatic as NaCl against *Staphylococcus aureus* (Rockwell & Ebertz, 1924). This research concluded that the factors involved in preservative properties of NaCl include the direct toxicity of Cl^-, removal of oxygen from the medium, sensitisation of the organisms to CO_2, and interference with the rapid action of proteolytic enzymes. The grade of fineness of NaCl was also related to its cell damaging effect (Hajmeer, Ceylan, Marsden & Fung, 2006). Researchers observed that extra coarse grade NaCl (e.g, sea salt) had a milder effect compared to fine grade salt on *Escherichia coli* and *S. aureus* cells.

The inhibiting effect of salt on pathogenic microorganisms has been observed in culture media and in food products. From studies on *S. typhimurium* in glucose-mineral salts medium at fixed temperature (19°C) and pH (7.0) levels, Thayer, Muller, Buchanan & Phillips (1987) observed decreasing aerobic growth with increasing NaCl concentrations. Other specific research focussed on pathogenic growth inhibition due to salt, confirmed plasmolysis and morphological changes due to growth in media supplemented with NaCl for *L. monocytogenes* (Zaika & Fanelli, 2003). NaCl also affected cellular progresses in *Clostridium sporogenes*, *Paracoccus denitrificans* and *S. aureus* (Erecinska & Deutsch, 1985; Smith, Maurer, Bencivengo & Kunsch, 1987; Woods & Wood, 1982). Desmond (2006) stated that salt was added to meat particularly as a deterrent to the growth of *Clostridium botulinum*. In cured meat products, the synergistic effect of nitrite and NaCl against *C. botulinum* has been reported (Sofos, 1983). The antimicrobial contribution of NaCl in a food system may also be influenced by other ingredients or food processing techniques. Synergistic effects or interactions of NaCl with benzoate, sorbate, phosphates, antioxidants (BHA), spices, liquid smoke, isoascorbate, etc. can be found in literature (Sofos, 1983). Smoking and NaCl were observed to inhibit *L. monocytogenes* in salmon (Niedziela, MacRae, Ogden & Nesvadba, 1998) and *C. botulinum* in fish (Eklund, Pelroy, Paranjpye, Peterson & Teeny, 1982). Similarly, drying and 3.64% (w/w) NaCl limited growth of coliforms and *S. aureus* toxin production during drying (10 days at 21°C) of air-dried fresh pork sausage (Bang, Hanson & Drake, 2008).

Microorganisms may tolerate salt stress in otherwise optimum conditions, however, this ability varies widely between species and will be reduced by suboptimal pH, temperature, redox potential, nutrient availability and the presence of other antimicrobial agents (Stringer et al., 2005).

4.3. Attempts to achieve the complete or partial exclusion of NaCl from the processing of dry-cured meat products

According to Ruusunen et al. (2005), different options exist to reduce the NaCl content in processed meat products, including

(i) the replacement (totally or partially) with other chloride salts, such as KCl, $CaCl_2$ and $MgCl_2$, of which KCl has been described to be the most used substitute for NaCl in low- or salt/sodium reduced foods (Desmond, 2006).

(ii) the replacement with non-chloride salts,

(iii) the use of new processing techniques or process modifications,

(iv) combinations of all the above approaches.

For dry-cured ham, techniques to accelerate salt distribution could be useful to reduce the amount of NaCl, including the reduction of ham thickness, using boned hams, trimming away subcutaneous and intermuscular fat and salting ham pieces with the curing mixture in a tumbler under vacuum (Arnau et al., 2007). In this type of products, the enzyme transglutaminase, which is used in developing restructured and low salt meat products (Verma & Banerjee, 2012), could be helpful to facilitate binding (Fulladosa, Serra, Gou & Arnau, 2009; Motoki et al., 1998).

The patent “Composición para la sustitución total o parcial del cloruro sódico en la elaboración de productos cárnicos crudos curados parcialmente deshidratados, uso de dicha composición, y proceso para la elaboración de productos cárnicos crudos curados parcialmente deshidratados en ausencia total o parcial de cloruro sódico” (Arnau et al.,

2011) allows for the first time, the production of dry-cured meat products without the addition of NaCl. "NaCl-free processing" could be developed by using KCl and potassium lactate (being K^+ at equalmolar concentrations as Na^+) and sugars for taste correction.

The following tables represent an overview of the published results on the partial or complete reduction of NaCl and its effect on physicochemical and organoleptic properties (Table 4) and microbiological stability (Table 5).

Table 4. Overview about the studies investigating the impact of partial or complete NaCl replacement on physicochemical and organoleptic properties of dry-cured meat products.

Main focus	Implementation of NaCl reduction	Important Observations	Reference
Studies using KCl and/or potassium lactate			
Physicochemical and organoleptic parameters of restructured dry-cured ham	I: Control: 30 g/kg NaCl II: 15 g/kg NaCl III: 15 g/kg NaCl + 39.7 g/kg potassium lactate	Potassium lactate had negative positive effect on colour, flavour or texture	(Fulladosa et al., 2009)
physicochemical and sensory parameters of restructured dry-cured hams	I: Control: 30 g/kg NaCl II: 15 g/kg NaCl III: 15 g/kg NaCl + 19.7 g/kg potassium lactate	II: Reduction of saltiness, increase of a_w, proteolysis and softness; III: the addition of potassium lactate contributed to reduce effects observed in II	(Costa-Corredor, Serra, Arnau & Gou, 2009)
consumer acceptability of small calibre fermented sausages	50% molar substitution of NaCl with 6 different mixtures of KCl (0-50%) and potassium lactate (0-50%)	The reduction of 50% of NaCl with a mixture of 40% KCl and 10% potassium lactate was sensorially accepted	(Guàrdia, Guerrero, Gelabert, Gou & Arnau, 2006; Guàrdia, Guerrero, Gelabert, Gou & Arnau, 2008)
texture and flavour of fermented sausages	9 different mixtures in which NaCl is partially (up to 60%) substituted by different combinations of KCl, potassium lactate and glycine	notable flavour and texture defects when using 40% KCl or 30% potassium lactate + 20% glycine; important flavour and texture defects when NaCl is replaced by > 40%	(Gelabert, Gou, Guerrero & Arnau, 2003)
texture, flavour and colour of fermented sausages texture, flavour and colour of dry-cured loin	Substitution of NaCl by KCl (0 - 60%), potassium lactate (1-100%) and glycine (0-100%)	Important flavour defects when NaCl was substituted at > 40%; loss of cohesiveness by using potassium lactate > 30% and glycine > 50% Important flavour defects when NaCl was substituted by KCl and potassium lactate > 40% and glycine > 30%; sensory analysis of substitution did not detect an effect on texture traits	(Gou, Guerrero, Gelabert & Arnau, 1996)
sensory parameters of Pasterma	NaCl was replaced at 30, 40 or 50% levels by KCl or potassium lactate	no significant changes in sensory properties when NaCl was substituted up to 40%	(Askar, El-Samahy & Tawfik, 1994)

Table 4. continued.

Main focus	Implementation of NaCl reduction	Important Observations	Reference
Studies using KCl and/or other chloride salts			
biochemical and sensory changes of dry-cured ham		III: no significant effect on proteolysis; all sensory attributes were affected; II: better evaluation but notable bitter taste of KCl	(Armenteros, Aristoy, Barat & Toldrá, 2012)
post salting stage of dry-cured ham	I: Control: 100% NaCl II: 50% NaCl+50% KCl III: 55% NaCl+25% KCl+15% $CaCl_2$+5% $MgCl_2$	II: 16 days longer post salting than I; III: 26 days longer post salting than I	(Aliño, Grau, Fuentes & Barat, 2010b)
physicochemical parameters of dry-cured ham		II: higher salt concentration than I and lower water contents than in I; III: lower salt concentration due to the difficulty of divalent cations to penetrate the muscle, which induced a higher water content and higher a_w values than in I	(Aliño, Grau, Toldrá & Barat, 2010)
physicochemical properties of dry-cured loin	I: Control: 100% NaCl II: 55% NaCl+25% KCl+15% $CaCl_2$+5% $MgCl_2$ III: 45% NaCl+25% KCl+20% $CaCl_2$+10% $MgCl_2$ IV: 30% NaCl+50% KCl+15% $CaCl_2$+ 5% $MgCl_2$	I-III: no significant differences in physicochemical characteristics; IV: significant increase in hardness and chewiness	(Aliño et al., 2010)
biochemical and sensory changes of dry-cured loin		II and IV: higher proteolytic acitivty than I; II: no significant differences in sensory traits when compared to I	(Armenteros, Aristoy, Barat & Toldrá, 2009b)
pile salting of dry-cured loin	I: Control: 100% NaCl II: 75% NaCl+25% KCl III: 65% NaCl+35% KCl IV: 50% NaCl+50% KCl V: 55% NaCl+25% KCl+15% $CaCl_2$+5% $MgCl_2$ VI: 45% NaCl+25% KCl+20% $CaCl_2$+10% $MgCl_2$	Strong effect of required salting time to reach comercial chloride concentration: presence of KCl decreased salting time while the addition of $CaCl_2$ and $MgCl_2$ had a contrary effect	(Aliño, Grau, Fuentes & Barat, 2010a)
compositional, physic-chemical and sensory parameters of Italian salami	I: Control: 27 g/kg NaCl II: 13.5 g/kg NaCl + 4.2 g/kg KCl + 2.4 g/kg $CaCl_2$ + 2.4 g/kg $MgCl_2$	II: Limited detrimental effects on sensory attributes, no effects on compositional parameters, pH, a_w and free fatty acid composition; significant increase in lipid oxidation	(Zanardi, Ghidini, Conter & Ianieri, 2010)
biochemical changes and sensory characteristics of dry-cured loin	I: Control: 100% NaCl II: 65% NaCl + 35% KCl III: 50% NaCl + 50% KCl IV: 30% NaCl + 70 % KCl	No significant differences in proteolysis, lipolysis and sensory analysis between I, II and III	(Armenteros, Aristoy, Barat & Toldrá, 2009a)
physicochemical parameters of dry-cured loin		Substitution of NaCl with KCl up to 50% without detrimental effects on physicochemical parameters was possible	(Aliño et al., 2009)
texture and colour of dry fermented sausages	I: Control: 2.6% NaCl II: 1% NaCl + 0.55% KCl + 0.74% $CaCl_2$	II: acceptable, but lower scores in texture and colour than in I	(Gimeno, Astiasarán & Bello, 1999)

Table 4. continued.

Main focus	Implementation of NaCl reduction	Important Observations	Reference
proteolysis and insolubilisation processes of dry fermented sausages		II higher percentage of insoluble protein fraction and higher intensity of proteolysis; softer and saltier taste than I	(Ibañez, Quintanilla, Cid, Astiasarán & Bello, 1997)
stability of the nitrosation process of dry fermented sausages	I: Control: 3% NaCl	II: more intense nitrosation process, lower pH and higher a_w than I	(Ibañez, Quintanilla, Cid, Astiasaran & Bello, 1996)
Lipid fraction of dry fermented sausages	II: 1.5% NaCl + 1% KCl	II: higher amount of volatile fatty acids than I and increased lipolytic activity	Quintanilla, Ibañez, Cid, Astiasarán & Bello 1996)
carbohydrate fermentation and nitrosation process of dry fermented sausages		Results suggested that II favoured the nitrosation process and carbohydrate heterofermentative activity of microorganisms	(Ibañez et al., 1995)
proteolysis, texture and flavour of Country-style ham	I: Control: 3% NaCl II: 1.5% NaCl + 1% KCl I: Control: 100% NaCl II: 33.3% KCl + 66.7% NaCl III: 66.7% KCl + 33.3% NaCl IV: 100% KCl. Complete (100%) or partial (50%) substitution of NaCl with equivalent ionic strengths of either KCl or LiCl	III and IV had higher levels of residual NO_3^-, less aged flavour, more cohesiveness and were unacceptable due to extreme bitterness. Hams II showed the same slight level of bitterness as I; hams with KCl had worst off-flavour, LiCl is not GRAS listed (toxic) but achieved sensory values more closely to NaCl	(Keeton, 1984)
Studies with simple NaCl content reduction			
sensory characteristics of Iberian dry-cured ham	2 different salt levels: I: 6% NaCl (w/w) II: 3% NaCl (w/w)	*Biceps femoris* muscles from I were harder, dryer and more fibrous than II	(Andrés, Cava, Ventanas, Thovar & Ruiz, 2004)
organoleptic, chemical, and physical parameters of dry sausages	I: 2.25% NaCl II: 2.5% NaCl III: 2.75% NaCl IV: 3.00% NaCl V: 3.25% NaCl	I: less acceptable flavour and softer texture than II-V; faster a_w decrease in V than in I	(Petäjä, Kukkonen & Puolanne, 1985)

From the published studies, the main findings on physichochemcial and organoleptic level were that NaCl could be reduced up to a maximum of 50%, by the combination of KCl (40%) and potassium lactate (10%) (Guàrdia et al., 2006; Guàrdia et al., 2008). A substitution of NaCl with KCl at levels higher than 40% resulted in sensorially unacceptable products. Potassium lactate was reported to improve colour, juiciness, tenderness and enhanced flavour (Terrell, Quintanilla, Vanderzant & Gardner, 1983) and was shown to extend shelf life of dry-cured meat products (Choi & Chin, 2003; Pipek et al., 2005; Prasai et al., 1992). However up to date, as can be seen from Table 4, no sensorially acceptable formulation for the complete NaCl replacement has been published.

In the NaCl-free processing strategy followed by Arnau et al. (2011) and used in the studies included in this PhD thesis, the texture and flavour problematic has been solved by using lactate together with the chloride salt KCl to reduce the a_w and to favour the protein solubilisation of the meat product. Additionally, authors found that the addition of an encapsulated acid or acid precursor (GDL) significantly contributes to correct the bitter and unpleasant taste of lactate and KCl and has a positive effect on the texture of the final product. This formulation, accordingly, can be used for the elaboration of dry-cured meat products without negatively affecting the organoleptic properties.

Table 5. Studies investigating the impact of partial or complete NaCl replacement microbiological stability of dry-cured meat products

Main focus	Implementation of NaCl reduction	Important Observations	Reference
	Studies using KCl and/or potassium lactate		
safety and quality of restructured dry-cured ham	I: Control: 30 g/kg NaCl II: Salt reduced: 15 g/kg NaCl III: Salt reduced + lactate: 15 g/kg NaCl + 39.7 g/kg potassium lactate	Produced products were safe; potassium lactate reduced a_w and microbiota mainly in the inner parts of the hams and had no effect on colour or sensory parameters	(Fulladosa, Sala, Gou, Garriga & Arnau, 2012)
microbiological parameters of fermented sausages	9 different mixtures in which NaCl is partially (up to 60%) substituted by different combinations of KCl, potassium lactate and glycine	Little effect on microbiologial stability	(Gelabert et al., 2003)
microbiological parameters of Pasterma	NaCl was replaced at 30, 40 or 50% levels by KCl or potassium lactate	No differences in bacteriological analysis	(Askar et al., 1994)
	Studies using KCl and/or other chloride salts		
Physicochemical properties of dry cured loin	I: Control: 100% NaCl II: 55% NaCl+25% KCl+15% $CaCl_2$+5% $MgCl_2$ III: 45% NaCl+25% KCl+20% $CaCl_2$+10% $MgCl_2$ IV: 30% NaCl+50% KCl+15% $CaCl_2$+ 5% $MgCl_2$	I-III: no significant differences in physicochemical characteristics; IV: significant increase in hardness and chewiness	(Aliño et al., 2010)
microbiological parameters of dry-cured loin	I: Control: 100% NaCl II: 65% NaCl + 35 % KCl III: 50% NaCl + 50% KCl IV: 30% NaCl + 70% KCl	no differences in microbial counts between all formulations	(Aliño et al., 2009)
physicochemical and microbiological changes during the post-salting stage of dry-cured ham	I: Control: 100% NaCl II: 50% NaCl + 50% KCl III: 55% NaCl + 25% KCl + 15% $CaCl_2$ + 5% $MgCl_2$	No differences in counts of mesophilic aerobic counts and salt tolerant microbiota between all formulations; II and III needed more time to reach similar a_w values than I, especially III	(Blesa et al., 2008)

Table 5. Continued.

Main focus	Implementation of NaCl reduction	Important Observations	Reference
microbiology of dry fermented sausages	I: Control: 2.6% NaCl II: 1% NaCl + 0.55% KCl + 0.74% $CaCl_2$	No effect on *Lactobacillus* and *Micrococcaceae* counts, indicating that modification did not affect starter culture development	(Gimeno, Astiasarán & Bello, 2001)
physicochemical and sensory parameters of dry fermented sausages	I: Control: 2.6% NaCl II: 1% NaCl, 0.55% KCl, 0.23% $MgCl_2$, and 0.46% $CaCl_2$	II: lower pH and higher a_w; lower *Micrococcaceae* counts; lower sensory acceptability than I	(Gimeno, Astiasarán & Bello, 1998)
physicochemical and microbiological parameters in dry fermented sausages	I: Control: 3% NaCl II: 1.5% NaCl + 1% KCl	Similar physicochemical parameter levels and microbiological results	(Ibañez et al., 1995)
quality and microflora of boneless dry-cured ham	I: Control: 100% NaCl II: 70% NaCl + 30% KCl III: 50% NaCl + 50% KCl	II: no effect on palatability or microbial quality (aerobic counts) III: important decrease in palatability and increase in microbial counts.	(Leak, Kemp, Fox & Langlois, 1987)
Studies with simple NaCl content reduction			
survival of *S. typhimurium* and *S. aureus* in Genoa salami	I: 2% NaCl II: 2.75% NaCl III: 3.3% NaCl	No differences in a_w and pH; *Salmonella* could no longer be detected from day 11 in none of the samples; Higher counts of *S. aureus*: were recorded in I than in II and III	(Messier, Smith & Tittiger, 1989)
microbiology of dry sausages	I: 2.25% NaCl II: 2.5% NaCl III: 2.75% NaCl IV: 3.00% NaCl V: 3.25% NaCl	similar levels of total bacteria counts, lactobacilli and staphylococci in I–V; highest level of gram-negative bacteria in V and lowest level in I	(Petäjä et al., 1985)

Changing salt concentrations will not affect the occurrence of organisms in foods but may affect their growth, survival or death (Stringer et al., 2005). Although it is known that the lowering of the salt content potentially reduces product safety unless alternative hurdles are included or shelf-life is reduced, only a few studies have focussed on microorganism behaviour, and only one has evaluated the fate of inoculated pathogens. In NaCl-free processing, the food safety primarily depends on the antimicrobial effects of KCl and potassium lactate used to substitute NaCl. The antimicrobial effects of equalmolar concentrations of NaCl and KCl at similar a_w values in broth were evaluated and both salts exerted similar effects on *L. monocytogenes* Scott A (Boziaris, Skandamis, Anastasiadi & Nychas, 2007). It was therefore concluded that NaCl could be replaced by KCl without risking the microbiological safety of the product (Bidlas & Lambert, 2008; Boziaris et al., 2007). However, Bautista-Gallego, Arroyo-Lopez, Duran-Quintana & Garrido-Fernandez (2008) showed in broth, that KCl was less inhibitory for *Lactobacillus pentosus* than NaCl.

The antimicrobial effect of lactate has been studied in broth (Chen & Shelef, 1992; de Wit & Rombouts, 1990). At equal a_w values, van Burik & de Koos (1990) showed in studies performed on culture media that sodium lactate provided better growth inhibition on *S. typhimurium* and *S. aureus* than NaCl. Moreover, in a wide range of meat products such as cooked ham (Jofré, Garriga & Aymerich, 2008; Stekelenburg & Kant-Muermans, 2001), frankfurter sausage (Stekelenburg, 2003), beef bologna (Mbandi & Shelef, 2002), cooked beef (Miller & Acuff, 1994), chicken dry fermented sausage (Deumier & Collignan, 2003) and restructured dry-cured ham with a reduced NaCl content (Fulladosa et al., 2012) the antimicrobial effect of lactate has been demonstrated. Additionally, the effect of lactate was reported to be enhanced by NaCl (Shelef, 1994; Taormina, 2010) and GDL (Garcia Zepeda et al., 1994; Juncher et al., 2000).

A considerable number of studies deal with the exclusion or reduction of salt in dry-cured meat products (Table 4 and 5), however, most of them investigated the effect of NaCl reduction on physicochemical and sensory traits. Up to date, no food safety studies have been performed on the complete exclusion of NaCl from dry-cured meat processing.

5. High pressure processing

Consumers increasingly demand high quality convenient foods with natural flavour and taste, free from additives and preservatives. With the aim to meet these requirements, several non-thermal technologies as alternatives and/or complementary technologies to traditional conservation treatments have been developed, among them high pressure (HP) processing (Garriga & Aymerich, 2009; Rastogi, Raghavarao, Balasubramaniam, Niranjan & Knorr, 2007). HP processing is defined as adiabatic compression, hold, and decompression of foods at pressures in the range of 100 to 800 MPa for holding times of 0.001 to 1200 seconds or longer (IFT, 2000). Nowadays, commercially available industrial HP equipments can reach up to 600-700 MPa and have capacities of up to 420 litres (Hiperbaric, 2012). At a

pressure level of 600 MPa, the economically reasonable time of treatment was estimated at 6 min (Garriga, Grèbol, Aymerich, Monfort & Hugas, 2004). Worldwide, more than 250 different HP-treated products are marketed. Roughly one-third of the HP processing machines are in use for processing RTE vegetables, primarily avocado products. A third of the installed HP machines are used to process meat products such as sliced or diced cooked pork, chicken, and turkey. The last third are used to process juices and beverages such as smoothies, seafood and fish, and other products such as dairy or for coprocessing or in tolling applications (Tonello, 2011). In Europe, HP technology is well accepted as an alternative technology and is industrially applied to a range of meat products, including dry-cured ones (Garriga et al., 2009). Spain as a pioneer in HP treated meat, first commercialized sliced cooked ham in 1998 (Tonello, 2011).

The effect of HP is based on two principles: as a general rule and first principle (Le Chatelier´s Principle), pressure enhances reactions that lead to volume decrease, and reactions involving increases in volume are generally inhibited or reduced by pressure application. According to the Arrhenius law, the reaction rate increases with increasing temperature. The second principle states (Principle of Pascal) that pressure is instantaneously and uniformly transmitted independent of the size and the geometry of the food. During pressurisation, the work of compression increases the temperature of foods through adiabatic heating approximately 3°C per 100 MPa, depending on the composition of the food (Smelt, 1998).

It is known that the key effects of HP include (i) the inactivation of microorganisms, (ii) the modification of biopolymers, such as protein denaturation, enzyme activation or inactivation, gel formation, influence on degradation, or extraction; (iii) quality retention (especially flavour and colour), due to the fact that only non-covalent bonds are affected by pressure; and (iv) product functionality, as exemplified by density changes, freezing and melting temperatures, or textural attributes (Knorr, 1993). The fact that nutritional values and quality are not affected by HP is viewed as an important benefit for food industry (Hoover, Metrick,

Papineau, Farkas & Knorr, 1989; Smelt, 1998; Téllez, Ramírez, Pérez, Vázquez & Simal, 2001). It can be applied as a final preservation measure, after slicing and packaging, as an in-package “cold” pasteurization step (Bover-Cid, Belletti, Garriga & Aymerich, 2011).

5.1. Effect on microorganisms

The greater the pressure level and time of application, the greater the potential for changes in the appearance of selected foods. These changes are usually undesirable for food but useful for the inactivation of pathogens.

High pressure induces several changes in the cell, including separation of the cell membrane from the cell wall, contraction of the cell membrane, compression of gas vacuoles, cell lengthening, and release of intracellular material (Patterson, 2005). Moderate levels of pressure decrease the rate of growth and reproduction, whereas very high pressures cause inactivation by completely destroying the functionalities of cell wall and cytoplasmic membrane, dissociation of the proteins and the ribosomal subunit structures and inactivation of some enzymes (Abe, 2007; Smelt, 1998). HP is similar to thermal processing in that there is a threshold value which depends on the microorganism and species and below which no inactivation occurs (Patterson, Linton & Doona, 2007). Above the threshold, cell death increases with pressure but it does not follow a first-order kinetics and sometimes there is a tailing off in inactivation (Garriga et al., 2004). Tailing may be a normal feature of the mechanism of resistance involving adaptation and recovery (Earnshaw, 1995). In practice, the non-logarithmic inactivation curves make it difficult to determine the appropriate kinetic parameters. Under favourable storage conditions sublethally injured microorganisms can recover and produce food-borne disease, as demonstrated in milk (Koseki, Mizuno & Yamamoto, 2008) and in chicken (Patterson, McKay, Connolly & Linton, 2010).

In general, gram-positive bacteria are usually more pressure resistant than gram-negative bacteria and the more developed the life form, the more sensitive it is to pressure (IFT, 2000). In general, cells in the exponential growth phase are more pressure-sensitive than

cells in the stationary phase (Mackey, Forestiere & Isaacs, 1995). Incomplete inactivation of microorganisms by pressure will result in injured cells capable of recovery under optimal growth conditions (IFT, 2000; Metrick, Hoover & Farkas, 1989).

Several studies have been performed on the gram-positive bacteria *L. monocytogenes* and *S.aureus* reporting their resistance against HP under various conditions (Chen, 2007; Garriga, Aymerich, Costa, Monfort & Hugas, 2002; Hayman, Baxter, O'Riordan & Stewart, 2004; Jofré, Aymerich, Grèbol & Garriga, 2009b; Jofré et al., 2008; Simpson & Gilmour, 1997b). Some strains of *Salmonella* spp. have also been demonstrated to have relatively high levels of pressure resistance (Jofré, Aymerich, Bover-Cid & Garriga, 2010). In phosphate and citrate buffer systems the inactivation of *L. monocytogenes* has been demonstrated to depend on the pH, duration and temperature of the treatment and pressure level (Ritz et al., 2000). Similarly, *Salmonella* was more inactivated at higher pressure levels in phosphate buffers (Patterson et al., 2007).

5.2. Studies on dry-cured meat products

The effect of pressure on several characteristics of meat and meat products has been published (Cheftel & Culioli, 1997; Ledward, 1998; Suzuki, Kim, Tanji, Nishiumi & Ikeuchi, 2006). Industry has found that an operating pressure of 600 MPa (87.000 psi) provides a satisfactory pasteurisation pressure and holding time (3-5 min) for most vegetative microbes (Tonello, 2011). Regarding pathogenic microorganisms of concern, HP has been recognized by the Codex Alimentarius (CAC, 2007) and the FDA, Health and Human Services (HHS, 2008) as a listericidal treatment for RTE products. A recently published model of HP induced inactivation of *L. monocytogenes* in dry-cured ham showed that considering the low contamination levels and the inability of *L. monocytogenes* to grow in dry-cured ham, 613 MPa for 5 min would be sufficient to achieve the U.S. “zero tolerance” policy (Bover-Cid et al., 2011). For *Salmonella*, a significantly reducing effect after pressurisation at 600 MPa has already been described (Bover-Cid, Belletti, Garriga & Aymerich, 2012; Jofré et al., 2009b).

Important findings from challenge tests with *L. monocytogenes* and *Salmonella* performed on different dry-cured meat products are summarized in Table 6.

Table 6. Important findings from HP studies focussed on the fate of *L. monocytogenes* and *Salmonella* in fermented sausages and dry-cured meat products

Product	Treatment Conditions P(MPa)/T(ºC)/t(min)	log cfu/ g	Storage cond.	Observations	Reference
Inactivation studies using high inoculum levels					
Sliced dry-cured ham pH: 5.85 aw: 0.891	600/12/5 500/12/5 400/12/5	6	8°C 60 days	*Salmonella* Enteritidis: 600 MPa: immediate reduction: 4.32 log, detectable in enrichment at end of storage. 500 MPa: immediate reduction: 2.54 log, further 2.66 log reduction after 60 days. 400 MPa: immediate reduction: 1.06 log, further reduction of 2.56 log after 60 days.	(de Alba, Montiel, Bravo, Gaya & Medina, 2012)
Sliced dry-cured ham pH: 5.84 a_w: 0.88	347-852/ 7.6-24.4/ 2.3-15.75 Modelling study	9	-	*Salmonella enterica:* Conditions proposed by authors to comply with the Food Safety Objective: 525 MPa (15.5 min/16ºC or 12 min/7.6ºC) to 600 MPa (12.1 min/16ºC or 5 min/23.5ºC).	(Bover-Cid et al., 2012)
				L. monocytogenes Conditions proposed by authors to achieve the U.S. "zero-tolerance: 613 MPa for 5 min.	(Bover-Cid et al., 2011)
Sliced dry-cured ham pH: 5.91 a_w: 0.92	600/15/5	7	8°C 60 days	*L. monocytogenes:* Immediate reduction: 3.85 log, decrease under LOD (10 CFU/g)at end of storage.	(Hereu, Bover-Cid, Garriga & Aymerich, 2012)
Sliced dry-cured ham pH: 5.84 a_w 0.88				*L. monocytogenes:* Immediate reduction: 1.82 log, further reduction of 3.34 log at end of storage.	
Genoa salami (65 mm-diameter) pH: 4.65 a_w: 0.92	600/19/5 483/19/12	7	4°C 28 days	*L. monocytogenes* Decrease to 6.08 log CFU/g before HP, 600MPa: immediate reduction: 3.94 log, further decrease to < 1 log CFU/g (=LOD) at the end of storage (absence in 5/5 enriched samples). 483MPa: immediate reduction:3.35 log, further decrease to < 1 log CFU/g (=LOD) at the end of storage (absence in 3/6 enriched samples).	(Porto-Fett et al., 2010)
				Salmonella Decrease to 2.21 log CFU/g before HP, 600MPa: immediate reduction to ≤ 0.3 log CFU/g (absence in 4/5 enriched samples). End of storage : absence in 6/6 enriched samples. 483MPa: immediate reduction to ≤ 0.3 log CFU/g (absence in 5/5 enriched samples) End of storage : absence in 6/6 enriched samples.	

Table 6. Continued.

Product	Treatment Conditions P(MPa)/T(ºC)/t(min)	log cfu/ g	Storage cond.	Observations	Reference
Sliced dry-cured ham pH: 5.91 a_w: 0.92	600/15/5	7	8°C 60 days	*Salmonella:* Immediate reduction of 4.18 log, decrease under LOD (10 CFU/g) during storage.	(Hereu, Bover-Cid, Rubio, Garriga & Aymerich, 2010)
Sliced dry-cured ham pH: 5.84 a_w: 0.88				*Salmonella:* Immediate decrease of 2.82 log, further reduction of0.65 logduring storage.	
Sliced dry-cured Serrano ham pH: 5.61 a_w: 0.88	450/12/10	6.78	4°C or 8°C 60 days	*L. monocytogenes* Scott A: Immediate reduction: 1.16 log, further decrease to 2.73 log CFU/g during storage.	(Morales, Calzada & Nuñez, 2006)
Sliced dry-cured Iberian ham pH: 5.9 a_w: 0.904				*L. monocytogenes* Scott A: Immediate reduction: 1.5 log, further decrease to 3.24 log CFU/g during storage.	
Growth inhibition studies using low inoculation levels					
Fuet pH: 5.92 a_w: 0.9	400/17/10	3	7°C 30 days	*L. monocytogenes:* grow to 6.5 log CFU/g before HP Immediate reduction: 0.58 log, decreasing trend during storage, 4 log decrease in last storagestage.	(Ananou et al., 2010)
				Salmonella: Immediate reduction: 2.08 log, furtherdecrease < 1 log CFU/g during storage.	
Cecina de Leon pH: 5.87 a_w: 0.909	500/18/5	4	6°C 120 days	*L.monocytogenes* Immediate reduction: 1.93 log, detectable at 2.56 log CFU/g until day 90.	(Rubio, Martínez, Garcia-Cachán, Rovira & Jaime, 2007a; Rubio, Martínez, Garcia-Cachán, Rovira & Jaime, 2007b; Rubio, Martínez, Garcia-Cachán, Rovira & Jaime, 2010)
Salchichón pH: 5.1 a_w: 0.827				*L.monocytogenes* Immediate reduction: 1 log, Further decrease <2 log CFU/g at day 15. No recovery recorded.	
Sliced dry-cured ham pH: 5.88 a_w: 0.91	600/31/6	3.5	4°C 120 days	*L. monocytogenes* and *Salmonella*: Immediate decrease under LOD (10 CFU/g) and no recovery during storage.	(Jofré et al., 2009b)
Fuet pH: 6.1 a_w: 0.93	400/17/10	2.7	7°C 30 days	*L. monocytogenes:* grow to 6.5 log CFU/g before HP, immediate reduction: 0.6 log, decreasing trend during storage, 4 log decrease in last storagestage.	(Jofré et al., 2009a)
				Salmonella: Immediate reduction: 2 log, further decrease to < 1 log CFU/g during storage.	

Table 6. Continued.

Product	Treatment Conditions P(MPa)/T(ºC)/t(min)	log cfu/ g	Storage cond.	Observations	Reference
Chorizo pH: 5.7* a_w: 0.98* (*at the moment of HP treatment)	300/17/10 Applied before ripening	2.8	Ripening cond.: 12°C 27 days	*L. monocytogenes*: Immediate reduction: 1 log, recovery of 1.8 log, which led to higher counts in pressurised than in non-pressurised samples.	(Marcos, Aymerich & Garriga, 2005)
				Salmonella: No immediate reduction, faster decrease during storage in HP treated samples.	
Fuet pH: 5.7* a_w: 0.98* (*at the moment of HP treatment)				*L. monocytogenes*: Immediate reduction: 1 log, recovery of 0.9 log, which led to higher counts in pressurised than in non-pressurised samples	
				Salmonella: No immediate reduction, faster decrease during storage in HP treated samples	
Chorizo pH: 5.7 a_w: 0.83-0.86	400/17/10	2.78	20°C 28 days	*L. monocytogenes:* Immediate reduction of 0.9 log, no information about behaviour during storage.	(Garriga et al., 2005)
				Salmonella: Immediate reduction to absence in 25 g, no recovery during storage.	
Fuet pH: 5.7 a_w: 0.83-0.86				*L. monocytogenes:* No immediatereduction, no information about behaviour during storage	
				Salmonella: Immediate reduction to absence in 25 g, no recovery during storage.	

In food products, two effects determine microbiological safety and stability: the effect of the food matrix during treatment and the effect after treatment (immediate and long term inactivation). In this context it should be taken into account that results from studies in buffers or laboratory media cannot be directly extrapolated to real food situations (Simpson & Gilmour, 1997a) because factors influencing threshold of inactivation not only include the pressure applied, the time and temperature of processing, pH and a_w, but also the composition of the food (Tewari, Jayas & Holley, 1999). In this sense, pressure resistance of microorganisms was reported to be reinforced in rich nutrient media (Hoover et al., 1989) and food constituents such as proteins, carbohydrates and lipids (Simpson et al., 1997a) or

cations such as Ca^{2+} could have a protective effect on microorganisms (Patterson et al., 2007).

Regarding performed studies listed in Table 6 and according to Jofré et al. (2009a), the effectiveness of pressurisation in dry-cured meat products not only depended on the treatment conditions (pressure and time) and the type of product, but also appeared to be highly related with the ripening stage at which the HP treatment was applied and the bacterial species. Used as a post processing treatment however, it can be assumed that HP could be used to eliminate low contamination levels of *L. monocytogenes* and *Salmonella* in dry-cured meat products.

Garriga et al. (2009) emphasized that low acid fermented sausages, due to the absence of the low pH hurdle, and sliced products, due to possible re-contamination during post handling (slicing), would benefit from the food safety enhancing effect of HP. For meat products with a low salt content, pressurisation was also proposed as potential complementary technology to enhance product shelf life (Verma et al., 2012).

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III. OBJECTIVES

The **main objective** of this PhD thesis was to evaluate the food safety impact of the QDS technology and/or NaCl-free processing on the fate of *Listeria monocytogenes* and *Salmonella* during the production and/or the refrigerated storage of dry-cured meat products. To that end the following specific objectives were proposed:

1. To evaluate the food safety impact of the QDS process® in acid and low acid chorizo;
2. To compare the food safety of NaCl-free processed acid and low acid chorizo produced with the QDS or the traditional drying method;
3. To evaluate the food safety impact of NaCl-free processing in traditionally dried smoked dry-cured ham;
4. To compare the food safety of QDS dried dry-cured hams produced with or without NaCl, acidification and smoking;
5. To assess the effectiveness of an industrial HP treatment (600 MPa at 13 ºC for 5 min) in all the above mentioned dry-cured meat products.

IV. EXPERIMENTAL DESIGN

The protocol of the challenge tests performed within the present PhD thesis was designed according to the recommendations from several challenge test guidelines documents published by different organisations/institutes (AFNOR, 2004; CRL/AFSSA, 2008; NACMCF, 2005; NACMCF, 2010; Scott et al., 2005) as follows:

- Products were handled under conditions that mimic as closely as possible the distribution, storage and use of a food product. Additionally in product preparation it was taken into account that the conditions (e.g. pH and a_w) within the range of variability, were most conducive to pathogen growth or survival ("worst-case scenario").

- The method of inoculation was consistent with how the food may be contaminated. Accordingly, the meat batter of comminuted products (products assumed to be homogenous) and the slices of whole piece products were spiked with *L. monocytogenes* and *Salmonella*.

- The choice of the pathogenic microorganisms was based on the likelihood of pathogen association with the specific food and pathogen resistance to inactivation. In this regard, the 2073/2005 regulation listed *L. monocytogenes* and *Salmonella* as pathogens that must be controlled in RTE-meat products.

- The chosen pathogenic microorganism strains were isolated from meat products and animal origin, in line with described desirable strain characteristics. In order to account for variations in growth and survival among strains, three different strains of each pathogen were used.

- Stationary phase cells were selected because the contaminating cells in a production environment are more likely to be in this phase than in the exponential phase.

- The level of inoculation reflected the contamination expected to occur in the food chain and was calculated regarding recommended levels of *ca*. 50-100 CFU/g to accurately represent the product's ability to support growth.

- The chosen temperature profile consisted of 1/3 of the storage at temperature 1 (T1) and 2/3 at temperature 2 (T2). T1 represented the temperature used under retail conditions (2-4ºC, restrictive temperature) and T2 the temperature common in the household (8ºC, abusive temperature).

- Storage times longer than commercial shelf-lifes were set for the evaluated products.

- Microbiological analysis of *L. monocytogenes* and *Salmonella* was performed directly after inoculation and periodically during subsequent processing and/or storage (five to seven samplings over the duration of the study were recommended). All experiments were repeated in two independent experiments and samples were analysed in duplicate (n=4). Recommendations about replicates indicate a minimum of two samples to be analysed at each sampling point, coming from different lots or batches to account for product variation.

- The determination of the physicochemical characteristics was necessary in order to compare the products submitted to challenge testing to the products routinely produced by the factory. Moreover, the determination of typical levels of competitive microbiota including starter cultures could provide useful information about possible interactions. For obvious safety reasons, no sensory assessment other than changes in appearance was performed on challenge test samples.

According to the objectives and the challenge test protocol, Figure 6 schematically represents the experimental design of all the assays performed in the framework of this thesis.

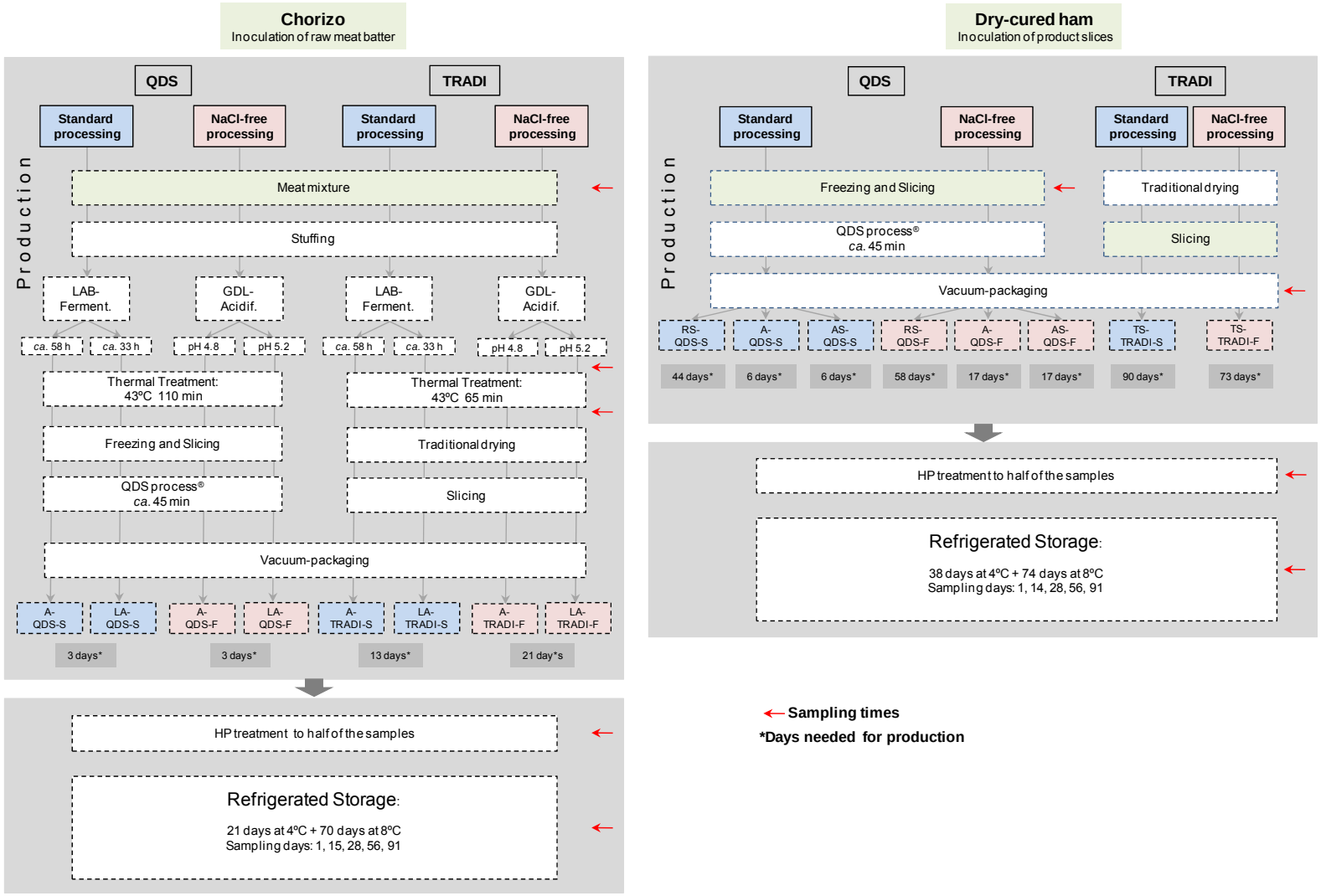

Figure 6. Schematic representation of experimental design

Table 7 lists the manufactured products, the abbreviations, which will be used in the general discussion and conclusions and the related sections of the "Results" in which the corresponding article is included.

Table 7. Overview about the products studied in the framework of this PhD thesis

Product	Specification	Processing	Drying	Abbreviation	Section
Chorizo	Acid	Standard	QDS process	A-QDS-S	V.1.
		Standard	Traditional	A-TRADI-S	
	Low acid	Standard	QDS process	LA-QDS-S	
		Standard	Traditional	LA-TRADI-S	
	Acid	NaCl-free	QDS process	A-QDS-F	V.2.
		NaCl-free	Traditional	A-TRADI-F	
	Low acid	NaCl-free	QDS process	LA-QDS-F	
		NaCl-free	Traditional	LA-TRADI-F	
Dry-cured ham	smoking	Standard	Traditional	TS-TRADI-S	V.3.
		NaCl-free	Traditional	TS-TRADI-F	
	Non-acidified smoked	Standard	QDS process	NS-QDS-S	V.42.
		NaCl-free	QDS process	NS-QDS-F	
	Acidified	Standard	QDS process	A-QDS-S	
		NaCl-free	QDS process	A-QDS-F	
	Acidified-smoked	Standard	QDS process	AS-QDS-S	
		NaCl-free	QDS process	AS-QDS-F	

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V. RESULTS

1. Ensuring food safety by an innovative fermented sausage manufacturing system

Katharina Stollewerk, Anna Jofré, Josep Comaposada, Gabriele Ferrini, Margarita Garriga. "Ensuring food safety by an innovative fermented sausage manufacturing system". *Food control*. Vol. 22, issue 12 (December 2011) : p. 1984–1991

http://dx.doi.org/10.1016/j.foodcont.2011.05.016

http://www.sciencedirect.com/science/article/pii/S0956713511002179

Abstract

Accelerated production of dry fermented sausages by shortening the drying-ripening process represents one of the new developments in meat product technology and is expected to have a promising future. However, food safety concerns, which could exist when processes are shortened, must be investigated in particular. In the present challenge test, the fate of *Listeria monocytogenes* and *Salmonella* was investigated in acid (pH 4.8) and low-acid (pH 5.3) chorizo that were fermented, thermally treated and dried either by the accelerated drying system QDS process® or the traditional process. Even though the innovative QDS process® substantially shortened the drying time when compared to the traditional drying system, results showed that in case of low level contamination of raw meat, the same product safety was achieved.

Highlights

► QDS process shortens the drying of fermented meat products. ► Chorizos were spiked with *L. monocytogenes* and *Salmonella* in the meat batter. ► We evaluated the impact of traditional and QDS drying on food safety of chorizo. ► Traditional and QDS process provided equal food safety.

Keywords

- Accelerated drying;
- Challenge test;
- Chorizo;
- High hydrostatic pressure;
- *Listeria monocytogenes*;
- *Salmonella*

2. The impact of fast drying (QDS process®) and high pressure on food safety of NaCl-free processed dry fermented sausages

Katharina Stollewerk, Anna Jofré, Josep Comaposada, Jacint Arnau, Margarita Garriga. “The impact of fast drying (QDS process®) and high pressure on food safety of NaCl-free processed dry fermented sausages”. *Innovative Food Science & Emerging Technologies*.

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http://www.sciencedirect.com/science/article/pii/S1466856412000598

ABSTRACT

In the present study the food safety impact of the QDS process® combined with a high pressure treatment at 600 MPa was evaluated in NaCl-free processed acid (pH 4.8) and low-acid (pH 5.2) chorizo. A challenge test was performed where the raw meat batter was spiked with low levels of *Listeria monocytogenes* and *Salmonella* (< 100 CFU/g) and chorizos were manufactured following either a traditional drying or a QDS process®. After drying, half of the sliced chorizo samples were pressurized (600 MPa, 5 min, 13 °C) and stored under refrigeration for 91 days. QDS processing proved to be adequate for the production of safe NaCl-free processed dry fermented sausages. Regarding pathogenic microorganisms elimination, it was as effective as traditional processing for acid chorizo and even safer for low-acid chorizo. The high pressure treatment assured absence of both pathogens in all samples during the whole storage time. Sausage reformulation to meet NaCl-free processing requirements modified the progress of pH and technological microbiota.

Industrial relevance: The QDS process® was designed to reduce the manufacturing time of sliced dry-cured meat products. It allows a just in time workflow, requires less space and energy and facilitates the rapid elaboration of new products, implying fast adaptation to marketing promotions. Among them, dry fermented sausages with reduced sodium chloride content is currently one of the major subjects investigated on the meat sector. Reduction of NaCl and the use of replacers, however, could negatively affect food safety and quality, which must therefore be properly evaluated. To extend shelf-life and improve safety, especially in products which are reformulated, high pressure processing could be a useful technology. The development of safe NaCl-free sliced dry fermented sausages in a short period of time is relevant for industry to meet consumer demands for convenient and healthy ready-to-eat products.

Highlights

► Safety of fast and traditionally dried NaCl-free processed chorizos was evaluated. ► A challenge test with *L. monocytogenes* and *Salmonella* was performed. ► The QDS process® was safer than the traditional process. ► Pathogen elimination from traditional low-acid chorizo required pressurization.

Keywords

- *L. monocytogenes*; *Salmonella*; Fast ripening;
- Chorizo; Pressurization; Food safety

3. The effect of NaCl-free processing and high pressure on the fate of *Listeria monocytogenes* and *Salmonella* on sliced smoked dry-cured ham

Katharina Stollewerk, Anna Jofré, Josep Comaposada, Jacint Arnau, Margarita Garriga. “The effect of NaCl-free processing and high pressure on the fate of *Listeria monocytogenes* and *Salmonella* on sliced smoked dry-cured ham”. *Meat science*. Vol. 90, issue 2 (February 2012) : p. 1984-1991

http://dx.doi.org/10.1016/j.meatsci.2011.09.009

http://www.sciencedirect.com/science/article/pii/S0309174011003147

Abstract

NaCl is an important multifunctional ingredient applied in dry-cured ham elaboration. However, its excessive intake has been linked to serious cardiovascular diseases causing a recent increase in the development of reduced salt products. In the present study *Listeria monocytogenes* and *Salmonella*, food-borne pathogens which can cross-contaminate post processed products, were spiked with < 100 CFU/g on slices of both standard (S) and NaCl-free processed (F) (elaborated with KCl + potassium lactate instead of NaCl) smoked dry-cured ham. Although *L. monocytogenes* and *Salmonella* counts decreased faster in S ham, pathogens were present in both types of non-pressure treated ham during the entire refrigerated storage period (112 days). Pressurisation at 600 MPa for 5 min caused the elimination of both pathogens in S ham after 14 days. In contrast, *Salmonella* and *L. monocytogenes* were present in F ham until days 28 and 56, respectively, indicating that the NaCl-free processed dry-cured ham had lower stability than standard smoked dry-cured ham.

Highlights

► We compare food safety of standard and NaCl-free processed dry-cured ham. ► NaCl was substituted by KCl and potassium lactate. ► A challenge test with low levels of *L. monocytogenes* and *Salmonella* was performed. ► NaCl-free ham had a higher food safety risk compared to the standard product. ► Pressurization was necessary to eliminate pathogens from both types of ham.

Keywords

- Food-borne pathogens;
- High hydrostatic pressure;
- KCl;
- Potassium lactate;
- Smoked dry-cured ham;
- Sodium reduction

4. NaCl-free processing, acidification, smoking and high pressure: effects on growth of *Listeria monocytogenes* and *Salmonella* in QDS processed® dry-cured ham

NaCl-free processing, acidification, smoking and high pressure: effects on growth of *L. monocytogenes* and *Salmonella* in QDS processed® dry-cured ham

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Abstract

To evaluate the food safety effect of NaCl-free processing, acidification, smoking and high pressure in QDS processed® dry-cured ham, 3 ham types (non-acidified smoked, acidified, and acidified smoked) were produced according to a standard (-S) and a new NaCl-free (-F) process. Slices were spiked with *Listeria monocytogenes* and *Salmonella* (<2 log CFU/g), dried by the QDS process®, vacuum packed, high pressure treated at 600 MPa and stored under refrigeration for 112 days. Results of the challenge test showed that *L. monocytogenes* could only be eliminated from acidified smoked (AS) -S and -F processed ham slices at the end of storage, while *Salmonella* was present in all non-pressurised slices. The safest hams were those pressurised, especially AS-S hams, where *L. monocytogenes* was eliminated from 25 g of product immediately after HP treatment and *Salmonella* after 14 days. Compared with standard processing, NaCl-free processing showed lower levels of pathogens in non-pressurised slices but their elimination was delayed in pressurised ham slices.

Keywords: *L. monocytogenes*; *Salmonella*; fast drying; meat products; sodium chloride reduction.

1. Introduction

In dry-cured ham production, ripening is the most time consuming step. It can last from a few weeks up to years, and various strategies have been described to accelerate the process (Arnau, Serra, Comaposada, Gou & Garriga, 2007). Among them the Quick Dry Slice process® (QDS process®) based on the patented technology from Comaposada, Arnau, Gou & Monfort (2004), is an innovative process which facilitates the reduction of the drying period of sliced products by direct drying of slices in a continuous system. Improved control of processing and product quality provides greater flexibility in production planning and allows a faster optimization of the development of new products. Additionally, less space and energy are required when compared to traditional systems (Comaposada et al., 2010).

Food safety and stability of traditional dry-cured meat products are based on a number of hurdles (pH, water activity (a_w), nitrite), which assure a long shelf-life through their combined effect (Leistner, 2000; Reynolds, Harrison, Rose-Morrow & Lyon, 2001). Likewise, they prevent the growth of pathogens, for example *L. monocytogenes* and *Salmonella*, which have been a concern in ready-to-eat (RTE) products (European Food Safety Authority, 2011). Nowadays, a_w values found in sliced and vacuum packed products are often higher than 0.92 (Hereu, 2009). Moreover, the fact that slicing represents a possible cross-contamination source for meat products (Talon et al., 2007) and the tendency to reduce the NaCl content require a redesigning in dry-cured ham manufacturing and proper food safety investigation. NaCl is an essential ingredient in processed meat products because of its multifunctional character, but the adverse cardiovascular effects of an excessive sodium intake on hypertension have provoked increased investigation on salt reduced products in recent years (FSAI, 2005; WHO/ISH, 2003). Therefore, for dry-cured ham, techniques to accelerate salt distribution, which could be useful to reduce the NaCl amount, have been described, such as reducing ham thickness, using boned hams, trimming away subcutaneous and intermuscular fat and salting ham pieces with the curing mixture in a tumbler under vacuum (Arnau et al., 2007). Transglutaminase, an enzyme, which is extensively used in developing restructured and low salt meat products (Verma & Banerjee, 2012) also facilitates binding to form restructured hams (Motoki & Seguro, 1998). In relation with this, Fulladosa, Serra, Gou & Arnau (2009) demonstrated that a 50% reduction of NaCl was possible in restructured transglutaminase added dry-cured hams. Regarding the use of NaCl substitutes in meat products, KCl and lactate, due to their technological properties, have been reported as suitable

compounds (Desmond, 2006; Gou, Guerrero, Gelabert & Arnau, 1996) and have been included recently in a process to produce NaCl-free dry-cured ham (Arnau et al., 2011).

The inclusion of antimicrobial ingredients (e.g. lactate) and/or additional (fermentation, smoking) or alternative techniques such as high pressure (HP) processing may be of great interest in the development of safe new products. The antimicrobial effect of lactate has been demonstrated in different meat products (Jofré, Garriga & Aymerich, 2008; Mbandi & Shelef, 2002; Miller & Acuff, 1994; Stekelenburg, 2003) against *Salmonella* and especially *L. monocytogenes*. Fermentation is not common in traditional dry-cured ham manufacturing (Arnau et al., 2007) but has been described to improve product stability, flavour and texture in fermented sausages. Smoking, mainly used in northern European meat processing has antibacterial and fungicide properties, attributed to the formaldehyde and phenolic compounds (Girard, 1988; Toth & Potthast, 1984). High pressure (HP) processing is a non-thermal food preservation technology that can be used for microbiological safety improvement and shelf life extension of RTE foods. Regarding *L. monocytogenes*, HP is recognized as a listericidal treatment by the Codex Alimentarius (CAC, 2007) and the FDA (HHS, 2008). For dry-cured ham, a recently published model for HP inactivation showed that pressurisation at 613 MPa for 5 min was sufficient to achieve the *L. monocytogenes* US "zero tolerance" policy (Bover-Cid, Belletti, Garriga & Aymerich, 2011a) considering the low contamination levels and the inability of *L. monocytogenes* to grow in this product. For *Salmonella*, a significantly reducing effect after pressurisation at 600 MPa has already been described (Bover-Cid, Belletti, Garriga & Aymerich, 2011b; Jofré, Aymerich, Grèbol & Garriga, 2009b; Stollewerk, Jofré, Comaposada, Arnau & Garriga, 2011). Moreover, the effects of HP have been studied at the physicochemical, sensorial and microbiological level in NaCl reduced ham (Fulladosa et al., 2009; Fulladosa, Sala, Gou, Garriga & Arnau, 2012) and on *L. monocytogenes* and *Salmonella* in traditionally dried NaCl-free processed ham (Stollewerk et al., 2012). Up to date the impact of HP on food safety of QDS dried NaCl-free processed hams during cold storage has not been evaluated.

As postulated by the hurdle technology, the combination of different preservative factors is more efficient for controlling microorganisms in food than using individual hurdles (Leistner, 2007). Based on this technology, the aim of the present study was to evaluate through a challenge test the fate of *L.*

monocytogenes and *Salmonella* spiked on QDS process® dried slices of dry-cured ham, manufactured with and without NaCl, acidification, smoking and pressurisation.

2. Materials and Methods

2.1. Manufacture of dry-cured hams and partial drying

Three ham types (non-acidified smoked (NS), acidified (A) and acidified smoked (AS)) were manufactured following different salting processes (dry salting or brine injection). Furthermore, composition of hams was adapted to the production process (standard (-S) and NaCl-free (-F)), based on previous sensorial results (Arnau, et al, 2011). The main differences between -S and -F processed hams included the substitution of NaCl by KCl and potassium lactate and the addition of more sugars to compensate the bitter taste of KCl and potassium lactate (Gou et al., 1996). Acidification to a pH of approximately 5.2 in A-S and AS-S hams was achieved by lactic acid bacteria (LAB) fermentation, while calculated amounts of gluconodeltalactone (GDL) were applied to A-F and AS-F hams to produce acidification because the addition of lactate delays the growth of LAB.

Figure 1 shows a schematic representation of the manufacturing process. All types of ham were elaborated from commercial raw boned hams trimmed of skin and subcutaneous fat with a $pH_{24} < 6.0$ in *Semimembranosus* muscle. Non-acidified smoked hams (NS-S and NS-F) were salted directly in the massaging unit with the addition of the ingredients (Table 1). The curing period was 48 h for NS-S and 72 h for NS-F hams to assure ingredient absorption. In the second massage, to help binding, 3 g/kg of transglutaminase (Denatex 100pur, Activa WM, Ajinomoto®, Impex Química, SA, Barcelona, Spain) and 3.5 g/kg of sodium lactate, were added, which were substituted by equal molar concentrations of potassium lactate (4 g/kg) in NS-F hams. Non-acidified smoked hams (NS-S and NS-F) were covered with a collagen film and then packed (Fig. 1). Acidified hams with (A-S) and without (A-F) NaCl and acidified smoked hams with (AS-S) and without NaCl (AS-F) were salted by injecting 15 g of brine in 100 g of meat (Table 1) and tumbling (25 min). Subsequently hams were wrapped in an elastic mesh (Euronet®-FRA®: Rete Spira AS 30 A 19) and vacuum packed (Cryovac bag CN330, 60 micron, 300x600mm). After pressing, all -F hams were repacked in drying bags (Tublin®, TUB-EX ApS, Taars, Denmark) allowing additive penetration and liquid evaporation up to a

weight loss of 8 %. Following, NS and AS hams were smoked for 3 h at 25-30 ºC by combustion of beech flakes using an oven (Doleschal, Steyr, Austria) connected to a smoker.

Non-acidified smoked hams (NS-S and NS-F) were subjected to a partial drying process, for 25 days at 5 ºC and for 8 days at 12 ºC until a final weight loss of 24 % was achieved. Continuous ventilation was applied for maintaining relative humidity (RH) at 65 %. After manufacturing all the hams were frozen at -20 ºC.

For each type of ham a total of eight hams were produced in two independent batches (4 hams per production).

2.2. Slicing, inoculation and QDS drying

Two challenge tests were performed on different days. For each challenge test and type of product two hams from two independent batches were sliced. Ham slices (2 mm thick, approximately 35 g/slice) were spiked with a mixture of *L. monocytogenes* and *Salmonella enterica* (3 strains each, Table 2) at the low inoculum levels of 50 CFU/g and 40 CFU/g respectively to simulate a recontamination during slicing (CRL/AFSSA, 2008; Hoz, Cambero, Cabeza, Herrero & Ordóñez, 2008). The mixture was prepared by diluting -80 ºC frozen cultures (previously grown overnight in BHI) of each strain in distilled water. The inoculation cocktail (0.2 ml) was spread on the surface of the slices with a Drigalsky spreader until it was completely absorbed.

Drying of ham slices was finished by applying the QDS drying, which was performed by convection of air at 30 ºC during approximately 50 min at a RH of 40 % until a product water content of 54 % was reached, calculated on basis of the water content measured before QDS and the drying weight loss. The maximum temperature of slices during the drying process was 20 ºC. Subsequently pairs of slices were vacuum packed in plastic bags of PA/PE (Sacoliva S.L., Castellar de Vallès, Spain) and stored for 12 h at 4 ºC until HP was applied.

2.3. High pressure treatment and storage

Half of the samples of each ham type were submitted to a HP treatment of 600 MPa for 5 min at 13 ºC in an industrial hydrostatic pressurisation unit (Wave 6000 from NCHiperbaric, Burgos, Spain). The chamber volume was 120 l, the come up time was 3.8 min and the pressure release was almost immediate. Subsequently, treated and non-treated samples were stored under refrigeration at 4 ºC for

38 days and afterwards at 8 ºC for 74 days, following the temperature profile recommended by guidance documents (AFNOR, 2004; CRL/AFSSA, 2008).

2.4. Microbiological analysis

Sampling was performed after inoculation and periodically (1, 14, 28 and 112 day(s) after drying) during storage under refrigeration. For plate counting, 25 g of the product were diluted 1/10 in BHI broth (Brain heart infusion, DB, NJ, USA) and homogenised in a Masticator Classic (IUL S.A., Barcelona, Spain). Appropriate dilutions of the homogenate were plated onto the following media: Chromogenic Listeria agar (Oxoid Ltd., Basingstoke, England) incubated for 48 h at 37 ºC for *L. monocytogenes*; CHROMagar™ *Salmonella* Plus (Scharlab, Barcelona, Spain) incubated for 48 h at 37 ºC for *Salmonella*; MRS agar (Merck KGaA, Darmstadt, Germany) incubated for 48-72 h at 30 ºC in anaerobiosis for LAB and MSA agar (Mannitol salt phenol-red agar, Merck KGaA) incubated 48-72 h at 30 ºC for grampositive catalase positive cocci (GCC+). When counts of *L. monocytogenes* and *Salmonella* were under 135 mm ∅ plate detection limit (10 CFU/g), presence or absence of viable cells in the enriched homogenates (48 h at 37 ºC) was investigated by seeding dots on selective media. For every *L. monocytogenes* enrichment, two 20-µl dots were seeded onto Chromogenic Listeria agar. For *Salmonella*, 200 µl of the enriched homogenate were transferred onto 10 ml of Rappaport-Vassiliadis Enrichment Broth (Oxoid). After incubation at 41.5 ºC for 48 h, 10 µl were seeded onto CHROMagar™ *Salmonella* Plus. Presumptive colonies of both *L. monocytogenes* and *Salmonella* were confirmed by real time PCR using the *hly* and *ttrBCA* genes for *L. monocytogenes* and *Salmonella*, respectively (Stollewerk et al., 2012).

2.5. Physico-chemical analysis

The pH of the minced slices was measured by using a portable Crison penetration electrode connected to a Crison pH metre PH25 (Crison Instruments S.A., Alella, Spain) and the a_w with an Aqualab S3TE dew point water activity meter (Decagon Devices, Inc. Pullman, Washington, USA).

2.6. Statistical analysis

Absence of the pathogens in 25 g of product was considered “N=0” and presence (counts below the plate detection limit (10 CFU/g) but presence in the enriched homogenate) “N=1”. To allow logarithmic transformation of zero values, log (N+1) was used. Data was analysed by analysis of variance

158 (ANOVA), followed by Tukey's test at the 0.05 level of significance using the Statistica 7.0 software
159 (Statsoft, Tulsa, UK).

160

161 **3. Results**

162 *3.1. Physicochemical parameters*

163 Differences in composition and processing had an impact on the physicochemical properties of the
164 hams. pH values of non-acidified smoked hams recorded before QDS drying were 5.45 ± 0.21 in NS-
165 S and 5.75 ± 0.05 in NS-F, however, after drying values increased (Table 3) and were comparable to
166 those found in commercially processed hams during storage. In standard hams, acidification due to
167 fermentation led to significantly lower pH levels in acidified hams when compared to NS hams, during
168 the whole study. In all NaCl-free processed hams, higher pH levels (ca. 0.3 units, $p<0.05$) were
169 observed before drying (Table 3). The QDS-drying process® produced a pH increase in all non-
170 pressurised samples, recording the highest one in A-S (0.37 units, $p<0.05$). During storage, pH values
171 of NS and A hams followed the same trend in -S and -F processed samples but not in AS hams
172 ($p<0.05$). Slightly lower a_w levels were recorded in NS than in acidified hams (0.014 units in –S and
173 0.006 units in –F hams, Table 4). Concerning manufacture and due to differences in composition of
174 the brine and length of the curing period (1 day shorter in –S hams), initial a_w values (before QDS) of -
175 S processed hams were higher than those of -F processed hams ($p<0.05$). However, after drying no
176 important differences were found among them and a_w values after 112 days of storage did not differ
177 ($p>0.05$). The application of a HP treatment did not affect or produced small changes in pH (an
178 increase of 0.29 in NS-F and 0.21 in A-F hams) and a_w (changes <0.005 a_w units) of dry-cured ham
179 slices (Tables 3 and 4).

180 *3.2. Technological microbiota*

181 At the time of slicing and spiking with pathogens, similar LAB counts were recorded in non-acidified
182 smoked (NS) –S and –F hams (ca. 10^7 CFU/g), while in acidified (A) and in acidified smoked (AS)
183 hams, counts were ca. 2.5 log higher in standard (-S) than in NaCl-free (-F) hams (Fig. 2). During the
184 following storage period of 112 days, LAB levels developed similarly in NS hams ($p>0.05$) and
185 significant differences remained between counts of -S and -F processed A and AS hams. The
186 application of a HP treatment of 600 MPa produced the highest LAB reductions in NS (>2 log) and

NaCl-free processed (>1.2 log) hams, although reduction was only statistically significant in NS-F samples (2.34 log). During subsequent storage LAB decreased an additional 1.5 log in all acidified hams and maintained in NS-F at a level of 4.5 log CFU/g. In NS-S, in contrast, LAB started growing from day 28 and reached the initial level after 112 days.

Initial GCC+ counts were 3.2 log lower ($p<0.05$) in NS-S than in the other hams (6-6.5 log CFU/g). During storage, differences in behaviour were only observed between GCC+ of NS-S and NS-F hams (Fig. 2), which led to a 1.6 log higher final GCC+ level of NS-S ham ($p<0.05$). HP did not affect GCC+ counts in A-S, AS-S and all -F ham slices and counts remained at the initial level or slightly decreased ($p>0.05$) during storage. In contrast, an increase of 3.5 log ($p<0.05$) to initial levels was observed in HP treated NS-S ham after 112 days.

3.3. Pathogenic microbiota

Dry-cured ham slices were spiked with *L. monocytogenes* and *Salmonella* at a level of < 2 log CFU/g. Subsequent QDS-drying did not affect the levels of pathogens in any of the -S and -F hams ($p>0.05$, Fig. 3). During refrigerated storage of vacuum packed ham slices *L. monocytogenes* counts decreased similarly (1.3-1.5 log) in all types of ham. At the end of storage, however, absence in 25 g of product was only recorded in AS-S and AS-F samples. Comparing non-pressurised -S and -F samples, equal or lower counts were recorded in -F hams during the whole experiment. A HP treatment of 600 MPa had an immediate bactericidal effect of 1.6 log reduction ($p<0.05$) in NS-S and A-S and eliminated the pathogen from all AS-S samples, while it took 112 days to achieve the same result in NS-S. Compared to -S ham samples, immediate reductions caused by HP were 0.22, 0.92 and 0.67 log lower in NS-F, A-F and AS-F, respectively. During storage, similarly, *L. monocytogenes* decreased slower in –F hams and pathogen absence was recorded in A-F and AS-F hams after 56 days. Regarding *Salmonella*, a similar decrease during storage was observed in all hams (Fig. 3). However, a HP treatment of 600 MPa was necessary to achieve absence in all -S and -F hams during storage. Taken together, *Salmonella* elimination from pressurised samples (25 g) was achieved faster in acidified and -S hams, for example, after 14 days the pathogen was absent from AS-S slices but its elimination from NS-F required 112 days.

4. Discussion

The decrease of pH and a_w during the ripening phase of dry-cured meat products due to fermentation and drying, respectively, are among the most important factors to assure food safety and stability (Leistner, 2000). New formulations or manufacturing procedures, which imply possible modifications of these factors, must therefore be properly evaluated. In the present study, differences in a_w observed before QDS drying could be attributed to variations in manufacturing processes (type of salting, curing period, ingredient composition, etc). However, after QDS drying, a_w values of different ham types were equalised to 0.93-0.94 and maintained at similar levels during storage. Hence, regarding described growth limits of *L. monocytogenes* and *Salmonella* (0.92 and 0.94, respectively, (ICMSF, 1996), theoretically a_w would not prevent their multiplication during storage. It must also be taken into account that similar a_w levels from hams with varying composition have been achieved by different solutes (NaCl *vs.* KCl + potassium lactate + sugars) and the type of solute has been shown to have an effect on microbial behaviour (Beuchat, 1974; Strong, Foster & Duncan, 1970). Evaluations of food safety as a consequence of the replacement of NaCl by KCl in broth demonstrated that KCl is a direct 1:1 molar replacer for the antimicrobial effect of common salt against *Aeromonas hydrophila*, *Enterobacter sakazakii*, *Shigella flexneri*, *Yersinia enterocolitica* and *Staphylococcus aureus* (Bidlas & Lambert, 2008). Regarding *L. monocytogenes*, Boziaris, Skandamis, Anastasiadi & Nychas (2007) demonstrated that NaCl could be replaced by equal-molar concentrations of KCl without risking microbiological safety in culture media. van Burik & de Koos (1990) showed that sodium lactate provided better growth inhibition on *S. typhimurium* and *S. aureus* than NaCl at equal a_w values in broth. Bacterial behaviour in foodstuff, however, cannot be directly extrapolated from studies in broth due to the significant effect of the food matrix composition (Brocklehurst, 2004).

From a technological point of view, acidification of dry-cured meat products increases hardness (Leroy, Verluyten & De Vuyst, 2006) when the pH decreases below the isoelectric point of myosin (pI 5.4) (Hamm, 1986). From a food safety point of view, a low pH represents an additional hurdle to the growth of pathogenic and spoilage bacteria and prolongs shelf life (Barbut, 2005). The stabilizing effect of a low pH in meat products is well known and reported from dry-fermented sausages (Leistner, 1995). Standard dry-cured ham production, however, does not include a fermentation step and the naturally occurring pH change is described to be unlikely a major factor in the microbial

245 stability (Reynolds et al., 2001). Nevertheless, the use of starter cultures in hams salted with brine
246 injection has been proposed to accelerate the production process (Jessen, 1995). In the present
247 study, starter cultures were added to A-S and AS-S hams, while in A-F and AS-F hams, due to the
248 possible growth delaying effect of lactate on LAB (Shelef, 1994), GDL together with GCC+ starter
249 were applied to improve flavour and colour. In this context, it has been observed on vacuum packed
250 beef that GDL enhanced the bactericidal effect of lactate on LAB (García Zepeda et al., 1994). In non-
251 acidified hams (NS hams), where no starter or GDL was applied, endogenous LAB grew during
252 manufacturing. Thus, differences in composition caused different levels of LAB between -S and -F
253 acidified hams at the moment of slicing and during subsequent storage. In contrast to the lower LAB
254 levels in -F hams, GCC+ starter behaved similar in -S and -F acidified (A and AS) hams and was not
255 affected by the presence of lactate. The application of a starter was necessary to achieve high levels
256 of GCC+ in NS-F, while in NS-S the same or higher levels were reached by endogenous GCC+
257 growth during storage.

258 Acidification was among the most important factors affecting the levels of pathogenic bacteria. In
259 general, acidified hams achieved higher proportion of samples with absence of *L. monocytogenes*
260 and *Salmonella*, which indicated that in dry-cured ham, pH reduction could provide additional food
261 safety. The antimicrobial activity of lactate against *L. monocytogenes* has been demonstrated in
262 various meat products, especially those cooked, such as frankfurter sausage (Stekelenburg, 2003),
263 beef bologna (Mbandi et al., 2002), comminuted cooked beef, cooked chicken roll and pork liver
264 sausage (Shelef, 1994). An enhanced effect of lactate in combination with GDL was also observed on
265 *L. monocytogenes* in a cooked cured emulsion type product, when 0.25 % GDL + 2 % lactate was
266 used instead of lactate alone (Juncher, Vestergaard, Soltoft-Jensen, Weber, Bertelsen & Skibsted,
267 2000). In contrast, studies on the inhibiting effect of lactate against *Salmonella* performed on chicken
268 dry fermented sausages, cooked ham and beef bologna only observed poor or no pathogen inhibition
269 (Deumier & Collignan, 2003; Jofré et al., 2008; Mbandi et al., 2002). Although in none of the dry-cured
270 hams *L. monocytogenes* and *Salmonella* could grow, absence of *L. monocytogenes* (in all the
271 replicates) was only achieved after 112 days in both acidified smoked (AS) -S and -F hams. Thus,
272 smoking in combination with acidification provided the best pathogen inhibition in non-pressurised
273 ham slices. The present results are in general agreement with literature, where the combined effect of
274 cold or liquid smoke together with low pH conditions and high salt concentrations have been observed

against *L. monocytogenes* and/or *L. innocua* in fish and meat products (Martin et al., 2010; Milly, Toledo & Chen, 2008; Montero, Gómez-Estaca & Gómez-Guillén, 2007). *Salmonella* has also been shown to be inhibited by smoke although its sensitivity is lower than the one observed for *L. monocytogenes* or other grampositive bacteria (Asita & Campbell, 1990; Suñen, 1998).

One of the major problems in the development of NaCl-free processed products is related to the multifunctional character of NaCl due to its flavouring and functional contributions and especially its antimicrobial activity (Sofos, 1983). In dry-cured ham, the substitution of 50 % NaCl by KCl did not affect mesophilic aerobic and salt tolerant flora (Blesa, Aliño, Barat, Grau, Toldrá & Pagán, 2008) and partial replacement of 40 % NaCl by KCl in dry fermented sausages maintained the microbiological stability of the product (Ibañez et al., 1995). However, food safety of traditionally dried dry-cured ham slices, spiked with *L. monocytogenes* and *Salmonella*, was compromised by NaCl substitution with the same ingredients as those reported in the present study (Stollewerk et al., 2012). Nevertheless, recorded differences could not only be related to the composition (presence of NaCl, KCl or lactate) but also to the acidification system (bacterial fermentation or GDL application) and the a_w of the product at the time of slicing (finished product in traditional hams (Stollewerk et al., 2012) *vs.* undried or partially dried product in QDS hams (this study)).

Pressurisation at 600 MPa is nowadays industrially applied on dry-cured meat products (Garriga & Aymerich, 2009), primarily because of its bactericidal and shelf-life extending effect while leaving important quality characteristics intact (Knorr, 1993). In the present study, the application of a HP treatment did not affect a_w and did not affect or increased slightly pH, as shown in previous studies performed on dry-fermented sausages (Jofré, Aymerich & Garriga, 2009a; Marcos, Aymerich & Garriga, 2005).

Regarding technological microbiota, pressurisation had an immediate lethal effect that was stronger on endogenous LAB than on starter LAB and GCC+. In pressurised NS-S ham, the only non-acidified product, recovery of endogenous LAB and GCC+ to levels of non-pressurised samples was observed. Similar behaviour was previously observed in traditionally dried dry-cured ham (Stollewerk et al., 2012).

The HP treatment of 600 MPa significantly affected both *L. monocytogenes* and *Salmonella* counts in all hams, confirming the listericidal and “anti-salmonella” effect of pressurisation, which has been

304 described before in traditional dry-cured ham (Bover-Cid et al., 2011a; Bover-Cid et al., 2011b; Hereu,
305 Bover-Cid, Garriga & Aymerich, 2012; Jofré et al., 2009b; Morales, Calzada & Nuñez, 2006;
306 Stollewerk et al., 2012). Nevertheless, ham type influenced the efficiency of pressurisation and
307 pathogen elimination, in particular *L. monocytogenes,* was considerably delayed in non-acidified
308 hams (112 days). The combination of low pH and HP has been described as an efficient way to
309 inactivate pathogenic microorganisms in foodstuff and to inhibit subsequent outgrowth of sublethally
310 injured cells (Smelt, 1998). However, it was the combination of acidification, smoking and HP that
311 provided the best protection and achieved immediately after processing absence of *L.*
312 *monocytogenes* and after 14 days of storage of slices under refrigeration absence of *Salmonella*.
313 Similarly, (Montero et al., 2007) observed that smoking and pressurisation, together with a high salt
314 concentration, kept *L. monocytogenes* counts under detection limit throughout 100 days of storage at
315 5 ºC in cold-smoked dolphinfish. Comparing the different manufacturings (-S and -F), pathogen
316 inhibition was lower in all NaCl-free processed hams, which we related to the protecting effect of
317 lactate on *L. monocytogenes* during pressurisation that has been previously reported from traditionally
318 dried dry-cured ham (Stollewerk et al., 2012) and cooked ham (Aymerich, Jofré, Garriga & Hugas,
319 2005; Jofré et al., 2008). Hence, the substitution of NaCl and its antimicrobial activity affected the
320 stability of NaCl-free processed HP treated hams and produced a significant delay in pathogen
321 elimination.

322 To sum up, the present study demonstrated for the first time that in case of a low-level
323 recontamination with *L. monocytogenes* and *Salmonella* during slicing, QDS dried cured ham
324 produced with a mixture of potassium lactate, KCl and sugars (-F) was safer than ham produced with
325 NaCl (-S). The combination of KCl together with potassium lactate, sugars and GDL in the case of A-
326 F and AS-F allowed a safe substitution of NaCl in dry-cured ham. The application of a HP treatment
327 was useful to produce an additional reduction of *Salmonella* and *L. monocytogenes*, however, the
328 inhibitory effect of pressurisation was higher in –S than in –F processed hams. New hurdle
329 combinations in reformulated food products must therefore be well considered and selected with
330 diligence.

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513 **Tables and Figure legends**

514 **Table 1.** Composition of the salting mixture applied to standard (-S) and NaCl-free processed (-F)
515 hams (in g per kg of raw meat).

516 **Table 2.** Description of the *L. monocytogenes* and *Salmonella* strains used to inoculate dry-cured
517 ham slices.

518 **Table 3.** pH values of non-pressurised (HP-) and high pressure-treated (HP+; 600 MPa/5 min/13 ºC)
519 standard (-S) and NaCl-free processed (-F) dry-cured ham slices before (day 0) and after (day 1)
520 drying and at the end (day 112) of storage.

521 **Table 4.** A_w values of non-pressurised (HP-) and high pressure-treated (HP+; 600 MPa/5 min/13 ºC)
522 standard (-S) and NaCl-free processed (-F) dry-cured ham slices before (day 0) and after (day 1)
523 drying and at the end (day 112) of storage.

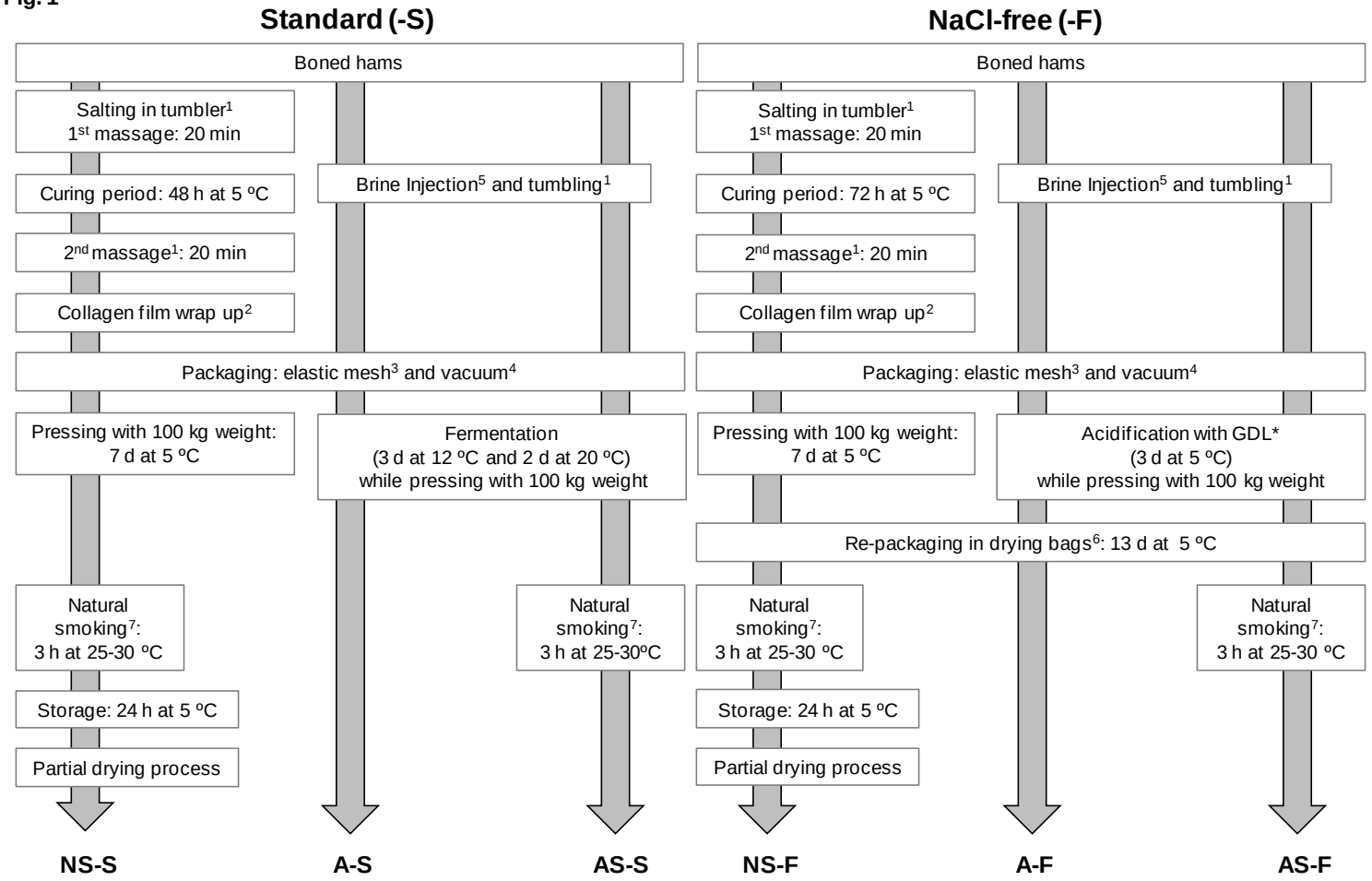
524 **Fig. 1.** Schematic representation of manufacturing processes of different ham types.

525 **Fig. 2.** Behaviour of (a) Lactic Acid Bacteria (LAB) and (b) Gram-positive catalase-positive cocci
526 (GCC+) in non-pressurised (HP-) and high pressure-treated (HP+; 600 MPa/5 min/13 ºC) standard (-
527 S) and NaCl-free (-F) processed dry-cured ham slices during the 112 days of refrigerated storage.
528 Data (mean and standard deviation) comes from 2 independent experiments performed in duplicate.

529 **Fig. 3.** Behaviour of (a) *L. monocytogenes* and (b) *Salmonella* in non-pressurised (HP-) and high
530 pressure treated (HP+; 600 MPa/5 min/13 ºC) standard (-S) and NaCl-free (-F) processed dry-cured
531 ham slices during the 112 days of refrigerated storage. Data (mean and standard deviation) comes
532 from 2 independent experiments performed in duplicate.

Figure

Fig. 1

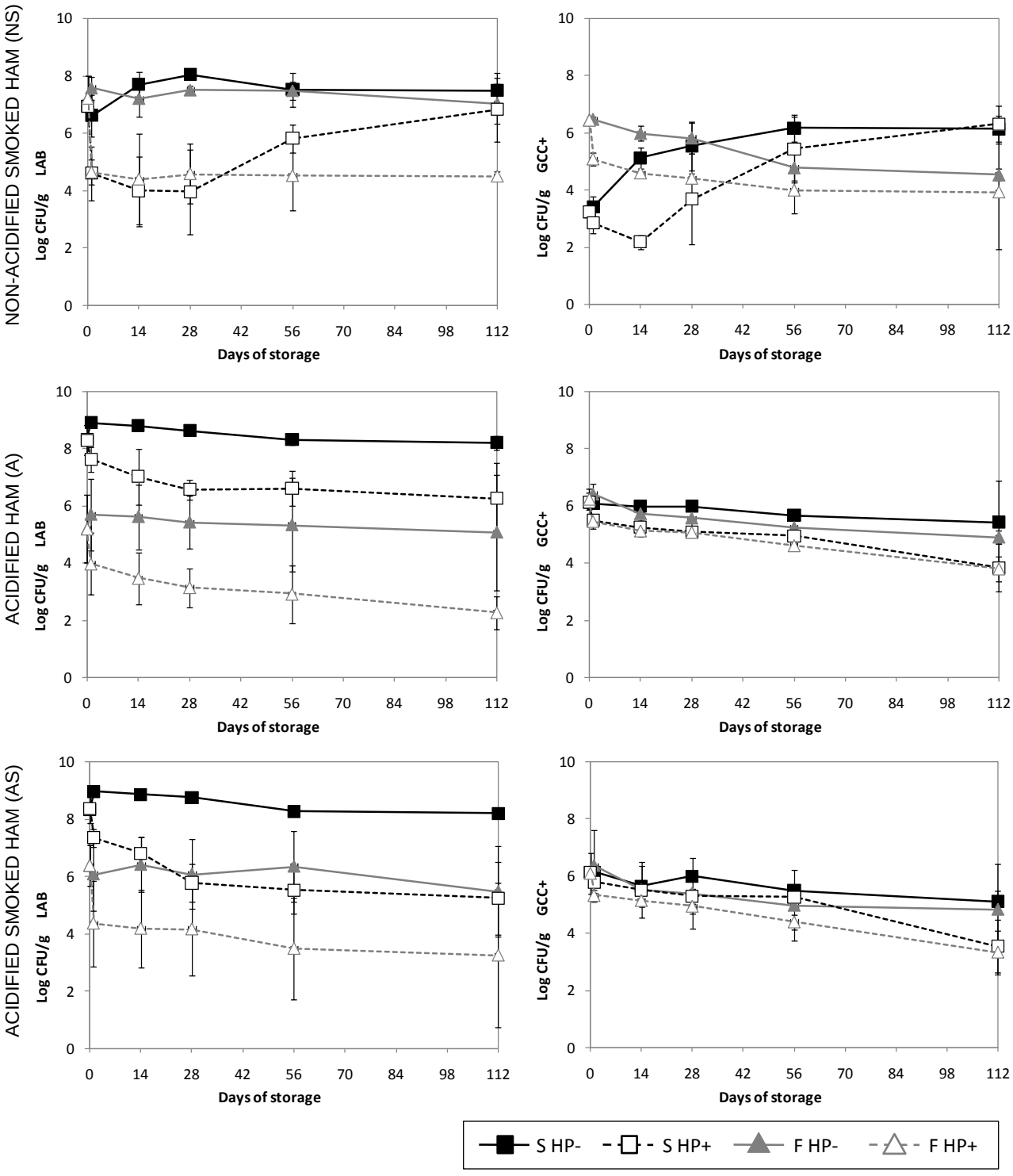


*pH decrease was due to the conversion of GDL to gluconic acid.

[1](Thermomat PX500, Metalquimia, Girona, Spain; velocity: 4 rpm); [2](Viscofan Naturin Coffi® Cal. 570, Tajonar-Navarra, España) [3](Euronet® -FRA®: Rete Spira AS 30 A 19); [4](Cryovac bag CN330, 60 micron, 300x600 mm); [5](high pressure spray effect multi needle injector Movistic 30PC, Metalquimia, Girona, Spain); [6](Tublin® 05, TUB-EX ApS, Taars, Denmark); [7](Doleschal, Steyr, Austria).

Figure

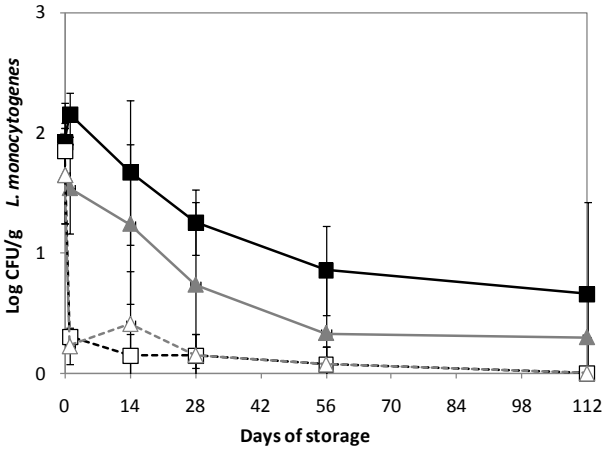
Fig. 2


NON-ACIDIFIED SMOKED HAM (NS)
ACIDIFIED HAM (A)
ACIDIFIED SMOKED HAM (AS)
Log CFU/g LAB
Log CFU/g GCC+
Days of storage
0
14
28
42
56
70
84
98
112
0
2
4
6
8
10
S HP-
S HP+
F HP-
F HP+

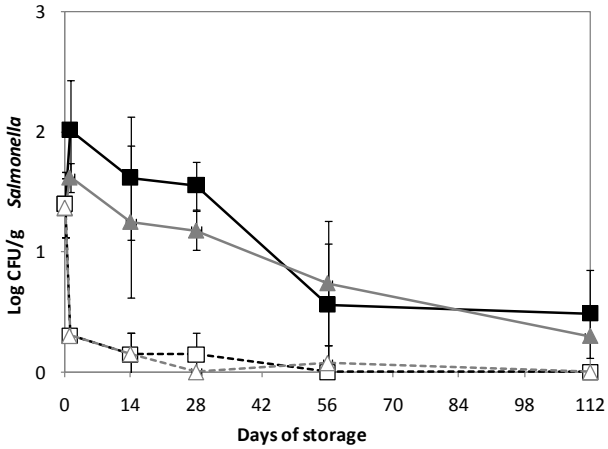
Figure

Fig. 3

NON-ACIDIFIED SMOKED HAM (NS)

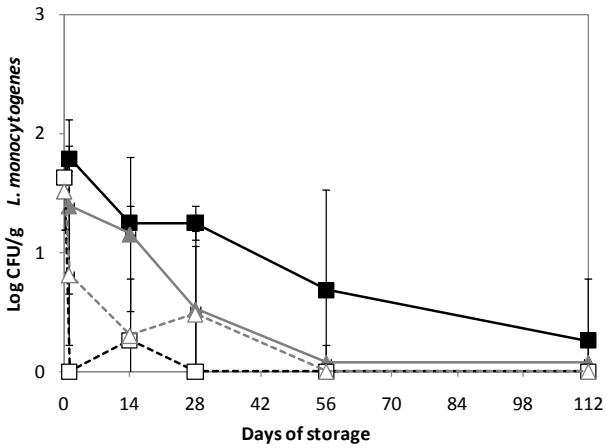


ham \ day	1	14	28	56	112
NS-S HP-	4 P	4 P	4 P	4 P	2 P/2 A
NS-F HP-	4 P	4 P	3 P/1 A	2 P/2 A	4 P
NS-S HP+	4 P	2 P/2 A	2 P/2 A	1 P/3 A	4 A
NS-F HP+	3 P/1 A	3 P/1 A	2 P/2 A	1 P/3 A	4 A

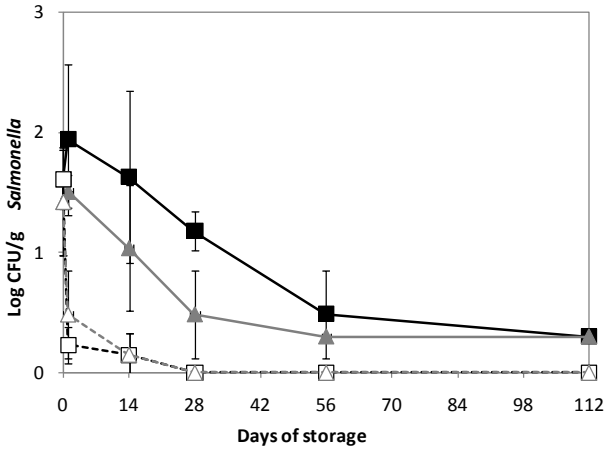


ham \ day	1	14	28	56	112
NS-S HP-	4 P	4 P	4 P	4 P	4 P
NS-F HP-	4 P	4 P	4 P	4 P	4 P
NS-S HP+	4 P	2 P/2 A	2 P/2 A	4 A	4 A
NS-F HP+	4 P	2 P/2 A	4 A	1 P/3 A	4 A

ACIDIFIED HAM (A)

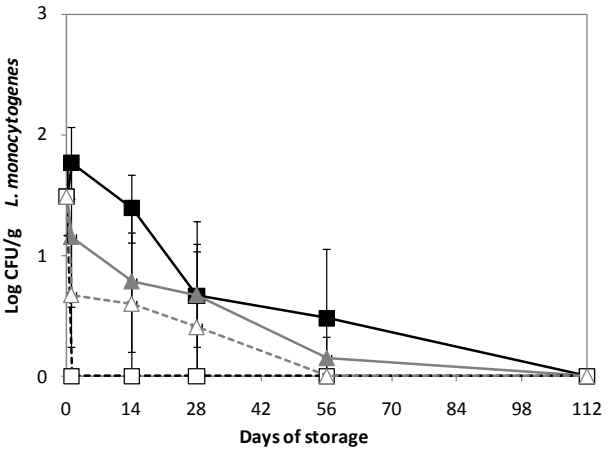


ham \ day	1	14	28	56	112
A-S HP-	4 P	4 P	4 P	2 P/2 A	1 P/3 A
A-F HP-	4 P	4 P	3 P/1 A	1 P/3 A	1 P/3 A
A-S HP+	4 A	1 P/3 A	4 A	4 A	4 A
A-F HP+	4 P	4 P	3 P/1 A	4 A	4 A

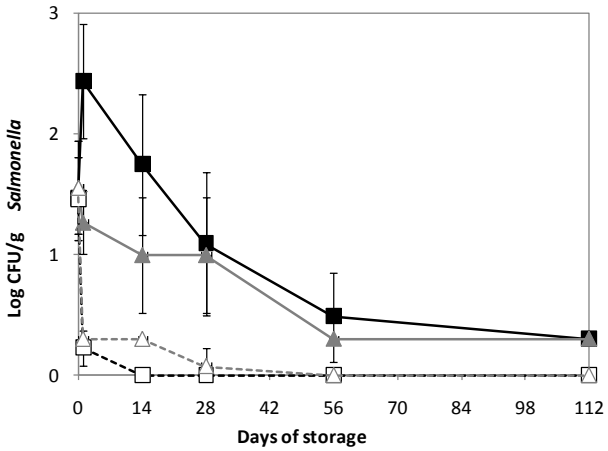


ham \ day	1	14	28	56	112
A-S HP-	4 P	4 P	4 P	4 P	4 P
A-F HP-	4 P	4 P	4 P	4 P	4 P
A-S HP+	3 P/1 A	2 P/2 A	4 A	4 A	4 A
A-F HP+	4 P	2 P/2 A	4 A	4 A	4 A

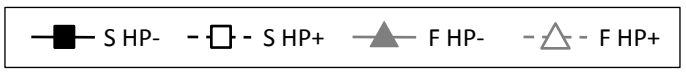
ACIDIFIED SMOKED HAM (AS)



ham \ day	1	14	28	56	112
AS-S HP-	4 P	4 P	3 P/1 A	3 P/1 A	4 A
AS-F HP-	4 P	4 P	4 P	2 P/2 A	4 A
AS-S HP+	4 A	4 A	4 A	4 A	4 A
AS-F HP+	4 P	4 P	2 P/2 A	4 A	4 A



ham \ day	1	14	28	56	112
AS-S HP-	4 P	4 P	4 P	4 P	4 P
AS-F HP-	4 P	4 P	4 P	4 P	4 P
AS-S HP+	3 P/1 A	4 A	4 A	4 A	4 A
AS-F HP+	4 P	4 P	1 P/3 A	4 A	4 A



Table

Table 1.

	Standard (-S)		NaCl-free (-F)	
Ingredients (g/kg of meat)	**NS**	**A and AS**	**NS**	**A and AS**
NaCl[a]	28	18	-	-
KCl[a]	-	-	15.31	15.31
Potassium lactate (77.8% v/v)[w,a]	-	-	33.83	33.83
Potassium lactate (60 % v/v)[x,b]	-	-	4[1]	-
Sodium lactate (60 % v/v)[y,b]	3.5[1]	-	-	-
Lactose	-	-	10	10
Water	-	124.57	10	47.31
GDL	-	-	-	10
Sucrose	-	-	7	7
Dextrose	3	3	3	3
Sodium ascorbate	0.5	0.5	-	-
Ascorbic acid	-	-	0.445	0.445
Potassium nitrite[c]	-	-	0.15	0.15
Sodium nitrite[c]	0.12	0.12	-	-
Potassium nitrate	0.15	0.15	0.15	0.15
Magnesium sulfate	-	0.05	-	-
Manganese sulfate	-	0.009	-	-
Starter culture (Lyocarni SXH-38 Sacco (Activa) *S. xylosus*	-	-	0.25	0.25
Starter cultura (Grama SE301: *L. sakei, S. xylosus, S. carnosus*)	-	0.1	-	-
Transglutaminase	3[1]		3[1]	

[w]Purasal® Hi Pure P Plus, [x]Purasal® Hi Pure P, [y]Purasal S, all from Purac bioquímica, S.A. Montmeló, Spain. Equal molar concentrations of Na^+ and K^+ ([a]), potassium lactate and sodium lactate ([b]) and nitrite ([c]) were used. [1]ingredients added in the 2nd massage.

Table

Table 2.

Species	**Reference**[a]	**Serotype**	**Origin**
L. monocytogenes	CTC1011	1/2c	Meat product
	CTC1034	4b	Meat product
	CECT 4031	1a	Rabbit
Species	**Reference**[a]	**Serovar**	**Origin**
S. enterica	CTC1003	London	Meat product
	CTC1022	Derby	Meat product
	GN-0006	Typhimurium	Pork gut

[a]CTC and CECT strains belong to collections from IRTA and Spanish type culture collection, respectively. GN strains were kindly provided by Dr. Badiola (CReSA, Bellaterra, Spain).

Table

Table 3.

Manufacturing		**Standard (-S)**						**NaCl-free (-F)**					
Ham type		**NS**		**A**		**AS**		**NS**		**A**		**AS**	
HP treatment		**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**
Before QDS process®		5.45±0.21^{Ab}		4.98±0.10^{Bb}		5.06±0.07^{Ba}		5.75±0.05^{Cab}		5.27±0.03^{Aa}		5.36±0.07^{Aa}	
Days of storage	1	5.82± 0.11^{BCGa}	5.87± 0.15^{BCa}	5.35± 0.11^{ADa}	5.43± 0.15^{ADEa}	5.19± 0.14^{Aa}	5.21± 0.22^{Aab}	5.85± 0.08^{BCb}	5.99± 0.04^{Cc}	5.37± 0.18^{Aab}	5.58± 0.13^{DEFb}	5.61± 0.09^{EFGb}	5.68± 0.17^{BFGb}
	112	5.77± 0.27^{DEa}	5.91± 0.17^{EFa}	5.42± 0.02^{ABa}	5.42± 0.10^{ABa}	5.38± 0.18^{ABb}	5.28± 0.11^{Bb}	5.65± 0.12^{CDa}	5.94± 0.12^{Fc}	5.42± 0.02^{ABb}	5.53± 0.06^{ACb}	5.52± 0.02^{ACc}	5.63± 0.05^{CDb}

Values are mean ± SD (n=4). For -S and –F processed hams, significant differences in rows are indicated by different capital letters and significant differences in columns are indicated by different small letters ($p<0.05$).

Table

Table 4.

Manufacturing		Standard (-S)						NaCl-free (-F)					
Ham type		**NS**		**A**		**AS**		**NS**		**A**		**AS**	
HP treatment		**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**	**HP-**	**HP+**
Before QDS process®		0.970±0.003^{Db}		0.985±0.007^{Cb}		0.982±0.004^{Cb}		0.953±0.001^{Ab}		0.958±0.002^{ABb}		0.960±0.001^{Bc}	
Days of storage	1	0.932± 0.012^{a}	0.932± 0.007^{a}	0.942± 0.014^{a}	0.941± 0.014^{a}	0.939± 0.015^{a}	0.938± 0.004^{a}	0.936± 0.014^{a}	0.941± 0.004^{c}	0.932± 0.007^{a}	0.930± 0.007^{a}	0.931± 0.003^{b}	0.926± 0.007^{a}
	112	0.926± 0.011^{a}	0.932± 0.009^{a}	0.933± 0.007^{a}	0.949± 0.001^{a}	0.926± 0.009^{a}	0.934± 0.010^{a}	0.934± 0.002^{a}	0.935± 0.001^{a}	0.930± 0.005^{a}	0.925± 0.008^{a}	0.924± 0.002^{a}	0.924± 0.006^{a}

Values are mean ± SD (n=4). For -S and –F processed hams, significant differences in rows are indicated by different capital letters and significant differences in columns are indicated by different small letters ($p<0.05$).

VI. GENERAL DISCUSSION

Fast ripening/drying and the complete or partial reduction of the NaCl content in the production of dry-cured meat products are two intensively investigated research areas in meat science, since there is much commercial interest in the rapid production of healthy foods. In general, dry-cured meat products are considered as shelf-stable and do not permit the growth of pathogens such *as L. monocytogenes* and *Salmonella* (Reynolds, Harrison, Rose-Morrow & Lyon, 2001); they owe their microbiological stability primarily to the decrease of a_w due to progressive desiccation and the decrease of pH during fermentation (Leistner, 2000). The European Commission Regulation 2073/2005 on microbiological criteria for foodstuffs as well as the U.S. FSIS regulation use these physicochemical parameters for the prediction of a food product to be supportive or non-supportive to *L. monocytogenes* growth (see Table 3, introduction). The achievement of the same a_w and pH levels in products of which the formulation or production process has been modified, could be one of the key prerequisites for innovative technologies to keep up with the food safety standards from traditional products. However, safe history of a food product is relevant only when all conditions remain the same. Even apparently minor changes in the product composition, process, or packaging method may have a large impact on the safety of the product (NACMCF, 2010). In this regard, similar levels of physicochemical parameters can only be considered as clues because in foods, the support or inhibition of pathogen growth is determined by many factors (NACMCF, 2010). To clarify food safety issues of innovative technologies, the performance of “pathogen growth inhibition studies” would be the most adequate. Accordingly, the AFNOR (2004), CRL/AFSSA (2008), European Commission (2005) and NACMCF (2010) recommend this type of challenge test, in addition to predictive mathematical modelling (European Commission, 2005), for the evaluation of the ability of a particular food product formulation with a specific type of processing and packaging to inhibit the growth of certain bacterial pathogens when held under specific storage conditions (time and temperature).

1. Effect of the QDS technology

The QDS process® is a technology that implies a fast drying step in order to speed up the production of sliced dry-cured meat products. The main differences between traditional and QDS processing consists of the product format and in the associated technologies; whereas traditional products are dried in whole pieces, QDS processing comprises as part of the technology the slicing before and the vacuum- or MAP packaging after drying. Both technologies (slicing and vacuum-packaging) are favourable for marketing and convenience reasons, however, slicing can cause recontaminations (Talon et al., 2007), which possibly lead to growth of pathogens and/or spoilage microorganisms during storage, especially in products with high a_w.

The challenge tests performed within the framework of this PhD thesis showed that the behaviour of the evaluated pathogens, *L. monocytogenes* and *Salmonella*, was similar in QDS and traditionally processed dry-cured meat products (dry-cured ham and chorizo).

In the case of standard dry-cured ham (processed with NaCl), the two pathogens showed the same trend in traditional (TS-TRADI-S) and QDS (NS-QDS-S) products (Figure 7).

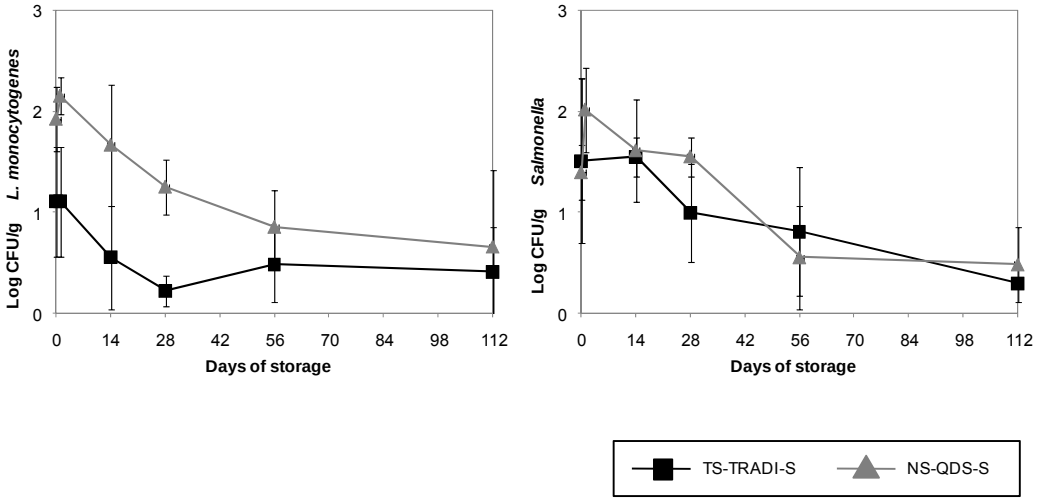


Figure 7. Comparison of the behaviour of *L. monocytogenes* and *Salmonella* in QDS and traditionally dried dry-cured ham

In both cases the product did not support pathogens growth but caused the progressive decrease of *L. monocytogenes* and *Salmonella* during storage. Hence, the QDS technology had no impact on the fate of the pathogens but assured a fast achievement of similar a_w (0.932 in TRADI and QDS) and pH (5.75 in TRADI and 5.82 in QDS) levels. The behaviour and levels of LAB and GCC+ during storage was also similar in both types of ham (Figure 8).

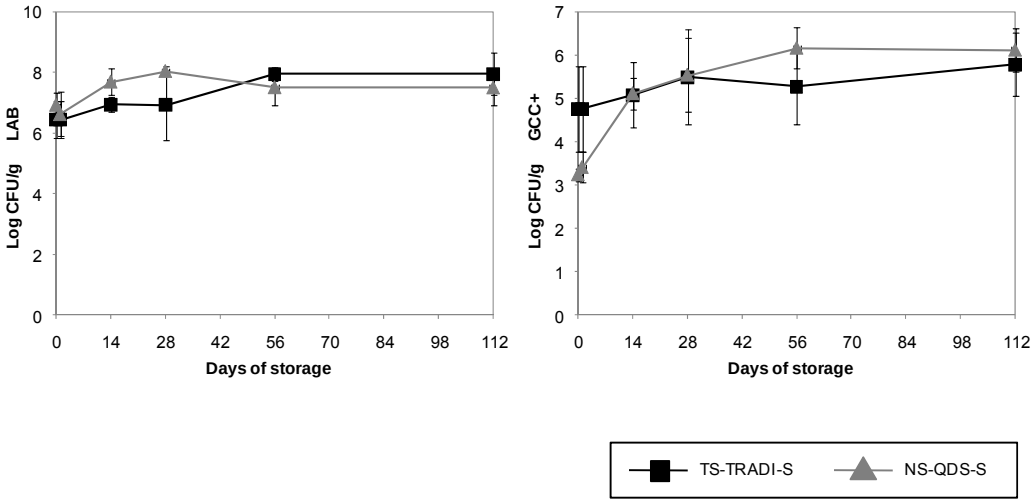


Figure 8. Comparison of the behaviour of *L. monocytogenes* and *Salmonella* in QDS and traditionally dried dry-cured ham

The challenge test performed to evaluate the suitability of the QDS technology for the production of safe acid and low acid chorizo also showed equivalence for the traditional and the QDS process® regarding the fate of *L. monocytogenes* and *Salmonella* during production and storage of 91 days under refrigeration (V.1.). However, in this type of product and due to the QDS technology, variations in pH evolution and technological microbiota were observed. Comaposada et al. (2010) and Comaposada et al. (2008) highlighted the better control of pH by QDS processing and its contribution to a consistent product appearance (Comaposada et al., 2010). The faster decrease of LAB and GCC+ in QDS than in traditional products could be primarily attributed to the more intense thermal treatment associated with the QDS process®. It is well known that technological microbiota influences the texture and flavour characteristics of the final product. From the obtained results it can therefore be derived that

differences in the behaviour of LAB and GCC+ together with differences in ripening time and drying format (whole piece or slices) would possibly affect the sensory characteristics of the final product.

From a food safety point of view, the QDS technology did not decrease the product stability in any of the dry-cured meat products. The decrease of *L. monocytogenes* and *Salmonella* in dry-cured ham during storage was due to the same preservative factors (low a_w, presence of curing salts and spices and/or smoking) as in the traditional product. In chorizo, the elimination of both pathogens during production was achieved by both the traditional method and the QDS drying, which includes a longer thermal treatment. The QDS technology hence, did not affect the microbiological hurdles responsible for the safe character of dry-cured meat products.

2. Effect of NaCl-free processing

One of the major problems in the development of NaCl-free processed products is related to the multifunctional character of NaCl due to its flavouring and functional contributions and especially its antimicrobial activity (Sofos, 1983). In literature, strategies to completely or partially reduce the NaCl content in dry-cured meat products have been reported, most by using other chloride salts. Regarding microbiological stability, a few studies investigated the implications of partial NaCl replacement in dry-cured meats on *Enterobacteriaceae*, total mesophilic aerobic, salt-tolerant flora, technological microbiota, etc. and did not show important differences (see Table 5, introduction). In non-inoculated dry-cured meat products, absence of pathogens such as *L. monocytogenes*, *Salmonella* spp. and *S. aureus* has been detected (Aliño et al., 2009; Aliño et al., 2010; Blesa et al., 2008; Fulladosa, Sala, Gou, Garriga & Arnau, 2012), however, from a food safety point of view, there is a lack of

challenge test studies focusing on pathogenic microorganism behaviour in NaCl-free processed products.

Differences between the performed challenge tests comparing standard (-S) and NaCl-free (-F) processing could be attributed to the reformulation of the product. The antimicrobial effects of KCl and potassium lactate, used to substitute NaCl, have been demonstrated separately in various studies performed in culture media (Boziaris, Skandamis, Anastasiadi & Nychas, 2007; Chen & Shelef, 1992; de Wit & Rombouts, 1990; van Burik & de Koos, 1990). In dry-cured meat products however, the antimicrobial effects of KCl and potassium lactate (separately or in combination) on inoculated *L. monocytogenes* and *Salmonella* has not been investigated yet. Apparently, in NaCl-free processed traditionally dried dry-cured ham (TS-TRADI-F), the combination of KCl, potassium lactate and sugars proved to be suitable to maintain the particular hostile environment of dry-cured ham (see Fig. 1, V.3.) and pathogenic microorganisms growth was inhibited. However, in the case of *L. monocytogenes,* a lower reduction of the pathogen during storage was observed when compared with standard (processed with NaCl) hams. It is known that results from broth based studies cannot be directly extrapolated to real food situations, because the efficacy of an antimicrobial agent may be dependent on the formulation of the product (NACMCF, 2010). Additionally, earlier studies showed that the type of solute chosen to control a_w had a definite effect on microbial behaviour (Beuchat, 1974; Strong, Foster & Duncan, 1970). Apart from the described differences in antimicrobial potential between NaCl and the combination of KCl, potassium lactate and sugars in traditional dry-cured ham, variations could also be detected in the achieved physicochemical levels. A_w values were higher in NaCl-free processed hams throughout the whole storage period under refrigeration, thus, at day 112 the a_w was recorded at 0.926 in standard and at 0.940 in NaCl-free processed products. Slight differences were detected in pH values (5.75-5.87 in –S *vs*. 5.95-5.79 in –F).

Regarding technological microbiota, the difference in initial GCC+ counts of *ca*. 2 log was due to differences in starter culture application (Fig. 2, V.3.).

In chorizo production, NaCl-free processing was accompanied with a modification in the acidification method, due to the presence of lactate which has been shown to affect LAB growth (Shelef, 1994) and which could compromise the pH decrease of the product: LAB fermentation was substituted by the application of a calculated amount of GDL. These changes in the formulation affected the decrease of the levels of pathogenic microorganisms, especially *L. monocytogenes* in low acid chorizo. LAB, apart from its competitive character, produced a stronger pH decrease during fermentation in LA-TRADI-S (to 4.82) than GDL acidification in LA-TRADI-F (5.14), which could explain the elimination of *L. monocytogenes* in fermented sausages but not in GDL acidified ones (Table 8).

Table 8. Behaviour of *L. monocytogenes* in standard and NaCl-free processed acid and low acid chorizo dried following the traditional process

	Chorizo type:	**ACID (A)**				**LOW ACID (LA)**			
	Processing:	**Standard (-S)**		**NaCl-free (-F)**		**Standard (-S)**		**NaCl-free (-F)**	
Production step	Before acidification	1.48 ± 0.22^{b}		1.08 ± 0.56^{a}		1.82 ± 0.26		1.65 ± 0.13^{a}	
	After acidification	0.59 ± 0.69^{a}		0.26 ± 0.52^{b}		ND		1.69 ± 0.34^{a}	
	After thermal treatment	0.26 ± 0.52^{a}		ND		ND		0.52 ± 0.60^{b}	
	After drying	ND		ND		ND		ND	
HP Treatment:		**0 MPa**	**600 MPa**	**0 MPa**	**600 MPa**	**0 MPa**	**600 MPa**	**0 MPa**	**600 MPa**
Days of storage	t0/after HP	ND	ND	ND	ND	ND	ND	ND	ND
	15 days	ND	ND	ND	ND	ND	ND	1PRE/3ND*	ND
	28 days	ND	ND	ND	ND	ND	ND	ND	ND
	56 days	ND	ND	ND	ND	ND	ND	ND	ND
	91 days	ND	ND	ND	ND	ND	ND	ND	ND

Values are mean ± SD of two independent experiments performed in duplicate. For acid and low acid chorizo, significant differences in columns are indicated by different small letters ($P < 0.05$). ND: not detected in 25 g; PRE: presence in 25 g. **L. monocytogenes* was only detected in 1 out of 4 replicates.

Similarly in acid chorizo, although *Salmonella* was not eliminated by the pH decrease, its decrease was higher in fermented than in GDL chorizos (Table 9).

Table 9. Behaviour of *Salmonella* in standard and NaCl-free processed acid and low acid chorizo dried following the traditional process

	Chorizo type:	ACID (A)		LOW ACID (LA)	
	Processing:	Standard (-S)	NaCl-free (-F)	Standard (-S)	NaCl-free (-F)
Production step	Before acidification	0.55 ± 0.43^{b}	1.04 ± 0.53^{a}	0.30 ± 0.00^{b}	1.04 ± 0.53^{a}
	After acidification	1PRE/3ND*	0.97 ± 0.49^{a}	0.23 ± 0.15^{ab}	0.86 ± 0.37^{ab}
	After thermal treatment	ND	1PRE/3ND*	0.15 ± 0.17^{ab}	0.30 ± 0.00^{b}
	After drying	ND	ND	ND	1PRE/3ND*

	HP Treatment:	0 MPa	600 MPa	0 MPa	600 MPa	0 MPa	600 MPa	0 MPa	600 MPa
Days of storage	t0/after HP	ND	ND	ND	ND	ND	ND	1PRE/3ND*	ND
	15 days	ND	ND	ND	ND	ND	ND	ND	ND
	28 days	ND	ND	ND	ND	ND	ND	1PRE/3ND*	ND
	56 days	ND	ND	ND	ND	ND	ND	ND	ND
	91 days	ND	ND	ND	ND	ND	ND	ND	ND

Values are mean ± SD of two independent experiments performed in duplicate. For acid and low acid chorizo, significant differences in columns are indicated by different small letters ($P < 0.05$). ND: not detected in 25 g. PRE: presence in 25 g. **Salmonella* was only detected in 1 out of 4 replicates.

These differences in the acidification method consequently affected the evolution of pH during the storage period under refrigeration. The present observations were in line with results from Lücke (1998), who attributed differences in pH courses to differences in composition and production processes.

NaCl-free processing decreased the food safety of low acid chorizo (LA-TRADI-F), where both pathogens could survive the production process (acidification and drying) (Table 8 and 9). This observation could be related to variations in the composition between standard (–S) and NaCl-free processed (–F) chorizo and the higher pH level of the latter (4.82 in -S *vs*. 5.14 in -F). Accordingly, the combination of higher pH and NaCl-free formulation hindered the elimination of *L. monocytogenes* and *Salmonella*. An important fact that must be considered at this point was the difference in the a_w values between –S and –F processed

chorizos: although higher levels were recorded in chorizos processed with NaCl (LA-TRADI-S: 0.938) than in the NaCl-free processed ones (LA-TRADI-F: 0.908), pathogenic microorganisms survived the production process of the latter. This observation highlights the importance of the matrix composition (presence of NaCl in this case) and the acidity hurdle in dry fermented sausages and the requirement of (an) additional preservative factor(s) (thermal treatment, HP) for low acid products. NaCl-free processed low acid chorizo produced with the QDS process® (LA-QDS-F), including a more intense thermal treatment and more time at $a_w \leq 0.9$, was safer than the traditionally processed NaCl-free product (LA-TRADI-F). In –F processed chorizos, these harsher conditions of QDS processing, however, not only affected pathogenic microorganisms, but also technological microbiota. In this sense, GCC+ counts only decreased in A-QDS-F (the product with the harshest conditions: lower pH and more intense thermal treatment), while endogenous LAB could only grow in LA-TRADI-F chorizo, due to the combination of higher pH, shorter thermal treatment, higher a_w during processing and longer drying period (the product with the mildest conditions).

To evaluate the safety of QDS processed dry-cured ham differing in acidity, smoking and NaCl content, three types of hams were produced including “non-acidified smoked” (NS), “acidified” (A) and “acidified smoked” (AS) according to the standard (-S) and the NaCl-free (-F) process.

L. monocytogenes and *Salmonella* counts decreased similarly (1.3-1.5 log) in all types of ham. Interestingly in –F hams equal or lower counts than those found in –S hams were observed throughout the whole storage period under refrigeration. In the case of *L. monocytogenes*, this result was in contrast to observations from traditionally dried hams (Fig. 1, TS-TRADI-S and TS-TRADI-F, V.3.) where lower levels of pathogens were recorded in the –S product. The differences in the product a_w at the time of slicing (finished product in traditional hams *vs*. undried or partially dried product in QDS) hams and the acidification system (bacterial fermentation *vs.* GDL application) could have caused differences in the

fate of the pathogens. Thus, the QDS technology in combination with NaCl-free processing had a positive effect on the food safety of dry-cured hams.

Moreover, differences in composition between –S and –F processed hams produced variations in pH values before QDS drying. In this sense, pH levels recorded from all NaCl-free processed dry-cured hams (NS: 5.75, A: 5.27, AS: 5.36) were 0.3 units higher than in the corresponding standard processed dry-cured hams (NS: 5.45, A: 4.98, AS: 5.06). This fact could be attributed to the presence of lactate as previously demonstrated in dry-cured meat products in which the NaCl amount was partially reduced (Costa-Corredor, Serra, Arnau & Gou, 2009; Fulladosa, Serra, Gou & Arnau, 2009; Gou, Guerrero, Gelabert & Arnau, 1996).

In A and AS products, acidification was achieved by adding LAB starter cultures to the salt brine of –S processed hams while GDL was added to NaCl-free processed dry-cured hams. This difference could have contributed to the observed variations in pH values before QDS drying, however, independent from the method, acidification was among the most important factors affecting the levels of pathogenic bacteria in the evaluated dry-cured hams. Accordingly, acidified hams achieved the highest proportion of samples with absence of *L. monocytogenes* and *Salmonella* (Fig. 3, V.4.). The only type of non-pressurised dry-cured ham in which absence of *L. monocytogenes* (in 25 g of sample) could be observed, however, was the acidified smoked one (both -S and –F processed). According to Leistner & Gorris (1995), it is more effective to use a combination of different microbial factors with low intensities that affect different microbial systems or act synergistically than to use a single preservative factor with a high intensity. In this sense, ham including more hurdles (smoking in combination with acidification) provided the best pathogen inhibition in both standard and NaCl-free processed (AS-QDS-S and AS-QDS-F) ham slices. These results are in general agreement with literature, where the combined effect of cold or liquid smoke together with low pH conditions and high salt concentrations have been observed against *L.*

monocytogenes and/or *L. innocua* in fish and meat products (Martin et al., 2010; Milly, Toledo & Chen, 2008; Montero, Gómez-Estaca & Gómez-Guillén, 2007).

To sum up, NaCl-free processing affected the stability of traditionally processed dry-cured meat products. By using the QDS technology for the production of NaCl-free processed chorizo and dry-cured ham, however, it became possible to produce dry-cured meat products without the use of NaCl that were safer than the traditional ones. The low pH hurdle is crucial for dry fermented sausage products and its incorporation in dry-cured ham production improves the product safety.

3. Effect of High Pressure

In all the studied dry-cured hams and in low acid traditionally dried chorizo (LA-TRADI-F, the only chorizo batch where *L. monocytogenes* and *Salmonella* were not eliminated during production), it was possible to achieve absence (in 25 g) of both pathogens with the application of a HP treatment of 600 MPa at 13ºC for 5 min. Pressurisation at these conditions is nowadays industrially applied to enhance the safety and to extend the shelf-life of a variety of meat products (Garriga & Aymerich, 2009). In relation to innovative production processes and product reformulations, which possibly bring along food safety implications, an HP treatment could be useful as complementary technology to guarantee equal food safety levels.

In pressurised samples, variations due to NaCl-free processing had the most important impact on pathogenic microorganism behaviour. Independently of the drying method (traditional or QDS) or inclusion of additional hurdles (low pH and/or smoking), a lower immediate bactericidal effect and slower elimination of *L. monocytogenes* and *Salmonella* throughout refrigerated storage was observed in all -F hams when compared with -S hams (Table 10 and Table 11).

Table 10. Elimination of *L. monocytogenes* during storage of HP treated dry-cured ham

ham \ day	1	14	28	56	112
TS-TRADI-S	4 ABS	4 ABS	4 ABS	4 ABS	4 ABS
TS-TRADI-F	3 PRE/ 1 ABS	2 PRE/2 ABS	3 PRE/ 1 ABS	1 PRE/ 3ABS	4 ABS
NS-QDS-S	4PRE	2 PRE/2 ABS	2 PRE/2 ABS	1 PRE/ 3ABS	4 ABS
NS-QDS-F	3 PRE/ 1 ABS	3 PRE/ 1 ABS	2 PRE/2 ABS	1 PRE/ 3ABS	4 ABS
A-QDS-S	4 ABS	1 PRE/ 3ABS	4 ABS	4 ABS	4 ABS
A-QDS-F	4PRE	4PRE	3 PRE/ 1 ABS	4 ABS	4 ABS
AS-QDS-S	4 ABS	4 ABS	4 ABS	4 ABS	4 ABS
AS-QDS-F	4PRE	4PRE	2 PRE/2 ABS	4 ABS	4 ABS

n=4; PRE: presence in 25 g; ABS: absence in 25 g.

Table 11. Elimination of *Salmonella* during storage of HP treated dry-cured ham

ham \ day	1	14	28	56	112
TS-TRADI-S	3 PRE/ 1 ABS	4 ABS	4 ABS	4 ABS	4 ABS
TS-TRADI-F	4PRE	1 PRE/ 3ABS	1 PRE/ 3ABS	4 ABS	4 ABS
NS-QDS-S	4PRE	2 PRE/2 ABS	2 PRE/2 ABS	4 ABS	4 ABS
NS-QDS-F	4PRE	2 PRE/2 ABS	4 ABS	1 PRE/ 3ABS	4 ABS
A-QDS-S	3 PRE/ 1 ABS	2 PRE/2 ABS	4 ABS	4 ABS	4 ABS
A-QDS-F	4PRE	2 PRE/2 ABS	4 ABS	4 ABS	4 ABS
AS-QDS-S	3 PRE/ 1 ABS	4 ABS	4 ABS	4 ABS	4 ABS
AS-QDS-F	4PRE	4PRE	1 PRE/ 3ABS	4 ABS	4 ABS

n=4; PRE: presence in 25 g; ABS: absence in 25 g.

The elimination of NaCl and its antimicrobial activity, hence, affected the stability of pressurised NaCl-free (-F) processed hams and produced a significant delay in pathogenic microorganism elimination (Fig. 1 in V.3. and Fig.3 in V.4.). This observation could be related to the previously observed protective effect of lactate on *L. monocytogenes* but not on *Salmonella* (Aymerich, Jofré, Garriga & Hugas, 2005; Jofré, Garriga & Aymerich, 2008).

Conversely, lactate had an inhibiting effect on technological microbiota, which could not recover after pressurisation in any of the batches were lactate was present. However, in traditionally dried and in non-acidified QDS dried hams with NaCl (TS-TRADI-S and NS-QDS-S), the only products where neither LAB nor GCC+ starter cultures were applied, endogenous microbiota could recover during refrigerated storage to initial or higher levels.

Studies evaluating the dependence of the pressurisation effect on other factors reported that physicochemical properties highly affect the impact of pressurisation and important differences in inactivation have been observed when different products were submitted to the same HP treatment (Garriga, Grèbol, Aymerich, Monfort & Hugas, 2004; Jofré, Aymerich, Grèbol & Garriga, 2009). Intermediate a_w for example, that is normally found in dry-cured meat products, was observed to have a protective effect on pathogenic microorganisms, which seems to be compensated by the inhibition of the recovery of the cells during storage, because microorganisms injured by HP are more sensitive to intermediate a_w (IFT, 2000). Most microorganisms tend to be more susceptible to pressure in low pH environments, and pressure-damaged cells are less likely to survive in acidic environments (Patterson, Linton & Doona, 2007). In addition, high salt concentrations and smoking compounds contribute to the particular hostile environment of dry-cured meat products (Flores, 1997; Verma & Banerjee, 2012) and could also hinder the recovery of sub-lethally injured cells.

Accordingly, the bactericidal effect of pressurisation observed in the present challenge tests varied between the products and was not only affected by compositional differences but also by physicochemical parameters. Among all NaCl-free processed (-F) hams, inactivation of pathogenic microorganisms was faster in acidified than in non-acidified dry-cured hams, especially in the case of *L. monocytogenes*. In this sense, the combination of low pH and HP has been described as an efficient way to inactivate pathogenic microorganisms in foodstuff and to inhibit subsequent outgrowth of sublethally injured cells (Smelt, 1998). In QDS dried

standard processed (-S) dry-cured hams, similarly, acidification achieved faster elimination of *L. monocytogenes* and *Salmonella*, although best results were achieved in the AS-QDS-S product, where elimination (absence of 25 g) of *L. monocytogenes* immediately after application and of *Salmonella* after 14 days was observed. The importance of smoking and presence of NaCl could also be observed in TS-TRADI-S ham, in which the same results as in AS-QDS-S were achieved. Similarly, Montero et al. (2007) demonstrated that smoking and pressurisation, together with a high salt concentration, kept *L. monocytogenes* counts under limit of detection throughout 100 days of storage at 5°C in cold-smoked dolphinfish.

To sum up, pressurisation produced a general decrease but not complete elimination in pathogenic microorganism levels in all dry-cured hams and assured absence of *L. monocytogenes* and *Salmonella* throughout the storage of LA-TRADI-F chorizo. In NaCl-free processed dry-cured ham, however, the bactericidal effect of HP was attenuated. This is a remarkable finding and should be kept in mind when applying HP processing to improve the microbiological safety of products with a complete or partial reduction in the NaCl content.

4. Differences between *L. monocytogenes* and *Salmonella* behaviour

From the challenge tests performed within the framework of the present PhD thesis, some differences were observed in the behaviour of the pathogens *L. monocytogenes* and *Salmonella*, which could be attributed to differences in their growth limits and tolerance to antimicrobial factors.

In dry-cured ham, *L. monocytogenes* was more affected than *Salmonella* counts by changes of a_w and NaCl-free processing could be observed, especially in traditionally dried products. In TS-TRADI-F, *L. monocytogenes* showed higher counts than in TS-TRADI-S ham whereas *Salmonella* behaved similar in both products. Observed differences may result from the different a_w growth optimums of the two pathogens (ICMSF, 1996) and their interaction with

temperature (psychrotrophic character of *L. monocytogenes*) and abilities to grow/survive in absence of NaCl.

In non-pressurised dry-cured ham, absence of *L. monocytogenes* could be achieved after 112 days in AS-QDS-S and AS-QDS-F whereas *Salmonella* could not be eliminated from any of the non-pressurised samples. In this context Asita & Campbell (1990) highlighted that smoke extracts are more active against gram-positive than gram-negative bacteria.

Elimination of *L. monocytogenes* could be achieved immediately after pressurization in TS-TRADI-S ham and in AS-QDS-S ham, while fastest inactivation of *Salmonella* through HP needed 14 days in the same samples (Fig. 1 in V.3. and Fig. 3 in V.4.). Although it is generally accepted that gram-positive bacteria are more resistant to pressurisation than gram-negative, strain and environmental conditions have an important effect and opposite results can be observed such as those found in the present studies and those reported by Chen, (2007) and Jofré, Aymerich, Bover-Cid & Garriga, (2010).

In standard chorizo, higher resistance of *Salmonella* than *L. monocytogenes* was observed in low acid samples, which could be related to the higher growth limits of the former to acid and high temperature conditions (minimum pH and maximum temperature growth limits are 3.8 and 49.5°C for *Salmonella* and 4.39 and 45°C for *L. monocytogenes* (ICMSF, 1996) (V.1.). Likewise in -F chorizo, *Salmonella* did not significantly decrease during acidification in the A batches such as *L. monocytogenes* (Table 8 and 9).

The observed differences in pathogenic microorganism behaviour point out the importance of the adequate choice of pathogens of concern for a challenge study in order to assess the food safety effect of new processes and/or reformulations of food products.

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VII. CONCLUSIONS

According to the studies enclosed to this PhD thesis, it can be **concluded:**

1. The QDS process® allows a fast achievement of an intermediate a_w without compromising food safety of dry-cured meat products.
2. The type of processing, traditional or QDS, has no influence on the behaviour of *L. monocytogenes* and *Salmonella*, which are eliminated after processing (fermentation and drying) and do not recover during storage of both acid and low acid chorizos and progressively decrease during storage of dry-cured ham.
3. In chorizo, the inactivation of lactic acid bacteria due to the thermal treatment included in the processing with QDS and the attained a_w level hinders further pH decrease during storage.
4. NaCl-free processing, where NaCl is substituted by KCl and potassium lactate, has no effect on the particular hostile environment of chorizo and dry-cured ham and, as in standard processing, *L. monocytogenes* and *Salmonella* cannot grow in any of the products.
5. NaCl-free processing affects the survival of *L. monocytogenes* and *Salmonella* and allows the survival of both pathogens during the production of traditionally processed low acid chorizo and produces a lower reduction of *L. monocytogenes* during the storage of traditionally dried dry-cured ham.
6. The combination of NaCl-free processing with the QDS technology allows the production of chorizo without the use of NaCl that are safer than the corresponding traditional products.
7. QDS dried NaCl-free processed dry-cured ham slices are safer than slices of QDS dried standard processed hams.
8. The application of a HP treatment produces a general decrease (below 10 CFU/g in most cases) but not complete elimination of *L. monocytogenes* and *Salmonella* in all dry-cured hams and assures their absence throughout 91 days of storage in NaCl-

free traditionally processed low acid chorizo, the only type of chorizo in which pathogens were detected during storage.

9. The inhibitory effect of pressurisation can be compromised by the product reformulation. Thus, when applying HP processing to improve the microbiological safety of products with a complete or partial reduction in the NaCl content, it must be considered that NaCl-free processing used in this study attenuates the bactericidal effect of pressurisation.
10. Lactate reduces the listericidal effect of HP in NaCl-free processed dry-cured ham and prevents the recovery of technological microbiota after pressurisation, during the storage period under refrigeration.
11. Acidity achieved by bacterial fermentation or GDL application improves the food safety of standard, NaCl-free processed and pressurised dry-cured meat products.