Reduction of *Listeria innocua* contamination in vacuum-packaged dry-cured Italian pork products after high hydrostatic pressure treatment

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Abstract

The present work aims to present the results of the application of a treatment with high hydrostatic pressure (HHP) on Italian fermented and dry-cured pork products. The products used in this study were portioned cured ham, portioned bacon and salami, vacuum-packaged and produced by a single processing company. Two studies were conducted on a single batch of the three products by means of an artificial contamination with *Listeria innocua* as a surrogate of *L. monocytogenes*. In the first trial a superficial contamination was obtained by immersion for 3 min in the culture broth with a concentration of approximately 9 log cfu/mL. At the end of the inoculum step, the pieces were dred at room temperature and vacuum packaged. In the second trial 50 kg of minced pork meat were contaminated before production of salami. In both cases the inoculum contained 5 strains of *L. innocua*. Subsequently, in both trials, 10 samples were randomly divided into two groups of 5 pieces each: i) TH group, samples treated with HHP; ii) group C, control samples, not subjected to any treatment. All samples were stored at refrigeration temperature at the end of HHP treatments (if applied), and analyzed for the determination of the surface (1st trial) and deep (2nd trial) quantitative contamination of *L. innocua*. pH and a_W were also determined on 3 pieces of each products belonging to group C. The difference between the medians of the log cfu/cm^2 or g established between controls and treated were compared using the non-parametric test (Kruskal-Wallis test) with $P<0.01$. In all products and in both trials the level of contamination detected in treatment groups was always significantly lower than in controls ($P<0.01$). In particular, in vacuum-packaged ham, bacon and salami viability logarithmic viability reductions equal to -2.29, -2.54 and -2.51 were observed, respectively. This study aimed to evaluate a not-thermal treatment on Italian cured or fermented pork products. The results of this study need to be confirmed in different products and in a greater number of lots, but they appear promising, also because of the considerable literature available for different categories of products (cheese, vegetables and fruit).

Introduction

The application of high hydrostatic pressure (HHP) in food preservation has received particular attention as a viable alternative (economically and technologically) to thermal processes (Patterson, 2005). High pressure processing (HPP) is a non-thermal food preservation method that has gained considerable interest in the last two decades because of its ability to preserve foods while maintaining their fresh-like qualities. Pressures of 300-700 MPa, which inactivate vegetative cells but not bacterial spores, are typically used for food preservation (Stewart and Cole, 2001). High-hydrostatic pressure processing is growing as a processing method or intervention technology of choice because of its demonstrated ability to economically extend shelf life and preserve the quality of food, as heat is not applied. Unlike interventions, such as antimicrobials or oils, which are usually applied to the surface of a food product, HPP operates according to the isostatic principle, in which the pressure applied to a sample through a pressurized medium such as water or oil is instantaneous and uniform throughout the sample, regardless of its volume or shape, thus inactivating microbes throughout a sample (Rastogi *et al*., 2007).

The application of pressure to a sample through a pressurized fluid via HPP was demonstrated as an effective method for inactivation of pathogens, such as *L. monocytogenes* and spoilage organisms under room temperature conditions, in a variety of food products such as guacamole, sauce, fruit juices, meats, seafood and cheese (Sandra *et al*., 2004; Rastogi *et al*., 2007; Zhang and Mittal, 2008; Van Hekken *et al*., 2013).

Inactivation of microorganisms by HPP has been attributed to cell death *i.e*. in cheese, has been shown that the treatment lead to a reorganization of water molecules around the ions, changing in the amount of free and unbound water molecules, which affect the mineral balance, enzyme interactions, and protein conformation (Knorr *et al*., 2006), loss of cytoplasmic membrane integrity and partial loss of membrane integrity. However HPP does not necessarily lead to cell death and a sub-lethal injury to the cells that accounts for the growth of cells after treatment has been observed (Ritz *et al*., 2001; López-Pedemonte *et al*., 2007).

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The extent of microbial inactivation using HPP is affected by applied pressure, initial substrate temperature, hold time, food substrate and composition, presence of antimicrobial compounds, and pressure resistance of the microorganisms (Smelt, 1998; Chen and Hoover, 2003; Patterson, 2005; Hayman *et al*., 2007). The water activity (a_w) could be an important factor that can influence the efficacy of HHP treatment. While reduced a_w can inhibit the growth of microorganisms, it can also protect them from other environmental stresses, such as heat (Gould, 1985). Decreasing a_w has also been found to increase the resistance of microorganisms to high pressures (Oxen and Knorr, 1993), however this effect depends on the solute used to depress a_w (Patterson, 2005; Koseki and Yamamoto, 2007).

In Italian typical pork productions such as salami, bacon and ham, lactic fermentation and/or a_w reduction are used to stabilize and transform the products, but survival or recontamination with *L. monocytogenes* can occur in different steps of the production process.

European Union food regulation admits the presence of *L. monocytogenes* below concentrations of 100 colony-forming units (cfu)/g in ready to eat (RTE) products if they do not sustain the growth of the pathogen above the limit during their shelf life. In USA a zero tolerance policy is applied and the presence of *L. monocytogenes* in RTE product is not admitted.

The aim of this research was to apply a treatment with HHP on Italian fermented and dry-cured products to evaluate the efficacy of the treatment on the reduction of superficial and deep (in the cases of salami) contamination of *L. innocua,* used as a surrogate for *L. monocytogenes* (Scott *et al*., 2005).

Materials and Methods

Two trials were conducted. The first was addressed to establish the efficacy of HHP treatment on superficial contamination of *L. innocua* as a surrogate for *L. monocytogenes* on vacuum-packaged portioned ham, bacon and salami. The second was addressed to establish the efficacy of HHP treatment on salami produced after contamination of minced pork meat with *L. innocua* as a surrogate for *L. monocytogenes*.

Products

Pork meat salami, bacon and ham coming from a single producer (one batch per each product) were used in the study (Table 1). For the first trial ten portion of 200 g of vacuum-packaged salami, bacon and ham were randomly divided into 2 groups of 5 pieces: i) high hydrostatic pressure treatment (TH group), samples treated with HHP; ii) Group C, control samples not subjected to any treatment. For the second trial, salami were produced from 50 kg of minced pork meat and after the curing period, were divided into the same 2 groups, TH and control groups, of 5 pieces each.

Inoculum

Five strains of *L. innocua* (IZSLER 111373/1 and IZSLER 111373/2 isolated in superficial swab collected in pork meat transformation plant, IZSLER 257529/1 isolated form pork fresh sausages, IZSLER 257529/2 isolated from fresh pork meat and a collection strain ATCC 33090) were streaked for colony isolation onto a non-selective agar plate (Trypticase soy agar) and incubated for 24-48h at 37°C. Revitalized strains were inoculated in a 1 L of non-selective nutrient broth (Brain Heart Infusion) and incubated at 37°C for 24-26h to obtain stationary cells at approximately $1x10^9$ cells/mL. At contamination time, a cocktail of the 5 broths culture was obtained and used for contamination. For the first trial, 5 pieces of the three products were surface inoculated by dipping the portioned foods into the inoculation suspension (cocktail of the 5 broth culture) for 3 min. A post-inoculation drying and attachment period of 30 min was applied. After that, each piece was re- vacuum-packaged and put at refrigeration temperature (4±2°C) until treatment. For the second trial, 100 mL of inoculation suspension (cocktail of the 5 broth culture) were added to a 50 kg of minced pork meat and fat suitable for salami production. The distribution of the inoculum was homogenized throughout 30 sec of mechanically homogenizer. Salami were dry-cured after 3 days of drying and ripened for 4 weeks following the producers' protocols.

High hydrostatic pressure treatment

The HHP treatment (TH group) consisted of 6000 bar for 360 sec. The pressure-holding treatment time in this study did not include the pressure increase time or the decompression time. The temperature reached by the product was not determined, while the water temperature during the process started from 12 to 13°C, grew until 31°C during the treatment, and immediately returned to 12°C after the end of pressure stress. The contribution of estimated highest sample temperature to the destruction of microorganisms by HHP was considered negligible.

Enumeration of *Listeria innocua*

Each vacuum-packaged piece was open after ethanol-sterilization. A superficial portion of approximately 100 cm^2 was sliced and an analytical portion of 25 cm^2 was obtained and submitted for *L. innocua* enumeration procedure. Due to the absence of a specific method for *L. innocua* enumeration, ISO11290-2:1998/Amd 1:2004 (ISO, 2004) for *L. monocytogenes* enumeration was used until spread plated onto ALOA agar. All plates were incubated at 37°C for 48 h and typical *L. innocua* colonies (green on ALOA agar not surrounded by any opaque halo) were counted manually and submitted to biochemical confirmatory test (API® listeria; bioMérieux Clinical Diagnostics, Marci l'Etoile, France). Bacterial numbers were expressed as the logarithm of cfu/g.

Statistical analysis

The difference between the medians of log cfu/cm^2 of *L. innocua* between control and treated groups was compared using the non-parametric test (Kruskal-Wallis test) with Intercooled Stata 7.0 software (Stata Corporation, College Station, TX, USA). Significance was established at $P<0.01$.

Results

In Table 2 the results obtained in the two trials are summarized. In particular, contamination in control group results in the whole experiment significantly ($P<0.01$) reduced after HHP treatment, regardless of the type of food product or the type of contamination (superficial or deep in the case of repined salami).

Discussion

The results show that the applied HHP treatment is able to reduce both superficial and deep contamination of *L. innocua* in all the products. A similar result was previously obtained for different types of food such as guacamole, sauce, fruit juices, meats, seafood and cheese (Sandra *et al*., 2004; Rastogi *et al*., 2007; Zhang and Mittal, 2008; Van Hekken *et al*., 2013). On salami and pork meat transformed products such as ham only few references are available. In particular in Bover-Cid *et al*. (2011) a model of *L. monocytogenes* inactivation on dry-cured ham by HHP processing is developed and the conclusion of this model fits exactly what is observed in our experiment. In particular the model predicts a treatment able to obtain 2.39D reduction through a HHP at 613 MPa for 5 min. In our study a similar treatment (6000bar - correspond to 600Mpa, per 360 sec) was able to obtain on average 2.44D reduction in portioned ham, bacon or salami and 2.76D reduction in deep contaminated salami.

The presence of *L. monocytogenes* in RTE Italian pork meat products is reported and the

Table 1. Products details.

Product	Fat content (%)	Ripening period
Pork meat salami	30.0	50 days at 12-13°C
Bacon	55.1	90 days at 15°C
Ham	13.7	80 days at 14-16°C followed by 9 months at 18°C

Table 2. Results of contamination of *Listeria innocua* in controls and vacuum-packaged products treated with high hydrostatic pressure.

	Control	TH group	D Log N/N_0	pH	a_w
Ham	5.108±0.099[a]	2.818±0.591[b]	-2.29	6.267±0.057	0.922±0.002
Bacon	5.632±0.115[a]	3.096±0.861[b]	-2.54	5.967±0.058	0.933±0.002
Salami (superficial)	4.924±0.201[a]	2.416±0.456[b]	-2.51	5.9±0.1	0.922±0.002
Salami (deep)	5.040±0.321[a]	2.280±0.164[b]	-2.76	5.867±0.115	0.923±0.002

TH group, high hydrostatic pressure treatment; a_w, water activity. Values are expressed as mean±standard deviation log 10 cfu/cm^2 or g.
[a,b]Means with different lowercase letter in the same rows differ significantly at $P<0.01$ (Kruskal-Wallis test).

microorganism can survive in cured products such as salami for a long period (Gianfransceschi *et al*., 2006). Moreover, post-producing manipulation, such as portioning, slicing and packaging of food products can enable cross-contamination and serve as a source for the spread of the pathogen (Vorst *et al*., 2006). When meat products are re-contaminated during post-processing and producing manipulation the contamination is assumed to be 10 cfu/g (*i.e*. 1 Log cfu/g) at the worst (ICMSF, 1996). In this way, Hoz *et al*. (2008) proposed a 2.39D process to meet the USA zero tolerance policy, which can be achieved in dry-cured ham, through a HHP at 613 MPa for 5 min according to the model proposed by Bover-Cid *et al*. (2011).

Taking into consideration the European Union microbiological criteria in relation to *L. monocytogenes*, a maximum tolerance of 100 cfu/g is admitted. The low concentration *of L. monocytogenes* in meat RTE products (Casadei *et al*., 2004; EFSA, 2013) considered and the effect recorded in this study given, it can be stated that HHP treatment can greatly contribute to reduce or eliminate the risk of exposure for consumers.

Conclusions

In conclusion, the results of this study indicate that HHP could be profitably applied to Italian typical pork products in order to reduce *L. monocytogenes* contamination as largely demonstrated for many other food products. Anyway, it is worthy to note that the results of this study need to be confirmed in different products and in a greater number of lots.

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