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Impact of cooking procedures and storage practices at home on consumer exposure to *Listeria monocytogenes* and *Salmonella* due to the consumption of pork meat

ABSTRACT

The objective of this research was to analyse the impact of different cooking procedures (i.e., gas hob and traditional static oven) and levels of cooking (i.e., rare, medium and well done) on inactivation of *Listeria monocytogenes* and *Salmonella* in pork loin chops. Moreover, the consumer's exposure to both microorganisms after simulation of meat leftover storage at home was assessed. The results showed that well-done cooking on static oven was the only treatment able to inactivate the tested pathogens. The other cooking combinations allowed to reach in the surface product temperatures always ≥ 73.6°C, decreasing both pathogens between 6 and 7 $\log_{10}$ cfu/g. However, according to simulation results, the few cells surviving cooking treatments can multiply during storage by consumers up to 1 $\log_{10}$ cfu/g, with probabilities of 0.059 (gas hob) and 0.035 (static oven) for *L. monocytogenes* and 0.049 (gas hob) and 0.031 (static oven) for *Salmonella*. The key factors affecting consumer exposure in relation to storage practices were probability of pathogens occurrence after cooking, doneness degree, time of storage and time of storage at room temperature. The results of this study can be combined with prevalence data and dose response models in risk assessment models and included in guidelines for consumers on practices to be followed to manage cooking of pork meat at home.

KEYWORDS: consumers, *Listeria monocytogenes*, *Salmonella,* exposure assessment, cooking practices

1. INTRODUCTION

Pork is the most frequently consumed meat in the European Union (1) and management of hazards transmitted to humans by pork consumption is of major health and economic significance. Twenty-seven biological hazards may be transmitted from pork to consumers (2). Among them, *Yersinia enterocolitica*, *Salmonella enterica*, thermophilic *Campylobacter* spp. and *Listeria monocytogenes* are characterized by the highest risk scores (2).

Pork meat, like loin chop, must be cooked before consumption. It is generally accepted that when meat (including hamburgers or any other minced meat) is subjected to a core temperature of 70°C for 2 min or to a heat treatment equivalent to 2 min at 70 °C, it will accomplish a substantial inactivation (i.e., 6 $\log_{10}$ reduction) of pathogens. The Food Safety and Inspection Service (FSIS) guidelines (3, 4) set that, for the production of ready-to-eat (RTE) foods, a reduction in *Listeria monocytogenes* and *Salmonella* spp concentrations of at least 5 $\log_{10}$ cfu/g must be ensured (7 $\log_{10}$ reduction for cooked poultry products). Moreover, the British Retail Consortium (BRC) standard defines "cook" as a thermal process which is designed to achieve a 6 $\log_{10}$ reduction in *Listeria monocytogenes* and correspond to 70°C for 2 minutes (5). However, if few pathogen cells persist during and after cooking, they might be able to multiply during storage in the refrigerator and when meat is left at room temperature. Endpoint internal temperature during cooking and treatment effectiveness depend directly on cooking uniformity and food characteristics, such as texture, level of fat, a_w, pH, thickness. Consequently, temperature and cooking time can vary considerably, depending on type of product and cooking procedures (6). Heat treatment produces physical-chemical modifications, causing changes in sensory properties. In particular, high temperatures have a negative influence on toughness, juiciness and tenderness (7-12). Crawford et al. (2010) (13) reported that pork loin becomes less tender and juicy as the cooking temperature increase from 62 to 79 ºC. Different studies have demonstrated that sensory changes are perceived by consumers and directly affect their preference for the cooking degree (14-16). In this context, the Advisory Committee on the Microbiological Safety of Foods (ACMSF) warns on the existence of potential risks for consumers associated with recent changes in cooking, in particular with the trend to serve food products in a "rare" state (17). In the same report, data from Public Health England refer to 22 outbreaks linked to incorrect cooking practices between 2009 and 2012, seven of them involving pork (17).

Consumers must be taken into consideration as a part of the food safety chain in the "Farm to fork" approach. Handling of leftover meat could represent a possible point of contamination and pathogen growth after processing. Gong, et al. (2011) (18), showed that 48.7% of Chinese consumers keep leftover meat at room temperature; 48.1% into the refrigerator immediately after

meals, and approximately 3% discard it. In relation to storage time, the same authors found differences between people who usually store leftovers into the fridge and those who do not. Overall, 27.5% of surveyed people keep the meat at room temperature less than 2 hours, 58% no more than one day, and 14.5% more than one day. People keeping leftovers in the fridge store leftover less than two hours in 74.6% of the cases, no more than 4 days in 18.8%, and more than four days in 6.6%. Specially, whether more than 50% do not re-heat the meat or just warm it before consumption (18).

Since degree of cooking and storage of leftovers at home have an impact on microbiological safety of pork meat at the time of consumption, the main objective of this paper was to analyse the dynamic of *Listeria monocytogenes* and *Salmonella enterica* inactivation in pork meat applying two of the most common cooking procedures (i.e., gas hob and traditional static oven) and three different levels of cooking (i.e., rare, medium, and well done). Moreover, the concentration of both pathogens in the product after cooking was taken as starting point to assess the consumer's exposure to both microorganisms at the time of consumption according to storage practices of meat leftovers.

2. MATERIAL AND METHODS

2.1 Product description

A total of 160 packs (samples) containing 300 ± 20 g of loin chop, vacuum packaged, were collected at the same processing facility during 10 different samplings (i.e., 16 packs/sampling). All samples collected during the same sampling were obtained from the same batch, meaning from animals reared in the same farm and slaughtered at the same age. After collection, samples were transported to the laboratory under refrigeration condition (0-4±0.5°C) and further processed.

2.2 Challenge test

All steps of the challenge tests (i.e., samples contamination with pathogens, samples cooking and analysis) were performed in rooms of the laboratory specifically dedicated to challenge tests and containing an experimental kitchen where challenge studies can be performed without health risks for the staff involved. During each sampling session, 16 samples were transported to the

laboratory under refrigeration condition (0-4 ± 0.5°C), contaminated and cooked within 24 hours from the arrival. In particular, 8 samples were contaminated with a pool of three strains of *L. monocytogenes* (i.e., *L. monocytogenes* ATCC 19111 and two field isolates from pig meat) and 8 samples were contaminated with a pool of four strains of *Salmonella enterica* (*Salmonella* Enteritidis ATCC 13076 and one *S.* Enteritidis field strain isolated from pig meat; *Salmonella* Typhimurium ATCC 14028 and one *S.* Typhimurium field strain isolated from pig meat). Samples were contaminated with *L. monocytogenes* and *Salmonella* at the concentration of 10^7 cfu/g to assess their reduction after cooking, according to FSIS and BRC specifications (3-5). This contamination level was higher than that expected to happen in nature, but it was selected to estimate the effectiveness of the heat treatment.

Each pathogen inoculum was prepared according to the European Union Reference Laboratory for *L. monocytogenes* Technical Guidance Document for conducting shelf-life studies on *L. monocytogenes* in ready-to-eat foods (19). *L. monocytogenes* and *Salmonella* spp strains were cultured on Tryptone Soy Broth (TSB, Oxoid, Milan) at 37°C for 15-18 hours, until the beginning of the stationary phase; then they were sub-cultured in TSB at 10°C for 3 days to reach the stationary phase. Finally, they were diluted and mixed to obtain the inoculum with the expected contamination level. The inoculum volumes corresponded to 1% of test unit weight (i.e., 3 ml for 300 gr of sample) to obtain a homogeneous contamination without affecting the intrinsic product properties. Samples were contaminated on the surfaces to mimic, as closely as possible, the natural contamination during slaughtering and cutting. After contamination, samples were kept at room temperature for 30 minutes and then divided into four groups, containing four samples each: two groups were contaminated with *L. monocytogenes* and two groups with *Salmonella* spp; then, for each pathogen, one group of samples was cooked on gas with a non-stick pan and the other in static oven, for a total of four possible combinations. Each group of samples was composed by four units: one was tested before cooking to verify the real contamination level; the other three were tested at different cooking levels: raw, medium and well-done.

2.3 Experimental design and simulation process

Figure 1 shows the different stages carried out in the present study, differentiating between experimental and simulation stages. Contaminated samples were cooked inside the dedicated experimental kitchen of the laboratory, by members of the staff. Considering the high contamination levels and pathogens used, for safety issues it was impossible to admit external people inside the laboratory. All cooks were family mothers and they were asked to cook according to their personal habits, as if they were at home: a "domestic scenario" was simulated and they turned the pieces of meat by using always the same tool to consider recontamination of the cooked product (i.e., worse scenario). Two different cooking protocols were used: cooking on gas with a non-stick pan for approximately 60 minutes at low heat; cooking in a static oven at 180°C for approximately 80 minutes. For each cooking protocol, three levels of cooking were achieved: raw, medium and well-done. For both cooking protocols, 300 g of loin chop (diameter about 6 cm and length 12-14 cm) were used along with half onion, two spoons of extra virgin olive oil, one sprig of rosemary, two sage leaves, 300 ml of milk, salt and pepper, 100 ml of water (for static oven only). As soon as the cook decided that the meat was ready according to each cooking scenario (i.e., gas rare, gas medium, gas, well-done, oven-rare, oven-medium, oven-well done), cooking time and surface temperature were registered with a calibrated thermometer. Finally, samples were transferred to the laboratory for analysis. For each combination (i.e., gas/static oven, and raw/medium/well-done) 40 repetitions were performed, for a total of 240 cooking tests. The heating kinetics (i.e., cooking procedure and level of cooking) were assumed as key factors affecting bacteria inactivation, beside a possible influence due to presence of salt, rosemary and milk in the recipes. Once the treatment was finished, the final contamination was the starting point for simulation stages (Figure 1). Leftover handling at home was simulated to assess the consumer exposure to both microorganisms.

2.4 *Salmonella* detection, enumeration, and confirmation

Each sample unit of 25 g was aseptically transferred to a BagFilter (Interscience), diluted 1:10 in Buffered Peptone Water (CM0895B, Oxoid, Milan, Italy) and homogenized by stomaching for 1 min in a Stomacher 400 (Seward, Worthington, UK) at normal speed. For *Salmonella* enumeration, after storage at 20±1°C for one hour, 1 ml of diluted sample was spread plated onto

three plates of Xylose Lysine Deoxycholate agar (XLD) (CM 0469, Oxoid) and three plates of Brilliant Green Agar (BGA) (CM 0263, Oxoid), respectively. All plates were incubated for 24 ± 3 h at 37 ± 1°C. For *Salmonella* detection, samples diluted in BPW were incubated for 18 ± 2 h at 37 ± 1°C for a pre-enrichment then following the reference culture method ISO 6579:2004. From the 18 h pre-enriched BPW, 0.1 ml was transferred into 10 ml of Rappaport Vassilliadis broth (CM 0669, Oxoid) incubated for 24 ± 3 h at 37 ± 1 °C. Moreover, 1 ml of BPW was transferred in 10 ml of Muller Kaufmann novobiocin tetrathionate broth (CM 1048, Oxoid) incubated for 24 ± 3 h at 41.5 ± 1 °C. After incubation, both enrichment broths were streaked in duplicate onto Xylose Lysine Deoxycholate agar (XLD) (CM 0469, Oxoid) and Brilliant Green Agar (BGA) (CM 0263, Oxoid). Plates were incubated for 24 ± 3 h at 37 ± 1 °C. Five suspected *Salmonella* colonies for each positive plate, refereed to both pathogen enumeration and detection, were sub cultured in Nutrient Agar (BO 0336, Oxoid) and tested by qualitative PCR as previously described (20). In each PCR run, *Salmonella enterica* ser. Typhimurium ATCC 14028 and *Listeria monocytogenes* ATCC 13932 were used as positive and negative controls, respectively.

2.5 *Listeria monocytogenes* detection, enumeration, and confirmation

Each sample unit of 25 g was aseptically transferred to a BagFilter (Interscience), diluted 1:10 in Fraser broth (CM0895B, Oxoid, Milan, Italy) without supplements and homogenized by stomaching for 1 min in a Stomacher 400 (Seward, Worthington, UK) at normal speed. For *L. monocytogenes* enumeration, after storage at 20 ± 1°C for one hour, 1 ml of diluted sample was spread plated onto three plates of Listeria Ottaviani Agosti agar (ALOA) (4016052, Biolife, Milan, Italy), in duplicate, incubated for 24 ± 3 h at 30 ± 1 °C. Moreover, 5 ml of half-strength Fraser supplement (SR0166E, Oxoid) were added to the Fraser broth, incubated for 24 ± 3 h at 30 ± 1 °C for a first enrichment. At the end of the incubation period, the broth was streaked in duplicate onto ALOA agar plates, incubated as previously described. Furthermore, 0.1 ml of the half-Fraser broth was transferred into 10 ml of Fraser broth supplemented with 1% of Fraser supplement (SR156E, Oxoid) before incubation for 48 ± 3 h at 37 ± 1 °C. At the end of the incubation period, a second streaking was carried out in duplicate onto ALOA agar plates, incubated as previously described. Identity confirmation for at least three suspected *L.*

monocytogenes colonies per plate was performed by PCR using the protocol published by Wesley et al., (2001) (21). In each PCR run, *L. monocytogenes* ATCC 13932 and *Escherichia coli* ATCC 25922 were used as positive and negative controls, respectively.

2.6 Definition of domestic scenarios

To map the consumer behaviours in terms of level of cooking, 40 people between 20 and 60 years old, consuming cooked pork at least once a month, were interviewed. During the interview, three different pictures (without any description) showing pork loins cooked "rare", "medium" and "well-done" were presented and each person selected one preference. In relation to the consumer behaviour, we considered the data published by Gong, et al. (2011) (18), for Chinese people, to define the probability to keep leftover meat at room temperature (0.487); into the refrigerator immediately after meals (0.481), and to discard it (approximately 0.03). In addition, to set the storage time of leftover meat we also took as reference the data obtained by the same author (18), who showed that it is less than 2 hours in 27.5% of the cases; less than one day in 58% and more than one day in 14.5%. When storage is performed in the refrigerator, it is less than 2 hours in 74.5% of the cases; less than one day in 18%; more than one day in 6.5%. Fitting these data (18) to a probability distribution function with the program @Risk 5.7 (Palisade Newfield) the leftover time of storage by consumers was calculated, (Table I). It its highlighting that the time distributions for both at room and refrigeration temperatures were limited to one, which implies that the minimum time considered was one hour of storage. Finally, to calculate temperatures in different positions inside the refrigerator, a non-representative study was carried out. In such study, 18 refrigerators were tested and 8000 data points collected during eight days using a data logger. The temperatures obtained for each position in the refrigerator were fitted to a distribution with the program @Risk 5.7 (Palisade Newfield) (Table I). However, the probability to store meat in each position was considered the same.

2.7 Growth model for *L. monocytogenes* and *Salmonella* during storage of leftovers

The predictive model used in this paper to estimate the evolution of the level of *L. monocytogenes* and *Salmonella* during storage of meat leftovers by consumers at home follows Equation 1, where "N_0" is the initial number of cells in $\log_{10}$ cfu/g, "N" is the final load in $\log_{10}$

cfu/g, "k" is the growth rate [log_{10} cfu/g]/h and "t" is the storage time, expressed in hours. In none of the cases, the lag-phase was considered in order to simulate the worst scenario.

$$Log_{10}(N) = Log_{10}(N_0) + [k(T)*t] \quad (1)$$

Growth rate data for each temperature were obtained with the software tool ComBase, considering pork meat and our product pH as well as water activity. ComBase is one of the most well-known open access databases for predicting microbial behaviours (22, 23), contains a large amount of raw data on bacteria inactivation and growth, is actively maintained and is largely reported in scientific literature. Recent publications concern *Bacillus cereus* group in ready-to-eat salads (24), *Salmonella* in pork (25) and *Listeria monocytogenes* in cooked-cured meat products (26). Once the growth rate data were obtained for each temperature, data were fitted to the most accurate parametric model with the program CurveExpert V3.1 (Hyams Development) (Table I). In order to assess the goodness of the fit, the accuracy factor (Af) and bias factor (Bf) were estimated (27). For both parameters, a value of 1 corresponds to a perfect fit. Af and Bf values for *Salmonella* at refrigeration temperature were 1.029 and 1.005, respectively, whereas those at room temperature were 1.130 and 1.087, respectively. For *L. monocytogenes* the values at refrigeration temperatures were 1.131 and 0.940, respectively, whereas those at room temperature were 1.001 for both Af and Bf. These results show that the regressions were reasonably accurate.

2.8 Exposure assessment to *L. monocytogenes* and *Salmonella* after storage of leftovers

In order to determine the human exposure to *L. monocytogenes* and *Salmonella* in leftover meat at the time of consumption, the starting point was to consider the occurrence of positive samples after cooking. This occurrence is reported as "Treatment effectiveness" (section 3.1) and was obtained as result of the experimental design described in section 2.3. The second step was to consider predictive microbiological models for growth of both pathogens, food storage conditions (i.e., storage at room temperature or in the refrigerator and time of storage between cooking and consumption) and probability of occurring of each storage condition. Finally, a simulation was made using the Monte Carlo technique. Overall, 10000 iterations per simulation were run using Latin Hypercube sampling. The simulation procedure was built as a spreadsheet model in Microsoft Excel with add on @Risk 5.7 (Palisade Newfield). In addition, a sensitivity

analysis was performed to study the effects that storage conditions have on the final output (i.e., human exposure to both microorganisms).

2.9 Statistical analysis

Descriptive analyses of the data were undertaken using Statgraphics Centurion XVI.II (Statpoint Technologies, Inc. Warrenton, Virginia). Relative proportions were compared using an analysis of variance (ANOVA). A probability value of less than 5% was deemed significant. The same program was used to carry out the box and whisker descriptive plot. This graph is a standardized way of displaying the distribution of data and it shows minimum, first quartile, median, third quartile, maximum value and possible outliners.

3. RESULTS

3.1 Treatment effectiveness

The temperature dataset for both cooking procedures (i.e., gas hob and static oven) and levels of cooking (i.e., rare, medium and well-done) was represented with a box and whiskers plot (Figure 2). For samples cooked on gas, mean and standard deviation temperatures corresponded to 74.9 ± 3.3 ºC for rare, to 85.8 ± 3 ºC for medium and to 88.5 ± 2 ºC for well-done. For samples cooked in static oven, mean and standard deviation temperatures were 73.6 ± 3.3 ºC, 87.7 ± 4.8 ºC and 96 ± 2.9 ºC for rare, medium and well-done, respectively. Nevertheless, outliers can be observed for the combinations “rare cooking in static oven” (i.e., 66, 69 and 80ºC) and “well-done cooking in static oven” (i.e., 89 and 90ºC). The results showed that after 45 minutes of heat treatment, corresponding to a rare cooking, almost 40% of the surface temperature values reached on gas were higher than those registered in the oven, which means faster cooking on gas. However, those temperature differences were not significant (p-value 0.1317). On the contrary, after 80 minutes (time used for medium cooking) more than 30% of data recorded for the oven were higher than those registered on gas. Nevertheless, neither these differences were significant (p-value 0.0600). However, after 120 minutes (used for well-done cooking), temperatures in the oven kept increasing on time, while temperatures on gas seem to be maintained or slightly decreased. Therefore, in this last case, the differences were significant (p-value 0.000).

Concerning the effectiveness of both heat treatments, samples positive for *L. monocytogenes* and *Salmonella* were only found with the qualitative analytical method (i.e., presence in 25g), while the quantitative results were always negative (< 10 cfu/g). Figure 3 shows the probability of occurrence of *L. monocytogenes* and *Salmonella* calculated for both cooking procedures (i.e., gas and oven) and levels of cooking applied (i.e., rare, medium and well-done) taking into account the positive samples over the total. For both pathogens, the probability of occurrence after cooking in oven was the same (p-value = 1.0000) and the only treatment able to completely inactivate the pathogens was oven well-done. These results demonstrated that a reduction between 6 and 7 $\log_{10}$ cfu/g of *L. monocytogenes* and *Salmonella* was reached after the application of all cooking treatments. The statistical analysis indicated that there were significant differences related to level of cooking (p-value = 0.0000) and cooking procedure (p-value = 0.0072). One of the main reasons for these differences is that heat penetration is not always uniform (28) and factors such as moisture, water activity, fat levels, salts, carbohydrates, pH and proteins can impact on treatment intensity and effectiveness (29). Velasquez et al. (30) showed a difference between *Salmonell*a heat resistances in whole muscle pork in comparison to ground pork. Overall, these studies emphasize the need to consider both the form of pork being cooked in specific conditions as well as the final temperature necessary to inactivate microorganisms in pork products.

3.2 Simulation and data model

The starting point to simulate storage of leftovers was obtained taking into account the occurrence of each pathogen after the application of each cooking procedure (i.e., gas or static oven) and level of cooking (i.e., rare, medium or well-done) (Figure 3) as well as the pathogen concentration in positive samples. As stated before, in all positive samples the pathogen concentration was lower than 1 $\log_{10}$ cfu/g. Therefore, it was assumed that the initial load of positive samples followed a Uniform distribution between 0 and 1 $\log_{10}$ cfu/g ($\log N_0$ = Uniform (0;1)) for both *L. monocytogenes* and *Salmonella* (Table I).

Starting from the initial load, different ways of storage of leftover meat at home were considered (Table I). The mean value of the temperatures obtained in the refrigerators was 7.1 ±

0.7 °C. In addition, taking into account the different locations within the refrigerators, the results showed that at the top, the mean temperature was 7.86 ºC, with a minimum value of 0.1 ºC and a maximum of 18.6 ºC. In the middle position, the mean temperature was 6.5 ºC, ranging between 0.1 and 13.9 ºC. Finally, the mean temperature value recorded for the lowest position was 6.9 ºC, ranging between 0.1 and 13 ºC. Concerning storage of leftovers at room temperature, directly related to environmental temperature, it was assumed between 20 and 30 ºC (Table I). For both *L. monocytogenes* and *Salmonella* the estimated growth rates for storage at refrigeration and room temperatures are reported in Table I. The same Table shows the survey's results about consumer preference in relation to level of cooking, indicating that 10% prefer "rare", 55% "medium", and 35% "well-done" cooked meat (Table I).

3.3 Exposure assessment

The consumer exposure to *L. monocytogenes* (Figure 4) was calculated taking into account pathogen occurrence and concentration after each cooking scenario and simulating different ways of consumer leftover storage. The results for the cooking combinations "gas_rare" and "oven_rare" showed that the probability of *L. monocytogenes* to multiply during leftover storage, up to the maximum concentration allowed at the time of consumption (i.e., 2 $\log_{10}$ cfu/g) (31), was 0.077 (Table II). The probabilities to reach higher concentrations, corresponding to 3 (extremely harmful for vulnerable consumers) (31) and 5 $\log_{10}$ cfu/g (extremely harmful even for healthy consumers) (31), were 0.047 and 0.015, respectively (Figure 4 and Table II). The cooking combinations "oven_medium" and "gas_well-done" also achieved the same inactivation levels. Therefore, the exposure, after simulation of storage of leftovers at home, was the same. In fact, for both combinations, the probability of *L. monocytogenes* to reach values as high as 2 $\log_{10}$ cfu/g at time of consumption was 0.026, whereas the probabilities to reach higher concentrations, corresponding to 3 and 5 $\log_{10}$ cfu/g, were 0.015 and 0.005, respectively (Figure 4). The combination "gas_medium" inactivated *L. monocytogenes* in 92.5% of the cases. For the 7.5% of cases of occurrence there was a probability of 0.039 to reach concentrations of 2 $\log_{10}$ cfu/g. Probabilities of *L. monocytogenes* to reach concentrations of 3 and 5 $\log_{10}$ cfu/g were 0.023 and 0.008, respectively (Table II). The cooking combination "oven_well done" was the only one

resulting in a complete pathogen inactivation after cooking and zero exposure after leftover storage (Figure 4).

The consumer exposure was also calculated for *Salmonella* (Figure 5), taking into account the probability of contamination after each cooking scenario and simulating different ways of consumer leftover storage. The probability of inactivation was slightly higher in static oven than on gas. The worst case was the combination "gas_rare", associated to a probability of inactivation after cooking of 0.825 (Table II). Simulating the leftover storage, the probability for *Salmonella* in the positive samples to reach concentrations of 2 $\log_{10}$ cfu/g at the time of consumption was 0.085, whereas the probability to reach concentrations of 3 and 5 log10 cfu/g were 0.069 and 0.046, respectively (Figure 5). For the combination "oven_rare", the probabilities to reach concentrations of 2, 3 and 5 $\log_{10}$ cfu/g at time of consumption, in the 15% of the samples in which *Salmonella* survived after cooking, were 0.073, 0.06 and 0.039, respectively. Combination "gas_medium" was effective in a 92.5% of the cases and the probabilities of *Salmonella* in positive samples to reach values of 2, 3 and 5 $\log_{10}$ cfu/g were 0.036, 0.029 and 0.019, respectively (Figure 5). The cooking combination "oven_medium" was effective in 95% of cases; the remaining 5% of samples in which *Salmonella* occurred after cooking, it showed a probability to reach a load as high as 2 $\log_{10}$ cfu/g corresponding to 0.024 (Table II). Moreover, the probabilities to reach concentrations of 3 and 5 $\log_{10}$ cfu/g were 0.02 and 0.013, respectively (Figure 5). Occurrence after cooking on "gas_well-done" was 2.5% and the probability of contamination at the time of consumption with concentrations of 2, 3 and 5 $\log_{10}$ cfu/g were 0.012, 0.01, 0.007, respectively. A total inactivation of *Salmonella* was reached after cooking with the combination "oven_well-done" and, therefore, any growth was obtained after storage simulation.

Figure 6 shows the mean values ($\log_{10}$ cfu/g) as well as 5^{th} and 95^{th} percentiles of exposure assessment to *L. monocytogenes* and *Salmonella* in meat leftover after simulation of storage by consumers at home, taking into account whether the meat was cooked on gas or oven. Although high pathogen concentrations were used in the challenge, in real conditions the contamination levels should not exceed 1-2 $\log_{10}$. Consequently, microbial load after cooking is expected to be around zero. Therefore, a second simulation was run, taking into account a uniform distribution

of concentrations after cooking, ranging between 1 cfu in 1 kg (-3 log) and 1 cfu in 10 g (-1 log) as starting point, supposing a higher effectiveness of the thermal treatment in comparison to the between 0 and 1 $\log_{10}$ cfu/g obtained in section 3.1. Figure 6 shows the simulation results considering to start from high (H) and low (L) contamination levels. The results show that the mean values corresponded to a slightly higher exposure to *Salmonella* compared to *L. monocytogenes* applying both cooking procedures (i.e., oven and gas) and both starting contamination levels (i.e., L and H). Values for cooking on gas were slightly higher than values for oven, especially at the 95th percentile. However, these differences were not significant (p-value 0.9435). Taking the high contamination level as starting value, the load at 95th percentile was around 1 $\log_{10}$ cfu/g for *Salmonella* and 1.5 $\log_{10}$ cfu/g for *L. monocytogenes*. Taking the low contamination level as starting point, almost no differences can be observed in the mean values and differences around 0.5 $\log_{10}$ cfu/g, can be observed at the 95th percentile, where the contamination was higher than 1.5 $\log_{10}$ cfu/g.

Integrating all consumer behaviours, an exposure distribution was obtained for each microorganism and starting level of contamination. The results show that the mean, 5th and 95th percentiles for *L. monocytogenes* were 0.16, 0 and 1.22 when the simulation starts considering high contamination levels after cooking. They corresponded to 0.13, 0 and 0.067 when a low contamination after cooking was taken into account. For *Salmonella*, the corresponding values at the same conditions were 0.20, 0, 0.77 and 0.18, 0 and 0.36, respectively. These results show similar values for both initial contamination levels, probably because the predictive model add the initial load to the growth rate.

The influence of different storage factors on consumer exposure was assessed for both pathogens performing a sensitivity analysis (Figure 7 a and b). The tornado diagram showed that the key factors affecting consumer exposure are the same for *L. monocytogenes* and *Salmonella* and correspond to probability of pathogen occurrence after cooking, doneness degree, time of storage and time at room temperature. The remaining factors considered, i.e., room temperature, type of cooking, initial load and position in the refrigerators, were less important in terms of consumer exposure and in different order of importance for each microorganism.

4. DISCUSSION

It is generally believed that when meat is heat treated to achieve the "safe harbour" process criterion of 70 °C for 2 min, or equivalent time/ temperature combination, a 6 $\log_{10}$ reduction of *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* is obtained and that the meat will be free of pathogens and thus safe to eat (6). In 2014, information on type of outbreak was reported for 216 of the strong-evidence *Salmonella* outbreaks and 124 of them were household/domestic kitchen outbreaks (32). Inadequate heat treatment was associated to 27 of those strong-evidence outbreaks.

In recent years, quantitative modelling has been used to estimate the risk of illness associated with different foodborne pathogens and safety criteria for specific food/hazards combinations have been calculated (33). Safety criteria should be implemented in risk assessment models and form the basis of food safety policies as recommended by FAO/WHO (34, 35). However, most quantitative risk assessments do not include the impact of cooking procedures and storage of leftovers in private homes in the calculation of human exposure to foodborne illnesses, even if they have been recognized important steps affecting such exposure (36, 37).

The results of this study provide data to be directly implemented in quantitative risk assessment models concerning *L. monocytogenes* and *Salmonella* inactivation in pork meat applying two of the most common cooking procedures (i.e., gas hob and traditional static oven) and three different levels of cooking (i.e., rare, medium, and well done). The concentration of both pathogens in the product after cooking was taken as starting point to assess the consumer's exposure to both microorganisms at the time of consumption, according to different storage time at room and refrigeration temperatures.

The results showed that for rare cooking there was a faster temperature increase on gas, whereas for medium and well-done cooking temperatures in the oven increased on time and on gas they seem to be maintained or slightly decrease. A reduction between 6 and 7 $\log_{10}$ cfu/g of *L. monocytogenes* and *Salmonella* was reached applying all cooking treatments always producing in the product temperatures ≥ 73.6°C. Therefore, the results of this study confirm the need to

reach a minimum temperature of 71ºC as suggested by several international organisations (38, 39).

Traditionally, it has been considered that inactivation follows log-linear kinetics, characterized by D and z-values. In this framework there are many studies defining both parameters for different foods and treatments. Ryser and Marth (40) concluded that the hardiness of *L. monocytogenes* to mild thermal processes at 60°C has substantially higher D-value than *Salmonella* in meat products. Juneja et al. (41) reported D-values for *Salmonella* in ground pork of 6.65, 1.62 and 0.87 minutes at temperatures of 60, 62.5 and 65°C, respectively. Quintavalla et al. (42), studied the thermal inactivation of the most heat resistant *Salmonella* serotype in pork meat containing curing additives and reported D-values of 4.80, 1.57 and 0.30 minutes at 58, 69 and 63°C, respectively. In relation to *L. monocytogenes*, Osaili et al. (43), found D values for breaded pork patties of 20.61 min at 60°C and 0.43 min at 70°C. These results show that processors should be careful in the evaluation of heat treatment intensities based on the assumption that microbial inactivation by heat is an irreversible first order event (44). In fact, different reports show that log-linear models, based on D-values, do not describe the death kinetics of microorganisms like *L. monocytogens* (45).

In a previous study on naturally contaminated loin chops, it has been assessed that *L. monocytogenes* in raw meat stored, before cooking, at refrigeration temperature do not exceed 2 $\log_{10}$ cfu/g. However, abuse temperatures in the refrigerators allow reaching higher concentrations in the meat before cooking (46). To consider the worse scenario, in this research the meat samples before cooking were challenged with 10^7 cfu/g of pathogen but after heat treatments, no sample produced positive results in the enumeration test. However, few cells of both *L. monocytogenes* and *Salmonella* survived all cooking treatments, except for combinations oven well-done.

Predictive microbiology represents a proactive approach for food quality and safety providing information on bacterial responses under different environmental conditions with on line databases (47, 48). Predictive models in food microbiology are classified as primary, secondary and tertiary (49). Commonly used primary models, describing the sigmoid bacterial growth curves, include Logistic, Gompertz and Baranyi models (50, 51). Secondary growth models

describe the effect that the environmental factors have on growth or inactivation kinetic rates (50). Finally, tertiary models are algorithms incorporated into software to integrate the effect of environmental variables on microbial responses and to provide predictions on the outcomes. With this aim, Combase as well as other pathogen modelling programs, like SymPrevious, Seafood Spoilage and Safety Predictor, are available on line.

According to the simulation results, the surviving cells can multiply during storage in the fridge and at room temperature to reach concentrations dangerous for regular, as well as vulnerable, consumers. The key factors affecting consumer exposure were the same for *L. monocytogenes* and *Salmonella* (Figure 7). They corresponded to probability of pathogen occurrence after cooking, doneness degree, time of storage and time at room temperature. On the contrary, room temperature, type of cooking, initial load after cooking and position in the refrigerators, were less important in terms of consumer exposure and in different order of importance for each microorganism. This paper took from bibliography most of the data used in the leftover handling simulation because we wanted to know if the prevalence after cooking could have exposure consequences. However, it is not easy to find information, which fits perfectly, which implies that for a more accurate simulation results, handling surveys from different cultural populations must be carried out.

The values recorded in the refrigerators tested in this study were slightly higher than those published by EFSA (52), concerning 11 surveys and 1924 samples. In the EFSA report, the mean temperatures ranged from 5 to 7.2°C, with minimum values between 0.2 and 3.8 and maximum values between 11.4 and 20.7°C (52). These and our data clearly show that storage temperatures at both retail and domestic level vary significantly between refrigerators. In relation to the different locations within the refrigerators, Xanthiakos (2006) (53) found differences inside the fridge with mean values of 7.6, 6.3 and 6.7°C for the top, middle and lowest shelf, respectively. The survey's results about consumer preference in relation to the degree of cooking indicated that 10% prefer rare meat, 55% medium, and 35 % well-done but these results largely depends on demographic and socio-cultural parameters (14-16).

Overall, the results of this study indicated a slightly higher exposure to *Salmonella* compared to *L. monocytogenes* applying both cooking procedures (i.e., oven and gas), with higher values for gas than oven (Figure 6). For both microorganisms and contamination levels after cooking, the probabilities to growth up to 1 $\log_{10}$ cfu/g were lower than 0.06. In particular, the best performance was achieved with the lower contamination level and cooking in the oven, resulting in only 2.5% of the samples reaching a contamination level of 1 $\log_{10}$ cfu/g. The worse performance was achieved in samples showing the highest level of contamination after cooking on gas, resulting in 5.9 % of the samples with contamination levels higher than 1 $\log_{10}$ cfu/g. Overall, the mean level of exposure to *L. monocytogenes* at the time of consumption was 0.1 $\log_{10}$ cfu/g and that to *Salmonella* 0.2 $\log_{10}$ cfu/g. The *L. monocytogens* value complies with the maximum concentration level allowed at the time of consumption in RTE products, corresponding to 2 $\log_{10}$ cfu/g. On the contrary, the value for *Salmonella* is not compliant because the pathogen should be absent in 25 g. **4.1 Conclusion**

The cooking combination "oven well done" was able to inactivate *L. monocytogens* and *Salmonella* in cooked pork meat, resulting in zero exposure for consumers after storage of meat leftovers. For all other combinations there was a probability to reach 1 $\log_{10}$ cfu/g, after cooking and storage of leftovers, ranging between 0.031 and 0.059. The results of this research show the inactivation dynamic of *L. monocytogenes* and *Salmonella* associated to different cooking scenarios as well as the impact of meat leftover storage on human exposure to those pathogens. The results obtained can be directly implemented in risk assessment models and guidelines for consumers on practises to follow in order to manage cooking of pork meat at home.

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