# UNIVERSITÀ DEGLI STUDI DI TORINO

**Risk-based control of food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* in the Italian fermented sausages Cacciatore and Felino**

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**Abstract**

Fermentation is the most important killing step during production of fermented meats to eliminate food-borne pathogens. The objective was to evaluate whether the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* may survive during the production of two Italian fermented sausages. Sausage batter was inoculated with five strains of *L. monocytogenes* or *S. enterica* (*ca*. $10^5$-$10^6$ cfu/g) and their kinetic behavior was monitored during production. Both pathogens survived relatively well (in Cacciatore *L. monocytogenes* and *S. enterica* inactivation was *ca*. 0.38 ± 0.23 and 1.10 ± 0.24 log cfu/g, respectively; in Felino was *ca*. 0.39 ± 0.25 and 1.62 ± 0.38 log cfu/g, respectively) due to the conditions prevailing during production (slow dehydration rate, small reduction of water activity and fermentation temperature mainly below 20$^o$C during the first 48h of fermentation). Quantitative analysis of data originating from challenge tests provide critical information on which combinations of the process parameters would potentially lead to better control of the pathogens.

Keywords: Non thermal inactivation; pathogens behavior; quantification; risk management; salami

## 1. Introduction

Although fermented meats are generally recognized as safe, some notable outbreaks of food-borne illness associated with fermented foods and presence of *Listeria monocytogenes* or *Salmonella* spp. have occurred (Adams & Mitchell, 2002; Comi, Frigerio, & Cantoni, 1992; Cowden, O'Mahony, et al., 1989; Norrung, Andersen, & Schlundt, 1999; Pontello, Sodano, et al., 1998; Taplin, 1982). Hence, the extent to which fermented meats are considered safe is primarily dependent on the way the fermentation process is conducted. Based on the manufacturing conditions, e.g., pH, water activity ($a_w$) and time-temperature profile applied for fermentation, food-borne pathogens may survive at the end of the process (Drosinos, Mataragas, Veskovic-Moracanin, Gasparik-Reichardt, Hadziosmanovic, & Alagic, 2006). *L. monocytogenes* and *Salmonella* spp. can be found in raw materials, e.g., pork, and can survive fermentation (Martin, Garriga, & Aymerich, 2011; Ockerman & Basu, 2007; Shay, 1993). Moreover, *L. monocytogenes* can contaminate the products during slicing and/or packaging due to its ubiquitous nature (Martin et al., 2011; Thevenot, Delignette-Muller, Christieans, & Vernozy-Rozand, 2005). *L. monocytogenes* has been detected in final products with concentration exceeding in some cases the limit of $10^2$ cfu/g (De Cesare, Mioni, & Manfreda, 2007; EFSA, 2013; Martin et al., 2011; Thevenot et al., 2005; USDA, 2003).

USDA risk assessment (USDA, 2003), regarding the relative risk of the *L. monocytogenes* presence in various ready-to-eat foods, classified the dry fermented sausages in the group designated as moderate risk. Mataragas, Skandamis, and Drosinos (2008) developed risk profiles for various combinations of hazard/food product based on data found in the literature and the Risk Ranger program (Sumner, Ross, Jenson, & Pointon, 2005). The authors found that the risk posed by *L.*

*monocytogenes* and *Salmonella* spp. for the preserved meats such as fermented sausages was moderate for the high-risk population (risk ranking: 45 and 38, respectively) and low for the rest (risk ranking: 13 and 16, respectively).

Safety of any food product, that is intended to be released in the market, must be assured. Therefore, the objective of this study was to evaluate if the food-borne pathogens *L. monocytogenes* and *S. enterica* are able to survive during the production of two Italian fermented sausages (Cacciatore and Felino) with different maturation times. Fermentation and ripening lasts up to 20 days (short-maturation time) in the case of Cacciatore and 40 days (long-maturation time) in the case of Felino. The quantification of the kinetic behavior of the food-borne pathogens in the foods can provide valuable information on the process parameters affecting their survival and also on the process parameters combinations leading to a better control. These results have the potential to provide important information for processors and researchers.

## 2. Materials and Methods

### *2.1. Sausage manufacture, pathogens inoculation and sampling*

Cacciatore (Hunter Style) or Salamini is a small, dry salami flavored with wine so-called because they were small enough to stuff in the hunter's pocket for lunch. Cacciatore, made from ground pork, is usually 15-18 cm in length and cured with the usual spices, wine, and herbs. It is considered a rapidly fermented sausage, with only 20 days of maturation. Salami Felino is a pure pork salami from Felino, a small town located near Parma. This special salami is the product of the best pork meat and the particular microclimate of the area of production. The fresh meat used to make the salami is carefully selected from deboned shoulder and belly that is ground with lean and fat cuts of ham. The meat is mixed with salt, spices and flavor compounds. The

salami should contain 25-30% fat and hard fat is preferred. After the meat is coarsely ground, salt, whole peppercorns and white wine are added. The salami is then stuffed by hand into a pork casing, which gives it its characteristic uneven width. It is then aged slowly for 40 days (slow fermented sausage) and the final product should be soft, with a sweet taste and delicate aroma.

Cacciatore and Felino sausages were prepared at the Istituto Zooprofilattico according to the standard recipe of each product. The ingredients of each product are shown in Table 1. A commercial starter culture, composed of lactobacilli and staphylococci was used. Starter cultures BITEC STARTER R4, B1 and R3 (Frutarom Savory Solutions GmbH, Korntal-Münchingen), were used to inoculate Cacciatore and Felino type salami. The cultures consist of strains of species *Lactobacillus sakei* and *Staphylococcus carnosus* (R4 and R3) as well as *Kocuria salsicia* (B1) (Mataragas, Bellio, Rovetto, Astegiano, Greci, Hertel, Decastelli, & Cocolin, 2015). The batter of each sausage was inoculated with a cocktail of five *L. monocytogenes* or *S. enterica* strains (Table 2). *L. monocytogenes* and *S. enterica* strains were grown twice in Brain Heart Infusion (BHI) broth (Oxoid, Basingstoke, Hampshire, UK) to resuscitate the culture (1% inoculum) and then incubated at 37$^{o}$C for 24 h to achieve a final concentration *ca*. $10^{9}$ cfu/ml. For inoculation purposes, the cells were harvested by centrifugation (13,400 ×*g* for 15 min at 4$^{o}$C), using an Eppendorf 5417R refrigerated centrifuge (Eppendorf, Milan, Italy), washed twice, and re-suspended with sterile Ringer solution (Oxoid) to give a similar final concentration. The cell concentration of the suspension was assessed with the McFarland scale, which is a series of standards of different opacity that can be used to estimate the density of bacterial suspensions (ref. 70,900, McFarland Standards, bioMerieux, Marcy l'Etoile, France). *L. monocytogenes* or *S. enterica* bacterial suspensions were gradually distributed into

the filling during mixing to achieve uniform distribution of the inoculum and a final concentration of $10^5$ to $10^6$ cfu/g.

After the preparation of the experimental batches, the mixtures were stuffed into casings and incubated following the ripening programs displayed in Table 3. For each pathogen and sausage, two independent trials (different batches of sausages prepared at different time periods) were carried out. In each trial, two sausage samples (replicates) were collected (2 trials/batches × 2 replicates = 4 samples per time point for each sausage/pathogen combination) on days 0, 2, 5, 10 and 20 for the combination Cacciatore/*L. monocytogenes* or *S. enterica* and 0, 3, 7, 10, 20, 40 for the combination Felino/*L. monocytogenes* or *S. enterica*. The samples were transported to the laboratory under refrigeration conditions (4°C) and subjected to microbiological and physicochemical analyses within 24 h.

*2.2. Microbiological and physicochemical analyses*

A 10-g sample of sausage was weighed and placed into a sterile stomacher bag with 90 ml of sterile Ringer solution. The sample was homogenized in a stomacher (BagMixer, Interscience, France) for 2 min at normal speed at room temperature. Serial decimal dilutions in sterile Ringer solution were prepared from this $10^{-1}$ dilution, and 1 or 0.1 ml samples from three appropriate solutions were poured in or spread on selective agar plates in duplicate. Lactic acid bacteria (LAB) were enumerated in de Man, Rogosa and Sharpe agar (MRS agar, LABM, Heywood, Lancashire, UK), overlayed with 5 ml of the same medium and incubated at 30°C for 72 h; Gram-positive/coagulase-negative cocci (CNC) on mannitol salt agar (MSA agar, LABM), incubated at 30°C for 72 h; *L. monocytogenes* on Agar Listeria acc. to Ottaviani and Agosti (ALOA, Oxoid), incubated at 37°C for 24-48 h; and *S. enterica*

on Xylose Lysine Deoxycholate agar (XLD agar, Oxoid), incubated at 37°C for 24-48 h.

*L. monocytogenes* and *S. enterica* detection (enrichment) was also performed in parallel with enumeration procedure. This was made in order to confirm pathogens presence in case *L. monocytogenes* and *S. enterica* concentration was below the detection limit ($10^2$ cfu/g). For *S. enterica*, pre-enrichment was done by suspending 10 g of sample in 90 ml of buffered peptone water (BPW, Oxoid) followed by incubation at 37°C for 16-20 h. Selective enrichment was done by transferring 0.1 ml of pre-enrichment culture in 10 ml of Rappaport-Vassiliadis Soya broth (RVS broth, Oxoid) followed by incubation at 42°C for 24 h. After incubation samples were streaked on XLD and modified Brilliant-green Phenol-red Lactose Sucrose (mBPLS agar, LABM) agars and incubated at 37°C for 24-48 h. For *L. monocytogenes* detection, primary enrichment was done by suspending 10 g of sample in 90 ml of half-concentrated Fraser broth (Oxoid) followed by incubation at 30°C for 24 h. Then, 0.1 ml of the primary culture were transferred in 10 ml of Fraser broth, incubated at 37°C for 48 h, 0.1 ml of the secondary enrichment were streaked on ALOA and OXFORD (Oxoid) agars and incubated at 37°C for 24-48 h. The plates were examined visually for typical colony characteristics associated with each growth medium. The selectivity of the growth media was also checked with rapid tests (e.g., Gram, catalase reaction or motility) for about 10% of the countable colonies (Harrigan & McCance, 1976).

The pH was measured by immersing the pH probe of a digital pH meter (micropH 2001, Crison, Barcelona, Spain) in a diluted and homogenized sample containing 10 g of sausage and 90 ml of distilled water. Water activity ($a_w$) was measured with a

calibrated electric hygrometer (HygroLab, Rotronic, Bassersdorf, Switzerland) according to the manufacturer's instructions.

*2.3. Inactivation models and statistical analysis*

The log-linear (equation 1) and Weibull (equation 2) models were tested with the data collected from the experiments. The log-linear model is given by the equation (Geeraerd, Valdramidis, & Van Impe, 2005):

$$N_t = N_0 \times e^{(-k_{max} \times t)} \qquad (1)$$

where $N_t$, the cell number (log cfu/g) at time $t$; $N_0$, the initial population (log cfu/g); $t$, the time (days); and $k_{max}$, the specific inactivation rate (ln cfu/day) of the *L. monocytogenes* or *S. enterica* population.

The Weibull model is given by (Mafart, Couvert, Gaillard, & Leguerinel, 2002; Geeraerd et al., 2005):

$$\frac{N_t}{N_0} = 10^{-\left(\frac{t}{\delta}\right)^p} \qquad (2)$$

where $\delta$, the time for the first log-unit decrease (days); and $p$, a shape parameter ($p < 1$ indicates a downward concave curve, $p > 1$ indicates an upward concave curve and $p = 1$ indicates that the Weibull equation is equal to the log-linear model).

The $D$-value for the log-linear model is derived from the $k_{max}$ parameter (equation 3):

$$D - value = \frac{ln(10)}{k_{max}} \qquad (3)$$

The equations were fitted to the log concentration data using the Microsoft Excel 2007 (Microsoft, Redmond, WA, USA) add-in GInaFiT v1.6 (Geeraerd et al., 2005) and the GraphPad Prism 5.04 (GraphPad Software, Inc., San Diego, CA, USA).

For calculating the kinetic parameters of the inactivation of each pathogen in the corresponding sausage, the model showed the best fit to the experimental data was selected based on the following statistical indices: i. number of model parameters, ii. Lack-of-Fit (*LoF*) based on the *F*-test (Zwietering, Cuppers, de Wit, & van't Riet, 1994), iii. normality of residuals using the D'Agostino and Pearson statistical test, iv. Root Mean Square Error (*RMSE*) (te Giffel & Zwietering, 1999, Ross, 1996), v. dependency between model parameters and vi. coefficient of multiple determination ($R^2$).

Dependency is reported by the GraphPad Prism program for each parameter and quantifies the degree to which that parameter is correlated with others. If it is high (> 0.90-0.95) then the same curve over the range of X values, for which data have been collected, can be constructed with multiple sets of parameter values. This means that the data do not define all the parameters in the model, i.e. the model has too many parameters (over-parameterized) and a simpler model should be checked.

## 3. Results

### *3.1 Starter culture growth and physicochemical changes in Cacciatore and Felino sausages*

LAB grew well displaying an increase of 2.3-2.8 and 1.6-1.9 log cfu/g in Cacciatore and Felino, respectively, in all experimental cases, i.e. *L. monocytogenes* and *S. enterica* challenge tests (Fig. 1a,c). LAB multiplied from *ca*. 6.5 log cfu/g at the first day of inoculation to 8.0-9.0 log cfu/g at the end of fermentation. At 5 and 3 days

after formulation for Cacciatore and Felino, respectively, LAB had almost reached their final population level. In Cacciatore sausage, CNC displayed a slight decrease (0.3-0.4 log cfu/g) within the first two days of fermentation from 6.4 log cfu/g to 6.0-6.1 log cfu/g and then remained constant (Fig. 1b). On the other hand, CNC were raised slightly by the same amount on the third day of fermentation of the Felino sausage and then remained unchanged (from 6.1-6.4 log cfu/g to 6.6-6.7 log cfu/g) (Fig. 1d). This was in accordance with the fermentation characteristics of each product, i.e. a rapidly (Cacciatore) against a slower (Felino) fermented sausage, as presented below.

Drop in the pH value by 0.6-1.0 unit(s) coincided with the increase of LAB (Fig. 2a,c). A rapid decrease of the pH during the first days of fermentation was observed for the Cacciatore sausage. From its initial value of 5.7, the pH value decreased to 4.9-4.8 on the second day of fermentation with an average decrease rate of 0.43 units/day and then remained almost constant (4.8-4.7) until the last day of the manufacture process. Slower fermentation as reflected by the pH drop was observed in the case of Felino. The initial pH value was 5.8-5.9, reached at 5.2 after three days of fermentation (decrease rate equal to 0.22 units/day), approaching its maximum decrease (pH=5.1) at the end of fermentation on day 7, whereas a slight increase (pH=5.3) at the end (on day 40) was observed. Water activity decreased constantly from its initial value of 0.976-0.978 to its final value of 0.922-0.923 as ripening of the Cacciatore fermented sausage was in progress, but this reduction (0.05-0.06 units) happened with a relatively slow rate (0.003 units/day) (Fig. 2b). Felino presented slow ripening as well. Water activity started to decline from day 10 after formulation. Drop in $a_w$ was of the order of 0.03-0.04 units (0.964-0.971 to 0.928-0.936) with a rate of 0.001 units/day (Fig. 2d).

*3.2. Non thermal inactivation of L. monocytogenes and S. enterica*

The quantitative data obtained from the challenge tests were analyzed to quantify the inactivation of *L. monocytogenes* and *S. enterica* in the Cacciatore and Felino sausages. Only two models were tested, the log-linear and Weibull, owing to curve shape displayed by both pathogens during their non thermal inactivation. Fitting was performed to all replicates and the statistics and estimated values are presented in Tables 4 and 5.

The log-linear model was chosen to describe *L. monocytogenes* reduction in the Cacciatore sausage (Fig. 3a), although the Weibull model seemed to perform better (lower *RMSE* and higher $R^2$). The Weibull equation, however, showed high (> 0.90-0.95) dependency between parameters indicating the model was not appropriate. Therefore, the simpler log-linear model was chosen instead. For *S. enterica*, both models performed equally and thus, the log-linear model was further selected due to the lower number of parameters (simpler model) (Fig. 3b).

*L. monocytogenes* and *S. enterica* non thermal inactivation in the Felino sausage was also described by the log-linear model (Fig. 3c,d). The Weibull model was inappropriate in both cases, i.e. use of a ambiguous model in the case of *L. monocytogenes* and the *LoF* was not accepted when *S. enterica* was considered. Table 6 shows the kinetic parameters of *L. monocytogenes* and *S. enterica* inactivation as calculated by the log-linear model. *L. monocytogenes* survived better than *S. enterica* during manufacture of both sausages as reflected by the much slower inactivation rate ($k_{max}$) and higher *D*-value. Within the time required for manufacture of Cacciatore and Felino sausages (20 and 40 days, respectively), *S. enterica* may decrease by 1 log or

more (*D*-value), but *L. monocytogenes* needs more time (52 and 111 days, respectively) to achieve 1 log reduction.

## 4. Discussion

In all cases, *L. monocytogenes* and *S. enterica* non thermal inactivation was linear and thus, the log-linear model was finally selected to determine the kinetic parameters. Especially for *L. monocytogenes*, although, $R^2$ was relatively low there was no evidence of bad fitting of the log-linear model considering the remaining statistical indices. When the objective of fitting is to determine the best fit parameters of a model, as it was in the current study, then $R^2$ should not be the main criterion for deciding whether a fit is reasonable or not. A high or low $R^2$ value does not necessarily imply the fit is good or bad, respectively. To evaluate a fit, all the results should be considered, not just the $R^2$ (Motulsky, 2007). *L. monocytogenes* and *S. enterica* responded differently to the environmental changes during production of the Cacciatore and Felino sausages. *S. enterica* proved to be more sensitive than *L. monocytogenes*. This observation is in line with the finding that Gram-negative bacteria such as *Salmonella* spp. are less resistant compared to Gram-positive bacteria during the dynamic processes of raw sausages (fermentation-ripening) (Adams & Nicolaides, 1997; Ockerman & Basu, 2007).

Depending on the fermentation temperature, the crucial factors for reduction of the pathogens are pH and $a_w$. At fermentation temperatures below or at 20$^o$C, $a_w$ reduction became more important than pH decrease (Gounadaki, Skandamis, Drosinos, & Nychas, 2005). If this reduction is slow, then pathogens may survive for longer. At higher fermentation temperatures (>20$^o$C), the crucial factor for *L. monocytogenes* and *Salmonella* spp. inhibition is the pH decrease, and its slow reduction results in

extended survival of the pathogens (Gounadaki et al., 2005). In the current study, although, pH decrease was rapid during the first 48h of fermentation, the temperature applied (mainly ≤20$^{o}$C), the slow dehydration of the sausages and the small change of $a_w$ did not contribute to rapid inactivation of the pathogens.

In the Serbian fermented sausage "sremska", inoculated with *L. monocytogenes*, the pH decrease was slow (from 6.0 to 5.4), but *L. monocytogenes* was rapidly inactivated by 3-4 logs during the 28 days of sausage production. Dehydration rate and $a_w$ change, however, were high (from 0.924 to 0.793) with fermentation temperature applied being below 20$^{o}$C (18 to 20$^{o}$C the first 24h and 14 to 16$^{o}$C the next 24h) (Drosinos et al., 2006). In another product from Hungary, although $a_w$ reduction was high and fast (from 0.960 to 0.830), *L. monocytogenes* survived well displaying a reduction of 1.0-1.5 logs at the end of the process. In this product, high fermentation temperature was applied (20 to 24$^{o}$C), but pH reduction was slow (reduction by only 0.1 unit at the end of fermentation on day 3, i.e. from 5.8 to 5.7) and small (from 5.8 to 5.4 at the end of the process) (Drosinos et al., 2006). In products from Croatia and Bosnia-Herzegovina, also fermented at temperatures above 20$^{o}$C, *L. monocytogenes* was inactivated by 4-5 logs at the end of the process, because a relatively rapid pH decrease (0.8-0.9 units) was observed during the first days of fermentation (Drosinos et al., 2006). In an Australian fermented sausage displaying similar characteristics to Cacciatore, i.e. small and slow reduction of $a_w$ (initial value equal to 0.960-0.970 and moisture loss rate equal to 2% per day on average), rapid reduction of pH (from 6.0 to 4.5 achieved already by the second day of fermentation) and short maturation time (14 days), *Salmonella* spp. was inactivated by 3 logs. The main difference, however, compared to Cacciatore sausage was the fermentation temperature applied in the Australian product (27$^{o}$C for 2 days and then dried and ripened for a further 12-14

days at 15$^{o}$C) (Shay, 1993). In another inactivation study performed by the current authors, *L. monocytogenes* and *S. enterica* were also able to survive during fermentation of Cacciatore, Felino and Milano sausages displaying a small reduction (<1 log cfu/g for both pathogens in Cacciatore and Felino, except of *S. enterica*/Felino combination in which the inactivation was around 2 log cfu/g) (Mataragas et al., 2015).

Lucke (2000) has proposed preventive measures to control growth of *S. enterica*, *Staphylococcus aureus* and *L. monocytogenes* during manufacture of fermented sausages indicating the Critical Control Points (CCPs) of the process. The CCPs of dry fermented sausages are: a) pH during butchering below or at 5.8, b) target $a_w$ during comminution and mixing 0.955-0.965, c) sugars concentration during comminution and mixing 0.3-0.5%, d) starters addition during comminution and mixing (LAB and CNC), e) fermentation at 18-22$^{o}$C and target pH ≤ 5.3 after *ca*. 3 days of fermentation and f) ripening at 10-15$^{o}$C and target $a_w \leq 0.90$. Based on Cacciatore and Felino characteristics, only those CCPs related to target $a_w$ (end of process, comminution and mixing) were out of limits confirming the previous observation about the role of $a_w$ in *L. monocytogenes* and *S. enterica* inactivation.

Predictive microbiology and modeling of experimental data obtained from challenge tests as performed in this study demonstrate the risk to consumers that products may have when contaminated with food-borne pathogens and therefore risk management measures for controlling such risks can be taken. The Food Business Operators can influence the prevalence and concentration of the food-borne pathogens in the product before its release to the market. The results from challenge tests can be used in risk assessment studies to evaluate the final risk (probability of infection or cases of disease) and the effect of various mitigation strategies.

## 5. Conclusions

Quantitative analysis of the data originating from challenge tests may provide critical information on the process and/or product parameters that have significant effect on inactivation of pathogens as well as how the change in these parameters can lead to better control of microorganisms. Challenge studies investigating the effect of fermentation processes on food-borne pathogens are very useful for the sausage industry. The current work has the potential to provide important information for processors and researchers. Based on the results obtained in this study, some indicative examples of control measures are given:

a) change of process and/or recipe to achieve a higher reduction of the pathogens during process (in combination with acceptable organoleptic characteristics), altering fermentation temperature, $a_w$ or NaCl content or combinations of these. The environmental conditions prevailing in the first 48h are critical with regard to the growth and subsequent survival rate of these pathogens. For instance, although decline of pH was rapid in the Cacciatore sausage, the pH contribution to *L. monocytogenes* and *S. enterica* inactivation was only marginal. Fermentation temperature above $20^oC$ is needed at least the first 2-3 days of fermentation, in order for the pH to become an important contributing factor for the inactivation of both pathogens. For such short maturation times and slow $a_w$ reduction, pH is a critical parameter for controlling the pathogens in the product (Shay, 1993). Any change in the process, however, should not alter the authenticity of the products and therefore more research must be performed regarding the allowable number and extent of changes, e.g. use of statistical techniques of the experimental design;

b) the implications of these findings is that if the initial contamination of raw materials with *L. monocytogenes* or *S. eneterica* is high then the final product may contain unacceptable levels of the food-borne pathogens posing a health risk for the potential consumers. Therefore, pre-requisite programs and Hazard Analysis at Critical Control Points (HACCP) implementation are required to assure supply of high quality raw materials, proper training of the personnel on hygiene issues and limited recontamination of the products. A combination of low initial contamination level in the raw materials, and consequently in the batter, and absence of recontamination during the manufacture of the products, even when the non thermal inactivation of *L. monocytogenes* and *S. enterica* is low like the one observed in Cacciatore and Felino (0.5-1.5D reduction), may assure their safety. Pre-requisite programs and HACCP will aid of preventing and controlling pathogens in these products; and

c) inclusion of an additional reduction step such as smoking during ripening, thermal treatment or holding of the final products at elevated temperatures for a time period to achieve an additional pathogens reduction (Drosinos et al., 2006; Hansen, Gunvig, Larsen, Hansen, & Aabo, 2011).

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**Table 1**

Ingredients used for production of the Cacciatore and Felino sausages.

| Ingredients | Cacciatore (%) | Felino (%) |
|---|---|---|
| *Meat and fat* | | |
| Defatted boneless pork shoulder | 50.0 | 67.0 |
| Pork leg (Rump) | 32.5 | |
| Pork neck fat | 17.5 | |
| Deskinned, defatted pork bacon (Belly) | | 30.0 |
| Pork lard (Fatback) | | 3.0 |
| | | |
| *Additives* | | |
| Salt | 2.200 | 2.400 |
| Sodium nitrite | 0.020 | |
| Potassium nitrate | 0.014 | 0.015 |
| Dextrose | 0.400 | 0.400 |
| Pepper 1/2 | 0.050 | 0.050 |
| Pepper powder | 0.040 | 0.050 |
| Ascorbate | 0.070 | 0.050 |
| Flavor | 0.030 | 0.200 |
| Garlic | 0.030 | 0.020 |
| Wine | 0.500 | 0.020 |

**Table 2**

*L. monocytogenes* and *S. enterica* strains used in the challenge tests of the Cacciatore and Felino sausages.

| Strain | Strain/Serotype | Source of isolation |
|---|---|---|
| *L. monocytogenes* | | |
| #5[a] | 4b | minced meat / beef |
| #19[a] | 1/2b | fresh salami |
| #36[a] | 1/2a | meat / pork |
| EGDe | 1/2a | collection |
| V7 | not serotyped | human clinical isolate |
| | | |
| *S. enterica* | | |
| 22754 | *S*. Derby | feces / pig |
| 56596 | *S*. Typhimurium | feces / pig |
| 57002 | *S*. Typhimurium | minced meat / pork |
| 54398 | *S*. Typhimurium | packaged meat |
| ATCC 14028 | *S*. Typhimurium | collection |

[a] Strains belonging to the culture collection of the laboratory isolated from various food products.

**Table 3**

Fermentation and ripening program followed during manufacture of the Cacciatore and Felino sausages.

| Phase | Time (h) | Temperature ($^{o}$C) | Relative Humidity (%) |
|---|---|---|---|
| *Cacciatore* | | | |
| Heating | - | 19-22 | Till 19$^{o}$C core temperature |
| Fermentation | 10 | 20-22 | 58-68 |
| Fermentation | 10 | 19-21 | 55-77 |
| Fermentation | 24 | 18-20 | 55-77 |
| Fermentation | 24 | 17-19 | 65-80 |
| Ripening | Till to end (20 d) | 15-17 | 72-82 |
| *Felino* | | | |
| Heating | 8 | 20-22 | 92-95 |
| Fermentation | 24 | 19-21 | 80-88 |
| Fermentation | 24 | 19-21 | 72-84 |
| Fermentation | 24 | 18-20 | 68-76 |
| Fermentation | 24 | 17-19 | 72-78 |
| Fermentation | 24 | 16-18 | 74-82 |
| Fermentation | 24 | 15-17 | 70-78 |
| Ripening | Till to end (40 d) | 12-14 | 74-88 |

**Table 4**

Statistical comparison between models used to describe *L. monocytogenes* and *S. enterica* inactivation in the Cacciatore sausage.

| Challenge test | Statistical indices | Model | |
|---|---|---|---|
| | | log-linear | Weibull |
| *L. monocytogenes* | No. of model parameters | 2 | 3 |
| | *LoF*[a] | Yes (*P*-value = 0.22) | Yes (*P*-value = 0.70) |
| | *RMSE*[b] | 0.232 | 0.212 |
| | $R^{2b}$ | 0.28 | 0.43 |
| | Residuals normality[c] | Yes (*P*-value = 0.26) | Yes (*P*-value = 0.63) |
| | Parameters dependency[d] | | |
| | $N_0$ | 0.52 | 0.80 |
| | $k_{max}$ | 0.52 | |
| | $\delta$ | | 0.97 |
| | $p$ | | 0.97 |
| *S. enterica* | No. of model parameters | 2 | 3 |
| | *LoF*[a] | Yes (*P*-value = 0.91) | Yes (*P*-value = 0.78) |
| | *RMSE*[b] | 0.251 | 0.258 |
| | $R^{2b}$ | 0.73 | 0.73 |
| | Residuals normality[c] | Yes (*P*-value = 0.26) | Yes (*P*-value = 0.25) |
| | Parameters dependency[d] | | |
| | $N_0$ | 0.52 | 0.75 |
| | $k_{max}$ | 0.52 | |
| | $\delta$ | | 0.58 |
| | $p$ | | 0.53 |

[a] *LoF* was either accepted (yes) or not accepted (no) based on the *F*-test. *P*-value < 0.05 indicates the use of an inadequate model

[b] The model with the lower *RMSE* and the higher $R^2$ indicates better fitting

[c] *P*-value < 0.05 indicates that residuals are not normally distributed

[d] A dependency above 0.90-0.95 is considered high indicating the use of an inadequate model and the fitting of a simpler model should be checked instead or it should be considered the collection of more data performing additional experiments or over a wider range of *X*, i.e. time.

**Table 5**

Statistical comparison between models used to describe *L. monocytogenes* and *S. enterica* inactivation in the Felino sausage.

| Challenge test | Statistical indices | Model | |
|---|---|---|---|
| | | log-linear | Weibull |
| *L. monocytogenes* | No. of model parameters | 2 | Ambiguous |
| | *LoF*[a] | Yes (*P*-value = 0.15) | |
| | *RMSE*[b] | 0.283 | |
| | $R^{2b}$ | 0.17 | |
| | Residuals normality[c] | Yes (*P*-value = 0.86) | |
| | Parameters dependency[d] | | |
| | $\log_{10}N_0$ | 0.49 | |
| | $k_{max}$ | 0.49 | |
| | $\delta$ | | |
| | $p$ | | |
| *S. enterica* | No. of model parameters | 2 | 3 |
| | *LoF*[a] | Yes (*P*-value = 0.07) | No (*P*-value = 0.04) |
| | *RMSE*[b] | 0.440 | 0.448 |
| | $R^{2b}$ | 0.63 | 0.64 |
| | Residuals normality[c] | Yes (*P*-value = 0.63) | Yes (*P*-value = 0.42) |
| | Parameters dependency[d] | | |
| | $\log_{10}N_0$ | 0.49 | 0.76 |
| | $k_{max}$ | 0.49 | |
| | $\delta$ | | 0.81 |
| | $p$ | | 0.67 |

[a] *LoF* was either accepted (yes) or not accepted (no) based on the *F*-test. *P*-value < 0.05 indicates the use of an inadequate model

[b] The model with the lower *RMSE* and the higher $R^2$ indicates better fitting

[c] *P*-value < 0.05 indicates that residuals are not normally distributed

[d] A dependency above 0.90-0.95 is considered high indicating the use of an inadequate model and the fitting of a simpler model should be checked instead or it should be considered the collection of more data performing additional experiments or over a wider range of *X*, i.e. time.

**Table 6**

Kinetic parameters estimation of *L. monocytogenes* and *S. enterica* non thermal inactivation in the Cacciatore and Felino sausages according to the log-linear model.

| Sausage/Challenge test | $k_{max}$ (95% CI)[a] (ln cfu/day)[b] | $N_0$ (95% CI)[a] (log cfu/g) | *D*-value (days) |
|---|---|---|---|
| Cacciatore | | | |
| *L. monocytogenes* | 0.04 (0.01-0.08) | 5.99 (5.83-6.15) | 52 |
| *S. enterica* | 0.13 (0.09-0.16) | 6.19 (6.02-6.36) | 18 |
| Felino | | | |
| *L. monocytogenes* | 0.02 (0.01-0.04) | 5.00 (4.83-5.17) | 111 |
| *S. enterica* | 0.09 (0.06-0.13) | 5.03 (4.77-5.29) | 24 |

[a] CI, confidence interval

[b] To convert $k_{max}$ units from ln cfu/day to log cfu/day, the $k_{max}$ value should be divided by ln(10)

**Figures Captions**

**Fig. 1.** Changes in LAB (a and c) and CNC (b and d) during manufacture of Cacciatore inoculated with *S. enterica* (a and b) and Felino inoculated with *L. monocytogenes* (c and d). The same trend was also observed for the batch inoculated with *L. monocytogenes* (Cacciatore) or *S. enterica* (Felino). The data points are mean values of four replicates and the vertical bars (if visible) represent the standard error (SE) of each mean value.

**Fig. 2.** Changes in pH (a and c) and $a_w$ (b and d) during manufacture of Cacciatore inoculated with *S. enterica* (a and b) and Felino inoculated with *L. monocytogenes* (c and d). The same trend was also observed for the batch inoculated with *L. monocytogenes* (Cacciatore) or *S. enterica* (Felino). The data points are mean values of four replicates and the vertical bars (if visible) represent the standard error (SE) of each mean value.

**Fig. 3.** Kinetic behavior of *L. monocytogenes* (a and c) and *S. enterica* (b and d) in Cacciatore (a and b) and Felino (c and d) as described by the log-linear model (solid line). Solid circles are the observed data; and dashed lines are the 95% confidence bands of the line, meaning that if the experiment is repeated again then the solid line should fall within the range of dashed lines.

**Figure 1**

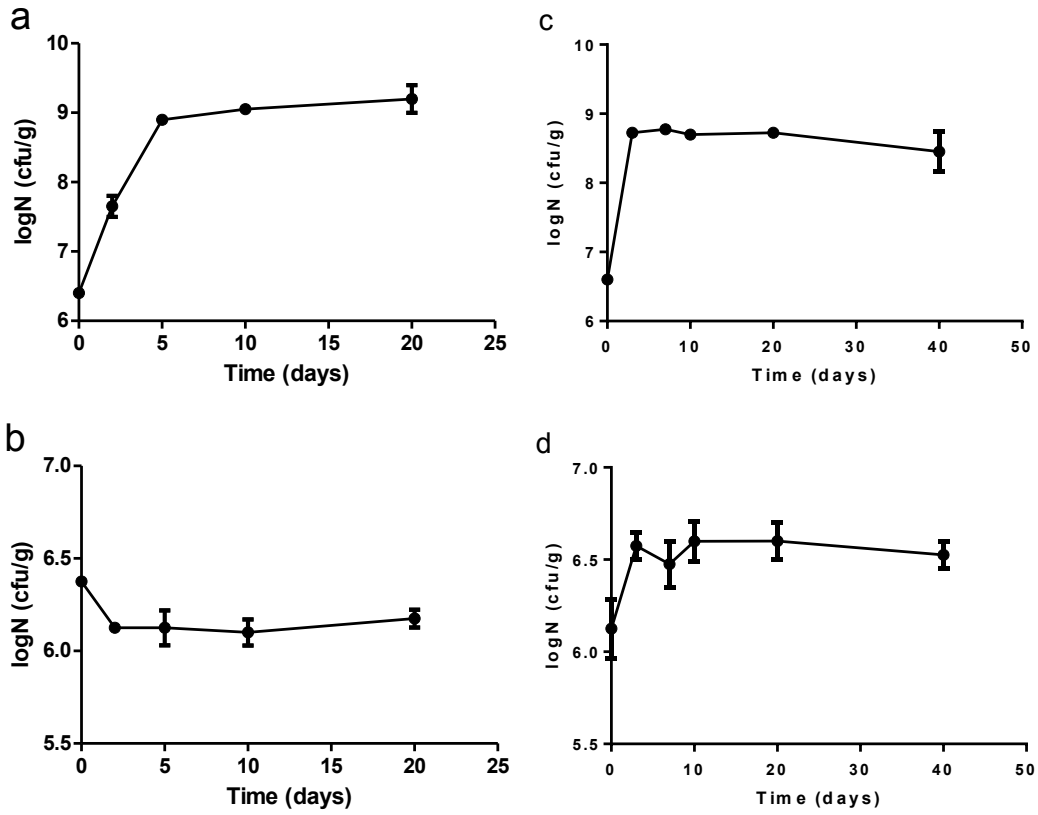
a
logN (cfu/g)
Time (days)
c
logN (cfu/g)
Time (days)
b
logN (cfu/g)
Time (days)
d
logN (cfu/g)
Time (days)

**Figure 2**

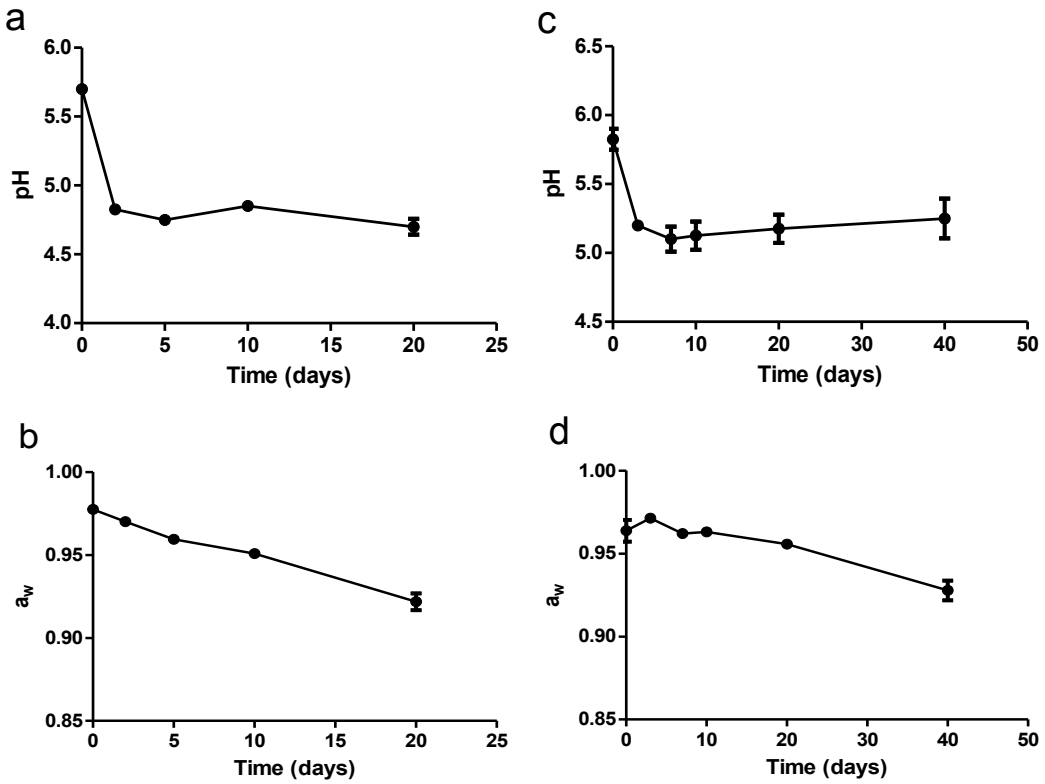

**Figure 3**