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Extending the gamma concept to non-thermal inactivation: A dynamic model to predict the fate of *Salmonella* during the dried sausages process

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ABSTRACT

The process of dried fermented sausages is recognized to be favourable to the reduction of the *Salmonella* population. The objective of this study was to develop a model describing the evolution of *Salmonella* during the fabrication process of dried sausages and to optimize the food formulation to prevent pathogen presence at the end of the process.

An experimental design was set to investigate the effects of the fermentation and drying process for several formulations, taking into account the type of starter culture, the sodium chloride concentration, the dextrose and lactose concentration on the *Salmonella* Typhimurium strain behaviour.

A growth-inactivation model based on the gamma concept was then developed to quantify *Salmonella* behaviour in dynamic process conditions of temperature, pH, lactic acid and water activity. This behaviour was characterized by a first growth step, followed by an inactivation step. The *Salmonella* fate was well described by the model in terms of population size variation and transition from growth to inactivation. The *Salmonella* behaviour was influenced by the initial sugar concentration and the starter type but not by sodium chloride content. This model can be a valuable tool to design the food process and formulation to control *Salmonella*.

1. Introduction

Salmonella is a zoonotic pathogen present in the intestinal tract of a wide range of domestic and wild animals. In 2011, 95 548 confirmed cases of human salmonellosis were reported in the European Union. Although a statistically significant decreasing trend was observed in the EU over the period 2008–2011, the European Food Safety Authority (EFSA) estimated the overall annual burden related to human salmonellosis in Europe to 3 billion Euros. Among the different food categories, the highest proportions of *Salmonella*-positive units were reported for fresh broiler meat and fresh pig meat. In the pork industry, slaughter equipments are often identified as the immediate source of *Salmonella* contamination (EFSA, (European Food Safety Authority), ECDC (European Centre for Disease Prevention and Control), 2013). The initial source remains a carrier pig that can contaminate the environment or cause pig-to-pig contamination during the transport and handling of pigs prior to slaughter (Berends et al., 1996; Newell and Williams, 1971). *Salmonella* is known to be heat sensitive (Doyle and Mazzotta, 2000), products intended to be consumed cooked can therefore be secured by cooking provided that recontamination is prevented. However, some processed products which are not thermally treated can cause real concern if manufactured with contaminated raw meat (Tomicka et al., 1997). Dry fermented sausages classified as ready to eat are an example of non-thermally treated meat products. They are produced by fermenting and then drying a batter made of lean and fat pork meat, added with starter cultures composed of Lactic Acid Bacteria (*Lactobacillus*) and Gram-positive catalase-positive cocci (*Staphylococcus*) (Hugas and Monfort, 1997), sugar, seasonings and spices and stuffed into natural or artificial casings (Thevenot et al., 2005). The starter cultures as well as the process may vary and some specific formulations may also include wine, rum and specific surface fungi (Thevenot et al., 2005).

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Salmonella tends to decrease significantly during the fermented dried sausage process, but, despite the different technological hurdles (pH, acid lactic, *aw*), *Salmonella* was shown to survive certain fermented dried sausages manufacturing processes (Barbuti and Parolari, 2002; Nissen and Holck, 1998). Recently, fermented dried sausages contaminated with *Salmonella* were epidemiologically linked to two outbreaks in France in 2008 with 132 cases of illnesses (Bone et al., 2010) and in 2010 with 337 cases of illnesses (Gossner et al., 2012). In 2011, the French network for *Salmonella* surveillance received 537 isolates from pork processed meat from which 110 isolates originated from fermented dried sausages and fermented dried sausages-like products (Moury et al., n.d.).

To better understand the effects of the process and the formulation on the behaviour of *Salmonella* or other pathogens, several models were proposed in the literature. A polynomial regression approach was proposed to predict the reductions of *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* during fermentation, drying, and storage of soudjouk-style fermented sausage, taking into account the effects of pH and water activity (Hwang et al., 2009; Porto-Fett et al., 2008). Pragalaki et al. (2013) modelled the inactivation of *Lactobacillus sakei*, *L. monocytogenes* and *E. coli* O157:H7 during fermented sausage process using a linear inactivation model adapted with a tail. Erkmen (2008) proposed a modelling of the survival of *L. monocytogenes*, aerobic bacteria, lactic acid bacteria, and yeasts and moulds during ripening and storage of a Turkish dry-fermented sausage using the modified-Gompertz or a logistic model. The inactivation of *E. coli*, *L. monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation and storage was also modelled by Lindqvist et al. (2002). These models are useful to estimate the risk of salmonellosis associated with the consumption of pork sausages, to evaluate different scenarii or strategies to reduce the risk of salmonellosis or the consumer's exposure to *Salmonella* (Gonzales-Barron et al. 2012; Alban et al. 2002).

The objectives of this study were to ascertain and to model the growth and the inactivation of *Salmonella* Typhimurium throughout the fermented dried sausage process for several formulations including: starter cultures type ("*fast*" or "*moderate*") and two levels of sugar and salt contents.

2. Materials and methods

2.1. Challenge test experiments

2.1.1. Inoculum preparation

Salmonella enterica serovar Typhimurium ADQP305, from the Sym'Previus collection, isolated from brine and kindly provided by ADRIA Développement (Quimper, France), was used in this study. The strain was stored at −80 °C using cryobeads (Mast Cryobank, Mast Diagnostics).

To prepare the inocula, a cryobead of the strain was first cultivated in Brain Heart Infusion broth (BHI) (Oxoid, Basingstoke Hampshire, United Kingdom), for 8 h at 37 °C, then subcultured by transferring 100 µl of the initial culture into 10 ml of BHI and incubating it for 18 h at 37 °C. The subculture was then submitted to a cold stress during which the microbial suspension was stored at 4 °C ± 1 °C for 24 h to place the microbial cells in a physiological state that is similar to that of naturally contaminating *Salmonella* cells encountered in the meat industry. Adequate dilutions were finally made to reach an inoculation level of 10^3 CFU/g in the batter.

2.1.2. Dried sausage manufacture and sampling

This study involved sixteen batches of fermented dried sausages corresponding to a full factorial design (four factors and two levels) in order to investigate the effects of the starter cultures, sugar concentrations of dextrose and lactose as well as salt concentration.

Raw pork meat was obtained from a local store and kept frozen until used. The batter was prepared by first grinding the raw meat (pork lean meat 80%, derinded backfat 20%) to obtain particles of 6 mm diameter before blending the mixture with starter cultures (fast or moderate acidifying power), dextrose (5 or 8 g kg^{-1}), lactose (0 or 10 g kg^{-1}), salt (24 or 28 g kg^{-1}), black pepper (1.5 g kg^{-1}) and nitrate (0.3 g kg^{-1}).

Commercial starter cultures Texel SA201 or Texel SA306 (Danisco, Dange Saint Romain, France) were added at a usual concentration of 10^6 CFU/g. The batter was then inoculated by the ADQP305 suspension to reach a contamination level of 10^3 CFU/g and stuffed into natural casings to obtain 450 g. For each formulation, a batch of non-inoculated sausages served as negative control. All fresh-made sausages were tied with strings before being dipped into a commercial *Penicillium* surface suspension (Texel Neo 2000, Danisco) and hung vertically in a temperature and humidity controlled incubator to undertake the ripening and drying process.

The temperature and relative humidity were set according to the average process conditions used in France by fermented dried sausages manufacturers and validated by a group of 6 industrial partners. This process involves a fermentation step (T°C: 22°C–24 °C; RH: 85%–90%) followed by a drying step (T°C: 13 °C; RH: 72%–80%) as shown in Table 1. During the fermentation step the temperature and the relative humidity evolve from 22 °C and 90–95% respectively to 16 °C and 82–90% respectively. During the drying step, the temperature is maintained at 13–14 °C and the relative humidity at 73–81%. Sausages from the different formulations were sampled for microbiological and physico-chemical analysis three times a day during fermentation (day 0–4) and then once a day during the drying stage (day 5–35).

2.1.3. Microbiological analysis

For each formulation and each sampling date, a sausage was taken from the climate controlled chamber. One half was ¼ diluted in buffered peptone water (BPW, Biorad) and homogenized for 1 min in a stomacher (BagMixer 400 W, Interscience). After a revivification of 1 h at room temperature, further decimal dilutions were made with tryptone salt (Biorad). Aliquots from appropriate serial dilutions were spread onto Brilliance Salmonella chromogenic media plates (Oxoid) and incubated at 37 °C for 24 h. The number of CFU per gram of dried sausage was converted in CFU per gram of "*fresh sausage*" by taking the loss of weight into account. Knowing the weight of the sausage at a given time, the number of *Salmonella* per sausage could be computed and then divided by the initial weight of the "*fresh sausage*". Samples with low *Salmonella* contamination (below the quantification limit of the method), were tested for the presence of *Salmonella*, according to the NF EN ISO 6579-1 standard protocol. Lactic Acid Bacteria representing the starter culture, were enumerated on the same samples using de Mann Rogosa Sharpe agar (MRS, Biomérieux) according to the ISO 15214 standard protocol.

Table 1
Fermented dried sausages process.

	Steps	Duration (h)	Temperature (°C)	Relative humidity (%)
Fermentation	1	1	17	92–96
	2	6	21	86–93
	3	20	22 à 24	82–90
	4	4	22 à 24	75–85
	5	6	20	75–82
	6	6	17	83–90
	7	3	16	82–90
Drying	8	3	14	73–82
	9	671	13	73–81