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Thermal inactivation of *Salmonella* spp. in pork burger patties

P.M. Gurman [a,b,*], T. Ross [b], G.L. Holds [a], R.G. Jarrett [c], A. Kiermeier [b,d]

[a] Food Safety and Innovation, South Australian Research and Development Institute, G.P.O. Box 397, Adelaide, South Australia 5001, Australia
[b] Tasmanian Institute of Agriculture, School of Land and Food, University of Tasmania, Private Bag 51, Hobart 7001, Tasmania, Australia
[c] P.O. Box 3059, Unley, South Australia 5061, Australia
[d] Statistical Process Improvement Consulting and Training Pty Ltd., Gumeracha, South Australia 5233, Australia

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ABSTRACT

Predictive models, to estimate the reduction in *Escherichia coli* O157:H7 concentration in beef burgers, have been developed to inform risk management decisions; no analogous model exists for *Salmonella* spp. in pork burgers. In this study, "Extra Lean" and "Regular" fat pork minces were inoculated with *Salmonella* spp. (*Salmonella* 4,[5],12,i:-, *Salmonella* Senftenberg and *Salmonella* Typhimurium) and formed into pork burger patties. Patties were cooked on an electric skillet (to imitate home cooking) to one of seven internal temperatures (46, 49, 52, 55, 58, 61, 64 °C) and *Salmonella* enumerated. A generalised linear logistic regression model was used to develop a predictive model for the *Salmonella* concentration based on the internal endpoint temperature. It was estimated that in pork mince with a fat content of 6.1%, *Salmonella* survival will be decreased by −0.2407 $\log_{10}$ CFU/g for a 1 °C increase in internal endpoint temperature, with a 5-$\log_{10}$ reduction in *Salmonella* concentration estimated to occur when the geometric centre temperature reaches 63 °C. The fat content influenced the rate of *Salmonella* inactivation ($P = 0.043$), with *Salmonella* survival increasing as fat content increased, though this effect became negligible as the temperature approached 62 °C. Fat content increased the time required for patties to achieve a specified internal temperature ($P = 0.0106$ and 0.0309 for linear and quadratic terms respectively), indicating that reduced fat pork mince may reduce the risk of salmonellosis from consumption of pork burgers. *Salmonella* serovar did not significantly affect the model intercepts ($P = 0.86$) or slopes ($P = 0.10$) of the fitted logistic curve. This predictive model can be applied to estimate the reduction in *Salmonella* in pork burgers after cooking to a specific endpoint temperature and hence to assess food safety risk.

1. Introduction

Salmonella is a major cause of foodborne illness worldwide. In 2011, there were 95,548 confirmed cases of salmonellosis in the European Union, exceeded in reported cases only by campylobacteriosis (EFSA, 2013). *Salmonella* spp. are responsible for the largest number of deaths from foodborne pathogens in the US, despite the relatively low death rate of 0.5% (Scallan et al., 2011). Similarly, in Australia in 2014, approximately 16,000 cases of salmonellosis were reported, an incidence exceeded only by campylobacteriosis (Department of Health Australia, 2015). The true number of cases of salmonellosis caused by foodborne contamination in Australia, circa 2010, was estimated at 39,600 (Kirk et al., 2014), resulting in an estimated 3500 cases of irritable bowel syndrome and 3250 cases of reactive arthritis (Ford et al., 2014). Common symptoms of salmonellosis include nausea, vomiting, abdominal cramps, diarrhoea, fever and headaches. Those at greatest risk of serious complications are the elderly, young and immuno-compromised (FDA, 2012). The identification of *Salmonella* spp. as the cause of illness can be difficult due to the onset of symptoms occurring 6 to 72 h after exposure (FDA, 2012). Some *Salmonella* serovars are of greater public health interest as they are more frequently identified as the cause of salmonellosis outbreaks. *Salmonella* Typhimurium accounts for the largest proportion of salmonellosis cases in Australia: 23.5% of outbreaks were attributed to this serovar in 2010 (OzFoodNet Working Group, 2012a).

Salmonella 4,[5],12:i:-, an emerging strain of public health interest, has risen to prominence in the last decade. The isolation rate, and number of human infections, from this strain is increasing in the European Union (EFSA, 2013) and United States of America (CDC, 2013). This strain has also caused foodborne illness in Australia (OzFoodNet Working Group, 2012b). *Salmonella* Senftenberg is also of interest because of reports of its unusually high heat resistance (Jay et al., 2003).

Source attribution studies for *Salmonella* spp. have identified pork products as causing a large proportion of salmonellosis cases (Mullner et al., 2009; Hald et al., 2004; Mughini-Gras et al., 2014). While no source attribution studies have been conducted for salmonellosis in Australia, pork products have been associated with salmonellosis outbreaks (OzFoodNet Working Group, 2012a; OzFoodNet Working Group, 2010; Delpech et al., 1998).

Pork burgers are currently being promoted to Australian consumers as a serving suggestion for pork mince, which has a relatively low

* Corresponding author at: Food Safety and Innovation, South Australian Research and Development Institute, P.O. Box 397, Adelaide, South Australia 5001, Australia.
E-mail address: Phillip.Gurman@gmail.com (P.M. Gurman).

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prevalence (1.4%) of *Salmonella* (Hamilton et al., 2011). Beef burgers have an increased risk of *Escherichia coli* (*E. coli*) O157:H7 survival after cooking compared with beef steaks (Smith et al., 2013). Similarly, salmonellosis risk from pork products in the European Union was greater from pork burgers compared with pork cuts in two of four member states examined (VLA/DTU/RIVM, 2011). The inactivation model in that risk assessment used *D*-values (time required for a 1-$\log_{10}$ reduction in *Salmonella* at a constant temperature) and *z*-values (temperature required for a 1-$\log_{10}$ reduction in the *D*-value) for beef mince, not pork mince. The fat content of pork, beef, chicken and turkey mince has been shown to have an effect on *Salmonella* inactivation (Juneja et al., 2000; Juneja et al., 2001; Smith et al., 2001) with increased fat content leading to increased *Salmonella* survival. This effect has not been quantified in pork burger patties. The risk of salmonellosis can also be influenced by the colour of the cooked burger patty if used as an indicator of the "doneness". Colour is a poor indicator of "doneness", with burgers cooked to 66 °C appearing as brown as burgers cooked to 71 °C (Hague et al., 1994). Factors linked to increased pinkness in cooked burgers include pH (Trout, 1989) and pigment concentration (Mendenhall, 1989). Unlike intact cuts of pork, where microbial contamination is limited to the surfaces of the food, burger patties are comminuted, with microbial pathogens potentially internalised. Heat from cooking surfaces needs to be transferred from the outer surfaces to the centre of the patty for thermal inactivation to occur. Juneja et al. (1997) presented a simple mathematical model for the reduction of *E. coli* O157:H7 during cooking of beef burgers, but no data exist for *Salmonella* thermal inactivation in pork burgers. An inactivation model for *Salmonella* reduction in pork burger patties cooked to mimic home cooking practices would provide information that can be used to assess food safety risk and, potentially, offer insights for food safety management. The aims of this study were to i) quantify the reduction in *Salmonella* caused by cooking pork burgers to various endpoint temperatures; ii) determine whether there are serovar differences in reduction due to cooking and iii) assess the influence of fat content on the rate of thermal inactivation of *Salmonella* in pork burgers.

2. Materials and methods

2.1. Salmonella strains

Three *Salmonella* serovars, of public health interest (see Introduction), were chosen for this experiment; *S.* 4,[5],12:i:-, *S.* Typhimurium and *S.* Senftenberg. All serovars used in this study were previously isolated from porcine sources, serotyped, and stored long-term in snap freeze medium (Oxoid, TM0171) at −80 °C. Prior to the experiment, isolates were removed from frozen storage, streaked onto nutrient agar slopes (Oxoid, TM0085) and stored at room temperature to provide working cultures for the duration of all experiments. Prior to the experiments, the viability of each strain was verified by streaking the cultures of each serovar onto nutrient agar (Oxoid, PP2036) and incubating at 37 °C for 18 ± 2 h. A single colony was picked off and inoculated into 100 ml of Tryptic Soy Broth (TSB, Bacto, Catalogue Number 211825) and incubated at 37 °C for 18 ± 2 h. Cultures were centrifuged (3667 *g*, 4 °C, 15 min) and rinsed twice with peptone saline solution (PSS, 0.1% trypticase, 0.85% NaCl, wt/vol) before being re-suspended in 5 ml of PSS to minimise the change in moisture of the mince upon inoculation, and thereby, water activity and texture of the mince.

2.2. Mince

Each batch of burgers required two packages of retail pork mince, which were purchased from supermarkets of the same chain in 500 g modified atmosphere packages (MAP). Pork mince was purchased with either a "Regular" fat level (nominally 17%, stated on packaging) or "Extra Lean" fat level (nominally 5%, stated on packaging), with the actual fat content of the mince determined analytically (see Section 2.6). The pork mince packages required for each week's experiments were purchased at the start of that week. Pork mince was transported to the laboratory by car, but without refrigeration. The transport time was up to 45 min at ambient temperature in the range of 19 °C to 29 °C. The "use-by" date and other information from the product labels were recorded and the packages placed into a refrigerator at 4 ± 2 °C. The number of days until the expiration of the product "use-by" period ranged from 2 to 5 days with four batches having 2, 3 or 4 days and six batches 5 days remaining.

This study consisted of 18 batches (labelled A to R) of 8 burger patties with 3 *Salmonella* serovars, 2 fat levels and 3 replicates of each fat-serovar combination (see Table 1).

2.3. Patty preparation

Pork mince was added to the bowl of a food mixer (Kambrook KSM500 Powermix Planetary Bench Mixer), and the leaf beater attached. The mixer was run for 1 min at the slowest speed setting to thoroughly mix the product. A 30 g sub-sample of the mince was collected for fat level determination and stored at −80 °C until analysis. Another 30 g sub-sample of the mince was taken for pH determination. The 5 ml re-suspension of *Salmonella* was poured onto the mince and the mixer was run at the lowest speed setting for 5 min to ensure that the *Salmonella* were homogeneously dispersed throughout the mince.

Mince was formed into patties using a circular mould of 8 cm diameter and 2 cm thickness. For each patty, 100 g of contaminated mince (determined by weighing) was pressed into the mould using a metal spoon. Each formed patty was placed inside a plastic, sealable container lined with baking paper and refrigerated overnight. The refrigeration served to "firm up" the patties after mixing and to condition the *Salmonella* cells to their environment, thus simulating contaminated pork mince purchased at retail.

2.4. Cooking of pork burger patties

Patties were cooked to one of seven nominal target temperatures (45, 48, 51, 54, 57, 60, 63 °C chosen to cover a realistic range of endpoint cooking temperatures at which some *Salmonella* survivors would still be able to be enumerated) measured by a type K thermocouple attached to a thermocouple thermometer (Model Number TFC-307P, OneTemp, Adelaide, Australia). Cooking times were expected to vary between burger patties cooked to the same internal endpoint temperature. For each batch of patties, the order of cooking and assigned endpoint temperature was randomised and recorded. An electric skillet

Table 1
Sequence that each fat level and serovar combination was cooked. Nominal fat levels are the descriptions on the mince packages.

Batch	Week	Nominal fat level	Serovar
A	1	Regular	*S.* 4,[5],12:i:-
B	1	Extra Lean	*S.* Senftenberg
C	2	Regular	*S.* Senftenberg
D	2	Extra Lean	*S.* 4,[5],12:i:-
E	2	Regular	*S.* Senftenberg
F	2	Regular	*S.* Typhimurium
G	3	Extra Lean	*S.* Typhimurium
H	3	Extra Lean	*S.* Senftenberg
I	3	Extra Lean	*S.* Typhimurium
J	3	Regular	*S.* Senftenberg
K	4	Regular	*S.* Typhimurium
L	4	Extra Lean	*S.* Typhimurium
M	4	Regular	*S.* 4,[5],12:i:-
N	4	Regular	*S.* 4,[5],12:i:-
O	5	Extra Lean	*S.* 4,[5],12:i:-
P	5	Extra Lean	*S.* 4,[5],12:i:-
Q	5	Regular	*S.* Typhimurium
R	5	Extra Lean	*S.* Senftenberg