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Behavior of *Salmonella typhimurium* DT104 during the manufacture and storage of pepperoni

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Abstract

Pepperoni batter (ca. 70% pork:30% beef) was prepared and subsequently inoculated with a six-strain cocktail (ca. 4.4×10^7 per gram batter) of *Salmonella typhimurium* DT104. After fermentation at 36°C and 92% relative humidity (RH) to $\leq$ pH 4.8, counts of the pathogen decreased by about 1.3 $\log_{10}$ units. An additional 1.6 $\log_{10}$ unit decrease was observed following drying at 13°C and 65% RH to a moisture protein ratio (M/Pr) of 1.6:1. After storage of pepperoni sticks for 56 days under vacuum at 4 or 21°C, counts of the pathogen were about 4.6 and 6.6 $\log_{10}$ units lower, respectively, compared with starting levels in the batter. These data establish that fermentation and drying result in about a 3.0 $\log_{10}$ reduction in numbers of *S typhimurium* DT104 in pepperoni sticks and that storage of pepperoni sticks under vacuum at ambient temperature is more severe on the pathogen than refrigerated storage. © 1998 Elsevier Science B.V.

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1. Introduction

Isolates of *Salmonella typhimurium* definitive phage type 104 (DT104) were first observed in 1984 in the United Kingdom (UK). Subsequently, the widespread and frequent association of DTlO4 isolates with farm animals or foods derived therefrom has been observed (Hollingsworth, 1997). Phage type 104 strains of *Salmonella* are resistant to multiple antibiotics, usually displaying resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT) (Threlfall et al., 1996). More recently, some strains have been isolated which also display resistance to trimethoprim and fluoroquinolones (Threlfall et al., 1996). In the UK, DT104 strains of *S. typhimurium* were second only to phage type 4 strains of *S. enteritidis* as the most prevalent *Salmonella* strains isolated (Threlfall et al., 1996). In the United States (US), *S. typhimurium* was also the second most commonly reported serotype of *Salmonella* (Hosek et al., 1997).

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Among *S. typhimurium* strains in the US, the DT104 phenotype was identified in 32% (90 of 282 isolates) of isolates tested during 1996, as compared to a prevalence of 28% (273 of 976 isolates) in 1995 and 7.0% in 1990 (Hosek et al., 1997).

Contact with ill farm animals and consumption of beef, chicken, pork sausage, and meat paste were identified as risk factors for DT104 infections in the UK (Davies et al., 1996; Wall et al., 1994). Although humans may have been the original source that subsequently spread this bacterium to animals (Anonymous, 1997), far less is known about the reservoir or risk factors for *S. typhimurium* in the US. A 1996 outbreak of salmonellosis among Nebraska elementary school children was attributed to *S. typhimurium* DT104 (Hosek et al., 1997). This US outbreak was linked to consumption of expired chocolate milk, but the organism was never cultured from the remaining cartons nor was the organism isolated from a kitten or turtle handled by children who became ill (Anonymous, 1997; Hosek et al., 1997).

In 1995, the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) began requiring manufacturers of dry and semi-dry fermented sausage to validate processes to insure a 5-$\log_{10}$-unit reduction in numbers of *Escherichia coli* O157:H7 (Reed, 1995). In mid-1997, the USDA/FSIS indicated that when a plant could not demonstrate compliance with that requirement, more frequent sampling of products from the plant for *E. coli* O157:H7, as well as for *Salmonella* species and *Listeria monocytogenes*, would be required (Billy, 1997). Although information on the behavior of *S. typhimurium* in fermented meats was published some years ago (Goepfert and Chung, 1970; Masters et al., 1981; Smith et al., 1975),no information exists on the fate of *S. typhimurium* DT104 in fermented meats. However, there has been at least one report confirming the recovery of *S.typhimurium* DT104 from a stick of salami that displayed a plasmid profile identical to the plasmid profile from an isolate from a patient who consumed the same salami and became ill (Wall et al., 1994). There has also been at least two recalls of fermented meats due to *Salmonella* contamination, a recall of Lebanon bologna in 1995 (United States Department of Agriculture, 1995) and a recall of cervelat in 1997 (United States Department of Agriculture, 1997). The association of *S. typhimurium* DT104 with exposure to live animals and foods derived therefrom and the higher hospitalization and mortality rates for DT104 isolates compared with non-typhoid salmonellae, as well as the likelihood of more aggressive testing of fermented meats for *Salmonella*, prompted us to evaluate the viability of this bacterium after fermentation, drying, and storage of pepperoni.

2. Materials and methods

Six clinical isolates of *Salmonella typhimurium* were used. Those were: (i) strains H3380 and H3402 (phage type DT104); (ii) strains H3278 and H2662 (phage type DTlO4b); and (iii) strains G8430 and G7601 (phage type U302). All six isolates displayed an indistinguishable genomic fingerprint as determined by pulsed-field gel electrophoresis (data not shown). The salmonellae were maintained at -20°C in brain heart infusion (BHI; Difco Laboratories Inc., Detroit, MI) broth plus 10% glycerol and transferred twice in BHI broth prior to use. The *Pediococcus acidilactici* starter culture (Saga 200, Quest International, Rochester, MN) was prepared by adding 12 ml of the thawed commercial culture, brought up to 100 ml with sterile double distilled water, to about 23 kg of raw meat to deliver about 10^7 cfu/g of batter.

The *S. typhimurium* strains were separately grown overnight at 37°C in 10 ml of BHI broth. A 750 μl portion of each of the overnight cultures was transferred to a separate flask containing 750 ml BHI broth and incubated for 18 h at 37°C. The cells were harvested by centrifugation, the six cell suspensions were combined, and the final volume was adjusted to 75 ml with 0.1% peptone (Difco).

Pepperoni was manufactured essentially as described (Faith et al., 1997; Hinkens et al., 1996) and outlined in Fig. 1. Meat was tested for viable *S. typhimurium* by direct plating prior to stuffing, after fermentation, during drying, and during storage using triplicate samples of meat and duplicate platings of each dilution. At each sampling interval, three 25-g portions of the batter or a 25-g cross section from the center of the three different sticks were aseptically transferred to separate Stomacher bags (Seward Medical, London, UK) containing 0.85% saline and

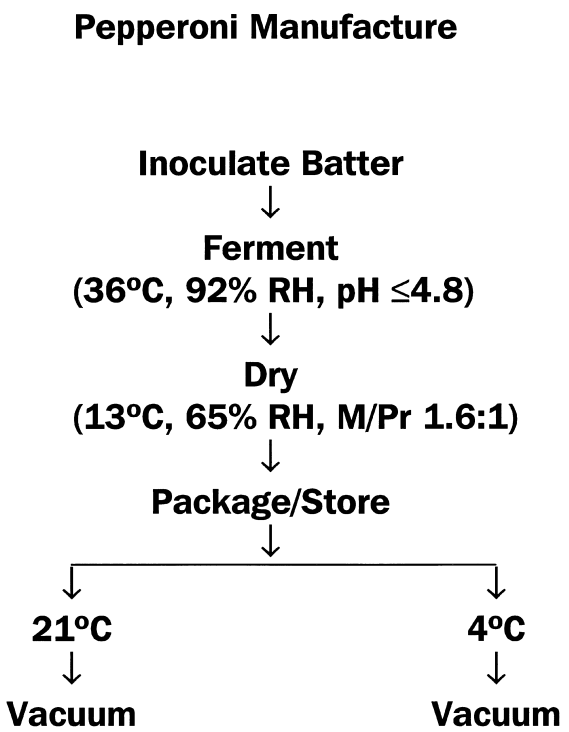

Fig. 1. Pepperoni manufacture. Flow diagram depicting the pepperoni processes which were evaluated.

macerated for 2 min in a stomacher (model 400; Tekmar, Cincinnati, OH). Next, 0.1 ml portions, with and without prior dilution were spread onto XLD (Remel, Ltd., Lenexa, KS) agar plates.

When numbers of the pathogen decreased below detection by direct plating ($<10^1$ cfu g), the presence/absence of the pathogen was determined by enrichment. A 25 g portion from each of three sticks was aseptically transferred to separate Stomacher bags containing 225 ml lactose broth (Difco). After maceration, the samples were incubated overnight at 37°C and an inoculation loop was used to streak a portion of the contents of each bag onto XLD agar plates that were then incubated at 37°C for 24 h. The non-inoculated batter was also macerated using a stomacher to test for *S. typhimurium* and total aerobic bacterial numbers by spreading 0.1 ml portions, with and without prior dilution, onto XLD and trypticase soy (Difco) agar plates, respectively. Immediately after grinding, the batter was also macerated using a Stomacher to test for viable pediococci by spreading 0.1 ml portions, with and without prior dilution, onto Lactobacilli MRS (Difco) agar plates. All plates were incubated at 37°C for 24 to 48 h before colonies were counted.

Chemical analyses were performed by a commercial testing laboratory on a 250-g composite sample from three sticks following Association of Official Analytical Chemists (AOAC) procedures to determine the moisture (AOAC procedure 950.46) and protein (AOAC procedure 928.06) contents as specified for meat products (McNeal, 1990).

The absence (<10 cfu/g) of the pathogen on XLD plates by direct plating was scored as 9 for regression analyses. When cells of the pathogen were not detected by enrichment, a value of 1.0 was used for regression analyses.

3. Results and discussion

Previous studies have evaluated the fate of *Salmonella* in fermented meats. As one example, Smith et al. (1975) reported about a 1.3 $\log_{10}$ reduction in numbers of *S. typhimurium* after fermentation (35°C, 85% RH, pH 4.5) and about a 3.0 $\log_{10}$ reduction in pathogen numbers after drying (12°C, 65% RH, 22 days) of pepperoni. As other examples, Goepfert and Chung (1970) reported a 0.75 to 2.5 $\log_{10}$ reduction of *S. typhimurium* after fermentation (30°C, 90% RH, $\leq$ pH 4.8) of Thuringer sausage and Masters et al. (1981) reported about a 2.0 $\log_{10}$ reduction of *S. typhimurium* after fermentation (35°C, 90% RH, 5.0 pH 5.0) of summer sausage. All three studies utilized a commercial lactic starter culture. These data are similar to results obtained in the present study for *S. typhimuriun* DT104. More specifically, fermentation and drying of pepperoni resulted in a 2.9 $\log_{10}$ unit decrease in numbers of *S. typhimurium*. In related experiments, monitoring the fate of *E. coli* O157:H7 in pepperoni, fermentation and drying resulted in about a 1.8 $\log_{10}$ unit decrease in pathogen numbers (Faith et al., 1997; Hinkens et al., 1996). As such, *S. typhimurium* DT104 appears less viable during fermentation and drying of pepperoni than *E. coli* O157:H7. Storage of pepperoni under vacuum for 56 days at 4°C resulted in an additional 1.7 $\log_{10}$ unit reduction of *S. typhimurium* DT104 over what was achieved by fermentation and drying. After storage for 56 days at 21°C under vacuum, pathogen numbers decreased an additional 3.7 $\log_{10}$ cfu/g over

what was achieved by fermentation and drying. After 28 days at 21°C, it was not possible to recover the pathogen by direct plating. However, it was possible to recover the pathogen by enrichment in 5 of 6 sticks from both trials after 28 days and in 1 of 6 sticks from both trials after 56 days. The greater decrease in numbers of DT104 at 21°C compared to 4°C during storage were similar to results obtained with *E. coli* O157:H7 in our previous study (Faith et al., 1997). Lastly, microbiological analyses of the raw meat revealed no indigenous *Salmonella* species and a total aerobic plate count and lactic acid bacteria count of 3.5×10^5 and 8.1×10^3 cfu/g, respectively. The LAB count after the addition of the starter culture was 5.9×10^7 cfu/g. (Fig. 2)

The incidence of salmonellae in over 1110 samples from 40 sausage-manufacturing plants in the US was estimated at 12 to 29% (Johnston et al., 1982). A recent survey in the UK of fresh sausage batter prior to fermentation identified a prevalence of 2 to 11% for salmonellae (reported by Wall et al., 1994), whereas a previous survey between 1969 and 1974 reported the prevalence for salmonellae in sausages at about 60% (Roberts et al., 1975). The results of the present study indicate that phage type 104 isolates of *S. typhimurium* are generally less viable in pepperoni than serotype O157:H7 isolates of *E. coli*. As such, manufacturing processes of fermented meats already validated for *E. coli* O157:H7 should be sufficient to also deliver a 5 $\log_{10}$ unit reduction in counts of *S. typhimurium* DT104.

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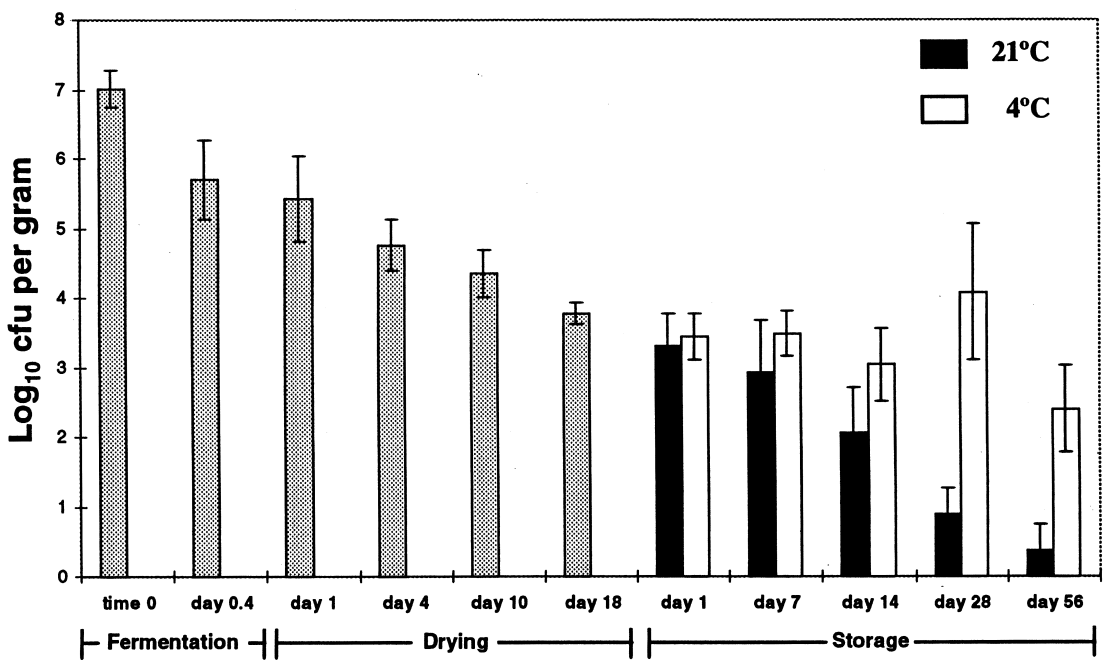

Fig. 2. *S. typhimurium* counts ($\log_{10}$ cfu per gram; $n = 2$) from pepperoni after fermentation at 36°C to pH 4.8, drying at l3°C and 65% RH to a M/Pr of $\leq$ 1.6:1, and storage under vacuum at room or refrigeration temperatures. Black bars are results for storage at 21°C and white bars are results for storage at 4°C.

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