



Survival of *Salmonella* spp. in low water activity chicken base paste and powder formulated at different salt levels



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ABSTRACT

Dry flavor ingredients such as black pepper and hydrolyzed vegetable protein (HVP) have been frequently recalled from the supply chain due to contamination with *Salmonella*. These spices are commonly used in preparation of savory meat flavor bases. This study evaluated the effect of various salt levels on the survival of *Salmonella* spp. in chicken bases formulated with pepper and HVP, in either paste or powder form. The salt content (% w/w), a_w and pH for regular-salt, 45% reduced-salt and 90% reduced-salt paste formulations were 33.0, 0.686, 5.75; 18.2, 0.691, 5.85; and 3.5, 0.751, 5.96, respectively, and powder formulations were 34.0, 0.254, 6.15; 18.5, 0.290, 6.28; and 3.1, 0.301, 6.50, respectively. To mimic natural contamination, a dry sand inoculation technique was used to prepare the samples. A five serovar cocktail of *Salmonella* was used and the final inoculation level in the samples was 7.5 ± 0.2 log CFU/g. Inoculated paste samples were stored at 21 °C for up to 12 weeks, and up to 42 weeks for powder formulations. *Salmonella* counts did not increase in either treatment during storage. The effect of salt levels on *Salmonella* survival was insignificant. There was a 5.2 ± 0.4 log reduction in paste formulations by 3 week. However, low *Salmonella* counts (<1.0 log CFU/g) were detected in samples from 6 to 12 weeks. In powder formulations, minimal log reductions of 1.3 ± 0.1 were observed over 42 weeks. When preparing a food product that does not undergo a kill step, it is necessary to ensure the absence of *Salmonella* in the ingredients, as well as practicing strict adherence to good manufacturing practices including proper environmental monitoring and sanitation in food processing. Furthermore, adequate consumer cooking instructions are important to ensure proper *Salmonella* lethality during preparation of reconstituted dry products.

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1. Introduction

Non-typhoidal *Salmonella* is the leading cause of bacterial foodborne illness (CDC, 2014). It is responsible for 35% of hospitalizations and 28% deaths attributed to the known agents of foodborne illness in the US (Scallan et al., 2011). It is also one of the few foodborne pathogens that has not significantly declined over the past 10 years (Batz, Hoffmann, & Morris, 2011). *Salmonella* is especially difficult to control due to the bacterium's high tolerance to environmental stress, widespread distribution, multiple drug resistance and adaptability to low water activity (a_w) environment (Humphrey, 2004; Warriner, 2011). In addition to the more traditional vehicles such as eggs and meat products, it has been linked to

high-profile outbreaks in a diverse group of foods including dry products, sprouts, and fresh produce. Although growth of *Salmonella* has not been observed in dry (low a_w) food products, its long-term survival in products such as peanut butter, dried egg, infant dried milk, hard cheese, chocolate, salami and dried fruits has been well documented (Bell & Kyriakides, 2002; Beuchat & Mann, 2014; Nummer, Shrestha, & Smith, 2012; Shrestha, Griener, McMahon, & Nummer, 2011). Survival of *Salmonella* spp. in low a_w environments for extended periods of time has been considered to be the contributing factor in the outbreak of salmonellosis associated with low a_w products (Gurtler et al., 2014; Komitopoulou & Peñaloza, 2009).

Dried spices and hydrolyzed vegetable protein (HVP) are two very low a_w products. Spices are periodically found to be *Salmonella* positive when tested. A surveillance study suggests that 7% of spices imported into the US between 2007 and 2009 were contaminated with *Salmonella* (US FDA, 2013). In 2009 and 2010, *Salmonella* contamination in dry pepper spice led to several

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products recalls (US FDA, 2010a). The CDC (2010) outbreak summary indicated that, from July 2009 through April 2010, at least 272 people in 44 states in the US were infected by *Salmonella* Montevideo as a result of consuming ready-to-eat salami products that were manufactured using contaminated black and red pepper spices. Numerous recalls of black peppers due to *Salmonella* contamination have been issued over the last several years. Likewise, *Salmonella* Tennessee contamination of HVP led to product recalls in 2010. No illnesses associated with the contaminated HVP were reported by the FDA (2010b). Any food product formulated with the contaminated pepper or HVP spices were recalled with the exception of products that were processed in a manner deemed lethal to *Salmonella* (US FDA, 2010a and 2010c). Additionally, products that were labeled with proper cooking instructions that would be sufficient to kill *Salmonella* were also exempted from the recall. Outbreaks and recalls from dried ingredients such as pepper and HVP have caused many food processors to consider the additional food safety risks associated with these ingredients.

Savory meat flavor bases, most often produced as either a dry powder or paste, is a product group that often uses black pepper, HVP, or both as ingredients. These low water activity products contain meat extracts, other spices and large percentages of salt and are often used as flavor bases in the preparation of soups, sauces, gravies, stews, dips or any dish that requires a meat flavor. When preparing such dishes, the temperature attained in the cooking step would likely be adequate to kill any *Salmonella* that comes with these dry ingredients. As the cooking instructions were provided in the package, savory flavor bases were not recalled in the *Salmonella* outbreaks of 2009–2010, even though they contained the contaminated pepper or HVP as an ingredient.

Savory meat flavor bases have a long, non-refrigerated shelf life. Currently, there is limited published data on the fate of *Salmonella*, if present, during storage of savory meat bases. The outbreaks of *Salmonella* spp. linked to dry spices have brought into question the safety of meat bases. Therefore, the purpose of this study was to determine the survival of a five-strain cocktail of *Salmonella* in chicken base paste (a_w 0.709 $\pm$ 0.029) and chicken base powder (a_w 0.282 $\pm$ 0.020), both formulated at regular-salt, 45% reduced-salt and 90% reduced-salt levels.

2. Materials and methods

2.1. Composition of chicken base paste and powder

A chicken flavor company based in Salt Lake City, UT supplied the chicken-flavor base paste and powder for this experiment. For proprietary reasons, the product manufacturer and the detail of ingredients in the chicken bases are not further discussed in this paper. To study the effect of salt on *Salmonella* survival, the bases were formulated at 3 salt levels, specifically, regular salt, 45% reduced-salt and 90% reduced-salt. The physicochemical characteristics of the chicken base paste and powder treatments are

presented in Table 1. To determine the pH, 5 g of base was combined with 50 mL distilled water in a glass beaker and stirred with a glass rod (AOAC, 1990). The pH was measured using an Accumet pH meter (Model AR 25, Fisher Scientific, Pittsburgh, PA, USA). Moisture content was determined in triplicate by weight loss after 16–18 h drying in a convection oven at 102 °C (AOAC, 1990). Water activity (a_w) was measured (AquaLab LITE; Decagon Devices Inc., Pullman, WA, USA) in triplicate. The fat and salt content were estimated as per the contribution made by individual ingredients in the recipe.

2.2. Inoculum preparation

Five serovars of *Salmonella* that were previously associated with outbreaks were used in this study. The serovars, Thompson FSIS 120 (chicken isolate), Enteritidis H3527 (clinical isolate, phage type 13a), Typhimurium H3380 (clinical isolate, phage type DT104), Heidelberg F5038BG1 (ham isolate) and Montevideo FSIS 051 (beef isolate), were obtained from the Utah State University culture collection of Dr. Jeff Broadbent. Stock cultures were maintained frozen (–80 °C). Individual inocula were prepared by scraping cells from an agar lawn as described by Danyluk, Uesugi, and Harris (2005) with slight adjustments. Frozen stock cultures were streaked onto tryptic soy agar (TSA; tryptic soy broth and 1.5% agar; Becton Dickinson, MD, USA) plates and incubated for 24 $\pm$ 2 h at 35 °C. A single isolated colony was transferred to 10 mL tryptic soy broth (TSB; Neogen, Lansing, MI, USA) and incubated at 35 °C for 21 $\pm$ 2 h. One milliliter of the overnight culture was then evenly spread (0.25 mL in each) over four TSA plates (100 by 15 mm) and incubated at 35 °C for 24 $\pm$ 2 h to produce a bacterial lawn. The *Salmonella* lawns grown on the TSA plates were overlaid with 0.1% peptone solution (about 4 mL each plate). A sterile plastic spreader was used to loosen/scrap off the bacterial lawn from the agar plates and collected (from 3 sets of 4-TSA plates) into a 50-mL conical centrifuge tube. Cells were pelleted by centrifugation (2100 $\times$ g for 15 min) and re-suspended in 40 mL fresh 0.1% peptone solution, twice. The mix of five serovars was prepared by combining equal volume of aliquots of each strain in a sterile, 250-mL glass bottle. To determine cell populations, appropriate serial dilutions (1:10 in 0.1% peptone solution) of the inoculum was made and plated onto *Salmonella*-Shigella agar (Acumedia Manufacturers Inc., Lansing, MI, USA). Colonies were counted after incubating the plates at 35 °C for 24 $\pm$ 2 h.

2.3. Dry sand inoculum preparation

Dry pepper or HVP could have been inoculated to simulate *Salmonella*-contaminated dry spices. However, these fine-sized and light-weight particulate spices could potentially generate airborne *Salmonella* during handling of the inoculated spices. In addition, a greater amount of liquid inoculum would be needed to completely and uniformly inoculate the samples. Also, the samples would from

Table 1

Physicochemical characteristics of chicken-base paste and powder treatments formulated at regular salt, 45% reduction, and 90% reduction salt levels (n = 3).

Parameters	Paste treatments			Powder treatments		
	90% reduction	45% reduction	Regular	90% reduction	45% reduction	Regular
Moisture (% w/w)	30.0 $\pm$ 2.9	24.4 $\pm$ 2.0	21.6 $\pm$ 2.1	8.6 $\pm$ 0.3	7.0 $\pm$ 0.3	6.1 $\pm$ 0.03
Water activity	0.751 $\pm$ 0.004	0.691 $\pm$ 0.003	0.686 $\pm$ 0.002	0.301 $\pm$ 0.004	0.290 $\pm$ 0.004	0.254 $\pm$ 0.006
pH	5.96 $\pm$ 0.01	5.85 $\pm$ 0.00	5.75 $\pm$ 0.01	6.50 $\pm$ 0.02	6.28 $\pm$ 0.00	6.15 $\pm$ 0.01
Estimated salt (%NaCl, w/w)	3.5	18.2	33.0	3.1	18.5	34.0
Cooked chicken meat added (% w/w)	41.8	34.6	29.5	NA ^a	NA	NA

Data are presented as mean values with standard deviations of three replicates of samples.

^a Not applicable.

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