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Short communication

Salmonella survival during thermal dehydration of fresh garlic and storage of dehydrated garlic products

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ABSTRACT

Salmonella survival was characterized and modeled during thermal dehydration of fresh garlic and storage of dehydrated garlic products. In our experiments that simulated commercial dehydration processing at 80 ± 5 °C, moderate level of *Salmonella* contamination (4–5 log CFU/g) on fresh garlic was reduced below the enumeration limit (1.7 log CFU/g) after 4.5 h of dehydration and not detectable by culture enrichment after 7 h. With high level of contamination (7–8 log CFU/g), the *Salmonella* population persisted at 3.6 log CFU/g after 8 h of processing. By increasing the dehydration temperature to 90 ± 5 °C, the moderate and high levels of initial *Salmonella* load on fresh garlic dropped below the enumeration limit after 1.5 and 3.75 h of processing and became undetectable by culture enrichment after 2.5 and 6 h, respectively. During the storage of dried garlic products, *Salmonella* was not able to grow under all tested combinations of temperature (25 and 35 °C) and water activity (0.56–0.98) levels, suggesting active inhibition. Storage temperature played a primary role in determining *Salmonella* survival on dehydrated garlic flakes. Under a typical storage condition at 25 °C and ambient relative humidity, *Salmonella* could persist over months with the population gradually declining (4.3 log reduction over 88 days). Granular size of dehydrated garlic had an impact on *Salmonella* survival, with better survival of the pathogen observed in bigger granules. At the early stage of dehydrated garlic storage (until 7 days), rising water activity appeared to initially promote but then inhibited *Salmonella* survival, resulting in a water activity threshold at 0.73 where *Salmonella* displayed strongest persistence. However, this phenomenon was less apparent during extended storage (after 14 days).

1. Introduction

There has been increasing concerns over microbial food safety of spices (Beuchat et al., 2013; Van Doren et al., 2013b). While typically used as minor ingredients for seasoning purpose, large-scale outbreaks can originate from contaminated spices. For example, between July 2009 and April 2010, a U.S. nationwide outbreak of *Salmonella enterica* serotype Montevideo infections due to the consumption of ready-to-eat salami products was traced back to the contamination of imported black and red pepper (Gieraltowski et al., 2013).

The supply chains of spices are often complex, involving foreign sourced products, long-term storage and further processing before distribution. *Salmonella* contamination associated with spices has been found at multiple stages along farm-to-table continuum including the point of import (Van Doren et al., 2013a), domestic processing facilities (Lienau et al., 2011), and retail products (Sagoo et al., 2009). In 2013, the U.S. Food and Drug Administration published a draft risk profile on pathogen and filth in spices (FDA, 2013) highlighting the importance of identifying, assessing and controlling food safety risks associated with spices.

Risk assessment and contamination control of *Salmonella* on dried spices requires quantitative understanding of the pathogen's survival on the herbal products that often feature natural antimicrobial properties. Data gaps still remain regarding survival kinetics of *Salmonella* on spices especially when a well characterized natural antimicrobial compound is present and effectively inhibits *Salmonella* in situ. Keller et al. demonstrated that at a permissive a_w (> 0.96) and 35 °C *Salmonella* could grow to maximum population densities (~9 log CFU/g) on ground black pepper within 24 h (Keller et al., 2013). With *Salmonella* generation time on black pepper similar to that on egg yolk (Bradshaw et al., 1990) and beef (Dickson et al., 1992), the study showed that antimicrobial compounds of black pepper had limited impact on *Salmonella* growth under the tested conditions (Keller et al., 2013).

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Dehydrated garlic accounts for 75% of U.S. garlic consumption (Boriss, 2006) and is commonly used in a wide variety of processed food. It is one of the few spices in the US that require both large scale domestic production and imports to meet the demand. Since 2006, garlic import has surpassed its U.S. domestic production (FDA, 2013). Garlic is known for its antimicrobial property because of the compound allicin (Ankri and Mirelman, 1999). However, *Salmonella* contamination has been associated with fresh and dehydrated garlic (Bennett et al., 2003; Van Doren et al., 2013a), including recent recalls of garlic powder (FDA, 2015a, 2015b).

Large-scale commercial production of dehydrated garlic typically includes four major steps: 1) wet preparation, 2) dehydration, 3) storage and 4) milling and packing (De La Cruz Medina and Garcia, 2007). Firstly, whole fresh garlic bulbs are cracked into cloves and peeled by machine. Peeled cloves are washed, sanitized and then sliced into approximately 3 mm garlic flakes. Secondly, wet fresh garlic flakes are dried by heated air at about 65–80 °C in dryers for about 5 h to remove > 90% of moisture. Thirdly, dehydrated garlic flakes are stored as semi-products until milling. Prior to milling, garlic flakes are often re-dehydrated to about 6% moisture. Lastly, re-dried garlic flakes are sorted and milled into different sizes, such as chopped (5–8 mesh), minced (8–16 mesh), and powder (> 80 mesh). The finished products are sifted and typically subjected to metal detection before packing and shipping.

Dehydrated garlic production does not typically include a validated kill step such as pasteurization, chemical treatment and steam sterilization. While thermal dehydration reduces the risk of microbial contamination, *Salmonella* has been found to contaminate low water activity foods such as peanut butter (Centers for Disease and Prevention, 2007; Ma et al., 2009) whose processing involves thermal treatment.

In this study, we characterized *Salmonella* inactivation and survival during thermal dehydration of fresh garlic and extended storage of dried garlic granules. While both processes are essential to dehydrated garlic production and may promote the inactivation of *Salmonella*, no quantitative data had been available to evaluate whether *Salmonella* could still pose a risk to dehydrated garlic known for its natural antimicrobial activity after such processing. We further investigated and modeled in situ kinetics of *Salmonella* under various processing and storage conditions of dehydrated garlic including temperature, moisture and granular sizes.

2. Methods and materials

2.1. Bacterial strains

The same *Salmonella* strains used in Keller et al. (2013) were obtained from L. Beuchat, University of Georgia, including *S. enterica* serotype Enteritidis strain 2415 (originally isolated from raw almonds), *S. enterica* serotype Oranienburg strain 1839 (originally isolated from raw pecans), *S. enterica* serotype Tennessee strain K4643 (clinical isolate from the 2006 US salmonellosis outbreak caused by contaminated peanut butter) and *S. enterica* serotype Anatum strain 6802 (originally isolated from raw peanuts). Inoculum cocktails were prepared similar to Keller et al. (2013). Briefly, each strain was grown in 10 ml tryptic soy broth with 0.6% yeast extract (TSBYE) (Becton, Dickinson and Company) for 24 h at 37 °C then spread on a TSBYE plate. After 24 h incubation at 37 °C, each TSBYE plate was flooded by 1 ml sodium phosphate buffer (0.5 M, pH 7.0) and cells were harvested by gentle scraping and mixing with a sterile plate spreader. Cell suspension of each strain (~0.5 ml/strain) was collected. An equal volume of each serotype culture suspension was combined to create inoculum cocktails at a final concentration of 10 to 11 log CFU/ml. Each cocktail was diluted in sodium phosphate buffer prior to inoculation of garlic samples.

2.2. Determination and modeling of Salmonella survival during thermal dehydration of fresh garlic

Fresh garlic (*Allium sativum*, α_w = 0.97) cloves were purchased locally in Griffin, GA. Cloves with no visual sign of germination were cut into approximately 3 mm slices per with a sterile knife. Each 5.0 ± 0.5 g aliquot of fresh garlic slices was placed in a plate and surface inoculated with 0.1 ml of *Salmonella* inoculum cocktail at 9 to 10 log FU/ml (high inoculum, or 7–8 log CFU per gram of garlic) or 6 to 7 log CFU/ml (moderate inoculum, or 4–5 log CFU per gram of garlic). Inoculated fresh garlic slices were placed in a countertop oven and dried at 80 ± 5 °C or 90 ± 5 °C between 15 and 480 min. During dehydration, 5 g samples in triplicate were removed from the oven at various time points (15, 30, 45, 60, 90, 120, 150, 180, 225, 270, 315, 360, 420 and 480 min) for analysis. Each sample was placed in a sterile 50 ml conical tubes containing 45 ml buffered peptone water (BPW) or BPW with 0.5% K_2SO_3 (Acros Organics) and vigorously vortexed for 60 s. After mixing, samples were appropriately diluted, plated on xylose lysine deoxycholate (XLD) (Becton, Dickinson and Company) agar, and incubated at 37 °C for 24 h prior to enumeration by plate count. When *Salmonella* population dropped below limit of detection (1.7 log CFU/g), triplicate samples (< 2 g per sample after thermal dehydration) were placed in 200 ml TSB with 0.5% K_2SO_3 and incubated at 37 °C for 24 h. If no growth was observed in all three cultures, the sample was determined to be *Salmonella* negative (i.e., below the detection limit at 1 CFU/5 g of fresh garlic). The survival of *Salmonella* on fresh garlic during thermal dehydration was modeled using a Weibull model with Origin 9.0 software (version 9.0, Origin Lab, Northampton, MA). After dehydration, the α_w of an uninoculated sample (5 g) that was dehydrated along with inoculated samples was measured using an AquaLab series 4TEV meter (Decagon Devices, Pullman, WA).

2.3. Determination of Salmonella survival on dehydrated garlic under storage conditions

Dehydrated garlic flakes (Harmoni International Spice Inc., City of Industry, CA) that are typically stored for long periods of time before further processing into granules of smaller sizes were used for this study. Garlic powder was not included in this study due its agglomeration in the presence of liquid, which made precise adjustment of water activity difficult. The garlic flakes had been stored for about 12 months before the experiments. Each 25 g aliquot of flakes was placed into a Whirlpak bag (Nasco, Fort Atkinson, WI) and inoculated with 100 µl of inoculum cocktail reach 7 to 8 log CFU per gram of dehydrated garlic. After thorough mixing, inoculated dehydrated garlic flakes were stored at 25 °C or 35 °C under ambient or high humidity similar to Keller et al. (2013). For ambient humidity (i.e., relative humidity of the laboratory measured between 32% and 48% during the experiment), Whirlpak bags were kept unsealed during storage. For high humidity, saturated potassium sulfate solutions were placed in desiccator jars along with inoculated samples to maintain relative humidity at 97% (Keller et al., 2013). At selected time points until *Salmonella* population dropped below the limit of detection (1.7 log CFU/g), three bags were removed and 225 ml of BPW was added into each bag. Then each bag of mixture was pummeled by a stomacher (Seward Medical, Ltd., London, UK) for 30 s at medium speed. The resulting suspension was appropriately diluted with BPW and plated in duplication on XLD. All plates were incubated at 37 °C for 24 h before enumeration. Representative colonies from XLD were confirmed to be *Salmonella* by PCR using primers that target a 94-bp segment of the *Salmonella*-specific *ttr* gene (GenBank accession no. AF 282268) (Malorny et al., 2004). At each time point, a forth bag of uninoculated sample was also removed to measure the α_w of garlic flakes.