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Review

An overview of *Salmonella* thermal destruction during food processing and preparation

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ABSTRACT

Each year there are an estimated one million non-typhoidal *Salmonella* infections in the U.S. and about 20,000 of those infected persons require hospitalization. These infections cost Americans almost $4 billion per year. Worldwide, there are more than 80 million cases of foodborne salmonellosis. Numerous food preservation methods have been developed for extending the shelf life of food and inhibiting the growth of foodborne pathogens such as *Salmonella*. Food processing and preparation methods using heat (thermal treatments) are considered to be the most effective methods for elimination of *Salmonella* in food. In this review we discuss the use of thermal treatments for elimination of *Salmonella* in or on many food products, including poultry, meats, eggs, produce and low water activity foods.

Contents

1. Introduction

The bacterial genus *Salmonella* has only 2 species, *enterica* and *bongori*; *Salmonella enterica* is further divided into 6 subspecies, one of which is *S. enterica* subspecies *enterica* (Issenhuth-Jeanjean et al., 2014). The *enterica* subspecies encompasses 1586 serovars

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and is the only subspecies which contains human and/or animal pathogens (Issenhuth-Jeanjean et al., 2014). Animals used for human food consumption can be carriers of numerous serovars of *Salmonella*, some of which can cause disease in humans, although they may not cause disease in the carrier animals (Kingsley & Bäumler, 2000). This poses a challenge for the production of wholesome food products of animal origin. When food products are contaminated with sufficient quantities of *Salmonella* or are handled in such a way as to allow for the outgrowth of the organism in the food that is consumed, then salmonellosis in humans is possible.

There are an estimated one million human *Salmonella* infections, not including typhoid fever, in the U.S. each year with about 20,000 of those cases resulting in hospitalization (Scallan et al., 2011). Globally, best estimates put the number of foodborne cases of salmonellosis at 80.3 million (Majowicz et al., 2010). Diarrhea is the most consistent symptom of salmonellosis, although combinations of diarrhea, fever, abdominal cramps, and/or vomiting occur regularly (CDC, 2014a; O'Mahony et al., 1990). Salmonellosis usually occurs within 6–48 h of consuming a contaminated food, although longer time periods have been reported (Abe, Saito, Kasuga, & Yamamoto, 2004). Outbreak analyses suggest that the infectious dose of *Salmonella* could be as low as 1 to 10 cells in some individuals (D'Aoust, Warburton, & Sewell, 1985; Kapperud et al., 1990; Lehmacher, Bockemühl, & Aleksic, 1995) although data from some outbreaks has indicated the number of cells consumed was on the order of 10^6 per person (Abe et al., 2004).

Unlike many other foodborne pathogens, *Salmonella* has been implicated in outbreaks from a wide variety of food products including raw poultry, fresh sprouts, peanut butter and chocolate (CDC, 2007; CDC, 2014b; Van Beneden et al., 1999; Werber et al., 2005). *Salmonella* has traditionally been associated with poultry and egg products, but in recent years fresh produce, especially alfalfa sprouts, baby spinach, basil, cantaloupe, lettuce, peppers, and tomatoes have been found to be contaminated with this organism (Finstad, O'Bryan, Marcy, Crandall, & Ricke, 2012; Foley, Johnson, Ricke, Nayak, & Danzeisen, 2011; Franz & van Bruggen, 2008; Hanning, Nutt, & Ricke, 2009; Howard, O'Bryan, Crandall, & Ricke, 2012; Lynch, Tauxe, & Hedberg, 2009; Nayak, O'Bryan, Kenney, Crandall, & Ricke, 2012). *Salmonella* has been identified as the causative agent in 17% of fresh produce foodborne illness outbreaks for the period between 1998 and 2007 (Olaimat & Holley, 2012). It was estimated by Scharff (2010) that produce, either fresh or processed, was the cause of 27% of reported *Salmonella* outbreaks. Foods can be contaminated at any point in the food chain from production through processing, distribution, preparation and consumption (CDC, 2015a).

Numerous food preservation methods have been developed for extending the shelf life and inhibiting the growth of foodborne pathogens such as *Salmonella* (Chen et al., 2012; Gil et al., 2015; Ricke, Kundinger, Miller, & Keeton, 2005; Wheeler, Kalchayanand, & Bosilevac, 2014). These intervention treatments can be categorized as either thermal or non-thermal, with thermal including heat applied either directly such as in grilling or by the use of a heating medium such as water or steam; foods can also be heated with the use of thermal radiation (infrared or microwave). Non-thermal preservation methods include chemical, physical, or biological treatments including electron beam irradiation, high pressure processing, pulsed electric fields and ozone or ultraviolet light (Warriner, 2011). Thermal treatment is considered to be one of the more effective food processing techniques to eliminate *Salmonella* and other foodborne pathogens from food products (Bermúdez-Aguirre & Corradini, 2012; Silva & Gibbs, 2012). However, some *Salmonella* strains are capable of growing at temperatures as high as 54 °C and thus may survive thermal processing of some foods (Droffner & Yamamoto, 1991; Park et al., 2014).

Studies of thermal inactivation of *Salmonella* in foods such as those reported in this review have traditionally assumed that inactivation adheres to first-order kinetics; in other words there is a log linear decline in survivors based on time (Blackburn, Curtis, Humpheson, Billon, & McClure, 1997). However, it has been known for several years that there are many deviations from the first-order kinetics, including survival curves that are sigmoidal in shape as well as those with shoulders or tails (Cerf, 1977; Jackson, Hardin, & Acuff, 1996; Juneja, Eblen, & Marks, 2001; Mafart, Couvert, Gaillard, & Leguérinel, 2002). In order to avoid over- or under-processing food these deviations from first-order kinetics should be taken into account when developing guidelines for thermal treatment. This has led many researchers to develop mathematical models to predict effects of thermal treatments as well as combination treatments. A discussion of the models that have been developed is outside of the scope of this review, but several of the models and references are listed in Table 1 for further investigation by the reader.

This review considers the more recent literature on thermal processing of food products, pulls from fundamental microbiology to draw connections and overarching principles between studies and food products, notes the limitations of previous research and research technologies, and finally makes observations and recommendations for future research.

2. Thermal destruction of *Salmonella* in poultry

The United States Department of Agriculture-Food Safety and Inspection Services (UDSA-FSIS) has implemented a 7 $\log_{10}$ relative reduction in viable counts of *Salmonella* for fully and partially cooked poultry products (USDA-FSIS, 1999). Results from thermal inactivation experiments are often expressed as the D-value, the time needed at a particular temperature to inactivate 90% of the exposed bacteria (Table 2). Juneja et al. (2001) compared the inactivation of *Salmonella* in ground turkey and ground chicken and determined that the D-values for ground turkey were higher (0.59 min) than for ground chicken (0.50 min) at the highest temperature examined (65 °C). Murphy, Duncan, Beard, and Driscoll (2003) also concluded that D-values varied by animal species at lower temperatures, although at the highest temperature studied (70 °C) no statistical differences were seen among the D-values for duck breast meat, chicken breast meat or turkey breast meat (0.11, 0.10 and 0.12 min respectively). These authors concluded that there can be considerable differences in the time required for inactivation of *Salmonella* between avian species as well as with different fat levels within the same bird species at lower processing temperatures, but at temperatures as high as 70 °C these differences become insignificant.

Several researchers have also focused on methods of cooking poultry used in commercial kitchens or in homes. Murphy, Johnson, Marcy, and Johnson (2001) inoculated ground chicken patties with a cocktail of *Salmonella* serovars and cooked the patties in a pilot-scale air convection oven at an air temperature of 177 °C with either low or high humidity. The patties were cooked to a final center temperature of 65–75 °C. They determined that humidity affected survival of the pathogen, with *Salmonella* populations 2 logs higher in patties cooked under low as compared to high humidity. After cooking, *Salmonella* populations were also up to 6 logs greater when patties were allowed to touch or partially overlap as opposed to being cooked in a single layer (Murphy, Duncan, Johnson, & Davis, 2001).

The unsuitability of microwave ovens for cooking raw or partially cooked poultry products was highlighted by several

Table 1
Models used to determine thermal treatment times for foodborne pathogens.

Name of model	Reference
Log linear biphasic	Cerf, 1977
Modified Gompertz	Linton, Carter, Pierson, & Hackney, 1995
Fermi equation	Peleg, 1996
Empirical sigmoidal	Augustin, Carlier, & Rozier, 1998
Log linear with tail	Geeraerd, Herremans, & Van Impe, 2000
Log linear with shoulder	Geeraerd et al., 2000
Weibull	Mafart et al., 2002
Weibull with tail	Albert & Mafart, 2005
Biphasic with shoulder	Geeraerd, Vladramidis, & Van Impe, 2005
Double Weibull	Coroller, Leguerinel, Mettler, Savy, & Mafart, 2006
Polynomial secondary model	Juneja, Gonzales-Barron, Butler, Yadav, & Friedman, 2013

notable foodborne outbreaks. MacDougall et al. (2004) investigated several cases of foodborne illness with *S.* Heidelberg that occurred in Canada in 2002 and discovered that many of the foodborne illnesses were linked to consumption of frozen chicken nuggets or chicken strips. Further investigation revealed that many people mistakenly regarded the nuggets and strips to be fully cooked although they were in fact raw; it was hypothesized that the commercial par-frying process led to the confusion since it lends a golden color to the breaded chicken pieces. In addition, many people also cooked these items in a microwave, which is well known to produce non-uniform heating and thus may not completely inactivate all the pathogens uniformly in the product (Vadivambal & Jayas, 2010). Subsequent to this outbreak product labels were modified to alert consumers that the products were in fact raw and cooking instructions were modified to include directions that consumers verify heating to an internal temperature of 74 °C (165 °F) (USDA-FSIS, 2006). In spite of this, outbreaks continue to occur associated with raw, ready to cook products, especially those prepared in home microwave ovens. In 2008, Smith et al. reported on four outbreaks of salmonellosis that occurred in Minnesota between 1998 and 2006 that were all associated with raw, frozen, microwaveable, breaded, pre-browned, stuffed chicken products. Since these products were breaded and pre-browned most of the consumers who became ill believed the product was precooked, most of them used a microwave oven to cook the product, most did not follow package cooking instructions, and none took the internal temperature of the cooked product.

Misunderstanding and confusion with package labels appears to be a fairly common problem with consumers. For example, although 90% of participants in one study self-reported that they did read and follow label directions in preparing frozen ready to cook foods, only 61% were observed reading and correctly following cooking directions (DeDonder et al., 2009). DeDonder et al. (2009) also stated that 73% of respondents reported that they owned a food thermometer but only 5% reported using one regularly and less than 1% (only three of 41 participants) were observed using a thermometer to measure the temperature of a frozen, uncooked, breaded chicken product. Since consumers often do not fully follow the cooking instructions on the package, processors could reduce the risk of foodborne illnesses by not putting microwave instructions on these raw, ready to cook products as well as stating on the package “do not cook in microwave”. Another option is to discontinue the sale of frozen, raw, ready to cook products and sell only frozen fully cooked poultry products. Additional consumer-based research is needed to examine these options and determine what would have the most effective impact.

3. Thermal destruction of *Salmonella* in beef

Cattle can also serve as a reservoir for a number of foodborne pathogens including *Salmonella* which increases the concern for foodborne illness from beef products. Ground meats are more likely than intact cuts to contain *Salmonella* and there have been outbreaks associated with ground beef (CDC, 2013). Juneja and Eblen (2000) determined that fat content affected thermal resistance of *Salmonella* in ground beef, with *Salmonella* Typhimurium DT104 being more resistant to heat at higher fat levels. Smith, Maurer, Orta-Ramirez, Ryser, and Smith (2001) confirmed this effect of fat content and also determined that freezing ground beef before cooking decreased the heat resistance of *Salmonella* inoculated into the beef prior to freezing (Table 3). Stopforth, Suhalim, Kottapalli, Hill, and Samadpour (2008) tested the theory that multi-drug resistant (MDR) *Salmonella* would be more resistant to thermal inactivation than their non-drug resistant (NDR) counterparts. They performed thermal death time studies and plotted survivor curves for several serotypes of *Salmonella* isolated from cattle or cattle environments and detected no statistically significant differences in heat resistance, although in general the NDR serotypes appeared to exhibit a slightly higher heat resistance than their MDR counterparts, especially at 55 and 60 °C. They also cooked inoculated ground beef patties in a George Foreman style grill (Next Grilleration GRP99, George Foreman Grilling Machine, Lake Forest, IL) to an internal temperature of 71 °C and determined that *S.* Agona was able to survive whether drug resistant or not (Stopforth et al., 2008) (see Table 3).

Other factors may influence *Salmonella* response to thermal treatments in beef products. *Salmonella* may undergo acid stress in the natural environment as well as in processing plants (Yousef & Courtney, 2003). Singh, Simpson, Mullins, and Dickson (2006) investigated the effects of acid adaptation and storage on the thermal resistance of *Salmonella* and determined that acid-adapted *Salmonella* exhibited greater heat resistance at lower temperatures but at higher temperatures there was no difference in heat resistance of acid-adapted or non-adapted *Salmonella* inoculated into refrigerated ground beef. Mogollón, Marks, Booren, Orta-Ramirez, and Ryser (2009) determined that particle size (coarsely ground, finely ground or pureed beef) did not affect thermal resistance of inoculated *Salmonella*. Juneja, Hwang, and Friedman (2010) concluded that *Salmonella* in ground beef could be made less resistant to thermal treatments by the addition of sodium lactate or oregano oil. Manios and Skandamis (2015) ascertained that length of frozen storage and method of thawing did not affect the survival of *Salmonella* in cooked ground beef patties, but found that using an oven broiler for cooking was more effective at thermal destruction of *Salmonella* than grilling in a pan. Results of these studies suggest that while drug resistance does not affect the heat resistance of *Salmonella*, serotype or strain is an important consideration in risk

Table 2
D values of *Salmonella* in poultry.

Serotype	Medium	D value (min) at Temp (°C)							Reference
		55	57.5	60	62.5	65	67.5	70	
4 serotype mix	Ground breast, thigh	6.08	4.77	3.00	0.66				Juneja, 2007
6 serotype mix	Chicken breast	24.0	9.60	3.83	1.53	0.61	0.24	0.10	Murphy et al., 2003
	Turkey breast	24.1	9.60	3.83	1.53	0.61	0.24	0.10	
	Duck muscle	28.6	14.3	6.79	2.09	0.58	0.20	0.11	
	Duck skin	25.3	14.5	4.30	2.46	1.21	0.49	0.17	
8 serotype mix	Ground chicken 2% fat			4.83	1.14	0.415			Juneja et al., 2001
	Ground chicken, 6.3% fat			4.68	1.16	0.314			
	Ground chicken, 9% fat			5.40	1.16	0.529			
	Ground chicken, 12% fat			5.50	1.30	0.502			
	Ground turkey, 1% fat			4.56	1.53	0.589			
	Ground turkey, 7% fat			4.94	1.85	0.552			
	Ground turkey, 10% fat			5.13	1.45	0.569			
	Ground turkey, 12% fat			5.43	1.78	0.592			
6 serotype mix	Cooked chicken breast, ground	24.1	9.6	3.83	1.53	0.609	0.243	0.097	Murphy, Duncan, Johnson, Davis, & Smith, 2002

Table 3
D values of *Salmonella* in meat.

Serotype	Medium	D value (min) at Temp (°C)										Reference
		55	57.5	58	60	61	62.5	63	64	65	70	
6 serovar cocktail	Beef patties	9.09	7.70		4.80		2.40			0.97		Murphy et al., 2002
Senftenberg	Ground beef 19.1% fat			21.8		3.38			0.92			Smith et al., 2001
Typhimurium DT104 (10127)	Ground beef 19.1% fat	21.98		2.63		0.65			0.16			
Typhimurium DT104 (10127)	Ground beef 4.8% fat	9.05		2.26		0.57			0.15			
Typhimurium DT104 (10601)	Ground beef 4.8% fat	10.6		2.15		0.41			0.07			
Typhimurium DT104 (01071)	Ground beef 4.8% fat	10.27		2.06		0.43			0.14			
8 serotype cocktail, log phase	Ground beef 19.1% fat	16.3		2.72		0.44			0.15			
8 serotype cocktail, stationary phase	Ground beef 19.1% fat	18.7		3.39		0.57			0.20			
8 serotype cocktail, log phase patties frozen	Ground beef 19.1% fat	9.85		1.43		0.29			0.14			
8 serotype cocktail, stationary phase patties frozen	Ground beef 19.1% fat	12.5		2.36		0.28			0.20			
Typhimurium 14028	Brined ham			3.35	1.30			0.31				Quintavalla et al., 2001
Typhimurium 133				3.49	1.25			0.27				
Typhimurium 1116				4.13	1.14			0.28				
Derby				2.89	1.18			0.23				
Potsdam				4.80	1.57			0.30				
Menston				3.41	1.02			0.25				
Eppendorf				4.23	1.14			0.27				
Kingston				2.79	0.92			0.24				
6 serotype cocktail	Breaded pork patties	69.5	29.99		15.2		7.71			2.64	0.29	Osaili et al., 2007

assessment of the pathogen with regard to survival at cooking temperatures. Additionally, additives and method of cooking are also important parameters to consider.

Although the interior of intact cuts of beef are usually considered to be sterile, it is known that *Salmonella* can penetrate into the interiors of these cuts of meat under some circumstances (Gill and Penney (1977). Orta-Ramirez, Marks, Warsow, Booren, and Ryser (2005) determined that *Salmonella* in whole muscle was more resistant to heat than was *Salmonella* in ground beef. Mann and Brashears (2007) conducted a study to determine the contribution of humidity to the lethality of salmonellae during thermal processing. They inoculated beef top round roasts externally with a cocktail of *Salmonella* and cooked the roasts to an internal temperature of 62.8 °C using a constant cooking temperature of 82.2 °C while varying humidity from 0 to 90%. When humidity was less than 30% *Salmonella* reductions were less than the required regulatory performance standard of 6.5 log CFU. Calle, Porto-Fett, Shoyer, Luchansky, and Thippareddi (2015) surface inoculated boneless rib eye roasts with *Salmonella* and subsequently blade tenderized a portion of the roasts prior to searing, slow roasting and warm holding (130 °F; 54 °C) procedures as are typically done in many restaurants. Searing resulted in a reduction of close to 1.0 log CFU/g on all roasts while slow cooking reduced *Salmonella* a further 3 log CFU/g for both types of roast; however, when tenderized roasts were held at an internal temperature of 37.8 °C (rare to medium rare) *Salmonella* populations increased nearly 2 logs. These studies emphasize the necessity of developing cooking parameters for intact roasts rather than relying on thermal destruction parameters developed for ground beef. Some additional parameters that should be considered are humidity, cooking temperature, final internal temperature and temperature and length of time for warm holding.

During the production of beef jerky, heat is used to achieve low water activity, desired texture, and shelf stability. In spite of the low water activity, outbreaks of salmonellosis have been attributed to eating beef jerky (Eidson, Sewell, Graves, & Olson, 2000). Goepfert, Iskander, and Amundson (1970) suggested that the drying conditions provide sublethal conditions for *Salmonella* leading to increased heat resistance of some serovars. Buege, Searls, and Ingham (2006) determined that ensuring high wet-bulb temperatures early in the process followed by drying at 76.7 °C (dry-bulb temperature) resulted in *Salmonella* reductions of ≥6.4 log CFU/g. Harper et al. (2009) assessed processes used by both large scale and small scale jerky producers and determined that both processes led to a greater than 5 log reduction of *Salmonella* in the jerky. Jerky is also a popular item produced in the home by consumers using kits and home dehydrators. Borowski, Ingham, and Ingham (2009) studied the lethality of these home processes on *Salmonella*

inoculated into beef jerky batter subjected to a range of dehydrators, temperatures, and seasoning mixes. Beef jerky from only one dehydrator across all three seasoning mixes resulted in a 5 log reduction at any of the administered temperatures, indicating the likely inadequacy of these home processes to produce a safe product.

4. Thermal destruction of *Salmonella* in pork

Pork can be a major vehicle of foodborne salmonellosis throughout the world and a 2000 study of pork in US retail stores found that nearly 10% of retail samples of pork tested positive for *Salmonella* (Duffy et al., 2000). Salmonellosis in the US is associated more often with foods other than pork possibly because people tend to cook pork more thoroughly due to historical fears of *Trichinella*. However, the presence of *Trichinella* in pork during production in the U.S. is so rare, that no retail studies have been done since 1961 when retail fresh bulk, fresh link, and processed link pork sausages were sampled and were found to have an incidence of *Trichinella* of 1%, 2.4%, and 0.2%, respectively (Zimmermann, Schwarte, & Biester, 1961). In 2011 the USDA revised the recommended internal temperature for cooking of whole cuts of pork downward from 160 F (71° C) to 145 F (63° C) with a 3 min rest time (USDA-FSIS, 2013).

In 2015 a multi-state outbreak of salmonellosis associated with pork occurred that sickened 192 people (CDC, 2015b). Quintavalla, Larini, Mutti, and Burbuti (2001) determined the heat resistance of six *Salmonella* serovars in pork meat containing curing additives. As expected, the eight *Salmonella* strains were more resistant to heat in pork meat than in a liquid. Of the serotypes tested, *S.* Potsdam was the most resistant strain in pork meat, exhibiting D-values of 4.80, 1.57 and 0.30 min, at 58 °C, 60 °C and 63 °C, respectively (Table 3). The authors concluded that heating processes for cured pork that result in an internal temperature of 60 °C for 9–10 min or 63 °C for 3–4 min would be expected to provide a $\geq$ 7 log kill of the studied serotypes of *Salmonella*. In contrast, Osaili et al. (2007) found that breaded pork patties would require 106 min at 60 °C for a 7 log kill, but only 2.03 min at 70 °C (Table 3). Velasquez et al. (2010) also noted that *Salmonella* was more heat resistant in whole muscle pork than in ground pork. These studies emphasize the need to consider both the form of pork being cooked in determining cooking conditions as well as the final internal temperature necessary to inactivate *Salmonella* in pork products.

5. Thermal destruction of *Salmonella* in eggs

From 1998 through 2014 there were 101 outbreaks of salmonellosis associated with eggs leading to 2347 illnesses (Foodborne Outbreak Online Database, 2016). Cooking methods and pasteurization of liquid whole eggs, egg whites and yolks have been extensively investigated but there are fewer studies of in-shell pasteurization of eggs using heat. USDA-FSIS (2011) requires a 5 log reduction of *Salmonella* in shell eggs for them to be labeled pasteurized. Brackett, Schuman, Ball, and Scouten (2001) investigated the use of a humidity controlled oven for pasteurizing shell eggs inoculated with a *Salmonella* cocktail. They determined that treating the eggs at 57.2 °C at an unspecified humidity for 70 min produced at least a 5 log decrease in inoculated *Salmonella*.

Many commercial food products such as desserts, shakes and beverages are made with pasteurized liquid egg products; ready-to-eat foods such as ice cream, eggnog, cookie dough, smoothies, mayonnaise, and Hollandaise sauce that contain these products are not subjected to further lethal treatment steps and so the egg ingredients must be *Salmonella* free (USDA-FSIS, 2011). Pasteurization of liquid egg whites must take place at lower temperatures than other liquid egg products so that the albumin does not coagulate (Robertson & Muriana, 2004). Undiluted egg white will begin to coagulate at 60 °C while egg yolk does not coagulate until it reaches approximately 70 °C (Froning et al., 2002).

Salmonella is also known to be more resistant to heat at lower pH levels in egg white (Palumbo, Beers, Bhaduri, & Palumbo, 1996). Pasteurization methods for liquid egg white were developed at a time when eggs were usually stored before being broken for liquid egg products and thus the pH of the egg whites was typically approximately 9.0 at the time of processing (Himathongkham, Riemann, & Ernst, 1999), whereas currently eggs may be used on the same day of lay when the pH of the white is much less alkaline, normally 7.8 (Robertson & Muriana, 2004). Robertson and Muriana (2004) investigated the effectiveness of two commercial liquid egg white pasteurization processes and concluded that the preheat portion of both methods did not contribute to *Salmonella* reduction. They also observed a greater reduction of *Salmonella* when egg white pH was 9.0 rather than at 8.2. Nemeth, Friedrich, Balla, Mraz, and Suhajda (2011) developed a method of treating liquid egg products using a low temperature treatment. Packaged liquid egg products were held at 55 °C for 24 h; when the products were tested no residual *Salmonella* was detected. The authors did note that when this treatment was applied to already pasteurized product the *Salmonella* was not inactivated because it had become thermally resistant in response to the pasteurization (Nemeth et al., 2011).

Commercial mayonnaise formulations usually contain pasteurized 10% salted liquid egg yolk as an emulsifier. Fats and water-binding solutes such as salt increase the thermal resistance of *Salmonella* in liquid egg products (Doyle & Mazzotta, 2000). Gurtler, Marks, Jones, Bailey, and Bauer (2011) examined the thermal inactivation kinetics of heat-resistant salmonellae in liquid egg yolk with 10% salt added in order to develop a model to estimate decimal reductions of *Salmonella* in this product. The model they developed predicted that a 5 log reduction of *Salmonella* would occur at a temperature of 67.3 °C for 3.5 min, whereas with the current USDA minimum required pasteurization regimen (63.33 °C for 3.5 min), their model predicted a reduction of only 2.7 log. Thus, manufacturers of commercial salted, liquid egg yolks may want to verify the lethality of their respective processes.

Hard-boiling of eggs effectively kills any *Salmonella* present (Chantarapanont, Slutsker, Tauxe, & Beuchat, 2000) but cooking eggs to a medium or soft boil presents a risk for salmonellosis. Grijspeerdt and Herman (2003) found no influence of egg size on time needed to inactivate *Salmonella* during boiling of eggs, although they did determine that eggs taken directly from refrigeration (6 °C) required a longer cooking time than did eggs that had been held at room temperature (approximately 22 °C). After inoculating the yolk of intact shell eggs with *Salmonella*, De Paula, Mariot, and Tondo (2005) allowed the pathogen to grow within the egg to a level of approximately 7 log CFU before evaluating cooking methods for inactivating the *Salmonella*. When inoculated eggs were placed in boiling water and allowed to boil for 1 min (yolk still visibly runny) *Salmonella* was reduced to 1.4 log CFU/ml. Three minutes in boiling water decreased *Salmonella* below the detection limit (1.0 log CFU/ml) and provided a visibly solid yolk. When eggs were placed in room temperature (approximately 21 °C) water which was subsequently brought to a boil, *Salmonella* was reduced below the detection limit after boiling for 1 min and yolks became solid. Vegetable oil was preheated in a frying pan and contaminated eggs were fried for 1.5, 2.0 and 2.5 min; *Salmonella* was reduced approximately 2.75 log CFU/ml at all times tested and there was no significant difference in reduction between cooking times (De Paula et al., 2005). Although a visibly solid yolk has often been used to indicate safe cooking of eggs, it should be noted that

the egg fried for 2.5 min and with a completely solid yolk still had greater than 2.0 log CFU/ml of detectable *Salmonella*.

6. Thermal treatment of produce to destroy *Salmonella*

6.1. Sprouts

Seed sprouts as a food originated in the Far East countries but have spread into the Western world within the past several years because of their image as a healthy food that contains protein, carbohydrate, vitamins and minerals (Bari, Inatsu, Isobe, & Kawamoto, 2008). In the Far East, sprouts are typically thoroughly cooked before consumption, but in many Western markets, sprouts are usually eaten raw as a component of salads or on sandwiches or lightly cooked in various dishes (Bari et al., 2008). In spite of their healthy image, sprouts have been associated with numerous outbreaks of foodborne illnesses, many caused by *Salmonella*. Between 1998 and 2014 in the US alone there were 35 sprout associated salmonellosis outbreaks with 1555 victims (Foodborne Outbreak Online Database, 2016). Most of the outbreaks associated with sprouts have been traced back to contaminated seeds; significant increases in *Salmonella* cell populations can develop during the storage, sprouting, and handling of seeds, potentially exceeding 7 log CFU/g (Jaquette, Beuchat, & Mahon, 1996).

Numerous chemical treatments have been studied for both seeds and sprouts, but many countries do not permit chemicals and therefore alternate means of disinfection need to be examined (Weiss & Hammes, 2005). Consequently non-chemical based interventions such as thermal treatments are attractive alternatives. However, optimizing the temperature and time of exposure requires determining the maximum *Salmonella* reduction while maintaining seed germination viability. Hot water treatment at 85 °C for 1 min was found to be ineffective in eliminating naturally occurring *S*. Mbandaka from alfalfa seeds that were to be used for sprouting, but reduced seed germination rates were observed following heat treatments of 65 °C for 6 min or 70 °C for 4 min (Suslow, Wu, Fett, & Harris, 2002). Weiss and Hammes (2005) inoculated alfalfa, mung bean and radish seeds with approximately 7 log CFU/g of *S*. Senftenberg W775 and *S*. Bovismorbificans then dried and stored the seeds at 2 °C for up to eight weeks. They determined that moist thermal treatment of mung bean seeds at 80 °C for 2 min reduced *S*. Bovismorbificans more than 5 log CFU/g but not *S*. Senftenberg W775. Temperatures of 62 °C for 2 min reduced *S*. Senftenberg W775 more than 5 log CFU/g on both radish and alfalfa seed and S. Bovismorbificans on alfalfa seed, but *S*. Bovismorbificans was only reduced by 3 log CFU/g on radish seed at that temperature. These thermal treatments still permitted at least 95% of the seeds to germinate. Studer, Heller, Hummerjohann, and Drissner (2013) investigated the use of steam for inactivating *S. Weltevreden* inoculated onto alfalfa or mung bean sprouts. Steam for 180 s reduced *Salmonella* on alfalfa seeds while only 90 s of treatment was needed to reduce *Salmonella* on mung bean seeds below the detection level. Germination rates of treated mung bean seeds were not lower than that of the controls while germination rates of treated alfalfa seeds were significantly lower than that of controls (Studer et al., 2013).

Feng, Churey, and Worobo (2007) inoculated alfalfa seeds with five strains of *Salmonella* at either a low (2 log CFU/g) or high (8 log CFU/g) level and subjected the seeds to dry heat at 55 °C for as long as 8 days. Five-log reductions in *Salmonella* were observed for both inoculation levels; however when the seeds with high inocula were sprouted, high levels of *Salmonella* were detected in the sprouts. Uninoculated sprouts heated at 55 °C for 8 days maintained a 75% germination rate as compared to 83% for non-heat treated seeds. Neetoo and Chen (2011) found dry heat at 55 °C and 60 °C for 10 days achieved no more than 2 log CFU reductions in a *Salmonella* cocktail artificially inoculated on alfalfa seeds; however, 65 °C for 10 days or 70 °C for 24 h could achieve a 5 log CFU reduction. The addition of high hydrostatic pressure increased the lethality of the treatment and allowed for a shorter period of thermal processing (Neetoo & Chen, 2011). Unfortunately germination yield was negatively influenced by these treatments; the least detrimental treatment on germination percentage and sprouting yield was 65 °C for 10 days (Neetoo & Chen, 2011).

A variety of factors can influence the efficacy of dry heat to eliminate *Salmonella* on sprouts, including water activity (a_w) and storage conditions (Neetoo & Chen, 2014). Seeds with an a_w of 0.3 or 0.5 were more resistant to thermal inactivation of *Salmonella* at 65 °C as compared with seeds with an a_w of 0.6 (Neetoo & Chen, 2014). However, freezing prior to heat treatment increased heat inactivation of *Salmonella* in seeds with higher (0.6) as compared to seeds with lower a_w (0.3–0.5) (Neetoo & Chen, 2014). Hong and Kang (2016) inoculated alfalfa seeds with *S*. Typhimurium and subjected them to dry heat at 60, 70 or 80 °C for up to 24 h and found that populations of *Salmonella* were reduced by 0.26–2.76 log CFU/g, germination rates of the heat treated seeds ranged from 88 to 94%. When heat treated seeds were soaked in distilled water for 10 min and dried at room temperature before germination the germination rates increased to between 91 and 94% (Hong & Kang, 2016).

Moist and dry heat as well as steam all appear to be effective for decontaminating certain seeds for sprouting. However, a single treatment does not appear to be suitable for all types of seeds and other factors including storage time and temperature, water activity and the type of seed itself can also impact the final results. All of these factors should be taken into account when developing a process to decontaminate a particular seed. Some of the major retailers in the US have refused to sell sprouts because of their concern as to the elevated risk (Weise, 2012).

6.2. Cantaloupe

Salmonella associated with cantaloupe has led to 21 foodborne illness outbreaks involving 897 illnesses in the US between 1998 and 2014 (Foodborne Outbreak Online Database, 2016). Chemical treatments of various forms have been investigated for use on cantaloupe with minimal success, especially when the contaminating bacteria had been on the rind of the melon for 24 h or more (Ukuku & Sapers, 2001). However, increasing the temperature does appear to improve efficacy of chemical treatments. Annous, Burke, and Sites (2004) obtained greater than 5 log reductions of inoculated *Salmonella* on the surface of cantaloupe with the use of water at 76 °C for 3 min. Likewise, Ukuku, Pilizota, and Sapers (2004) achieved a 3-log reduction with a treatment of 60 s at 97 °C using a laboratory-scale water bath. However, fresh cut pieces of melon from the treated melons were still positive for *Salmonella* after enrichment when the inoculum was 4.7 log CFU/cm^2 (Ukuku et al., 2004). Melons with a starting inoculum of 3.5 CFU/cm^2 when treated with 97 °C did not generate positive enrichments, but those with only a 70 °C treatment were positive. Lastly, enrichment failed to recover *Salmonella* from melons with an initial inoculum of 1.9 log CFU/cm^2 regardless of treatment (Ukuku et al., 2004). Solomon, Huang, Sites, and Annous (2006) used water (room temperature, 65 °C, 75 °C, and 85 °C) for 10, 30, 60, and 90 s to treat experimentally inoculated cantaloupes (inoculated and stored for 24 h at 4 °C prior to heat treatment). A temperature of 75 °C elicited a 3 log reduction with 90 s of treatment, and exposure to 85 °C achieved approximately a 4.5 log reduction within 60–90 (Solomon et al., 2006). However, even 90 at 65 °C only led to a 1 log reduction

(Solomon et al., 2006). Annous, Burke, Sites, and Phillips (2013) reported that 60 or 90 in 92 °C hot water resulted in at least a 5 log reduction in a laboratory scale pasteurizer, while 82 °C caused slightly less than a 3 log reduction. The a_w or state of desiccation were not evaluated for this study, however the cantaloupes were inoculated, air-dried in a biosafety hood for 2 h, and stored at 32 °C in plastic tubs lined with absorbent paper for 24 h prior to hot water treatment (Annous et al., 2013). In commercial scale equipment, approximately 5 log reductions were observed with either of the temperatures after either 60 or 90 of treatment (Annous et al., 2013). However, these treatments did not completely destroy all viable *S*. Poona because some melons remained positive after enrichment (Annous et al., 2013). They also evaluated the distribution of bacteria on the melon rind, finding that it was unequally distributed with the stem end supporting more growth during storage than the blossom end or the rest of the rind and bacteria at the ends may have been more resistant to hot water treatments (Annous et al., 2013).

The potential downside to using hot water is degradation of the cantaloupe flesh due to elevated temperatures. Solomon et al. (2006) noted rind softness after 90 at 85 °C. Darkening of the flesh was previously reported after 4 min at 96 °C, but not after 1–3 min (Annous et al., 2004). However, unlike other products where thermal treatments may produce unwanted side effects, some produce may actually benefit from treatment. In this case, cantaloupe shelf-life appeared to increase due to hot water treatments (Annous et al., 2004). Annous et al. (2004) found that treatments for 2 min at 76, 86, or 96 °C all achieved at least a 2 log reduction of the endogenous microbial populations on the uninoculated melon rind. In addition, after 21 days of storage at 4 °C the heat treated cantaloupes were still firm whereas the controls were softening and becoming moldy. Ukuku et al. (2004) found that 60 at 50 °C had no effect on indigenous bacteria while 60 °C produced approximately a 1 log reduction, 70 and 80 °C generated approximately a 4 log reduction, and 90 °C produced a 4.5 log reduction of indigenous mesophilic bacteria on cantaloupe rinds. Sixty seconds at either 70 or 90 °C were also effective at reducing indigenous yeasts and molds (Ukuku et al., 2004). Thus the extended shelf-life is potentially mediated through a reduced microbial load on the cantaloupe rind. Many restaurants now use water circulators on a regular basis (Baldwin, 2012) making such hot water treatments a possibility. This leads into a potentially intriguing concept for future work. While multiple hurdles are often employed at processing/manufacturing they are not generally used in retail establishments beyond just simple cooking. Potentially some of these procedures commonly used in earlier production steps could be adapted for restaurant or retail use, thus introducing another hurdle. A 5 log reduction at the packing shed followed by another 5 log reduction at the restaurant, prior to storage or cutting, could increase product safety, assuming no re-contamination.

7. Thermal destruction of *Salmonella* in low water activity (a_w) foods

Reducing the available water in foods to prevent bacterial growth is one of the oldest forms of food preservation (Brown, 1976). Water activity ranges from 0 (absolutely dry) to 1.0 (pure water). *Salmonella* grows best at an a_w of 0.99 but can survive for long periods of time in low a_w foods, that is those foods with an a_w below 0.85 (Tamminga, Beumer & Kampelmacher, 1976). Low a_w foods do not support the growth of foodborne pathogens, including *Salmonella* spp. (Podolak, Enache, Stone, Black, & Elliott, 2010). Nevertheless, foodborne outbreaks of salmonellosis have originated from these foods (CDC, 1993, 1998; Craven, Mackel, Baine, Barker, & Gangarosa, 1975; Rushdy et al., 1998). These outbreaks occurred because even though low a_w foods do not support the growth of *Salmonella*, apparently any contaminating bacterial cells are still able to survive on these foods.

7.1. Nuts

7.1.1. Almonds

Outbreaks of salmonellosis have been associated with consumption of contaminated almonds (CDC, 2004; Isaacs et al., 2005; Ledet-Müller et al., 2007) and peanuts (Killalea et al., 1996; Kirk et al., 2004; Shohat et al., 1996). The first outbreak of salmonellosis associated with raw almonds occurred in 2000 resulting in 168 illnesses (Isaacs et al., 2005). Prior to this outbreak, raw almonds were not considered a potential vehicle for *Salmonella* transmission (Isaacs et al., 2005). These documented outbreaks led to US regulations, implemented in 2007, which require all California-grown almonds sold in North America to be processed with a treatment capable of achieving a minimum 4-log reduction in *Salmonella* (Federal Register, 2007). Survival of *Salmonella* on almond kernels depends on several factors, including storage temperature. Uesugi, Danyluk, and Harris (2006) inoculated almond kernels with *S. Enteritidis* at quantities varying from 1 log CFU to 8 log CFU/kernel that were stored at room temperature (23 °C) for up to 161 days. All levels of inocula exhibited the same rate of reduction, approximately 0.20 log CFU per month. They further evaluated kernels inoculated with 7 or 8°log°CFU/kernel and stored at freezing (−20 °C), refrigeration (4 °C), room temperature (23 °C) or with heat (35 °C) for 171 or 550 days. Neither freezing nor refrigeration resulted in any significant reductions in *Salmonella* at 550 days. With heat, *Salmonella* decreased at a rate of 1.1°log CFU/month until 59 days, but there was no significant reduction thereafter. Infrared (IR) heating of dry kernels for 30, 35 and 45 s followed by immediate cooling at room temperature, yielded a 0.63-, 1.03-, and 1.51-log reduction in inoculated *S. Enteritidis* populations, respectively (Brandl, Pan, Huynh, Zhu, Y. & McHugh, 2008). When kernels were exposed to IR heating then held at a warm temperature (above 80 °C) for 60 min, a greater than 7.5-log reduction was achieved and moisture loss from the kernels was low (Brandl, Pan, Huynh, Zhu, & McHugh, 2008).

Jeong, Marks, and Orta-Ramirez (2009) evaluated moist air convection as a means to inactivate *Salmonella* on almonds across the parameters of temperature, air moisture content, and time. They found that the moisture content at the surface of the almond had a greater impact on the inactivation kinetics than the air moisture; using this information they modified the traditional D- and z-value model to include the changes in moisture content at the surface of the product under humid heating conditions (Jeong et al., 2009). Bari et al. (2009) inoculated raw almonds with a cocktail of *Salmonella* and evaluated dry heat or hot water followed by IR heating for inactivation of the *Salmonella*. Initial levels of *Salmonella* were 5.7 log CFU/g with dry heat (60 °C for 4 days) followed by IR drying for 70 s reducing the population levels approximately 1 log CFU/g. Hot water (85 °C for 40 s) followed by IR drying for 70 s reduced the levels below the detection limit (10/g) by direct plating, but not when determined by enrichment. Du, Abd, McCarthy, and Harris (2010) inoculated whole almond kernels with *S. Enteritidis* PT 30 or *S.* Senftenberg 775W and subsequently heated them in oil. Rapid reductions of 2.9, 3.0, or 3.6 log CFU/g for *S. Enteritidis* were observed after 30 s of exposure to oil at 116, 121, or 127 °C, respectively and for *S.* Senftenberg at 127 °C. Both *Salmonella* serovars were undetectable by enrichment when almonds were inoculated at 5 log CFU/g followed by heating at 127 °C for 1.5 min, and the authors concluded that standard hot oil roasting times that exhibit acceptable color and texture would inactivate

more than 5 log CFU/g of *Salmonella* (Du et al., 2010).

Yang, Bingol, Pan, Brandl, McHugh, and Wang (2010) evaluated the effect of traditional hot air roasting, IR roasting or a combination of sequential IR treatment with traditional hot air technology (SIRHA) on *Pediococcus* (as a surrogate for *S. Enteritidis* PT30). SIRHA roasting at 130, 140 and 150 °C to generate a medium roasted almond product was the only method to deliver the required 4 log reduction, producing 4.10-, 5.82- and 6.96-log reductions of bacteria. Another group used IR treatments at 100, 110, and 120 °C alone or immediately followed by holding at 70, 80, or 90 °C for up to 60 min on *Pediococcus* inoculated almonds (Bingol et al., 2011). They found that IR alone produced no more than a 0.62 log reduction, but they observed that holding almonds at 90 °C for 10–15 min reduced the *Pediococcus* population greater than 5-logs and holding at 80 °C for longer than 22 min provided more than the required 4-log reduction (Bingol et al., 2011). They speculated that the IR treatment injured the cells which in turn made them more susceptible to holding temperatures (Bingol et al. 2011). Abd, McCarthy, and Harris (2012) inoculated almonds with *S. Enteritidis* PT 30 and stored them at 4 or 23 °C for up to 48 weeks. At varying times almonds were removed from storage, immersed in oil at 121 °C for 0.5–2.5 min, drained, and direct plated for *Salmonella* enumeration. *Salmonella* declined by approximately 2 log CFU/g at 23 °C but less than 1 log CFU/g at 4 °C after 48 weeks. They developed a model to determine the amount of time needed in hot oil to produce a 4 log CFU/g reduction of *Salmonella* on the stored almonds and predicted 0.85 min for almonds stored at 4 °C and 1.6 min for almonds stored at 23 °C. Harris, Uesugi, Abd, and McCarthy (2012) evaluated the kinetics of artificially inoculated raw almonds in a water bath treatment as might be used during almond blanching. The inactivation curves were also upwardly concave, however they chose to use traditionally calculated D-values with linear regression. Processing at 88 °C for 2 min achieved a 7-log reduction, and neither *Salmonella* serovar could be recovered by enrichment (Harris et al., 2012). Lambertini, Danyluk, Schaffner, Winter, and Harris (2012) have emphasized that raw almonds are currently rarely sold, most almonds having been treated with at least one validated process making the current regulations effective in maintaining the risk of salmonellosis from almonds less than one case per year.

7.1.2. Pecans

Routine testing of pecans for *Salmonella* has led to several recalls when nuts were found to be contaminated (Palumbo, Beuchat, Danyluk, & Harris, 2015). While *Salmonella* has been found to be able to penetrate the shells of pecans (Beuchat & Mann, 2010a), it can also survive on nut surfaces during storage for at least 18 months (Beuchat & Mann, 2010b). The survival of *Salmonella* on pecans after either hot air or hot oil treatments was investigated and it was determined that the use of hot oil greatly decreased the time required to reduce bacterial populations by 4 or 5 log units, indicating that typical times used to oil roast pecans are sufficient (Beuchat & Mann, 2011). Hot air treatment at 120 °C for 20 min reduced the number of *Salmonella* by approximately 2 log CFU/g, but did not completely eliminate *Salmonella*. The authors concluded that hot air treatment alone cannot be relied upon to reduce *Salmonella* by 5 log CFU/g without changing sensory qualities.

7.2. Peanut butter

In the 1990s an international outbreak of salmonellosis was associated with peanut butter coated snacks followed by an outbreak in Australia implicating nine different brands of peanut butter (Killalea et al., 1996; Scheil, Cameron, Dalton, Murray, & Wilson, 1998; Shohat et al., 1996). Peanut butter is pasteurized at temperatures of 70–75 °C before packaging, but several researchers suggested that *Salmonella* would exhibit increased heat resistance in this food since it possessed low a_w and high fat content (Juneja & Eblen, 2000; Juneja et al., 2001; Mattick, Jorgenson, Logan Lappin-Scott & Humphry, 2000). Shachar and Yaron (2006) set out to determine if the parameters for pasteurizing contaminated peanut butter were adequate. They concluded that serovars Agona, Enteritidis, and Typhimurium, recovered from contaminated peanut butter, were highly resistant to heat, thus the pasteurization process did not consistently inactivate all *Salmonella* when peanut butter was highly contaminated, and longer exposure times or higher temperatures did not improve the process (Shachar & Yaron, 2006). Park, Oh, and Kang (2008) inoculated five commercial brands of peanut butter with *S.* Tennessee and examined survival when stored for up to 14 days at refrigeration (4 °C) and room temperature (22 °C). *Salmonella* was inoculated at 10^{6-7} CFU/g and decreased by 0.15–0.65 and 0.34 to 1.29 log CFU/g at 4 °C and 22 °C, respectively, depending on the brand.

Ma et al. (2009) compared the thermal inactivation rates of *S.* Tennessee strains recovered from peanut butter involved in an outbreak to *S.* Tennessee strains not associated with the outbreak as well as to serovars Enteritidis, Typhimurium and Heidelberg. They inoculated commercial peanut butter with the isolates and heated the peanut butter to temperatures of 71, 77, 83, or 90 °C. A composite of the three outbreak strains required a significantly longer time (120 min) at 90 °C to provide a 7 log reduction than did the non-outbreak strains (55–86 min). The authors concluded that although the outbreak strains were more thermotolerant, it was not a characteristic of the serovar, since non outbreak *S.* Tennessee were less heat tolerant (Ma et al., 2009).

He et al. (2013) determined that increasing the water activity of peanut butter prior to pasteurization reduced the heat resistance of inoculated *Salmonella*. They also compared low fat and regular fat formulations of peanut butter and found that higher fat and lower carbohydrate contents may lead to reduced heat resistance (He et al., 2013). Li, Huang, and Chen (2014) also observed that there was higher heat resistance in the low fat peanut butter and peanut spreads. Kataoka et al. (2014) however did not observe a difference in survival of *Salmonella* in peanut pastes related to fat level, although *Salmonella* did survive better in pastes with a lower a_w. *Salmonella* survived in the pastes for 12 months, much longer than the shelf life of typical peanut butter products (Kataoka et al., 2014). Song and Kang (2016) evaluated the use of microwave heating to inactivate *Salmonella* in peanut butter. Inoculated peanut butter was treated using a 915 MHz microwave at 2, 4 and 6 kW power. The six kW 915 MHz microwave treatment for 5 min reduced the *Salmonella* levels 3.24 to 4.26 log CFU/g, without resulting in any deleterious effects on quality parameters of the peanut butter.

8. Conclusions

Doyle and Mazzotta published a review of the thermal resistance of *Salmonella* in foods in 2000. As expected, since their review was published there have been many more studies done both on commercial thermal processing as well as cooking methods to inactivate *Salmonella* in contaminated foods. Since their review there have been more than 2000 peer reviewed papers written dealing with the thermal resistance of various *Salmonella* both in foods as well as nonfood products. In this review we have covered some of the more recent information on thermal treatment for certain foods contaminated with *Salmonella* in meats, eggs and some low a_w foods.

In examining more recent research efforts, several common themes emerge. In addition several factors influence the

effectiveness of thermal intervention and to some extent are independent of temperature and time of exposure. For example, while meats and some non-meats have been thoroughly investigated, often in response to an outbreak, information is still lacking on heat resistance in many seafoods and fruits and vegetables. Differences in the reaction of different *Salmonella* serovars to heat as well as strain differences within serovars have also been observed. Highly heat resistant strains and serovars should be identified and used in further studies to identify the range for safe processing criteria. Food components also influence heat resistance indicating a need for further research in different formulations as well as in foods that contain other preservatives such as bacteriocins. The most effective methods of processing may prove to be combinations of thermal and other technologies to provide a multiple hurdle effect. Finally, a more in-depth understanding of the biology of *Salmonella* and the mechanistic capacity of different serovars to respond to thermal stress is needed. This understanding is becoming more of a possibility given the recent advances made at the molecular level and should provide explanations and even potentially predict the potential of variable responses to thermal applications.

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