AGRICULTURE, FOOD & ANALYTICAL BACTERIOLOGY

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Agriculture, Food and Analytical Bacteriology

CONFERENCE PROCEEDINGS*- REVIEW
Physical and Chemical Control of *Salmonella* in Ready-To-Eat Products

O. K. Koo[1], S. A. Sirsat[2], P. G. Crandall[1] and S. C. Ricke[1]

[1] Center for Food Safety, Dept. of Food Science, University of Arkansas, Fayetteville, AR 72704
[2] Conrad N. Hilton College of Hotel and Restaurant Management, University of Houston, Houston, TX 77204-3028

*Arkansas Association for Food Protection (AAFP) Conference, Enhancing Food Protection from Farm to Fork, held on Sept. 28-29, 2010, Springdale, AR.

ABSTRACT

The focus on post-processing contamination of foodborne pathogens in ready-to-eat (RTE) products has been mostly associated with outbreaks by *Listeria monocytogenes*. However, recently USDA-FSIS announced a guideline for small plants on RTE meat to control pathogens including *L. monocytogenes* as well as *Salmonella* due to an increase in outbreaks. *Salmonella* causes the second highest illness among foodborne pathogens and the growth of this pathogen needs to be inhibited since RTE products are minimally cooked. For physical control methods, heating is the most common method employed but irradiation has also been actively studied for RTE products. Organic acids are a common method for chemical control of *Salmonella*. Essential oils are recommended as a natural antimicrobial agent and they are effective against foodborne pathogens. However, they can change the flavor and texture of the food product. Multiple hurdle technology with the combination of physical, chemical or biological agents can be more effective if combinations can be optimized for maximum effect. Hurdle technology can also reduce the chances of microbial resistance against antimicrobial agents. Microarray analysis of gene expression profile by antimicrobial treatments may help to identify the most applicable treatments to target pathogens and maximize the effectiveness of hurdle technology. Application of appropriate control methods to RTE products is required for effective control of target pathogens without affecting organoleptic properties.

Keywords: ready-to-eat, *Salmonella* spp, antimicrobial, hurdle technology

Agric. Food Anal. Bacteriol. 2: 56-68, 2012

FOODBORNE PATHOGENS

Contamination of pathogen in food products has been a constant concern for humans due to severe health threats and economical loss. Annually, 9.4 million illnesses are caused by foodborne pathogens with 55,961 hospitalizations and 1,351 deaths. Among the number of illness, 5.5 million (58%) people are infected by norovirus and the second highest is 1.0 million illness (11%) by nontyphoidal *Salmonella* spp (Scallan *et al.*, 2011). Nontyphoidal *Salmonella* spp, norovirus, *Campylobacter* spp and *T. gondii* caused the most hospitalizations; and nontyphoidal *Salmonella* spp, *T. gondii*, *L. monocytogenes* and norovirus caused the most deaths (Scallan *et al.*,

Released Online Advance Publication: July 1, 2011.
Correspondence: Steven C. Ricke, sricke@uark.edu
Tel: +1 -479-575-4678 Fax: +1-479-575-6936

2011). Overall cost for foodborne illness in the US was estimated to be 152 billion dollars annually, out of which 39 billion dollars was associated with fresh, canned and processed produce (Moran, 2010).

SALMONELLA PATHOGENESIS

Salmonella is Gram-negative, non-spore forming bacillus, and facultative anaerobe that can grow at 5 to 45°C and form long filamentous chains at extreme conditions, such as 4 to 8°C or 44°C and pH 4.4 or 9.4 (Bhunia, 2008). Poultry is a major source of contamination particularly in high-density farms where transfer of pathogens can occur rapidly between birds. Moreover, *Salmonella* colonizes the intestine of the bird and can cross-contaminate the carcass during slaughter (Bryan and Doyle, 1995; Park *et al.* 2008). Salmonellosis is an animal origin foodborne disease including poultry, meat, milk and eggs; however more recent *Salmonella* outbreaks have also involved in other foods such as fruits, vegetables, and ready to eat (RTE) products. These outbreaks are mostly due to nontyphoidal *Salmonella* and the symptoms are self-limiting and subside within 3 to 4 days for healthy individuals. However, the symptoms can be severe and potentially fatal in young children and the elderly (Pegues and Miller, 2010). *Salmonella* can cause systemic disease by invading intestinal cells, and subsequently be transported to liver, spleen and mesenteric lymph nodes. The pathogen further causes neutrophil infiltration, tissue injury, fluid accumulation and diarrhea (Bhunia, 2008). A septic shock can develop as well. Infectious doses can be vary from 1 to 10^9 CFU/g. A human subject based study demonstrated on infectious dose with at least 10^5 cells, however outbreaks have been associated with as low as 10 cells (Todd *et al.*, 2008).

READY TO EAT PRODUCTS

Ready to eat (RTE) food products have been increasingly popular in recent years since they involve very little preparation time and the consumer does not require extensive cooking skills. RTE products include seafood, meat and poultry, dairy products, confectionaries, fruits and vegetables and RTE meal segment. USDA-FSIS defined the RTE meat and poultry product as "a product that is in a form that is edible without additional preparation to achieve food safety and can include frozen meat and poultry products" (9 CFR part 430) (USDA-FSIS, 1999). This meat product as described by USDA-FSIS represents as popular and easily consumed component of the human diet. However, RTE products can be a food-safety concern, since consumers eat the products without further cooking. Without any proper process to eliminate foodborne pathogens, the pathogens in the contaminated food product will be able to survive and grow during storage. Contamination can occur during packaging or further processing after cooking the product at the manufacturing facility, retail store or at the domestic environment.

RTE meats have often been implicated with *L. monocytogenes* contamination, a foodborne pathogen that can survive under extreme conditions such as low temperature and high sodium concentration. *L. monocytogenes* causes severe disease with high mortality rate and is particularly deadly to the immunocompromised and pregnant women by causing spontaneous abortion (Lecuit, 2007). There have been several outbreaks due to listeriosis; delicatessen turkey in 2000 and 2002 for 30 and 54 cases, hot dogs in 1998 to 1999 for 108 cases in multistate in the US, 279 cases in France in 1992 by pork tongue in jelly, and 366 cases in 1987 to 1989 by Paté in the UK (Swaminathan and Gerner-Smidt, 2007). Recently, *Salmonella* has also been implicated in contamination in RTE products. For instance, salami contaminated with *Salmonella* Montevideo resulted in 272 cases in 44 states during 2009 to 2010 (CDC, 2010). The origin of contamination was found to be the dried black pepper spice in the salami. Further investigation revealed that the contamination occurred after the salami underwent lethality steps, the raw ingredients (i.e. in this case the black pepper spice) were added to the salami (CDC, 2010). The Risk Ranger program assessed high on *Salmonella* risk in all food categories in pork and poultry meat products, raw, partially cooked and processed meat (Matargas *et al.*, 2008). *Salmonella* was shown to have a high-risk

score for both high and low risk population, while *L. monocytogenes* exhibited a high-risk score mostly for high-risk population such as immuno-compromised individuals (Matargas *et al*., 2008).

Recently, USDA-FSIS released updated information on the *Salmonella* compliance guide for small plants on RTE meat products (USDA-FSIS, 2011). USDA-FSIS tests for *Salmonella* positive RTE products using two test programs; the random testing program and the risk-based testing program. Half of all positive products were found to be from headcheese, pork barbecue, and sausage products. The source of contamination in pork barbeque could be either from the meat or the sauce which raw ingredients were mixed with. Even though the incidence of *Salmonella* contamination is lower than *L. monocytogenes*, *Salmonella* contamination can be indicative of not only under-processing but also serious deficiencies in sanitary practices. For the production of RTE meat products, USDA-FSIS requires *Salmonella* lethality performance standards to be a 6.5 log reduction for roasted, cooked and corned beef products (9 CFR 318.17) and a 7 log reduction for fully cooked poultry products (9 CFR 381.150) (USDA-FSIS, 1999). For other types of RTE meat products such as cooked meat patties, dried, fermented sausages, and salt-cured products, FSIS recommends at least a 5 log reduction of *Salmonella* (USDA-FSIS, 2011).

Other types of RTE products such as fruits and vegetables as well as foods with low water content including nuts and cereals are receiving more attention due to the recent outbreaks. For example, celery was contaminated with *L. monocytogenes* causing 7 illness and 5 deaths in 2010 (Outbreak database, 2010) and bagged spinach was contaminated with *E. coli* O157:H7 causing 238 cases and 5 deaths in 2006 (CDC, 2006). In 2008, jalapeno and serrano pepper imported from Mexico caused approximately 1400-reported illness due to *Salmonella*. Specifically the peppers were contaminated with *S*. Saintpaul (Klontz *et al*., 2010). Tomato related *Salmonella* Newport outbreaks in 2002 (510 cases) in 26 states and 2005 (72 cases) in 16 states were caused by persistence of the pathogen in tomato fields (Greene *et al*., 2008). *S*. Wandsworth was found in commercial RTE vegetable-coated snack food with 69 patients from 23 states and 93% were aged 10 months to 3 years (Sotir *et al*., 2009). A more detailed discussion on *Salmonella* contamination in fresh produce can be found in a comprehensive review by Hanning *et al*. (2009). Other types of RTE products such as peanut butter caused illness in 628 persons in 47 states (during 2006-2007) due to *S*. Tennessee contamination (CDC, 2007). Salmonellosis was reported in 41 states (401 cases) due to the presence of *Salmonella* in frozen potpies and failure to kill the pathogen during cooking (CDC, 2008). A savory snack imported from Israel in 1994 to 1995 was implicated in a *S*. agona outbreak affected young children in the US and UK (Killalea *et al*., 1996). Tainted German chocolates resulted in 439 cases due to *S*. Oranienburg contamination over several European countries mostly affecting young children (Werber *et al*., 2005). Multi ingredient RTE foods with a high fat content such as cheese, chocolate, ice cream and egg-based foods are more likely to be a vector for foodborne pathogens since the fats may protect the pathogen that Traverse the gastrointestinal tract (Todd *et al*., 2008).

These *Salmonella* related outbreaks demonstrate that RTE products should not be considered safe from a food safety standpoint and require more efficient and targeted control to minimize pathogen contamination. This environmentally persistent pathogen is highly morbid and can cause huge economic losses worldwide. Several physical and chemical treatments may be employed to combat this pathogen in RTE foods and are discussed in the following sections.

PHYSICAL CONTROL ON RTE

Thermal treatments

Physical methods include exposure to heat, cold, and packaging methods. Temperature control is one of the more conventional approaches for limiting the growth of microorganisms in a food product. Under refrigerated condition, the growth rate of the spoilage and pathogenic bacteria is reduced. However,

some bacteria such as *L. monocytogenes* and *Yersinia enterocolitica* can survive at 1°C. In these cases, shelf life and sell-by dates play an important role (Herbert *et al*. 2000). Heat has been used in the form of pasteurization at various times and temperatures to inactivate certain microorganisms. Thermal inactivation of target bacteria varies based on strain, food product, and environmental factors (Doyle and Beuchat, 2007). Sterilization techniques are employed to inactivate bacterial spores. Heat is considered one of the standard commercial methods and is one of the most efficient methods for inactivating microorganisms in foods (Gould, 2000). Trials performed using hot water on poultry carcass have been shown to reduce *Salmonella* numbers substantially (Morrison *et al*. 1985). The use of hot water as a hurdle is based on the principle of increasing the surface temperature of the carcass. For instance, dipping meat samples in 95°C hot water for 3 s increased the surface temperature of meat to 82°C (Ellebracht *et al.* 1999). Hot water at various temperatures has been used to study log reductions of pathogenic bacteria on meat surfaces. Immersion at 70°C for 20 s resulted in less than 1 log cycle reduction in the total microflora with about 2 log cycles reduction in numbers of Enterobacteriaceae. Water used at 74°C reduced *E. coli* O157:H7 by 2.6 log colony forming unit (CFU) (Dorsa *et al*., 1997), whereas water at 95°C reduced *E. coli* O157:H7 by 3.7 log CFU (Castillo *et al*., 1998).

Heat treatment of RTE meat product may be more challenging to inactivate bacteria. This is because the product has already gone through a cooking process, which promotes resistance to the survived pathogens by food ingredients that protect the bacteria from heat treatment. Osaili and others (2007) performed thermal inactivation experiments (at 55 to 70°C) on *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in breaded pork patties. The study showed that salts added to the product enabled water molecules to bind and caused poor heat penetration for bacteria to survive in the product. In addition, the breading ingredients, which consist mostly of carbohydrates and coat the pork patties, also increased the chances for bacteria to be thermal resistant. Fat content of the product did not have any effect on the thermal resistance for this study. D-values at 55 to 70°C were 69.48 to 0.29 min and the z-values were 6.2°C for pork patties. In order to achieve a 7 log reduction of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, the heat treatment time at 70°C must be 0.56 min or higher, 2.03 min or higher, and 3.01 min or higher, respectively (Osaili *et al*., 2007). Thermal treatment in chicken-fried beef patties evaluated the D-values at 55 to 70°C to be 67.68 to 0.22 min and z-value to be 6.0°C for *Salmonella*. The process lethality to achieve a 6.5 log reduction at a reference temperature of 70°C for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* was 0.26 min or higher, 1.43 min or higher, and 2.02 min or higher, respectively (Osaili *et al*., 2006). These two studies demonstrate that an appropriate heat treatment is necessary depending on the food ingredients to kill target bacteria.

Thermal resistance can also be different between whole-muscle and ground meat. A *Salmonella* cocktail showed stronger resistance in whole-muscle with D-value of 2.7 min than ground meat with a D-value of 1.2 min when treated at 60°C (Mogollon *et al*., 2009). This study revealed that increasing fat content increased the heat resistant of *Salmonella*. Also, segregated fat tissue in the whole-muscle was able to protect *Salmonella* from the heat. In the ground beef samples, thermal protection may have been lost by the homogenous distribution of fat as well as increased osmotic potential in muscle cells members even though the moisture content would be considered the same as the whole-muscle. Thermal resistance can increase significantly by lower water activity of meat (Mogollon *et al*., 2009).

Nonthermal treatments

Relatively newer physical methods are the use of high hydrostatic pressure (HPP), ultrasonication, electroporation, high intensity light, and irradiation and these methods have been employed to inactivate microorganisms mostly at ambient temperatures. The advantage of using a nonthermal process is that it preserves the flavor, color and nutrient value of the food products. HPP and high voltage electric

discharges are routinely used to inactivate bacteria, yeast, and molds in foods. This method has been used for jams, juices, avocado dip, and salad dressings and other RTE products. The use of HPP for poultry can be combined with other treatments, and hence prevent the growth of pathogenic bacteria and increase the shelf life of the product (Raso and Barbosa-Canovas 2003). A combination of pressure at 20 MPa with carbon dioxide reduced *Salmonella* by 6 log cycles while *E. coli* was reduced 3 log cycles in orange juice and apple cider (Balaban *et al*., 2001). When used at high intensities, ultrasonication technology has proven to be able to inactivate vegetative bacteria and heat-resistant spores.

High intensity light such as UV radiation has been used to effectively sterilize packaging materials used for foods. UV radiation induces the formation of thymine dimers and enables polymerase to replicate new DNA strands (Rastogi *et al*., 2010). Kuo et al (1997) contaminated the surface of shell eggs with *S*. Typhimurium, treated with UV radiation (620 μW/cm^2) and the result indicated a 3 log reduction after 1 min. In addition, the UV radiation for 15 min was able to significantly reduce mold and yeast population (Kuo *et al*., 1997). Irradiation has been approved for variety of foods to reduce pathogens and to extend the shelf life. Foods that have been examined include; wheat flour, white potatoes, fruits and vegetables, herbs and spices, fresh meat, pork and poultry and the dose range is from 0.05 to 0.15 kGy for potatoes to 4.5 kGy for fresh meat (Tauxe, 2001). It was reported that low doses of radiation could kill 99.9% of *Salmonella* in poultry and *E. coli* O157:H7 in ground beef (Olson, 1998). The WHO stated that no toxins or other hazards were associated with high doses of irradiation when used to decontaminate food surfaces. However, the quality of the food has been a concern due to off-odors of meat with high fat content, texture change of egg white and grapefruit.

There have been limited studies on the nonthermal processing to inactivate *Salmonella* in RTE products and most of these studies were focused on irradiation. Studies in RTE products such as carrot, cucumber, sprouts and pineapple have demonstrated that 2 kGy of radiation worked effectively to reduce *Salmonella* and did not have any adverse effects on texture, nutritional, or organoleptic properties of the produce (Dhokane *et al*., 2006; Saroj *et al*., 2006). Gamma radiation processing uses radioactive materials such as cobalt-60 and cesium-137. This process was used for *S*. Typhimurium in RTE pineapples with 2-kGy dose to reduce 5 log CFU/g of *Salmonella*. No growth was detected for 12 days at 4 and 10°C (Shashidhar *et al*., 2007). In sprouts, D-values of *S*. Typhimurium ranged from 0.192 to 0.208 kGy and with 2 kGy demonstrated complete elimination of 4 log CFU/g of *S*. Typhimurium (Saroj *et al*., 2006). Electron beam irradiation can only penetrate limited depth without any radioactivity involved and hence is mostly used for thin layers of food products. Cabeza and others (2009) tested E-beam irradiation in vacuum-packed RTE dry fermented sausages to inactivate *S*. Enteritidis and *S*. Typhimurium without any sensory change. At 1 kGy, the odor and taste did not exhibit detectable differences when compared with untreated sausages, however off-odors and off-taste increased significantly at 2 and 3 kGy. Meanwhile, color change occurred especially on the redness, which was reduced significantly due to the production of heme-pigment with carbon monoxide ligand formation, and the lightness increased while yellowness was not affected. Also the off-color by irradiation could increase the concern on using irradiation on RTE meat products (Cabeza *et al*., 2009). X-ray treatment, which is an alternative to gamma rays and penetrates foods in greater depth than E-beam irradiation, was used on *S. enterica*, *E. coli* O157:H7, *Shigella flexneri* and *Vibrio parahaemolyticus* in frozen RTE shrimp. The D-values for *E. coli* O157:H7, *S. enterica*, *Sh. flexneri* and *V. parahaemolyticus* were 1.1, 1.3, 1.2 and 1.2 kGy, respectively. In order to reduce 5 log CFU, the shrimp samples had to be treated with 2.0, 3.0, 2.0, and 2.0 kGy for *E. coli* O157:H7, *S. enterica*, *Sh. flexneri* and *V. parahaemolyticus*, respectively. Overall results demonstrated that *S. enterica* had stronger resistance to X-ray treatment than other pathogens (Mahmoud, 2009).

CHEMICAL CONTROL ON RTE

Chemical methods include use of organic acids,

salts, chlorine, spices, or oils. Organic acids, which are generally recognized as safe (GRAS), are the most common method to control the growth of microorganisms in foods. In the meat industry, chemicals rinses using organic acids are employed to rinse animal carcasses. Acetic acid, lactic acids, and citric acids at concentrations of 1.5 to 2.5% are applied as sprays for carcass decontamination (USDA-FSIS, 1996). These acids reduce the pH of the food and hence lower the internal pH of bacteria to control the growth of microorganisms. At a low pH environment, the membrane of the bacteria is saturated with hydrogen ions, which alter the permeability of the cell or reduce the proton motive force, and this eventually affects the ability of bacteria to reproduce (Banwart, 1989; Ricke, 2003). Organic acids are the most effective when applied over the carcass shortly after hide removal (Huffman, 2002). Organic acids have been used for low pH sauces, mayonnaises, salads dressings, and fruit juices. Weak acids and esters such as sorbate, benzoate, and propionate are used to preserve pickles, soft drinks, breads, cakes, and grains. Nitrite is used routinely to preserve cured meats (and Gould, 2003). Lactic acid is most effective when applied at higher temperatures and a concentration of 2 to 4%. Studies have been conducted using a 4% L-lactic acid solution at 55°C on chilled beef carcass in order to reduce bacterial contamination on meat surfaces. *E. coli* O157:H7 and *S.* Typhimurium exhibited 2.0 to 2.4 log cycles and 1.6 to 1.9 log cycles reduction after postchill acid treatment (Castillo *et al.*, 2001). Min and Yoon treated potassium lactate (PL) and sodium diacetate (SDA) to reduce *S.* Typhimurium and *Staphylococcus aureus* and evaluated the shelf life in RTE pork. Combinations of PL and SDA (1.46% PL and 0.10% SDA and 2.18% PL and 0.16% SDA) were able to delay the growth of pathogens by causing a significant increase in lag time and a significant decrease in growth rate at 10, 17, 24 and 30°C. This study showed the potential to store RTE pork at room temperature (Min and Yoon, 2010). However, there are growing concerns in the food industry about increasing number of acid-resistant bacteria due to use of organic acids as well as the disposal of the wastewater for environmental reasons (Dickens *et al.*, 1994; Dickens and Whittemore, 1994; Kwon and Ricke, 1998).

Chlorine is another compound, which is routinely used as an antimicrobial. The most effective form of chlorine is hypochlorous acid, which can penetrate bacterial cell wall and react with key respiratory enzymes to prevent normal functioning of the cell (Lillard, 1980). Yang and others (1998) studied the effect of four different antimicrobial treatments on poultry carcass after inoculating the carcass with *Salmonella*. They used 10% trisodium phosphate, 2% lactic acid, 0.5% cetylpyridinium chloride (CPC), and 5% sodium bisulfate treatments at 35°C and a pressure of 413 kPa for 17 s. They found that 0.5% CPC was the most effective treatment for reducing *Salmonella* on the carcasses (Yang *et al.*, 1998). CPC is a quaternary ammonium compound that has been shown to reduce bacterial counts on beef carcasses by up to 6 log CFU (Cutter *et al.*, 2000). On fresh-cut vegetables such as broccoli, cauliflower and radishes, 0.5% CPC treatments significantly reduced *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 by 3.7, 3.15 and 1.56 log CFU/g, respectively (Wang *et al.*, 2001). The effects of chlorine have only been evaluated on RTE vegetables. Trisodium phosphate (TSP) has an extremely high pH of 10 to 13. This is detrimental to the pathogen as it is not able to carry out its normal cellular functions at this pH. Scientists conducted a study to check the effect of 10% TSP on *S.* Typhimurium attached to chicken skin (Kim *et al.* 1994). The results demonstrated that TSP was successful in reducing 2 log CFU/cm^2 *Salmonella*. It has been suggested that TSP is effective in reducing *Salmonella* on chicken since it affects the binding kinetics of the bacteria on the carcass (Kim *et al.*, 1994).

Plant essential oils (EO) have been a growing interest as natural and safe preservatives with a broad spectrum of antimicrobial activity. EO generally works more effectively against Gram-positive than Gram-negative bacteria. However, Guiterez and others (2008) showed that EO in RTE vegetables was effective against *Salmonella*. Among a variety of EOs, marjoram and basil showed some activity against Gram-negative organisms. EOs have hydroxyl groups and allylic side chains, which may

increase the antimicrobial effect on *Salmonella* contaminated iceberg lettuce and carrots. Oregano and thyme also showed the strongest antimicrobial activity due to the high phenolic compounds, however these compounds exhibited strong flavor (Gutierrez *et al*., 2008). Another study revealed reduction on spoilage bacteria, *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* in RTE fruit salads using pure citral and citron essential oil (Belletti *et al*., 2008). Minimally processed fruits and vegetables are required protection during storage and especially low-acid fruits can be more of a concern since they can allow the growth of pathogen easily. *L. monocytogenes* was reduced by 5 log CFU/g during 9 days of storage at 9°C, *S.* Enteritidis E4 was reduced by 2.5 log CFU/g and *E. coli* O157:H7 exhibited a 1.2 log CFU/g reduction (Belletti *et al*., 2008).

In general, EOs have an organoleptic impact on food products. However, citron did not cause undesirable change on color and damage to the fruit tissues but a demonstrated negative effect on taste. Interestingly, different EO extraction fractions from the same origin can target different pathogens. In orange oil, orange terpenes, d-limonene and terpenes from orange essence showed inhibition against 11 different *Salmonella* serotypes by a disk diffusion assay (O'Bryan *et al*., 2008). Limonene also exhibited inhibitory effects against *E. coli* O157:H7 (Nannapaneni *et al*., 2008) while cold pressed terpeneless Valencia orange oil was effective against *E. coli* O157:H7 and *L. monocytogenes* but not against *Salmonella* (Friedly *et al*., 2009). Studies on EO indicates that further investigation is required to find a suitable EO at an appropriate concentration against *Salmonella* that will not negatively impact the organoleptic properties of the product.

Chemical antimicrobial methods are often combined with other control methods including biological agents such as bacteriocins. Biological methods may be classified as natural antimicrobial agents and a detailed review has been provided by Sirsat and others (2009). A natural aromatic organic compound, ρ-cymene was added to RTE pork sausage to control the growth of *S.* Typhi. This compound is a constituent of EO from oregano and thyme and it was combined with nisin to achieve a synergistic effect on target pathogen. There were no antimicrobial effects by individual compound, however with a minimal concentration of 0.3 ppm of nisin and 2.5 ppm of ρ-cymene, it was able to eliminate the pathogens at 4°C (Rattanachaikunsopon and Phumkhachorn, 2010).

Nisin is a bacteriocin that is mostly effective against Gram-positive bacteria. However when combined with a chelating agent such as ethylenediamine tetraacetic acid (EDTA), it can increase the antimicrobial efficiency against Gram-negative bacteria. Synergistic effects of whey protein isolate coating incorporated with grape seed extract (GSE), nisin, malic acid (MA), and EDTA in turkey frankfurter was studied for their potential to inhibit *L. monocytogenes*, *E. coli* O157:H7, *S.* Typhimurium (Gadang *et al*., 2008). The results demonstrated a 1 log reduction of *S.* Typhimurium after 28 days at 4°C when a combination of nisin, GSE and MA was used. However, MA alone showed a 3.3 log reduction of *S.* Typhimurium. The combination showed an additive rather than synergistic effect on *Salmonella*. The treatments were more effective against *L. monocytogenes* and showed a 4.8 log reduction when the frankfurters were stored for 28 days at 4°C. In order to kill or inhibit the growth of *Salmonella* with acids, the disruption of outer membrane is prerequisite and MA has low molecular weight compared to nisin and GSE (Alakomi *et al*., 2000). Therefore MA was more effective alone than in combination of all compounds (Gadang *et al*., 2008). Other studies added lysozyme and nisin to calcium arginate coated RTE smoked salmon, which resulted in a 2.7 log CFU/g reduction of *L. monocytogenes*. Nisin alone was not effective against *S.* Anatum, however when combined with lysozyme and nisin with calcium alginate coating showed 2.25 log CFU/g reduction after 35 days at 4°C (Datta *et al*., 2008).

MULTIPLE HURDLE TECHNOLOGY

In food safety, multiple hurdle technology is an important approach to consider. Sequential sublethal stress treatments cause the target microorganism to

face the challenge of a hostile environment leading to metabolic exhaustion and death. Hurdle technology can target a single function of a microbial cell such as cell membrane, DNA, pH, and water activity for additive effects or target multiple elements of the cells, which can cause synergistic effects by disturbing homeostasis of the cell in several aspects. With additive effects, the cells could lose the ability to recover and the damage becomes irreversible (Leistner, 2000). This approach can be beneficial for the food product since the dosage of antimicrobial agent or treatment may require less than a single treatment. The treatment can be a combination of three different methods; physical, chemical or biological method. Examples of the combination of chemical and biological methods are indicated in the earlier section. Irradiation can be combined with other chemical methods to enhance the reduction of target pathogens. For instance, without SDA and PL treatment, *L. monocytogenes* exhibited radiation resistance and it was able to grow during storage under refrigerated condition. In order to reduce 1 log of *L. monocytogenes*, 0.56 kGy of irradiation without SDA and PL was required in bologna. However, the combination of SDA 0.07% and PL 1% increased the sensitivity of pathogen to irradiation to 0.46 kGy for 1 log reduction and 3 kGy with SDA 0.07% and PL 1% prevented the growth of radiation-damaged pathogens during storage up to 8 weeks at 9°C (Sommers *et al.*, 2003). Milillo and others investigated the combinational effect of thermal and acidified organic acid treatment on *S.* Typhimurium (Milillo and Ricke, 2010; Milillo *et al.*, 2011). Sodium propionate 2.5% at pH 4 was able to exhibit a 4 log reduction of *S.* Typhimurium at 55 and 60°C within 1 min. The synergistic reduction was primarily caused by cell membrane disruption and microarray analysis revealed the specific genes involved during the combination treatment (Milillo *et al.*, 2011).

Microarray assays can be a great source to understand the hurdle technology for the gene expression profiling of *Salmonella* to identify the most effective combinations of different antimicrobial treatments (Sirsat *et al.*, 2010). Transcriptomics to study the gene expression will provide information on which treatment on *Salmonella* would regulate the survival pathway and biochemical mechanism with identifying and quantifying different genes. For the broad range of gene and protein level analysis, microarrays are advantageous since we can analyze the whole gene profile of target pathogen (Sirsat *et al.*, 2009). Dowd *et al.* (2007) exposed *S.* Typhimurium to nalidixic acid and evaluated differential regulation of SPI-1 and 2 and induction of multidrug resistance efflux pumps and outer membrane lipoproteins using microarray. Microarrays have also been utilized to access *S.* Enteritidis and *S.* Typhimurium responses when these microorganisms were exposed to butyric acid and have shown to exhibit down-regulation of SPI-1 genes including *hilA* and *hilD* (Gantois *et al.*, 2006). Detection of different *Salmonella* serovars have also been employed with this technique (Alvarez *et al.*, 2003) as well as real-time PCR for detecting *Salmonella* from RTE meats (Patel and Bhagwat, 2008). Understanding the pathogen responses to the stressors would be effective to minimize cross protection of target pathogen, which in turn would potentially decrease the level of virulence expressed by these organisms when RTE foods are consumed.

CONCLUSIONS

The major cause of contamination in RTE food products appears to be after cooking or after processing the product. Therefore, proper handling practices are required to minimize any cross-contamination and for the safe consumption after purchase. It is important for the workers in the processing plant to apply the appropriate sanitary practices. Pathogens can easily be transmitted by food workers, especially when handling raw food to generate RTE products such as potato salad, and sliced ham where bare hand contact may happen. Along with proper hygiene, it is also important to be careful to avoid temperature abuse during storage. In this review, physical and chemical control methods to inhibit the growth of *Salmonella* in RTE products were discussed. Antimicrobial agent coated packages may also be a great strategy to protect the RTE product from post-processing contamination. However, bac-

terial resistance against antimicrobial agents and cross-protection against different hurdle treatment are also an increasing issue since this may limit handling *Salmonella* in RTE products. As discussed in the review, hurdle technology with the combination of the physical, chemical or biological methods may be an ideal tool for efficient control against bacterial resistance for individual control methods. Future research should focus on developing strategies such as genomic screening to design optimal multi-hurdle conditions to lower the potential growth of *Salmonella* in RTE food.

ACKNOWLEDGEMENTS

This review was supported by National Integrated Food Safety Initiative grants (NIFSI) (2008-51110-04339) to S. C. Ricke and to P. G. Crandall (2010-51110-21004) and a USDA Food Safety Consortium grant to S. C. Ricke.

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