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Recent Advances in the Application of Non Thermal Methods for the Prevention of *Salmonella* in Foods

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1. Introduction

Food-borne illness as a result of consumption of foods contaminated with pathogenic bacteria is a world-wide concern. The presence and subsequent growth of micro-organisms in food in addition to improper storage not only results in spoilage but also in a reduction of food quality. The microbiological safety in ready to eat products is a cause of big concern not only for the consumers and food industries but also for the regulatory agencies. The number of documented outbreaks of foodborne diseases has increased in the last decade with *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* being responsible for the largest number of outbreaks and deaths.

The European Food Safety Authority (EFSA) reported *Salmonella* to be the most common cause of food-borne outbreaks in the EU (EFSA, 2009). As high as 50,000 and 35,000 people were reported to be suffering from salmonellosis in the Netherlands during 1999-2000 and 2002, respectively (Bouwknegt et al., 2003). The symptoms include diarrhoea, vomiting, nausea, abdominal pain and fever. *Salmonella enterica* Typhimurium and *Salmonella enterica* Enteritidis are the most frequently isolated serovars in the EU which are responsible for diarrhoea and fever (EFSA-ECDC, 2007). Some strains of *Salmonella* such as *S*. Senftenberg are more heat resistant than other strains. Even in the United States, *Salmonella* is considered to be one of the most prevalent bacteria amongst the foodborne pathogens, causing an estimated 1.6 million foodborne illnesses with annual cost of ~$14 billion. *Salmonella* Typhimurium has been implicated in the US as the major causative agent for food borne salmonellosis.

2. *Salmonella*

Salmonella is a gram negative, non-spore forming bacilli belonging to the family Enterobacteriaceae and is one of the most prominent food pathogenic bacteria. This pathogen has the ability to grow at a wide range of temperatures (8-45 °C), pH (4 to 9) and foods with high moisture content (thus high water activity). Since the organism is heat sensitive, it is more prevalent in raw and under-cooked foods. In general, consumption of

contaminated foods such as raw or under-cooked eggs, meat, poultry or even dairy products can act as vehicles for salmonellosis in humans. Because of the ability of *Salmonella* cells to exist under dormant conditions and regain active growth phase when favourable conditions return, it also has the ability to survive in dry products. As fresh cut fruits lack any skin barrier they are also likely to be contaminated by *Salmonella*. Storage of raw or pasteurized foods under refrigerated conditions or with treatments that reduce pH can help to increase the shelf life by retarding or avoiding the growth but certain strains of *Salmonella* have been reported to survive even under chilling conditions. It is now evident that these conditions cannot stop the chromosomal replication and are only bacteriostatic in nature (Tahergorabi et al., 2011). Risco (2009) reported survival of *Salmonella* inoculated into chicken nuggets during 16 weeks at -20ºC. This further adds to the problems that can arise by the consumption of ready to eat frozen products that are just pre-warmed in a microwave prior to consumption.

Although any person can contract food poisoning due to *Salmonella*, the disease can be more serious in infants, elderly and people with weak immune system. Treatment with antibiotics becomes essential for the eradication of this bacterial species. However, excessive use of antibiotics has made several strains to develop resistance against such drugs resulting in increased prevalence of these resistant strains in humans and animals. In order to minimize its presence in foods, synthetic antimicrobial agents such as sodium benzoate and sodium nitrite were used. However, these are also losing popularity due to consumer demand for food products with natural preservatives. Thermal processing is the most efficient way for eliminating *Salmonella* from foods. However, consumer's demand for minimally processed foods in addition to the negative effect of heat on nutritional properties of foods is making this technology less popular in the food industry. Novel remedies for safe and efficient removal of this bacterium from foods are becoming vital. Nowadays, non thermal techniques such as the addition of naturally occurring compounds having antibacterial activity, the use of high pressure carbon dioxide (HPCD), use of electrolysed water, high intensity pulsed electric field (PEF) or irraditation are increasingly gaining attention as a means of food preservation. In addition, it is imperative for the non thermal applications to have similar or higher inactivation as compared to the traditional heat treatments. According to US-FDA guidelines, the main requirement is to reduce the pathogen load by 5 logs (FDA, 2001). The major advantage of these non-thermal technologies (table 1) is that they are environmentally friendly and act at ambient or sub-lethal temperatures resulting in minimal impact on color, flavor and nutritional quality of foods. These techniques help in retaining the "fresh-like" characteristics of food and may also help to preserve functionalities.

However, the use of essential oils and other plant extracts is often limited by organoleptical criteria. Moreover, high pressures can cause cell wall breakdown and result in loss of cell turgidity. Thus, under these conditions, it might be necessary to combine two or more technologies in order to achieve the desired preservative effect. The technique of combination or "hurdle technology" is slowly becoming eminent. Thus, the use of natural antimicrobials along with pulsed electric field, ozone or super critical carbon dioxide can be used to curtail the growth of *Salmonella* with a minimal effect on the sensory characteristics such as flavor.

	Advantages	**Limitations**
Irradiation	Effective for several foods Many different sources available (Gamma rays, electron beam)	Limited public acceptance Lipid oxidation of meat products
UV radiation	No chemicals are used Non-heat related method Lesser changes in quality attributes of food	Long term exposure can be harmful to the industry workers
HPP	Independent of the shape of food Can be used for both solid and liquid samples.	Changes in quality of food has been observed
HPCD	Can be used in a batch or continuous process CO_2 is GRAS, nonflammable and non-toxic	Not very successful for solid foods Commercial application is still not a success
PEF	Pulse applied for a short period so no generation of heat Less usage of energy	Cannot be applied to foods which cannot withstand high fields Cannot be applied to foods that form bubbles
Natural antimicrobials	Natural "green" preservatives Have "GRAS" status	Can have a negative effect on the sensory properties of foods

Table 1. Limitations and advantages of non thermal processing techniques

3. Thermal processing

Heating of food is the most common and effective method for eliminating pathogens. Thermal pasteurization, involving the reduction or inactivation of micro-organisms, was traditionally the most common method for the production of microbiologically safe food products. The method involves generation of heat outside the food which gets transferred into the food through conduction or convection. Although the method is inexpensive, preservative free and environmental friendly, it does result in undesirable changes related to the nutritional and organoleptical properties of foods. At the same time, the content or bioavailability of some bioactive compounds such as ascorbic acid, phenolic compounds or carotenoids may be severely diminished. The case becomes even worse if the food product is heat sensitive. Nonetheless the extent of destruction depends on the temperature used for processing in addition to the time for which it is applied. In order to circumvent the shortcomings of thermal processing, several non-thermal methods such as the use of radiation, high pressure processing and natural antimicrobials are receiving considerable attention (table 2).

Food Product	Strain	Condition	Reduction	Reference	Technique
Chicken meat	*S.* Typhimurium	13.7 MPa, 35 °C , 2 h	94-98%	Wei et al., 1991	*hpcd*
Beef Trimmings	*Salmonella spp.*	10.3 MPa, 36 °C , 15 min	0.83 log	Meurehg, 2006	*hpcd*
Ground beef	*Salmonella spp.*	10.3 MPa, 36 °C , 15 min	1.23 log	Meurehg, 2006	*hpcd*
Physiological saline	*S.* Typhimurium	6 MPa, 35 °C , 15 min	7 log	Erkmen and Karaman 2001	*hpcd*
Orange juice	*S.* Typhimurium	38 MPa, 25 °C , 10 min	6 log	Kincal et al., 2005	*hpcd*
Melon juice	*S.* Enteritidis	2000 µs and 100 Hz	4.27 log	Mosqueda-Melgar et al., 2007	PEF
Watermelon juice	*S.* Enteritidis	1250 µs and 175 Hz	3.75 log	Mosqueda-Melgar et al., 2007	PEF
Orange juice	*S.* Typhimurium	90 kV/cm and 55 °C	5.0 log	Liang et al., (2002)	PEF
UHT Milk	*Salmonella spp.*	600 MPa for 10 min and 21.5 °C	6.5-8.2 log	Chen et al., 2006	HPP
Orange juice	*Salmonella spp.*	600 MPa and 20 °C	7 log	Bull et al., 2004	HPP
Sliced Ham	*S.* Typhimurium	2 kGy	3.78	Song et al., 2011	Electron beam
Sliced Ham	*S.* Typhimurium	8000 J/ m^2	2.02 logs	Chun et al., 2009	UV-C

Table 2. Inactivation of *Salmonella* spp. achieved by application of non-thermal techniques in foods

4. Non thermal approaches

4.1 Application of radiation

4.1.1 Irradiation

The use of ionizing radiation as a means of food preservation is being extensively researched and is approved in many countries such as the United States, France, Netherlands and Canada. The use of radiation dose up to 7 kiloGray (kGy) has been sanctioned by WHO as safe. The critical target of ionizing radiation is the bacterial DNA. Gamma rays, X-rays and electron beam are the most common types of ionizing radiation. Gamma radiation is generated using radioactive isotopes such as cobalt-60 or Cesium-137 (FDA approved) whereas for electron beam high speed electrons are generated using electricity. Generation of X-rays involves interposition of a metal target between the food and the electron beam. The choice of use between e-beam and X-ray is typically made as an exchange between efficiency and product penetration depth. Unlike gamma radiation, the

processing time using electron beam is very short and the technique does not produce radioactive waste. The effect of both techniques on the quality is minimal as no heat is generated during the process. However, electron beam can penetrate only up to 8 cm in foods which is its major limitation. Nonetheless both these techniques are being studied for eliminating *Salmonella*. Irradiation in the range of 2-3 kGy has been used for the elimination of *Salmonella* in meat products. Park et al. (2010) reported lower total aerobic counts in gamma rays treated beef sausage patties as compared to electron beam treated samples. Reduction of 3.78 and 2.04 logs has been reported using electron beam irradiation (2 kGy) for *S.* Typhimurium inoculated in sliced ham (Song et al., 2011) and powdered weaning foods (Hong et al., 2008), respectively whereas Martins et al., (2004) reported a 4 log reduction in a cocktail of *Salmonella* strains using 1.7 kGy in watercress thereby showing the applicability of gamma radiation in salad vegetables. Application of 3 kGy electron beam resulted in a reduction of 6.75 and 4.85 logs of *S*. Tennessee and *S*. Typhimurium inoculated in Peanut butter (Hvizdzak et al., 2010). In contrast, irradiation by electron beam was found to be an unacceptable method for destroying *Salmonella* on raw almonds (Prakash et al., 2010). A dose of 5 kGy was reported to be required for achieving a 4 log reduction whereas radiation intensity higher than 2.98 kGy induced significant sensory changes in raw almonds (Prakash et al., 2010). Mahmoud (2010) reported 3.7 logs reduction in *S*. enterica per tomato upon the application of 0.75 kGy X-rays. Increasing the dose to more than 1 kGy resulted in more than 5 logs reduction. X-ray has shown to result in more than 6 logs reduction in ready to eat shrimps (Mahmoud, 2009) and spinach leaves and shredded iceberg lettuce (Mahmoud et al., 2010). However, several adverse effects (lipid oxidation, textural degradation) caused by ionizing radiation have prevented this technology from being extended. Especially, lipid oxidation of meat products by irradiation is the most important factor for quality decline. An increase in the off-odors of irradiated ground pork and pork chops upon refrigerated storage were observed (Ohene-Adjei et al., 2004). The negative effects of gamma radiation on the appearance and color of chicken breasts, pork loin and beef loin, has also been reported (Kim et al., 2002). Additionally just like other inactivation techniques, *S*. Typhimurium has been reported to develop resistance against the radiation if the cells are repeatedly processed with electron beam at sub-lethal doses (Tesfai et al., 2011). Although irradiation has a high potential to be used for food preservation, its use is limited by an uncorroborated view that irradiated foods are not well accepted by the public as safe and desirable.

4.1.2 Ultravoilet radiation

Irradiation using non-ionizing rays, especially ultraviolet (UV)-C (wavelengths of 220–300 nm with 90% emission at 253.7 nm) has been approved as a non thermal method by the U.S. Food and Drug Administration (FDA) for surface sterilization (US Food and Drug Administration (2007)). This technique has been used extensively to decontaminate food surfaces directly or other materials which come in contact with food surfaces. The main industrial application of UV is its use in disinfection of drinking water. The mechanism of action of UV light involves the interruption of bacterial replication due to the formation of thymine dimers in the bacterial chromosome either killing them or making them unable to reproduce.

Chun et al., (2009) reported a reduction of 2.02 logs of *S*. Typhimurium in sliced ham upon the application of 8000 J/m^2 of UV-C whereas in the case of chicken breasts a reduction of only 1.19 logs were observed upon the application of 5 kJ/m^2 UV-C radiation (Chun et al.,

2010). At the same time, storage of UV-C treated chicken breasts resulted in an increase in the TBARS values and a negligible change in the Hunter L, a and b values for the product. The effects of UV-C on the quality attributes and decontamination efficiency against *Salmonella* Enteritidis were evaluated in different egg fractions (de Souza and Fernández, 2011). In terms of quality attributes, UV-C did not affect the viscosity and the pH however, browning due to maillard reaction was detectable in egg yolk and whole egg at low UV-C doses. The TBARS value was not significantly different to untreated samples. At the same time, a reduction of 5.3, 3.3 and 3.8 log was achieved under dynamic conditions (9.22 J/cm^2, 39 min) in egg white, egg yolk and whole egg, respectively.

The main drawback of UV irradiation is that it is a surface sterilization method. The efficiency of the treatment will strongly depend on the actual location of the bacterial contaminant as well as the composition, surface topography and transmissivity of the food (Allende et al., 2006). Moreover, the penetration of UV in liquid foods will strongly depend on the characteristics of the liquid product. The presence of solid particles and other components can seriously hinder the penetration. In addition the actual physical arrangement, power and wavelength of the UV source will also play a significant role. Besides, care has to be taken while using short wave UV regarding the damage that it can cause to human eyes in addition to being a cause of skin cancers and burns in humans upon excessive exposure.

4.2 Application of pressure

4.2.1 High pressure processing (HPP)

High pressure processing (HPP) is a food processing method involving the application of pressure throughout the food. The technique is independent of the shape of food and can be used for both solid and liquid samples. Pressures in the range of 100-800 MPa are generally applied with temperatures ranging from 0-100 °C. The main target for HPP is the bacterial cytoplasmic membrane. In addition to the loss of solute, enzyme inactivation and protein coagulation might also occur as a result of excess pressure. HPP technique has been used for reducing or eliminating *Salmonella* in foods or culture media. Reduction of 6.5-8.2 logs in *Salmonella* inoculated in UHT whole milk was achieved at a pressure of 600 MPa for 10 min and 21.5 °C (Chen et al., 2006). Several instances regarding the growth of *Salmonella* spp. on the surface of tomatoes have been reported. HPP has been applied for the removal of this bacterium from the tomatoes surface. Application of pressures in the range of 350-550 MPa has been reported to result in 0.46-3.67 log reduction in *S. enterica* serovar Braenderup inoculated on diced and whole tomatoes (Maitland et al., 2011). Exposure to a pressure of 550 MPa for 2 min resulted in a reduction of several *S. enterica* serovars (Baildon, Gaminara, Michigan and Typhimurium) in the range of 4 log cfu/ml or greater for broth, water and apple juice (Whitney et al., 2007). Time did not seem to be an important factor when HPP was applied in a chicken meat model system. Treatment at 400 MPa for 2 min and 20 °C resulted in an inactivation between 3.26 and 4.35 log in a chicken meat model system (Escriu and Mor-Mur, 2009). The applicability of HPP as a preservation method against *Salmonella* has also been evaluated for products with lower water activity such as raw almonds. Goodridge et al. (2006) studied the effect of continuous and oscillatory HPP treatment on the viability of two *Salmonella* Enteriditis strains (FDA and PT30) inoculated onto raw almonds. Continuous pressurization of raw almonds resulted in less than one log reduction whereas the oscillatory process provided 1.27 and 1.16 log reduction for FDA and PT30 strains, respectively. However, a reduction of 3.37 logs was achieved when the almonds were

directly suspended in water and then given the treatment. The effect was attributed to the fact that low water activity provided a protective effect to the bacterial cells. Application of HPP to orange juice resulted in 7-log inactivation of *Salmonella* at 600 MPa and 20 °C (Bull et al., 2004) and 615MPa and 15 °C (Teo et al., 2001) for 60 s. At the same time, HPP was reported not to have any significant effect on the quality parameters of orange juice such as titratable acid content, °Brix, viscosity, alcohol insoluble acids, color, ascorbic acid and β-carotene concentrations (Bull et al., 2004).

However, the application of high pressure at high temperatures may result in undesirable changes in the quality of many foods. Moreover, in the case of meat products, high pressure can increase the susceptibility of meat products to attack by oxygen thus resulting in increased lipid oxidation. For instance, Ma et al. (2007) reported almost 5-fold increase in TBARS values after 7 days storage at 4 °C in beef exposed to a pressure ≥400MPa. In other studies, pressures higher than 300 or 400 MPa (at ambient temperatures) caused increased rate of oxidation in pork (Cheah and Ledward, 1996) and cod muscles (Angsupanich and Ledward, 1998), respectively. McArdle et al. (2011) reported detrimental effect of HPP at 600MPa on texture, oxidation and water binding properties of beef. However lower TBARS and cook loss for beef processed by HPP were obtained as compared to raw or conventional heat processed samples. Besides, HPP carried out at high temperatures can cause cell wall breakdown and result in loss of cell turgidity. In addition, large-scale industrial application will only be possible if the technique becomes economical. The treatment time and the pressures applied are the major factors involved in deciding the cost and in achieving the desirable microbial inactivation. Hence, it is important to optimize conditions wherein minimal pressure is applied for the shortest time so that a food product with a reasonable cost is obtained.

4.2.2 High pressure carbon dioxide (HPCD)

High pressure carbon dioxide (HPCD) is another upcoming treatment that is being extensively used as a non-thermal technique for food pasteurization. The process is not only environmentally friendly due to the non-toxic nature of carbon dioxide but also involves application of lower CO_2 pressure as compared to those employed for HPP. The use of lower pressures makes this technique an energy-saving process. The major factor involved in the destruction is CO_2 although pressure helps in greater penetration of CO_2 in the cells. Lethality imparted by pressurized CO_2 is a result of disassociation of CO_2 (in foods with high water content) into reactive ions such as carbonates (CO_3^{2-}), bicarbonates (HCO_3^-) and hydrogen (H^+). These reactive ionic species can then have an effect on the permeability of the cell membrane and properties of cell constituents. In addition, generation of carbonic acid (H_2CO_3) in the water present in food products further results in a reduction in the pH of the food products enhancing the penetration of CO_2 (Wei et al., 1991).

Studies involving the use of HPCD for the inactivation of *S.* Typhimurium (Kim et al., 2007; Erkmen and Karaman, 2001; Erkmen 2000; Wei et al., 1991) have clearly reported the microbial strain, pressure applied, pH of the medium, type of medium and temperature to be important factors for the inactivation. *S.* Typhimurium in orange juice was effectively reduced by 5-6 logs when subjected to continuous dense phase carbon dioxide (DPCD) for 10 min at 21–107 MPa and 25 °C (Kincal et al., 2005) whereas in another study reduction as high as 8 logs was achieved when the growth media was changed to physiological saline (PS) or phosphate buffer solution (Kim et al., 2007). Kim et al. (2007) also analyzed the structural changes in *S.* Typhimurium cells upon the application of super-critical CO_2. A

complete loss of colony forming activity was observed for the treated cells with a formation of veins and small vesicles on the surface. TEM images showed the inner areas to be highly disrupted accompanied by a membrane deformation. In addition, shrinking and uneven dispersion of cytoplasmic materials was also observed (Figure 1). Liao et al. (2010) obtained a remarkable reduction of 5 logs for *S.* Typhimurium when carrot juice was subjected to DPCD treatment. Both temperature and pressure had a noticeable effect as the inactivation was enhanced with increasing pressure at a constant temperature or increasing temperature at a constant pressure. In contrast, inactivation of *S.* Typhimurium in PS or PS containing 10% brain–heart infusion (PS-BHI) broth was completed in 35 min in PS whereas it took 140 min in the case of PS-BHI (Erkmen, 2000). Besides, the previous study reported the presence of two phases during the destruction characterized by a slow rate of reduction in the cell number which increased sharply at the later stage. Erkmen and Karaman (2001) observed that the exposure time required to achieve the same level of *Salmonella* inactivation was drastically reduced as the pressure during the inactivation increased. Complete inactivation of *Salmonella* was reported in egg yolk, 94-98% in chicken meat strips and limited inactivation in whole egg at a pressure of 13.7 MPa at 35 °C for 2 h (Wei et al., 1991). The variation in the results clearly indicates the complex nature of food systems. A treatment of 14 MPa at 45 °C for 40 min resulted in a 34.48% and 32.74% reduction for *S.* Typhimurium in soy sauce and hot-pepper paste marinated pork products, respectively (Choi et al., 2009a). However, the technique is more suitable for liquid foods as the diffusion of CO_2 into solid samples becomes a limitation due to the absence of agitation in solid foods. Also, high concentrations of CO_2 can cause darkening of color of certain animal products due to the formation of metmyoglobin. Due to the complex nature of foods conflicting results are available on the effect of HPCD on sensory, chemical and physical properties of foods. In spite of the potential advantages of HPCD more research is needed to monitor and quantify sensory and chemical characteristics of foods undergoing this preservation technique.

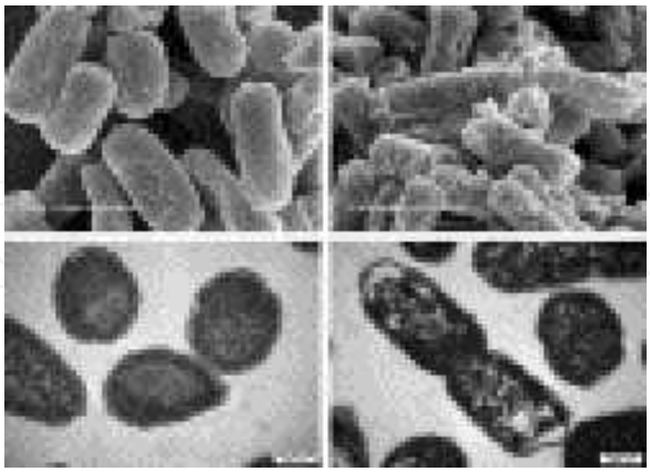

Fig. 1. Scanning electron micrograph (upper; magnification: 20,000) and Transmission electron micrograph (lower; magnification: 50,000) images of *S.* Typhimurium cells (left: untreated; right: treated) upon application of super critical carbon dioxide at 35°C and 100 bar for 30 min (Kim et al., 2007)

4.3 Pulsed electric field (PEF)

Pulsed electric field (PEF) is another non-thermal technology that can be used to inactivate bacterial cells at ambient temperatures. The process involves placing the food material between two electrodes and passing pulses of high electric field (1-50 kV/cm) strengths. Since the pulses are applied for short durations (2µs to 1 ms) the negative impact on food quality due to heat processing is highly diminished (Barbosa-Cánovas et al., 2001). The technique is more suitable for liquid or semi-liquid foods which can be easily pumped. It can be used to increase the shelf life of soups, milk, whole liquid eggs and fruit juices. PEF as a non-thermal preservation method has been implemented by Genesis Juices, Oregon, USA. The application of electric field results in cellular death due to generation of pores (electroporation) in the bacterial cell membrane without having an effect on enzymes or proteins present in foods (Wouters et al., 2001). The effectiveness of the technique will strongly depend on the treatment time, electric field strength and specific energy of the pulses. For instance, Monfort et al., (2010) achieved an inactivation of 4 log for *Salmonella* Typhimurium when 45 kV/cm of electric field was applied for 30 µs. Higher number of pulses and electric field was reported to be a stronger factor for reducing the number of *S.* Typhimurium population in orange juice (Liang et al., 2002) whereas in another study on melon and water melon juices, treatment time was found be a more important factor (Mosqueda-Melgar et al., 2007). Treatment of watermelon and melon juice with PEF resulted in a reduction of 4.27 log (at 2000 µs and 100 Hz) and 3.75 log (at 1250 µs and 175 Hz) of *S.* Enteritidis, respectively (Mosqueda-Melgar et al., 2007). In contrast, Liang et al. (2002) reported a 5 log reduction of *S.* Typhimurium in orange juice exposed to a PEF of 90 kV/cm at a temperature of 55 °C. However, the higher reduction could be a result of combination of higher acidity of orange juice in addition to relatively higher temperature and high intensity of the PEF applied. Although the technique is useful, inactivation has only been achieved in the range of 3-4 logs.

4.4 Natural antimicrobials

Since ancient times, spices and herbs have been used for preventing food spoilage and deterioration, and also for extending food shelf life. The antimicrobial effect of these components is a result of an increase in the permeability of the cytoplasmic membrane which leads to the loss of cellular constituents. At the same time, plant secondary metabolites such as essential oils and natural plant extracts have also been reported to have antibacterial, antifungal and anti-insecticidal properties. Extracts from capsicum, seaweeds and green tea have been found to inhibit the growth of *Salmonella* spp. in-vitro. Studies are also available wherein inhibitory effect of plant extracts was evaluated against *Salmonella* inoculated in minced beef, salad vegetables, fresh cut apples and minced sheep meat.

4.4.1 Extracts from vegetables

Vegetable extracts have shown a good potential when applied under laboratory conditions in culture media. For instance, application of 6% seaweed extract was shown to result in complete inhibition of *S. abony* whereas 3% extract resulted in 93% inhibition (Gupta et al., 2011). In contrast, 2.8% methanolic extract from Irish York cabbage was shown to result in only 64% inhibition of *S. abony* (Jaiswal et al., 2011). Xu et al. (2007) reported a minimal inhibitory concentration (MIC) of 15µl of grapefruit seed extract to

inhibit *Salmonella*. Careaga et al. (2003) reported that a minimum concentration of 1.5 ml of capsicum extract per 100g of meat was needed in order to prevent the growth of *S.* Typhimurium inoculated in minced beef. Karapinar and Sengun (2007) evaluated the antimicrobial activity of koruk (unripe grape—*Vitis vinifera*) juice against *S*. Typhimurium on cucumber and parsley samples which resulted in 1-1.5 log reduction upon immediate contact with korak juice and the reduction increased as the time of exposure of the vegetables to the juice increased.

The antimicrobial efficacy of plant extracts has been attributed to the presence of phenolic compounds, quinones, alkaloids, flavanols/flavonoids and lectins. Solubility of the extract in the food systems and the pH of the extract are important factors determining their efficacy in foods. Mechanism of action of these phenolic compounds involves alteration in the cell morphology which results in a disruption of the cytoplasmic membrane and leakage of cell constituents. Although the use of vegetable extracts for controlling the growth of *Salmonella* is promising the actual application in foods is in its budding stage.

4.4.2 Extracts of herbs and spices

In addition to providing flavor and fragrance, spices and herbs have also antimicrobial potential and thus can be used for preventing food deterioration and shelf life extension. Sumac, rosemary, sage, basil and ginger are some of the spices commonly being used for imparting antimicrobial effects on food. The flower, buds, leaf, stem or bark of these plants contains aromatic oily liquid which is the essential oil (EO). These EO are rich in phytochemicals such as terpenoids, polyphenols, flavonoids, antocyanin and organic acids which are responsible for the antimicrobial activity. Compounds such as carvacrol, citral, thymol, eugenol and citric acid have been shown to inhibit the growth of *Salmonella*. Eugenol has been reported to strongly inhibit the growth of *Listeria monocytogenes*, *Salmonella Enteritidis*, *Escherichia coli* and *Staphylococcus aureus.* Carvacrol and thymol are reported to be the principal constituents of EO of certain herbs. Burt et al. (2007) evaluated the antimicrobial activity of carvacrol vapour against *S.* Enteritidis on pieces of raw chicken. UV sterilized chicken pieces treated with carvacrol vapour (2 µl) showed reduced viable numbers of salmonellae at 4, 20 and 37 °C and a concentration of 4 µl resulted in a complete elimination of all viable cells in a minimum of 3 h at 37 °C. Govaris et al. (2010) studied the antimicrobial effect of oregano EO, nisin and their combination against *S.* Enteritidis in minced sheep meat during refrigerated storage (4 or 10 °C) for 12 days. Addition of nisin, at 500 or 1000 IU/g, proved insufficient to inhibit *S.* Enteritidis. The addition of oregano EO at 0.9% caused the population of *S.* Enteritidis to be maintained below 1 log cfu/g whereas a combination of 0.9% oregano EO and nisin at 500 or 1000 IU/g showed a bactericidal effect. The addition of 0.6% or 0.9% EO was found to be organoleptically acceptable also. EOs have also been applied for the elimination of *Salmonella* on fresh tomatoes. Gündüz et al. (2010) tested the antimicrobial potential of essential oil extracts on tomatoes. The tomatoes were inoculated with the nalidixic acid resistant strain of *Salmonella* Typhimurium ATCC 13311 and treated for 5-20 min with water extracts of sumac or oregano oil. Tomatoes treated with 100 ppm oregano or 4% sumac extract resulted in 2.78 and 2.38 log reduction, respectively. Hayouni et al. (2008) studied the antimicrobial effect of extracts from *Salvia officinalis* L. and berries of *Schinus molle* L against *S. anatum* or *S.* Enteritidis inoculated on minced beef meat. Concentrations in the range of 0.02-0.1% showed bacteriostatic effect against both the bacteria by the

extracts from *S. officinalis* and *S. molle* for over 15 days. In case of *S. molle*, the bacteriostatic effect was seen up to a concentration of 1%. At concentrations higher than 1.5% for *S. officinalis* and 2% for *S. molle*, immediate bactericidal effect was observed with a 2.6 log cfu /g reduction at 1.5% *S. officinalis* and 1 log cfu/g at 2% *S. molle*. However, sensory analysis of meat containing more than 2% of *S. molle* and 1.5% of *S. officinalis* showed a distinguished effect on the flavour and taste. In order to reduce the amount of EO being used, combinations of EO with NaCl were studied. The use of 0.1% or 1.5% *S. officinalis* with 6% or 4% NaCl or 0.1% or 1.5% *S. molle* with 4 or 8% Nacl could effectively eliminate *S. anatum* from refrigerated raw beef (Hayouni et al., 2008). The positive effect of spices on the inactivation of *S.* Typhimurium DT104 was observed when in direct contact, however, the activity reduced when added to food system such as ground beef (Uhart et al., 2006). Utilization of packaging materials containing these antimicrobial compounds is also becoming an attractive option in the food industry. However, a major limitation in using the EO in foods is the effect they have on the sensory properties of foods. At times, the concentration required to show the antimicrobial effect can surpass the organoleptically levels resulting in alteration in the flavor of foods.

5. Hurdle technology or synergism

Hurdle approach or the process of using multiple technologies is an effective approach to improve microbial decontamination in comparison to that of a single technology alone. Deliberate and intelligent combination of preservative treatments can help in maintaining the quality of food and delivering almost similar levels of microbial destruction as conventional methods alone. At the same time it warranties to counteract the negative effect of individual technologies on food quality. The choice of hurdles will strongly depend on the type of food it is being applied to in addition to the mode of inactivation. Potential synergistic effects among different technologies have been reported to be more effective than individual technologies applied alone. The outer membrane of gram negative cells prevents the entry of hydrophobic compounds. A combined treatment of heat and irradiation can result in sub-lethal injury to the cells. The sublethally injured cells can be more vulnerable to attack by antimicrobial compounds thereby reducing the dose of each individual technique.

For instance, combined effect of UV-C (0.5 J/cm^2) and potassium lactate, lauric arginate ester and sodium diacetate (FDA approved) resulted in a 3.6-4.1 log reduction of *Salmonella*, *L. monocytogenes* and *Staphylococcus aureus* on the surface of frankfurters (12 weeks storage at 10 °C). In addition, UV-C and antimicrobials had no significant impact on frankfurter color or texture (Sommers et al., 2010). Amiali et al. (2007) studied the synergistic effects of temperature, treatment time and electric field strength on inactivation of *S.* Enteritidis and *Escherichia coli* O157:H7 in egg yolk. A 5 log reduction in the population of *E. coli* O157:H7 and *S. enteritidis* was observed at an electric field of 30 kV cm^{-1} and 40 °C.

Exposure of egg shells contaminated with *S.* Enteritidis with UV radiation (1,500 to 2,500 μW/cm^2) followed by ozone (5 lb/in^2 gauge for 1 min) resulted in an inactivation of 4.6 logs or more in a total treatment time of 2 min (Roriguez-Romo and Yousef, 2005). Although the individual treatments resulted in similar reductions, however exposure time and pressure were comparatively higher. Combined treatment of lactic and acetic acid with super critical CO_2 resulted in 2.33 log cfu/cm^2 reduction in *S.* Typhimurium in fresh pork which was higher as compared to these treatments being applied individually (Choi et al., 2009b).

Application of PEF (25kV/cm, 250 µs in pulses of 2.12 µs) followed by heat treatment at 55 °C for 3.5 min increased the inactivation of *Salmonella* Enteritidis inoculated into liquid whole egg from 1 logs to 4.3 logs (Hermawan et al., 2004). The combination treatment had no effect on the color, pH, viscosity and brix of the treated samples and had a longer shelf life in comparison to heat treated samples.

High pressure applied in combination with other agents such as heat or antimicrobial agents can be effectively used to increase microbial inactivation. Individual and combined effects of HPP and nisin treatment on relative resistance, viability and cellular components on *S.* Enteritidis (strains: FDA and OSU 799) was evaluated in culture media. High pressure up to 200MPa and nisin (200 IU/ml) when applied separately did not have any effect on the viability of either strain. However, application of high pressure (500 MPa) or a combination of nisin with a pressure of 350MPa (OSU 799 strain) and 400 MPa (FDA strain) resulted in an 8 log reduction (Lee and Kaletunç, 2010). Penetration of nisin into the cells was assisted by the pressure and thereafter the additive effect of two hurdles resulted in inactivation to be achieved at a lower value than when the technique was applied separately. Viedma et al. (2008) studied the synergistic effects of antimicrobial peptide enterocin AS-48 and high-intensity-PEF treatment (35 kV/cm, 150 Hz, 4 µs and bipolar mode) on the inhibition of *S. enterica* CECT 915 in apple juice. A combination of high intensity PEF (1000 µs) and AS-48 (60 µg/ml) and a treatment temperature of 40 °C resulted in 4.5 log reduction. The sequence of the synergistic treatments was an important factor as the inhibition was observed only when HIPEF was applied in the presence of previously-added bacteriocin. Since both, enterocin AS-48 and high pressure PEF, act on the bacterial cytoplasmic membrane, synergism between them could be a result of enhanced permeability of bacterial cytoplasmic membrane.

6. Conclusion

With the rise of the concept of "green consumerism", meeting the consumer demand for nutritious and fresh food in addition to providing food safety has increased interest in non thermal preservation methods. The literature described herein gives an account of some of the non-thermal methods used for the elimination of *Salmonella* from foods. Considering the wide range of conditions under which *Salmonella* can easily grow, it is imperative to apply a combination of intervention technologies. With the advent of these novel methods of food preservation, it is hoped that issues of spoilage and contamination of food products, not only with *Salmonella* spp. but also with many other food spoilage or pathogenic micro-organisms could be effectively controlled. Besides, a major impediment in the acceptance of foods processed by these emerging technologies is a lack of information among the consumers. Thus, it is very important to provide proper knowledge to the consumers regarding the benefits of these technologies as a means of food preservation.

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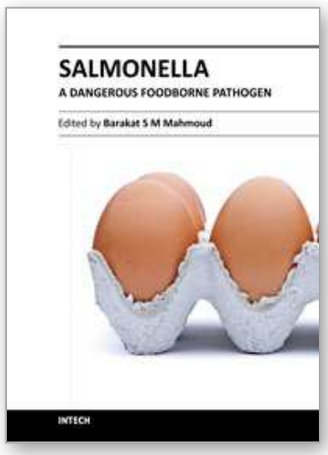

Salmonella - A Dangerous Foodborne Pathogen
Edited by Dr. Dr. Barakat S M Mahmoud

ISBN 978-953-307-782-6
Hard cover, 450 pages
Publisher InTech
Published online 20, January, 2012
Published in print edition January, 2012

More than 2,500 serotypes of Salmonella exist. However, only some of these serotypes have been frequently associated with food-borne illnesses. Salmonella is the second most dominant bacterial cause of food-borne gastroenteritis worldwide. Often, most people who suffer from Salmonella infections have temporary gastroenteritis, which usually does not require treatment. However, when infection becomes invasive, antimicrobial treatment is mandatory. Symptoms generally occur 8 to 72 hours after ingestion of the pathogen and can last 3 to 5 days. Children, the elderly, and immunocompromised individuals are the most susceptible to salmonellosis infections. The annual economic cost due to food-borne Salmonella infections in the United States alone is estimated at $2.4 billion, with an estimated 1.4 million cases of salmonellosis and more than 500 deaths annually. This book contains nineteen chapters which cover a range of different topics, such as the role of foods in Salmonella infections, food-borne outbreaks caused by Salmonella, biofilm formation, antimicrobial drug resistance of Salmonella isolates, methods for controlling Salmonella in food, and Salmonella isolation and identification methods.

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In order to correctly reference this scholarly work, feel free to copy and paste the following:

Shilpi Gupta and Nissreen Abu-Ghannam (2012). Recent Advances in the Application of Non Thermal Methods for the Prevention of Salmonella in Foods, Salmonella - A Dangerous Foodborne Pathogen, Dr. Dr. Barakat S M Mahmoud (Ed.), ISBN: 978-953-307-782-6, InTech, Available from: http://www.intechopen.com/books/salmonella-a-dangerous-foodborne-pathogen/recent-advances-in-the-application-of-non-thermal-methods-for-the-prevention-of-salmonella-in-foods

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