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The Emerging Technology in The Sector of Food Technology- The Non-Thermal Technology

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ABSTRACT: This papers details the different non-thermal technology and processing impact on food. The different technology included are ultraviolet light, ionizing radiation, pulse electric field, natural antimicrobials, hurdle technology, high pressure processing, carbon dioxide treatment, plasmas technology and pulse X-rays.

KEYWORDS: Ultraviolet light, Pulse Electric Field, Hurdle technology, High Pressure Processing, Plasmas Technology and Pulse X-rays.

1 INTRODUCTION

Non-thermal processing (NTP) treatments are gentler than thermal treatments, with targeted function for certain microbial inactivation and quality of products. The ability to retain flavor and aroma after NTP application is a huge advantage for Asian foods which often have stronger taste and aroma compared to Western foods. The vast variety of flavorful food in Asia such as fruits and fruit juices, sauces and seasonings, cereals, grains, flour and starches, seafood and meat products, snacks, and traditionally preserved foods (fermented, salted, added sugar) creates potential for the commercial application of NTP technologies. Some of these technologies are suitable for small scale entrepreneurs and some are suitable for large businesses. Besides cost, suitability of the technology and the targeted objective of processing the product must also be evaluated.

1.1 ULTRAVIOLET LIGHT

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/m2 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/m2 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/cm2, 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.

Ultraviolet radiation has been used for several years as a physical disinfection medium for air, surfaces and liquids. During processing and storage of liquid foods, contamination can take place in many different contact points (incoming raw materials, storage vessels, air in storage vessels, equipments, rinse water, drinking water and water to be added to foods). UV irradiation can and has been used at these contact points to reduce microbial load and minimize contamination (Ngadi *et al*., 2004). UV light (254 nm) can be used to inactivate many types of organisms, including viruses and has been used for many years in pharmaceutical, electronic, and aquaculture industries as a disinfection medium. Microorganisms exposed to UV light are affected at the DNA (deoxyribonucleic acid) level. Thus, the injured reproduction systems of cells lead to their death. Exposure to UV light can be applied at different doses for pasteurization of liquid foods or disinfection of solid foods (Guerrero-Beltr·n and Barbosa- C·novas, 2004). Fresh fruit and vegetable products in pumpable form can be processed using UV light to reduce food-borne microbial load. Most of these products are commonly pasteurized but unfortunately, heat from thermal pasteurisation can negatively change the taste and flavor of such products. However, when using very high UV doses for food disinfection, loss of nutritional value and undesirable appearance, may take place (Mohd Adzahan, 2006; Gardner and Shama, 2000). FDA has approved UV irradiation (21 CFR 179.39) for use on fruit and vegetable juices and juice products to achieve the 5-log reduction of target microorganisms such as *E.coli* O157:H7 or *Cryptosporidium parvum* as part of HACCP rule compliance (CFR, 2000). Processors also may use chemical antimicrobial agents, such as certain sanitizers, on the surface of citrus fruit as long as FDA has approved the chemical agent. It has a relatively lower initial investment compared to heat pasteurization and the equipment is easy to operate (Worobo *et al.*, 1998; Choi and Nielsen, 2004). In addition, UV pasteurizers do not take up a lot of floor space and losses of ascorbic acid, thiamine, riboflavin, pyridoxine and nicotinic acid due to UV exposure at 14 mJ/cm2 are comparable to those in heat treated juices (Mohd Adzahan, 2006). In the cases of quality and consumer acceptability tests, there seemed to be little difference between cider samples that were untreated and those that were UV-pasteurized (Choi and Nielsen, 2004; Tandon *et al*., 2003), which is an indication of the fresh-like product quality. Guava, papaya, and pineapple juices are high in ascorbic acid and exposure to heat treatment will diminish their nutritional value. These juices may be suitable for UV processing as long as they do not have high initial microbial populations, particulate materials and organic compounds. Such properties will prevent low transmissivity of UV light (Guerrero-Beltran and Barbosa-C·novas, 2004) and hamper consistent exposure of UV energy throughout the juice. Processing helps to reduce initial counts of yeast and mold in the juice or filtering the juice through membrane technologies before UV irradiation will result in higher efficiency for microbial inactivation by UV light (254 nm). Clear and less turbid juices such as coconut water, sugarcane and chrysanthemum juice all have the potential for UV application as a more economical alternative to heat pasteurization. Rice and soy milk are also potential products for UV treatment but no data has been published on UV treatment of these drinks. A greater than 5-log reduction of *Listeria monocytogenes* was achieved when goat milk is exposed to a cumulative UV dose of 1.6 mJ/cm2 (Matak *et al*., 2005). Shelf life of UV treated mango nectar lasted for 20 days with almost no microbial growth. The nectar maintained yellow and orange-yellow colors, after 26 days of storage and polyphenoloxidase activity remained constant after 30 days. However, the lower the flow rate during UV treatment, the higher the browning of nectar during storage. The maximum log reduction (CFU/mL) in UV treated (30 min of UV exposure at 0.451 liter/ min) mango nectar was 2.71 and 2.94 for total microbial count and yeast count, respectively (Guerreo-Beltr·n and Barbosa-C·novas, 2006). There is still a lot of room for research and improvement in this area and it is crucial that studies are conducted on variables such as flow rate, exposure time, type of fruit product, color of fluid and composition of the beverage in order to ensure reduced microbial load, increased shelf life and nutritious with acceptable taste.

1.2 IONIZING RADIATION

New regulations by USDA-APHIS allow countries such as Chile, Brazil Philippines, Malaysia and Thailand to export fruits and vegetables to the United States which increases the capability of these countries in food irradiation (CFR, 2001). Gamma and electron beam irradiation are used to inhibit the sprouting of vegetables, extension of shelf life of fresh produce, control of pathogenic organisms, insect and microbial disinfestations and sterilization of food and food packaging materials (Kanatt *et al*., 2005; Chouliara *et al*., 2006; Javanmard *et al*., 2006). Irradiation of food up to an overall dose of 10 kGy is accepted in several countries for commercial food processing (Lacroix and Quattara, 2000). However, the formation of metmyoglobin when meat was exposed to higher dose was reported (Brewer, 2004). Sausages made from fermented, uncooked pork

wrapped in a banana leaf (Nham) are considered a great delicacy in Thailand which has frequently caused intestinal illness due to bacterial contamination. Fortunately, this is no longer the case as irradiation has provided an efficient way of making Nham safe for Thai consumers. Som tam is a popular spicy papaya salad originating from Laos and the Isan region of northeastern Thailand. The main ingredients are fermented fish and salted crab. The ingredients are now irradiated for export. Onions are a highly marketed produce in Thailand, and because of the climate, shelflife is fairly limited during storage in markets and households. An alternative to systemic chemical treatment (e.g. maleic hydrazide) for sprouting inhibition is the application of absorbed doses of ionizing radiation of about 1 kGy (Biramontri *et al*., 1989). Effect of gamma irradiation combined with hot water dip and transportation from Thailand to Canada improved the shelfe-life, biochemical and physical characteristics of Thai mangoes (Nahng Glahng Wahn variety) (Gagnon *et al*., 1993). Reibroy *et al*. (2007) studied the effect of irradiation on properties and storage stability of Som-fug produced from bigeye snapper. It was found that the irradiation at low dose (2 kGy) could be used to control the over fermentation of Som-fug up to 20 days at 4oC without adverse effects on quality and acceptability.

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.

1.3 PULSED LIGHT PROCESSING

The FDA has approved pulsed light for the treatment of food (CFR Title 21, Sec. 179.41) for surface microorganism control, with the total cumulative treatment not exceeding 12.0 (J/cm2) and the pulse duration is no longer than 2 milliseconds (CFR, 2000). The application is suitable for surface treatment of food packaging materials, egg shells, processing equipments and production lines, sliced meats and raw or fresh cut fruits and vegetables (Szabo *et al*., 2006). Fishermen producing salted seafood products can use this technology to prevent growth of resistant microorganisms; especially so that the intensity of pulsed light is more than the sunlight. The germicidal effect appears to be due to both the high UV content and the brief heating effects which come from the infrared portion of the light. While UV causes damage to the nucleic acid and other components of the cell, the instantaneous heating of the cell results in the rupture of the cell wall, or lysing (Wekhof, 2000).

1.4 PULSED ELECTRIC FIELD (PEF)

PEF is the application of short-duration pulses (1-100ms) of field strength (10-50 kV cm-1) to a product placed between two electrodes. Recently, the continuous flow-through chambers has developed and upgrading offer possibilities (Qin *et al*., 1998). Microbial inactivation by PEF is believed to be caused by the PEF on the cell envelopes. PEF can cause formation of pores affecting the integrity and functionality of the membrane. These pores can be reversible or irreversible, depending on the degree of membrane damage (Ho and Mittal, 1996; Weaver and Chizmadzhev, 1996). Under mild pulsation conditions, the pore formation in the membrane will be reversible, whereas more drastic conditions will lead to irreversibility of this phenomenon, which will eventually result in cell death (Barbosa- C·novas *et al*., 1999). The optimum conditions of inactivation of microorganisms depend on species and processing conditions (Abram *et al*., 2003; Rodriguez-Calleja *et al*., 2006). The main parameters are electric field strength, pulsed length, number of pulses, pulse shape and starting temperature (Wouters *et al*. 2001). Physical and chemical characteristics such as pH, conductivity and ionic strength of the medium in which the microorganisms are PEF treated can influence microbial inactivation (Alvarez *et al*. 2000). Sub-lethal injury caused by PEF in *Escherichia coli* (Garcia *et al*., 2003) and the effectiveness of PEF to inactivate enzymes has been reported (Giner Segui *et al*., 2006).

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.

1.5 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.

1.5.1 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.

1.5.2 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.

1.6 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/cm2) 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/cm2) followed by ozone (5 lb/in2 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 CO2 resulted in 2.33 log cfu/cm2 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.

1.7 HIGH PRESSURE PROCESSING (HP)

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.

Sauces and seasonings play a significant role in Asian dishes. Most of these products have been either fermented, salted, dried or added sugar or acid for preservation purposes. Despite that, due to microbial resistance to traditionally harsh conditions, further preservation is needed. A method which is suitable for reducing microbial counts in sauces and seasonings is high pressure processing (HP). Research on HP applications have been done on juices, seafood, pork, milk, fruit jams and eggs (Berlin *et al*., 1999; Linton *et al*., 1999; Patterson and Kilpatrick., 1998; PrÈstamo *et al*., 1999; Mussa *et al*., 1999; Yuste *et al*., 1999; Ponce *et al*., 1999). Surimi gels from Pacific whiting obtained by HP were less opaque than traditionally heat set gels and had higher stress and strain values than those of heat set gels (Chung *et al*., 1994). Guacamole, a product made from avocado has high fat content and very low acid and has been processed using HP and commercialized (San Martin *et al*., 2002). The durian fruit has high fat content and low acid just like guacamole. 'Tempoyak', a fermented Malay side dish made from this fruit may be suitable for commercialization using HP processing. Durian has a variety of volatile flavor compounds or/and aroma and its texture, taste and smell can be altered by heat (Voon *et al.,* 2006), which is the reason why NTP should be seriously considered as an alternative to a thermal process. Nuts have the potential of causing harmful diseases such as salmonellosis as well as intoxication from aflatoxins. Reports on raw almonds treated with high hydrostatic pressure to reduce *Salmonella enteriditis* have proven effective and the same application can be used on peanuts and other nuts, soybeans and legumes. This is done by suspending the food to be pressurized directly in the pressurizing medium (water) which would increase the Aw of the food, leading to an improved reduction in the concentration of vegetative bacteria (Goodridge *et al*., 2006). A type of soybean vegetable protein that Asians have long consumed and its intake is increasing in other countries is known as tofu. High pressure treatment of tofu at 400 MPa at 5oC for 5, 30, and 45 min showed reduction of the Gram-negative and Gram-positive microorganisms without causing any negative effects in terms of its sensory properties. Micrographs on the cryofracture observed with a cryoscanning electron microscope revealed a more compact structure after pressure compared with that of untreated samples, but the aggregates in the treated samples were more disperse (PrÈstamo *et al*., 2000). One study reported that soybean-towater ratio of 1:8 ratio at pH 7 led to pressurized soymilks which have color and viscosity properties similar to the untreated soymilk, with enhanced emulsion stability (Lakshmanan *et al*., 2006).

1.8 CARBON DIOXIDE TREATMENT

1.8.1 DENSE PHASE CARBON DIOXIDE (DP-CO2)

DP-CO2 or supercritical and liquid CO2 is a cold pasteurization method that affects microorganisms and enzymes through molecular effects of CO2 under pressure and suitable for juices and dairy based beverages. Pozo-Insfran *et al*. (2006) reported that thermal pasteurization decreased anthocyanins (16%), soluble phenolics (26%), and antioxidant capacity (10%) whereas no changes were observed for DP-CO2 Muscadine grape juices including its sensory attributes. Application of DP-CO2 extraction includes extraction of lycopenes from tomatoes, removal of caffeine from tea or coffee, extraction of flavor and aroma from hops in breweries, freeze-drying of vegetables and removing fat from animal products such as powdered eggs. The pharmaceutical industry also uses DP-CO2 extraction to remove medicinal compounds from herbs.

1.8.2 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 CO2 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 CO2 although pressure helps in greater penetration of CO2 in the cells. Lethality imparted by pressurized CO2 is a result of disassociation of CO2 (in foods with high water content) into reactive ions such as carbonates (CO32-), bicarbonates (HCO3-) 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 (H2CO3) in the water present in food products further results in a reduction in the pH of the food products enhancing the penetration of CO2 (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 CO2. 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 CO2 into solid samples becomes a limitation due to the absence of agitation in solid foods. Also, high concentrations of CO2 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.

1.9 ULTRASOUND

1.9.1 INTRODUCTION

Ultrasound is energy in the form of sound waves with a frequency greater than 20 kHz. It has proven bactericidal effects, especially when combined with other microbial reduction strategies such as mild heating or pressure. A combination of pressure, thermal and ultrasound is called mano-thermosonification (MTS) and this combination has been proven to be more effective in reducing pathogenic microorganisms and in retaining quality of juices (Kuldiloke, 2002). Ultrasound treatment (sonifier probe at 20 kHz, 100% power level, 150 W acoustic power, 118 W/cm2 acoustic intensity) combined with mild heat (57oC) for 18 min resulted in a 5-log reduction of *L. monocytogenes* in ultrahigh-temperature milk, a 5-log reduction in total aerobic bacteria in raw milk, and a 6-log reduction in *E. coli* O157:H7 in pasteurized cider (D'Amico *et al*., 2005). Ultrasound is now applied to food products for microbial an enzyme inactivation and for food extraction via mechanical actions. Ultrasonic cavitation produces shear forces which breaks cells mechanically and allows material transfer from cell into solvents. Particle size reduction by the cavitation increases the surface area in contact between liquid and solid phases, an advantage when extraction of compounds is expected. The technology has been used for extraction of phenolic compounds from vacuolar structures by disrupting plant tissues, extraction of lipids and proteins from seeds, emulsification, viscosity improvement, homogenization and improvement of dispersion stability in liquid foods. This technology has also been used for salads, vegetables, fresh poultry, sauces, confectionaries, beverages, juices and purees, dairy products, as well as for sanitized washing and for waste water treatment. For poultry products, ozone in combination with ultrasound is an option to reduce microbial counts. Ozone can be used for both the products and also the equipment and facility, doesn't leave a residue, eliminates the storage problems associated with peroxide, chlorine dioxide and other sanitizers and is more effective in reducing pathogen loads (Ngadi *et al*., 2004).

Ultrasound when propagated through a biological structure, induces compressions and depressions of the medium particles and a high amount of energy can be imparted. In dependence of the frequency used and the sound wave amplitude applied a number of physical, chemical and biochemical effects can be observed which enables a variety of applications [Got et al. 1999, Knorr et al. 2004, Ultrasound... 1998]. Ultrasound has been used for a variety of purposes that includes areas as diverse as communication with animals (dog whistles), the detection of flaws in concrete buildings, the synthesis of fine chemicals and the treatment of disease. Despite its wide-ranging uses and exciting developments the study of ultrasound is a

young science. The oldest application, the exploitation of diagnostic ultrasound only dates back to the beginning of the 20th century and ultrasound in processing is even more recent in origin [Mason 2003]. In nature bats and dolphins use low-intensity ultrasound pulses to locate prey; while certain marine animals use high-intensity pulses of ultrasound to stun their victims before capture. In the food industry, a similar division into two distinct categories of ultrasound applications is made [Fellows 2000, McClements 1995]. For the classification of ultrasound applications the energy amount of the generated sound field, characterised by sound power (W), sound intensity (W·m-2) or sound energy density (Ws·m-3), is the most important criterion [Knorr et al. 2004]. The uses of ultrasound are broadly classified into two groups. Low energy (low power, low-intensity) ultrasound applications involve the use of frequencies higher than 100 kHz at intensities below 1 W·cm-2. Low-intensity ultrasound uses a so small power level that the ultrasonic waves cause no physical or chemical alterations in the properties of the material through which the wave passes, that is it is generally non-destructive. They are successfully used for non-invasive monitoring of food processes. The most widespread application of low- intensity ultrasound in the food industry is as an analytical technique for providing information about the physicochemical properties of foods, such as composition, structure and physical state [Fellows 2000, Jayasooriya et al. 2004, Knorr et al. 2004, McClements 1995]. Ultrasound has advantages over other traditional analytical techniques because measurements are rapid, non-destructive, precise, fully automated and might be performed either in a laboratory or on line. One of the most widespread and most promising ultrasonic applications is the utilization of ultrasound for composition measurement. Ultrasonic velocity in fish tissues, chicken and raw meat mixtures can be related to its composition using semi-empirical equations [Simal et al. 2003]. The other group is high energy (high power, high-intensity) ultrasound which uses intensities higher than 1 W·cm-2 (typically in the range 10-1000 W·cm-2) at frequencies between 18 and 100 kHz [McClements 1995, Ultrasound... 1998]. Physical, mechanical or chemical effects of ultrasonic waves at this range are capable of altering material properties (e.g. physical disruption, acceleration of certain chemical reactions) [Jayasooriya et al. 2004]. High-intensity ultrasound has been used for many years to generate emulsions, disrupt cells and disperse aggregated materials. More recently various areas have been identified with greater potential for future development, e.g. modification and control of crystallization processes, degassing of liquid foods, enzymes inactivation, enhanced drying and filtration and the induction of oxidation reactions [Knorr et al. 2004, McClements 1995, Roberts 1993, Zheng and Sun 2006]. The beneficial use of the sound energy is realized through the various effects the ultrasound generates upon the medium where it transmits. Physical, mechanical or chemical effects of ultrasonic waves at this range are capable of altering material properties through generation of immense pressure, shear and temperature gradient in the medium through which they propagate. During the sonication process, longitudinal waves are created when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion. These regions of pressure change cause cavitation to occur, and gas bubbles are formed in the medium. These bubbles have a larger surface area during the expansion cycle, which increases the diffusion of gas, causing the bubble to expand. A point is reached where the ultrasonic energy provided is not sufficient to retain the vapour phase in the bubble; therefore, rapid condensation occurs. The condensed molecules collide violently, creating shock waves. These shock waves create regions of very high temperature and pressure, reaching up to 5500°C and 50 MPa. Cavitation can result in the occurrence of microstreaming which is able to enhance heat and mass transfer [Jayasooriya et al. 2004, Zheng and Sun 2006]. The ability of ultrasound to cause cavitation depends on ultrasound characteristics (e.g. frequency, intensity), product properties (e.g. viscosity, surface tension) and ambient conditions (e.g. temperature, pressure) The ultrasound intensity required to cause cavitation increases markedly above about 100 kHz [Williams 1983].

1.9.2 APPLICATIONS IN THE FOOD INDUSTRY

Developments in the application of ultrasound in processing began in the years preceding the Second World War when it was being investigated for a range of technologies including emulsification and surface cleaning. By the 1960s the industrial uses of power ultrasound were accepted and being used in cleaning and plastic welding which continue to be major applications [Mason 2003]. The possibility of using low-intensity ultrasound to characterize foods was first realized over 60 years ago; however, it is only recently that the full potential of the technique has been realized [Povey and McClements 1988]. There are a number of reasons for the current interest in ultrasound. The food industry is becoming increasingly aware of the importance of developing new analytical techniques to study complex food materials, and to monitor properties of foods during processing; ultrasonic techniques are ideally suited to both of these applications. Ultrasonic instrumentation can be fully automated and make rapid and precise measurements. Ultrasound is non-destructive and non-invasive, can easily be adapted for on-line applications, and used to analyse systems that are optically opaque [McClements 1995]. Within food technology we can find almost all of the examples of processing to which ultrasound can be applied. Until recently the majority of applications of ultrasound in food technology involved non-invasive analysis with particular reference to quality assessment. Such applications use techniques that are similar to those developed in diagnostic medicine, or non-destructive testing, using high frequency low power ultrasound. Examples of the use of such technologies are found in the location of foreign bodies in food, the analysis of droplet size in emulsions of edible fats and oils and the determination of the extent of

crystallization in dispersed emulsion droplets [Mason et al. 1996]. The relationship between measurable ultrasonic properties of foods (velocity, attenuation coefficient and impedance) and their physicochemical properties (composition, structure and physical state) is the basis of the ultrasonic analysis. This relationship can be established either empirically by preparing a calibration curve relating the property of interest to the measured ultrasonic property, or theoretically by using equations describing the propagation of ultrasound through materials [McClements 1995]. By monitoring the attenuation of an ultrasound pulse has proved possible to determine the degree of homogenisation of fat within milk. The measurement of ultrasound velocity in conjunction with attenuation can be used to estimate the degree of emulsification in such materials. It is possible to determine factors such as the degree of "creaming" of a sample, i.e. the movement of solid particles/fat droplets to the surface. Such information gives details, for example, of the long term stability of fruit juices and the stability of emulsions such as mayonnaise. The combination of velocity and attenuation measurements shows promise as a method for the analysis of edible fats and oil as well as for the determination of the extent of crystallization and melting in dispersed emulsion droplets [Mason et al. 1996].

In recent years food technologists have turned their attention to employment of power ultrasound in processing. Its history can be traced back to 1927 when a paper entitled "The chemical effects of high frequency sound waves I. A preliminary survey" was published [Richards and Loomis 1927]. Physical, mechanical, or chemical effects of ultrasonic waves at this range are capable of altering material properties (e.g., disrupting the physical integrity, acceleration of certain chemical reactions) through generation of immense pressure, shear, and temperature gradient in the medium through which they propagate [Ultrasound... 1998]. High power ultrasonic applications generally depend on complex vibration induced effects in the propagating media, which produces cavitation in liquids or biological tissue. In addition to cavitation, ultrasound is able to weaken the physical structure of the material or medium, provided the dimensions of the media are similar to those of the ultrasonic wavelength used [Got et al. 1999]. One of the major long-established industrial applications of power ultrasound is for cleaning and it has proved to be an extremely efficient technology [Mason et al. 1996]. Surface cleaning is applicable to a wide range of disciplines and applications (e.g. sensors, filters, substrates, reactors, catalysers and heat exchangers). Ultrasound has been shown to be particularly effective for in situ cleaning in conjunction with chemical treatment and offers such advantages as: reduced chemical consumption, reduction of direct worker contact with hazardous cleaning chemicals/substances, enhanced cleaning speed, cleaning consistency – the ultrasonic activity is micro in nature and reaches all areas of complex configurations for uniform cleaning, automatic operation and control savings in energy costs, labour and floor space [Mason 2003].

Possible applications of power ultrasound in the food industry are very wide ranging. One of the earliest uses of power ultrasound in processing was in emulsification. Emulsions generated with ultrasound are often more stable than those produced conventionally and often require little, if any, surfactant [Mason et al. 1996]. Investigations have shown that the use of ultrasound as a processing aid can reduce the production time of yoghurt of up to 40%. Moreover, sonication reduced the normal dependence of the process on the origin of milk as well as improved both the consistency and the texture of the product. It was also found that fish egg exposure to ultrasound of frequency 1 MHz for 35 min, three times a day resulted in the reduction in hatch time for loach from 72 to 60 hours. Several reports in the literature suggest that ultrasonic treatment of seeds before sowing is an effective method of improving crop yield [Mason et al. 1996]. One of the original uses of power ultrasound in biochemistry was to break down biological cell walls to liberate the contents. Subsequently it has been shown that power ultrasound can be used to activate immobilized enzymes by increasing the transport of substrate to the enzyme. As far as enzymes are concerned, ultrasound can also be employed as a method of their inhibition [Mason et al. 1996]. Chambers [1937] reported that pure pepsin was inactivated by sonication probably as a result of cavitation. By applying ultrasound for over three hours, the original activity of peroxidase, responsible for the development of off-flavours and brown pigments, was progressively reduced by 90% [Mason et al. 1996]. The use of power ultrasound significantly improves the extraction of organic compounds contained within the body of plants and seeds. The mechanical effects of ultrasound provide a greater penetration of solvent into cellular materials and improve mass transfer [Mason et al. 1996]. Additional benefit results from the disruption of biological cell walls to facilitate the release of contents. Combined with this effect is enhanced mass transfer, due to the effects of microstreaming which results in a more efficient method for sugar extraction [Chendke and Fogler 1975]. The sonication accelerated sugar diffusion and gave the higher level of dry matter content and sugar content in juice [Stasiak 2005]. In some cases sonication increased the efficiency of extraction at lower temperatures producing a purer product in a shorter time [Mason et al. 1996]. By using of ultrasound extraction of tea solids from leaves was improved by nearly 20%. Authors noticed that the majority of material was extracted in the first 10 minutes of sonication [Mason and Zhao 1994]. Zayas [1986] reported that an increased yield of the enzyme rennin from calf stomachs has been achieved by using ultrasound. Moreover, the activity of ultrasonic extract was found to be slightly increased in comparison with normal technology. Power ultrasound has proved to be extremely useful in crystallization processes. It serves a number of roles in the initiation of seeding and subsequent crystal formation and growth [Mason et al. 1996, Stasiak and Dolatowski 2007]. Ultrasound has also been applied to filtration. As a result, the moisture content of slurry containing 50% water was

rapidly reduced to 25%; whereas conventional filtration achieves a limit of only 40% [Mason et al. 1996]. Another example of ultrasound application of potentially great commercial importance is acoustic drying. Ultrasonically enhanced drying can be carried out at lower temperatures than the conventional methodology which reduces the probability of oxidation or degradation in the material. By employing ultrasound the heat transfer between a solid heated surface and a liquid is increased by approximately 30-60% [Ensminger 1988].

Power ultrasound has proved itself an effective method in assisting food freezing and its benefits are wide-ranged. In addition to its traditional application in accelerating ice nucleation process, it can also be applied to freeze concentration and freeze drying processes in order to control crystal size distribution in the frozen products. If it is applied to the process of freezing fresh foodstuffs, ultrasound can not only increase the freezing rate, but also improve the quality of the frozen products. Application of power ultrasound can also benefit ice cream manufacture by reducing crystal size, preventing incrustation on freezing surface, etc. [Zheng and Sun 2006]. Among other applications are improvements in the extraction of flavourings, filtration, mixing and homogenization and the precipitation of airborne powders, destruction of foams which cause general difficulties in process control e.g. in fermentation [Ultrasound... 1998]. As a result of continued research interest and development in instrumentation, novel applications such as oxidation of unsaturated oils, aging of alcoholic beverages, hydration of acetylene, decalcification of bone, hydrolysis of esters have been developed [Mason 1999, McClements 1995].

1.9.3 ULTRASONIC INACTIVATION OF MICROORGANISMS

The most common techniques currently used to inactivate microorganisms in food products are conventional thermal pasteurization and sterilization. Thermal processing does kill vegetative microorganisms and some spores; however, its effectiveness is dependent on the treatment temperature and time. However, the magnitude of treatment, time and process temperature is also proportional to the amount of nutrient loss, development of undesirable flavours and deterioration of functional properties of food products. High power ultrasound is known to damage or disrupt biological cell walls which will result in the destruction of living cells. Unfortunately very high intensities are needed if ultrasound alone is to be used for permanent sterilization. However, the use of ultrasound coupled with other decontamination techniques, such as pressure, heat or extremes of pH is promising. Thermosonic (heat plus sonication), manosonic (pressure plus sonication), and manothermosonic (heat plus pressure plus sonication) treatments are likely the best methods to inactivate microbes, as they are more energy – efficient and effective in killing microorganisms. The advantages of ultrasound over heat pasteurization include: the minimizing of flavour loss, greater homogeneity and significant energy savings [Mason et al. 1996, Piyasena et al. 2003]. A considerable amount of data exists regarding the impact of ultrasound on the inactivation of microorganisms [Piyasena et al. 2003]. The effectiveness of an ultrasound treatment is dependent on the type of bacteria being tested. Other factors are amplitude of the ultrasonic waves, exposure time, volume of food being processed, the composition of food and the treatment temperature. Bactericidal effects of ultrasound were observed while suspended in culture medium [Davies 1959]. According to Lillard [1993] Salmonellae attached to broiler skin were reduced upon sonication in peptone at 20 kHz for 30 min. Results of research carried out by Dolatowski and Stasiak [2002] proved that ultrasound processing was having a significant influence on microbiological contamination of meat. There are a large number of potential applications of high intensity ultrasound in the food industry. Applications of both high- and low-frequency ultrasound in the food industry have already been shown to have considerable potential for either modifying or characterising the properties of foods. In many instances, techniques based on ultrasound have considerable advantages over existing technologies.

1.10 OZONE PROCESSING

Ozone has a highly biocidal effect and a wide antimicrobial spectrum for food preservation technology. In the food industry, ozone has been routinely used for washing and storage of fruits and vegetables by gaseous treatment. With the recent FDA approval of ozone as a direct additive to food, the potential of ozonation in liquid food applications has increased (Cullen *et al.*, 2009). Ozone as an antimicrobial agent has numerous potential applications in the food industry because of its advantages over traditional antimicrobial agents such as chlorine and potassium sorbates. Ozone processing within the food industry has been carried out on solid foods by either gaseous treatment or by washing with ozonated water. However, with the FDA approval of ozone as a direct additive to food, the potential of ozonation in liquid food applications has begun to be exploited. A number of commercial fruit-juice processors in the USA have begun to employ ozone to meet the recent FDA mandatory 5 log reduction of the most resistant pathogens in their finished products. This practice has resulted in industry guidelines being issued by the FDA for the ozonation of apple juice (FDA, 2004). The FDA's approval of ozone as a direct additive to food in 2001 triggered interest in ozone applications, with a number of commercial fruit juice processors in the US and Europe employing ozone for pasteurization, resulting in industry guidelines being issued by the United States Food and

Drug Administration (USFDA, 2004). The use of ozone application for the disinfection or storage of various exotic fruits or their products, including kiwi fruit, has been reported (Graham & Tyman, 2002; Hur *et al.*, 2005; Oztekin *et al.*, 2006; Whangchai *et al.*, 2006; Akbas & Ozdemir, 2008; Zorlugenc *et al.*, 2008; Barboni *et al.*, 2010; Meyvaci *et al.*, 2010). However, most of the reported studies are limited to the microbiological analysis of exotic fruits. Ozone at concentrations of 0.15–5.0 ppm has been shown to inhibit the growth of spoilage bacteria as well as yeasts (Jay *et al.*, 2005). Ozone has been investigated for fruit juice processing applications, including apple cider (Steenstrup & Floros, 2004; Choi & Nielsen, 2005). Torres *et al.* (2011) reported that apple juice color, rheological properties and phenolic content were significantly influenced by ozonation. Thus, although ozonation can be employed as a preservation technique for processing apple juice, its impact on the nutritional and quality parameters of the juice should be considered.

Willams *et al.* (2005) studied the effect of ozone in combination with dimethyl dicarbonate and hydrogen peroxide for orange juice preservation. They reported that a 5-log reduction of *E. coli* O157:H7 could be achieved by using ozone in combination with dimethyl dicarbonate. Similarly, Patil *et al.* (2009) reported a 5-log reduction of *E. coli* NCTC 12900 in <7 min in orange juice. Steenstrup and Floros (2004) reported that the overall inactivation of *E. coli* O157:H7 by ozone is rapid enough for practical application in apple juice production. Excess ozone auto-decomposes rapidly to produce oxygen, and thus, it leaves no residues in food. The half-life of ozone in distilled water at 20 °C is approximately 20-30 min (Khadre *et al.*, 2001). The applications of ozone in fruit and vegetable processing were extensively reviewed by Karaca and Velioglu (2007). Tiwari *et al.* (2008a), Tiwari *et al.* (2009a) and Tiwari *et al.* (2009b) recently highlighted that the nutritional quality depends on the ozone control parameters of concentration and gas flow rate. Achieving rapid microbial inactivation using optimized control parameters while retaining the nutritional quality is important. Patial *et al.* (2010) stated that overall, the gaseous ozone treatment applied to orange juice resulted in a population reduction of 5 log cycles within a time range that varied between 5 and 9 min. Table 2 lists recent studies on ozone application in fruit juices, whole fruits and vegetables. Although the ozonation of liquid foods is still in its infancy, it has been reported for the processing of various fruit juices, including apple cider (Steenstrup & Floros, 2004; Choi & Nielsen, 2005; Williams *et al.*, 2005) and orange juice (Angelino *et al.*, 2003; Tiwari *et al.*, 2008a). Ozone is extensively applied in the treatment of water and wastewater due to its powerful oxidation and disinfection capabilities. Ozone as an oxidant is used in natural water treatment, washing and disinfecting of fruits and vegetables and juice processing to inactivate pathogenic and spoilage microorganisms (Muthukumarappan *et al.*, 2000).

Increasing consumer demand for fresh products, which are usually refrigerated, has led the food industry to develop alternative processing technologies. The goal is to produce food with minimal nutritional, physicochemical or organoleptic changes induced by these technologies (Esteve & Frígola, 2007) while maintaining safety profiles with respect to the pathogens of concern. Ozone is a triatomic allotrope of oxygen and is characterized by a high oxidation potential that conveys bactericidal and virucidal properties (Burleson *et al*., 1975; Kim *et al.*, 1999). Ozone inactivates microorganisms through oxidization, and residual ozone decomposes to nontoxic products (i.e., oxygen), making it an environmentally friendly antimicrobial agent for use in the food industry (Kim *et al.*, 1999). In the gas or aqueous phase, ozone has been used to inactivate microorganisms and decontaminate meat, poultry, eggs, fish, fruits, vegetables and dry foods (Fan *et al.*, 2007).

1.11 PLASMA TECHNOLOGY

1.11.1 PLASMA DEFINITION, GENERATION AND CLASSIFICATION

Plasma is ionized gas, that consists of a large number of different species such as electrons, positive and negative ions, free radicals, gas, atoms, molecules in the ground or excited state and quanta of electromagnetic radiation (photons). It is considered to be the forth state of matter in the world. It can be generated in the large range of temperature and pressure by means of coupling energy to gaseous medium. This energy can be mechanical, thermal, nuclear, radian or carried by an electric current. These energies dissociate the gaseous molecules into collection of ions, electrons, charge – neutral gas molecules and other species. Depending on the type of energy supply and amount of energy transferred to the plasma, density and temperature of the electrons are changed. These lead Plasma to be distinguished into two groups, high temperature plasma and low temperature plasma (given in table 1). High temperature plasma implies that electron, ions and neutral species are in a thermal equilibrium state. Low temperature plasma is subdivided to thermal plasma, also called local thermodynamic equilibrium plasmas (LTE) and non thermal plasma (NTP), also called non-local thermodynamic equilibrium plasmas (non-LTE). An equilibrium or near equality between electrons, ions and neutrals is the main characterization of thermal plasmas (TP). Frequently employed thermal plasma generating devices are those produced by plasma torches, and microwave devices. In generation of cold plasma most of the coupled electrical energy is channeled to electron component instead of heating entire gas stream so the temperature of heavy particle remains near the room temperature, these characteristics make it suitable to be used in processes which high temperature is not desirable.

Table 1. Classification of Plasma

Plasma	Properties	Example
High temperature plasma (Equilibrium plasma)	Te ≈ Ti ≈ Tg , Tp = $10^6 - 10^8$ K	Laser fusion plasma
	Low temperature plasma	
Thermal plasma (Quasi-equilibrium plasma)	Te = Tg = T≤ 2×10^4 K $n_e \geq 10^{20}$ m^{-3}	Arc plasma
Non-thermal plasma (Non-equilibrium plasma)	Te > > Ti ≈ Tg = 300 – 10^3 K $n_e \approx 10^{10}$ m^{-3}	Glow discharges

Table 2. Recent findings in the area of nonthermal plasmas for inactivation of micro-organisms and Spores

Organism	Plasma conditions	Treatment surface/ medium	Salient result	References
Escherichia coli, Staphylococcus aureus	Atmospheric plasma corona discharge, with high voltage (20kV) DC power supply	On agar plates	Changes of pH levels from alkaline to acid, upon plasma application to bacteria in water, does not play a predominant role in cell death.	Korachi et al. (2010)
Staphylococcus aureus	DC cold- atmospheric-pressure plasma microjet with compressed air as the working gas	Aqueous suspensions of the organism	First 10 min treatment led to insignificant inactivation. After 16 min *S. aureus* was completely inactivated. Effective inactivation of *S. aureus* was found to start after the pH values decreased to about 4.5.	Liu et al. (2010)
Bacillus atrophaeus, Geobacillus sterathermophilus, Clostridium sporogenes, Kocuria rhizophila, Staphylococcus aureus, Aspergillus niger	low- pressure inductively-coupled plasma (ICP) with different mixtures of gases	Glass substrates and silicon wafers coated with the organism by spraying.	All the organisms were found to be reduced by at least 4 orders of magnitude under optimized low-pressure argon plasma. Efficiency of inactivation was variable for different strains of a given species.	Von Keudell et al. (2010)
Bacillus subtilis	Oxygen and nitrogen treated using 8 Duo-Plasmalines driven by microwaves power	Microscopic slides stacked with spores	Plasma treatment of the spores caused release of DPA, generation of auxotrophic mutants, reduction in Kat X activity and damage to DNA. A biphasic model for the inactivation kinetics was proposed.	Roth et al. (2010)

1.11.2 ATMOSPHERIC PRESSURE PLASMAS (APP)

Low pressure glow discharge plasmas are of great interest in microelectronic industries but their vacuum equipment limits their application. Therefore one of the recent challenges was developing new plasma sources that can operate at or near 1 atmospheric pressure. Power sources of atmospheric pressure plasma generation can be microwave, RF (radio frequency), pulsed, AC (alternating current) or DC (direct current). Devices that have been used for plasma generation are the corona discharges, micro hollow cathode discharges, gliding arc discharge, one atmospheric uniform glow discharge, dielectric plasma needle barrier discharge (DBD), atmospheric pressure plasma jet (APPJ). Among all. DBD and APPJ are commonly used in industrial application like lightening, surface modification, etching and deposition.

1.11.3 MICROBIAL INACTIVATION MECHANISM OF PLASMA

The use of plasma as a sterilization method was first patented in 1968 and the plasma made from oxygen was first applied in 1989 after that considerable researches have been done on mechanisms of microbial inactivation by plasma agent. Interacting plasma agent with biological mater contributed to lethal action. Plasma treatment can effectively inactivate a wide range microorganisms including spores [6-8] and viruses. Effect of plasma on different microorganisms can be completely selective, meaning that it can damage pathogenic microorganisms without damaging host or it can activate different pathways in different organisms. The reactive spices in plasma have been caused the oxidative effects on the outer surface of microbial cells. Nitrogen and oxygen gas plasma are good sources of reactive oxygen- based and nitrogen-based spices such as O., O2, O3, OH, NO., NO2. Chemical rate constant of atomic oxygen for oxidation at room temperature is so higher than molecular oxygen. These act on the doubled bond of unsaturated fatty acid of membrane cell, thereby disturbing the transport of bimolecules across it. In spite of oxidation of the lipids, amino acids and nucleic acids of cells and spores are vulnerable to the action of these spices and oxidation of them cause changes that lead to microbial death or injury. In addition to reactive spices, UV photons can modify the DNA of the microorganisms and as a result disturb cell replication. The role of UV photons in inactivation of microorganisms when they are subjected to plasma was reviewed in detailed by

Boudam et al.. Many studies have found that reactive spices had the most important role in inactivation of microorganisms and the role of UV photons in plasma was minor, but these studies demonstrated that more researches need to be done over the role of UV photons in plasma. Contribution of each of the above mentioned mechanisms in inactivation microorganism depends on plasma characteristics and to the type of microorganisms. The former includes device set up (reactor geometry), voltage, gas pressure, gas composition, water content in the gas, and distance of the microorganism from the discharge glow, where the latter takes account of Gram-positive, Gram -negative, spores and other types. To cite an example, Hury et al., compared the destructive efficiency of different gas composition and temperatures on Bossilus spp. Spores. They found that oxygen-based plasma is more efficient than pure argon plasma. The other criterion that was considered is the direct exposure or remote exposure of substances from the plasma sources, The recent findings demonstrated that if the substrate which is sterelizied to be in indirect contact (remote exposure) with the plasma, the quantum of heat transmitted to a sample is reduced and many of the short-lived reactive spices do not reach the sample so the treatment cannot be efficient in microorganism inactivation.

1.11.4 COLD PLASMA

Cold plasma is a novel non-thermal food processing technology designed for the inactivation of pathogenic microoganisms and food safety improvement. CP is a ionized gas that comprises a large number of different species such as electrons, positive and negative ions, free radicals, electrons and gas atoms, photons and it is suitable to be used in processes for which high temperature is not recommended. CP could be employed in inactivation of the microorganisms on the surface of fresh and processed foods. The accumulation of charged particles can rupture the cell membrane. Oxidation of the lipids, amino acids and nucleic acids with reactive oxygen species and nitrogen species cause changes that lead to microbial death or injury. Contribution of mentioned mechanisms depends on plasma characteristics and on the type of microorganisms. CP has been applied in the food industry including for decontamination of raw agricultural products (apple, lettuce, almond, mangoes and melon), egg surface and real food system (cooked meat, cheese). However, there are few studies on the application of this technology in real food systems and on the effects of cold plasma on nutritional and chemical properties of food are not known well. Key limitations for cold plasma are the relatively early state of the technology development, the variety and complexity of the necessary equipment, and the largely unexplored impacts of cold plasma treatment on the sensory and nutritional qualities of treated foods. The treatment must be proven not to have negative impact on the organoleptic and nutritional properties of foods. Hence, it is necessary for further studies to specify the extent in which CP affect the chemical and the nutritional properties of foods and its shelf-life. Furthermore, the studies which explore the safety and cost aspects to apply into practice the CP and for scaling up this technology in food industry should be addressed to determine the applicability of this method. Combining the CP treatment with other non-thermal processes could be a possible future breakthrough in this field. In this case, synergistic effects may be more considerable, however scaling up this technology remains for the moment a challenge to be solved.

1.11.5 BENEFITS AND APPLICATIONS

Cool (or nonthermal) plasma has many potential applications for the food industry, including the dry disinfection of food surfaces (meat, poultry, fish, nuts, fruit and vegetables), powders (dried milk components, flour, herbs and spices) and seeds for sprouting (alfalfa and mung beans). The technology can also be used to disinfect surfaces of processing equipment and packaging materials. The gaseous atoms and ions of plasma are effective against bacteria, viruses, moulds and fungi. The plasma can penetrate cracks and crevices unlike other potential surface treatments such as ultraviolet light. Therefore, the technology functions more effectively over uneven or cracked surfaces such as those found on many foods (seeds, meats etc). Cool plasma has several advantages over competing preservation technologies such as irradiation, chemical sterilization (e.g. ethylene oxide) or disinfection treatments (e.g. chlorine). These can have detrimental sensory effects on treated food products or they are being phased out because of safety concerns. Although cool plasma technology is not yet used commercially on a large scale in the food industry, it has been successfully used for decades in many other industries, and is readily scaleable. Cool plasma systems for treating foods at atmospheric pressure are currently being developed and tested. Target foods include nuts, spices, flour, and horticultural produce.

1.12 PULSE X-RAYS

Pulsed X-ray is a technology that utilizes a solid state opening switch to generate electron beam x-ray pulses of high intensity. Electrons have limited penetration depth of about 5cm in food while x-ray have significantly higher penetration depths (60-400cm) depending upon the energy used.

Curry and others used a system consists of an x-ray accelerator with a thyristor charging unit, a magnetic pulse compressor, a solid state opening switch, an electron beam diode load and an x-ray convertor. The thyristor charging unit converts 3 phase current to direct current. A thyristor capacitor charging circuit is used to charge the magnetic pulse compressor. A 2-stage circuit compresses and sequentially steps up the voltage pulse before it is used to charge an inductive load. Energy from capacitor is transferred from the inductive load in approximately 100ns. A convertor is installed on the accelerator and the electron beam is converted to pulsed x-rays to allow thick samples to be processed. Curry and others used pulsed x-rays to produce up to a 3 log reduction of e-coli O157:H7 in ground beef.

1.12.1 APPLICATIONS OF PULSED X-RAYS

Use in examination of packaged food for tramp materials, inactivation of e-coli in meat also used to eliminate Salmonella from foods.

2 CONCLUSION

The non-thermal technology is the emerging technology, in demand for the processing and preservation of food in recent and coming years, in the area of food processing, food engineering and food technology. Above mentioned NTT retains the fresh quality of food for longer time, only the point of consideration is the consumer's knowledge about these technology and safety of the product they need to consume. With the increasing demand of these technology there implementation cost and the finally the product cost is going to get reduced, benefiting human kind.

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