International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 6; November 2017; Page No. 171-180

Non-thermal processing in food applications: A review

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Abstract
The food-processing industry has made large investments in processing facilities relying mostly on conventional thermal processing technologies with well-established reliability and efficacy Food contains many heat sensitive nutrients which include vitamins, minerals, and nutrients having functional properties such as pigments, antioxidant, Bioactive compounds. Many processes during manufacturing of food cause detrimental effects on these nutrients. Retention of these nutrients in food products requires innovative approaches for process design because of their sensitivity to a variety of physical and chemical factors, which causes either loss of biological functionality, chemical degradation and premature or incomplete release. Alternative methods for thermal processing of food are gaining importance, due to increased consumer demand for new methods of food processing that have a reduced impact on nutritional content and overall food quality. Also as a result of the increasing consumer demand for minimally-processed fresh-like food products with high sensory and nutritional qualities, there is a growing interest in non-thermal processes for food processing and preservation. Non-thermal food processing/preservation methods interest food and food packaging scientists, manufacturers and consumers because they exert a minimal impact on the nutritional and sensory properties of foods, and extend shelf life by inhibiting or killing microorganisms. They are also considered to be more energy efficient and to preserve better quality attributes than conventional thermally based processes. Non-thermal physical processes are evolving as potential alternatives to thermal and chemical unit operations in food processing. Non-thermal methods allow the processing of foods below temperatures used during thermal pasteurisation, so flavours, essential nutrients, and vitamins undergo minimal or no changes. These processes also meet industry needs by offering value- added products, new market opportunities and added safety margins.

Keywords: preservation, thermal, bioactive, antioxidants

Introduction
Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. Within the disposable arsenal of preservation techniques, the food industry investigates more and more the replacement of traditional food preservation techniques (intense heat treatments, salting, acidification, drying and chemical preservation) by new preservation techniques due to the increased consumer demand for tasty, nutritious, natural and easy-to-handle food products. The most investigated new preservation technologies are non-thermal inactivation technologies such as high hydrostatic pressure (HHP) and pulsed electric fields (PEF), new packaging systems such as modified atmosphere packaging (MAP) and active packaging, natural antimicrobial compounds and bio preservation. In spite of the intensive research efforts and investments, very few of these new preservation methods are until now implemented by the food industry. Despite the recognized advantages of the pressurizedproducts, a positive consumer attitude towards them isnecessary, to guarantee the success of the product in today's competitive global market, where the new food product innovation is required for survival. In this sense,emerging and improved technologies are increasinglybeing used in food innovation to successfully differentiateproducts [1]. Theknowledge that a product success is dependent uponthe product being unique and superior; good understandingof consumer wants, needs and preferenceswas established during the late 1970s [2] and needs to be kept in the food processors mind.

Non-thermal inactivation technologies
The last decade, non-thermal inactivation techniques have been a major research issue, driven by an increased consumer demand for nutritious, fresh like food products with a high organoleptical quality and an acceptable shelf life. Investigated inactivation technologies are ionisation radiation, HHP, pulsed electrical fields, high pressure homogenisation, UV decontamination, pulsed high intensity light, high intensity laser, pulsed white light, high power ultrasound, oscillating magnetic fields, high voltage arc discharge and streamer plasma.

Pulsed X-Ray
Electrons have a limited penetration depth of about 5 cm in food, while X-rays have significantly higher penetration depths (60 - 400 cm) depending upon the energy used. Pulsed X-rays are generated using radionuclide sources that utilizes a solid state-opening switch to generate electron beam X-ray pulses of high intensity. The radionuclides Co-60 and Cs-137 are produced by neutron bombardment of Co-59 and Cs-136 as a fission fragment of a nuclear power reactor operation. They emit γ-radiation of discrete energy. These radionuclide

sources require permanent massive concrete shielding to protect workers and the environment from their permanent radiation. Second approach is electrically driven radiation sources that switch off when the radiation is no longer needed are easier to incorporate into existing food processing plant. Linear Induction Electron Acceleration (LIEA) generates broad spectrum ionizing radiation by targeting the accelerated electron beam to collide with a heavy metal converter plate. This plate converts the electron beam in X-rays with a broad-band photon-energy spectrum. Then, by filtering the energy spectrum of the radiation, high-energy, highly penetrating radiation is produced, resulting in smaller variations in dose uniformity of food packages and higher quality. LIEA can deliver dose rates many orders of magnitude higher than possible with Co-60 sources. Consequently, ultra-short, high-intensity radiation treatments can be applied, resulting in higher local radical concentrations and favouring radical-radical recombination reactions. This reduces the diffusion of radical species, which are thought to be responsible for undesirable effects of irradiation on food quality.

Salient Features of this technology are (1) flexibility of controlling the direction of the electrically produced radiation; (2) the flexibility of shaping the geometry of the radiation field to accommodate different package sizes; and (3) its high reproducibility and versatility. The kinetic energy limit for X-ray irradiation is 5MeV (Codex Alimentarius General Standard on Food Irradiation (2003).X-ray treatment reduces or eliminates Salmonella serovars in poultry, mold growth on strawberries and sprout development in potatoes. Salmonella serovars have been found to be the most radiation sensitive of all pathogenic organisms on foods. As a method of food preservation, X-ray treatment has low energy requirements. Microbial inactivation by all types of ionizing radiation is thought to happen through 2 main mechanisms:

Direct interaction of the radiation with cell components and indirect action from radiolytic products, such as the water radicals. The primary target of ionizing radiation appears to be chromosomal DNA, although effects on the cytoplasmic membrane may also play a role. Changes to chromosomal DNA and/or cytoplasmic membrane can cause microbial inactivation or growth inhibition. Many studies have shown that ions, excited atoms and molecules generated during irradiation have no toxic effect on humans (USA - FDA Center for Food Safety and Applied Nutrition (2011)

Pulsed Visible Light

The technique of pulsed light food processing was developed as a non-thermal food processing technique, that involves discharge of high voltage electric pulses (upto 70 Kilovolt/cm) into the food product placed between two electrodes for few seconds [5]. It is one of the emerging technologies which are used for the replacement of traditional thermal pasteurization among non thermal processes [26]. It is a decontamination technique which aims at reducing the pests, spoilage microorganisms and pathogens from food without much effect on its quality [10]. It is recognized by several names in scientific literature i.e., Pulsed ultraviolet light [47], high intensity broad-spectrum pulsed light [45], Pulsed light [46] and pulsed white light [37]. The pulsed light processing can be described as a sterilization or decontamination technique used mainly to inactivate surface micro-organisms on foods, packaging material and equipments. This technique uses light energy in concentrated form and exposes the substrate to intense short bursts of light (pulses). Typically for food processing about one to twenty flashes per second are applied.

Mechanism of microbial inactivation

The lethality of Pulsed Light may be attributed to its rich broad spectrum ultraviolet content, its short duration, high peak power and the ability to regulate the pulse duration and frequency output of flash lamps [21, 54]. As a substantial portion of the Pulsed light spectrum covers ultraviolet light, it is considered that ultraviolet plays a vital role in the microbial cell inactivation. It was also found that that there is no killing effect if a filter is used to remove ultraviolet (UV) wavelength region lower than 320 nm [54]. The ultraviolet spectrum comprises of three wave ranges: Long-wave ultraviolet -A (320-400 nm), Medium- wave ultraviolet -B (280-320 nm) and Short-wave ultraviolet -C (200-280 nm). Mechanisms that have been proposed to explain the lethality of pulsed light treatment are related to ultraviolet (UV) part of the spectrum which include photochemical and photothermal effect [4, 54, 63]. The lethal effect of pulsed light can be due to photochemical or photothermal mechanism or both may exist simultaneously. However their relative importance depends on the fluence and target microorganism. The lethal effect of pulsed light was explained by most of the authors on the basis of photochemical mechanism e.g., the inactivation achieved by [46] was associated with less than 1^0C rise in temperature concluded that the lethality can be attributed to the photochemical action of the shorter ultraviolet wavelengths. The primary target cell of pulsed light in photochemical mechanism is nucleic acid as DNA is the target cell for these ultraviolet wavelengths [17, 38]. Ultraviolet light absorbed by the conjugated carbon-carbon double bonds in proteins and nucleic acids induces the antimicrobial effect as it changes the DNA and RNA structures. The bactericidal effect is attributed to the high energy short wave ultraviolet-C range. In the ultraviolet-C range of 250-260 nm, alterations in DNA take place due to pyrimidine dimers mainly thymine dimers [24]. These dimers inhibit the formation of new DNA chains in the process of cell replication resulting in the chologenic death of affected microorganisms by ultraviolet [13]. The ultraviolet-C treatment of bacterial spores may result in the formation of spore photo-product 5-thyminyl-5, 6-dihydrothymine and in single-strand breaks, double-strand breaks and cyclobutane pyrimidine dimers [48]. It was also found by experiments that enzymatic repair of DNA does not occur after damaged by pulsed light. The lethal effect of Pulsed light can also be due to photothermal effect [60]. Proposed that with a fluence exceeding 0.5 Joule/cm2, the disinfection is achieved through a rupture of bacteria during their temporary overheating caused by absorption of all ultraviolet light from a flash lamp. This hypothesis become evident by [61] when they showed electron-microscope photographs of flashed Aspergillusniger spores presenting severe deformation and rupture. The ruptured top of spore become evident of an escape of an overheated content of the spore, which became empty after such an internal ‘‘explosion’’ and ‘‘evacuation’’ of its content took place during the light pulse. As Pulsed light causes cell

membrane damage, it could be considered as a technique for sterilization [54, 12]. [22], reported that PL treatments achieves high levels of microbial inactivation on relatively simple surfaces, while generally showed only 1–3 log reductions on complex surfaces such as meats. Part of the radiation may have been absorbed by proteins and lipids, thus decreasing the effective radiation dose on microorganisms [25]. Proteins have strong absorption of UV at about 280 nm as well as at higher wavelengths of the UV-B region, while lipids with isolated or conjugated double bonds also absorb UV [22] demonstrated that beef steaks treated with PL using 5 J cm−2 to each side and stored 3 days at 4–5 °C exhibited 2 log reductions in microbial counts. Listeria innocua was reported to be reduced by 2 log cycles on hot dogs after a PL treatment [22]. Milk was efficiently cold pasteurized by the exposure to PL at a minimum dose of 12.6 J cm−2 delivered in 56 s [50]. Complete inactivation of S. aureus was obtained when processing milk in a continuous system applying PL [32].

Advantages The intensity of light, that lasts for only a second, is 20,000 times brighter than sunlight, but there is no thermal effect, so quality and nutrient content are retained The xenon-flash lamps used in pulsed light treatment are more eco-friendly than the mercury vapour lamps used in ultraviolet (UV) treatment [25].

Disadvantages a possible problem of this preservation method is that folds or fissures in the food may protect microbes from being exposed to the pulsed light (Brown, 2008). There might be some strains of micro-organisms which are resistant to the pulsed light treatment, for example Listeria monocytogenes. This technique for decontamination of micro-organisms is useful mostly in case of liquid foods and surface of solid foods and hence limiting its application.

Pulsed Electric Field (PEF)

During PEF processing, energy is stored in a capacitor, retrieved from a high-voltage power supply, and is discharged through foods that are either static or are flowing through a treatment chamber. PEF uses short bursts of electricity (sub-microseconds to milliseconds), yielding few to no detrimental affects on quality attributes in pumpable foods. This process pulses high voltage (10–80 kV/cm) into foods placed between two electrodes, for less than one second, near ambient temperature, then packaged aseptically and distributed refrigerated. This process attains a 5 log reduction on most pathogenic bacteria by rupturing the cell membranes in liquid media. It causes only minimal detrimental changes to the physical and sensory properties in foods, helps retain 'fresh' quality and assists in nutrient retention. PEF can be applied to the pasteurization of liquid products, in continuous systems, such as milk, yogurt, juices, liquid eggs, soups, brines and other products that can withstand high electric fields. High electric field pulses can be employed to aid in the extraction of polysaccharides and peptides. PEF has limited effects on microbial spores, cannot be used on products that contain or could form air bubbles, and cannot be used on foods that have higher or variable electrical conductivity. 'Pressure is applied to inhibit the formation of air bubbles in which electrical arcing could occur with fields above 20000V/cm'. Since PEF kills cells and impairs water retention, it can aid in filtration methods and can also be used for the extraction of sugars and starches from root vegetables. PEF only affects a few enzymes, a concern in the juice industry. Enzymes negatively affect juice processing by reducing pectin, which aids in fruit particle suspension, and may cause sedimentation, discolouration and flavour degradation. Critical factors that can affect the inactivation of microorganisms using PEF include process variables, media and microbial factors. PEF processing variables include pulse wave and width, electric field intensity, temperature and time of exposure. Electric fields are produced on equipment that can be compared to that of radar. 'The most typical equipment generates a short square wave and reverses polarity, in part to avoid erosion of electrodes [19]. Two other wave forms can be produced, which include sinusoidal and exponential decay. In reverse polarity, bipolar generators are twice the cost of non-polar units, making this an expensive process at this time. One operation has received FDA approval and is being used by Genesis Juice Corp. for fresh juice processing. The sinusoidal wave form uses equipment comparable to that of a radio and is less difficult to generate. A square wave can deliver more energy per cycle because the sinusoidal only reaches maximum power for a split second. Although PEF is a non-thermal process, an increase in temperature occurs in the processing chamber.

A typical temperature change is about 30°C for orange juice and less for apple juice. Processes typically operate at 35–50°C'.4 Time of exposure depends on several factors, first of which is the chamber design of which there are two categories: flowing and non-flowing. Flowing processes include co-axial, parallel plate or co- field. In the co-field method, the electric field is cycled 1000 times a second through various treat- ment chambers, separated by ceramic or polymer insulators, while receiving multiple pulses. In non-flowing, the process is static and can be applied to solids. PEF imposes a strong electric field on pumpable foods for a very short time to kill vegetative cells. Critical field strengths of about 15000V/cm are used on foods, whereas at 35000V/cm, PEF is used as a disinfectant. Under PEF, cell membrane pores develop or enlarge, and can be reversible or irreversible. Pores affect membrane permeability by allowing external matter to enter, causing a loss of cellular content, thereby killing the cell. Perforation of cell membranes caused by PEF in fruit and vegetable cell walls can yield improved extraction of juice from cells.

Disadvantages that must be overcome in order to commercialize PEF

a. Scale up of the system in such a way that profitable production is possible
b. The presence of bubbles, which may lead to non-uniform treatment as well as operational and safety issues
c. Treatment of suspensions with solid particles, with a minimum risk of breakdown
d. Availability of commercial units [11]

If bubbles are present in the PEF treatment chamber, dielectric breakdown will occur. This happens because the spherical gas bubbles elongate, causing the ends to have up to a five times more intense electric field. The bubbles grow larger as the electric field overcomes the dielectric strength of the bubbles,

causing partial discharge, and eventually connecting the two electrodes, causing a spark. Vacuum de-gassing and pressurized treatment during processing can minimize the presence of gas bubbles. Concerns must be addressed when considering PEF for the treatment of suspensions with particulates: include the potential for dielectric breakdown on the surface of particulates; uniform treatment distribution of the applied electric field; the manufacturing of a treatment chamber and feed pump system designed for particulates; control of heat induced by the process; and the particle size must be smaller than the gap in the treatment region to ensure proper processing [11].

Applications of pulsed electric fields technology

PEF is a continuous processing method, which is not suitable for solid food products that are not pump able. Pulsed electric fields technology has been successfully demonstrated for the pasteurization of foods such as juices, milk, yogurt, soups, and liquid eggs. Application of PEF processing is restricted to food products with no air bubbles and with low electrical conductivity. The maximum particle size in the liquid must be smaller than the gap of the treatment region in the chamber in order to ensure proper treatment. PEF processing has been successful in a variety of fruit juices with low viscosity and electrical conductivity such as orange, apple, and cranberry juice. Additionally, the color change in fruit juices (subject to prolonged storage) was reportedly less in juices treated by PEF, as in a recent study of PEF-treated orange juice stored at 4°C for 112 days; there was less browning than thermally pasteurized juice, which was attributed to conversion of ascorbic acid to furfural. Considering the effectiveness of PEF treatment on liquid products, such as milk, fruit juices, liquid egg, and any other pumpable food products, extensive research has been done to implement the process at an industrial level. Flavor freshness, economic feasibility, improvements in functional and textural attributes and extended shelf life are some of the main points of interest besides achievement of microbiological safety of food products [21].

Ultrasound

Ultrasonic waves (energy generated by sound waves of 20,000 Hz or more) generate gas bubbles in liquid media, that produce a high temperature and pressure increase when they immediately burst [59]. The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating and production of free radicals [15]. 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 dif- fusion 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 ^{0}C and 50,000 kPa. The pressure changes resulting from these implosions are the main bactericidal effect in ultra- sound. The hot zones can kill some bacteria, but they are very localized and do not affect a large enough area. The cavitation threshold of a medium (that is, the minimum oscillation of pressure that is required to produce cavitation) is determined by a number of factors. Among these are dissolved gas, hydrostatic pressure, specific heat of the liquid and the gas in the bubble, and the tensile strength of the liquid. Another extremely important variable is temperature, which is inversely proportional to cav- itation threshold. The ultrasonic frequency used must be under 2.5 MHz, as cavitation will not occur above that level [3].

Mechanism and effect of ultrasonics

When sound energy passes to the medium resulting in a continuous wave-type motion, longitudinal waves will be generated with the result that the motion creates alternative compression and rarefaction of the medium particles. For food processing purposes it is important to address the generation of heat due to ultrasound applications and the related cavitation (implosion of gas bubbles) caused by a rapid change of heating to 5500_C and pressure increase to 50 Mpa. The temperature and pressure indicated are generated during a very short periods of time at the point were cavitation occurs with an order of temperature variation of 109 _C/s [52]. Shock waves are generated due to cavitation, which are contributed to the ultrasound effect.

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High pressure processing

The technology of high pressure processing (HPP) also referred to as ultra high pressure UHP) or high hydrostatic pressure (HHP) has been known to be a potential preservation technique for more than a century (e.g. Hite, 1899); for instance, microbial spoilage of milk could be delayed by high pressure. Technical-scientific progress has led to a renaissance of food pasteurisation by hydrostatic high pressure recently [18]. A range of pressure-treated products has already been introduced into the markets of Japan, France, Spain and USA. HPP subjects liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa. Process temperature during pressure treatment can be from below 0 C to above 100 C. Exposure times can range from a few seconds to over 20 min.

Food treated in this way has been shown to keep its original freshness, colour, flavour and taste. HPP acts instantaneously and uniformly throughout a mass of food independent of size, shape and food composition. Compression will increase the temperature of foods approximately 3 C per 100 MPa and may also shift the pH of the food as a function of imposed pressure. Pres- sure pasteurisation is feasible also at room temperature and energy saving as compared to heat treatment. Water activity and pH are critical process factors in the inactivation of microbes by HPP. An increase in food temperature above room temperature and to a lesser extent a decrease below room temperature in some cases increases the inactivation rate of micro-organisms during HPP treatment. Temperatures in the range of 45– 50 C appear to increase the rate of inactivation of food pathogens and spoilage microbes.

Temperatures ran- ging from 90 to 110 C in conjunction with pressures of 500–700 MPa have been used to inactivate spore-form- ing bacteria such as Clostridium botulinum. Current pressure processes include batch and semi-continuous systems. Besides destruction of micro-organisms there are further influences of pressure on food materials to be expected: protein denaturation or mod- ification, enzyme activation or inactivation, changes in enzyme–substrate interactions, changes in the properties of polymer carbohydrates and fats [27]. Generally any process and any reaction in food to which the principle of Le Chatelier applies are of interest. According to this principle, under equilibrium conditions, a process associated with a decrease in volume is favoured by pressure, and vice versa. An increase of pressure has been found to change the reaction rate of chemical reactions in solution. But this effect is small as compared to the influence of temperature. The renewed interest in high-pressure pasteurisation of food has raised questions e.g. on the pressure– temperature behaviour of macromolecular food components such as proteins, lipids and polysaccharides. For example, the mechanism of protein gelation and of the sol/gel behaviour of polysaccharides are not well understood. Little is known so far about chemical reactions of low-molecular weight compounds in the food matrix under pressure, usually in aqueous media. High pressure, on the other hand, has for long been a means of manipulating organic-chemical reactions [35]. So at pressures >500 MPa which are employed for food sterilisation chemical reactions in the food are to be expected which may be of desirable character or not.

Effect of HPP on food quality

HPP has the potential to produce high-quality foods that display characteristics of fresh products, are microbiologically safe and have an extended shelf life. HPP foods are currently considered novel foods as they fullfil two criteria: a new manufacturing process has been employed in their production, and their history of human consumption, to date, has been minimal. Consumer perception of food quality depends not only on microbial quality but also on other food factors such as biochemical and enzymatic reactions and structural changes [18].

HPP can have an effect on food yield and on sensory qualities such as food colour and texture. Chemical changes in HPP-processed foods are minimal because the break of covalent bonds does not occur [43]. Therefore, sensory properties, nutrients, and particularly bioactive compounds of current high commercial interest, suffer no significant losses. Pressure affects weaker bonds such as van der Waals forces, electrostatic interactions and hydrogen bridges. Changes to them explain the preservation effect of HPP treatments [57]. They cause changes in membrane structures resulting in micro bial in activation effects but they can also facilitate the access of an enzyme to its substrate causing product deterioration during storage.

The appearance and colour of food has been shown to significantly influence consumer sales. While some degree of protein denaturation can take place during HPP treatment of certain high-protein foods, the resulting changes in physical functionality and/or changes in raw product colour are significantly less than those experienced using conventional thermal processing techniques

Ionizing Radiation

Ionizing radiation is a non-thermal food pasteurization process that reduces or eliminates spoilage and pathogenic microorganisms, such as Salmonella, Escherichia coli O157:H7, L. monocytegenes and Campylobacter jejuni, by fragmenting DNA.

Irradiation processes minimize post-harvest loss, decrease perishability and inhibit sprout formation in products such as potatoes. Post-packaging potentials for irradiation includes the disinfection of grains, legumes, spices, fruits, melons, lettuces, vegetables and tubers; colour retention in fresh meats; and microbiological control in eggs, pork, poultry and meat. Not all foods are suitable for irradiation processes. Milk and other protein foods can develop off-flavour, odour and colour, and some fruits may exhibit softening and discolouration, especially at higher dose levels. All radiation processes must obtain approval from the Food and Drug Administration (FDA) because they are defined as a food additive. This ruling included package materials, all of which are subject to the regulation. In 1990, the FDA approved the use of irradiation of poultry prod- ucts at the level of 1.5–3.0 kilogray (kGy). In 1997, the FDA approved its use for fresh or frozen meats including beef, lamb and pork. The use of irradiation, at a dose of 1.5kGy, in conjunction with reduced oxygen packaging and refrigeration, can increase the shelf life of ground beef to more than 15 days, compared to a four-refrigerated-day life of non-irradiated. A dose of 1.0kGy, however, is recommended for ground beef to minimize the deterioration of sensory qualities. More than 40 countries have approved irradiation in over 100 food items. 'Of the freshness-enhancing non-thermal technologies, many consider irradiation to be the most effective approach to eliminating pathogens and spoilage microorganisms from the food supply.

Irradiation processes can be gamma from radioisotopic sources such as Cobalt60 or Cesium137, electrons, X-rays from electron beam accelerators, or ultraviolet (UV) sources.In irradiation, foods are exposed to a form of energy, which produces free radicals that then reacts with food biochemical alternatively, the radiation directly attacks the cellular nuclei. Forms of ionizing radiation include UV, gamma and beta ray or electron beam. High dose (10–74kGy) is required for sterilization, which usually damages the food, but a lower dose (0.1kGy) may be employed for pasteurization. 'Microbial inactivation by all types of ionizing radiation is believed to happen through two main mechanisms: direct interaction of the radiation with cell components and indirect action from radiolytic products such as water radicals H+, OH−. Ionizing radiation's primary target is the chromosomal DNA, and it exerts a secondary effect on the cytoplasmic membrane, either of which can cause microbial inhibition or inactivation. Gamma rays are photons or electromagnetic waves that are emitted from the nucleus of the atom. This energy dislodges electrons from food molecules, converting them to electrically charged particles or ions. Since gamma rays do not have enough energy to affect the neutrons in the nuclei, they are incapable of inducing radioactivity. Gamma radiation has high penetrating power. Optimally, irradiation is used in post-packaging where pallets of

packaged products are conveyed into a chamber behind a labyrinth. The Co^{60} gamma ray source is raised from a water pool, allowing the products to absorb gamma radiation. The source then returns to the pool for shielding, and the product exits the chamber [14].The World Health Organization has declared that irradiation of any food commodity, up to 10 kGy, is not a toxicological hazard. Gamma irradiation has been shown to preserve nutritive content and prolong shelf life by preventing post-harvest insect and pest infestation of beans and grains. The advantage of gamma radiation over chemicals, e.g. fumigation with ethylene oxide, is that the irradiation does not leave a chemical residue or induce other adverse effects in the quality of products being treated such as spices [7]. Irradiation is widely used for sterilization of package structures for aseptic packaging. Examples include laminated pouches for bag-in-box for food service and industrial containment of fluid foods, and thermoformed unit portion-size plastic cups for liquid coffee lighteners packaged on aseptic deposit/fill/seal equipment. Ionizing radiation is or has been used commercially in the USAfor spice sterilization or reduction of infestation, microbial reduction in strawberries and some other fruits, and pathogen reduction on poultry and ground beef. Gamma irradiation provides enhanced microbial safety in green onions with a dose as low as 1kGy, and cilantro with 2 kGy, for retention of sensory attributes and increased shelf life of 14 days, and extends the shelf life of minimally processed gourds by 7 days. It has been found that at low doses, irradiation has little effect on nutritional and organoleptic food qualities, but can minimally affect some vitamins such as thiamin.

Effect of irradiation on anthocyanins

Irradiation induces negligible or subtle losses of nutrients and sensory qualities in food compared to thermal processing as it does not substantially raise the temperature of food during processing [62]. However, [2] reported a significant reduction in the total and individual anthocyanin content in pomegranate juice after irradiation at higher doses (3.5e10 kGy). Irradiation effects on anthocyanin pigments depend upon the nature of anthocyanin for example; diglycosides are relatively stable towards irradiation dose compared to monoglycosides. This increase in measured content may be due to the extraction of bound pigments by degradation of the cell wall. Apart from gamma radiation, UV radiation is also reported to have a negative influence on anthocyanins. [9, 33] reported a strong negative influence of UV irradiation on the complex of cyanidin-3-glucoside with co pigment compared to thermal treatment at 80 ^{0}C. However, the presence of certain copigments can inhibit the degradation effect of UV on anthocyanins improving the cyanidine pigment complex [33]. Literature reveals that most of the reported applications of irradiation are limited to solid foods and there is scarcity of information regarding treatment of fruit juices. Application of gamma radiation to pomegranate juice [2], carrot and kale juice [29] and UV radiation to orange, guava-and-pineapple juice [28] has been reported for the inactivation of microorganisms

Oscillating magnetic fields

Static Magnetic Field (SMF) and oscillating Magnetic Fields (OMF) are used for their potential as microbial inactivation techniques. For SMF, the magnetic field intensity is constant with time, while an OMF is applied in the form of constant amplitude or decaying amplitude sinusoidal waves. The magnetic fields may be homogeneous (uniform magnetic field intensity) or heterogeneous (magnetic field intensity is inversely proportional to distance from coil) (USA-FDA Center for Food Safety and Applied Nutrition (2011).OMF is used in the form of pulses reverses the charge for each pulse, and the intensity of each pulse decreases with time to about 10% of the initial [11]. Preservation of foods with OMF involves sealing food in a plastic bag and subjecting it to 1 to 100 pulses in an OMF with a frequency between 5 to 500 kHz at temperatures in the range of 0 to 50^0 C for a total exposure time ranging from 25 to 100 milli-seconds. Frequencies higher than 500 kHz are less effective for microbial inactivation and tend to heat the food material [11]. Magnetic field treatments are carried out at atmospheric pressure and at moderate temperatures. The temperature of the food increases 2-5 ^{0} C(USA-FDA Center for Food Safety and Applied Nutrition (2011). Studies have proposed two theories to explain the inactivation mechanisms for micro-organism and pathogenic cells placed in SMF or OMF. The first theory states that OMF loose the bonds between ions and proteins. Many proteins vital to the cell metabolism contain ions such as enzymes, hormones, pre-cursors which get damaged by OMF. A second theory considers the effect of SMF and OMF on calcium ions bound in calcium-binding proteins, such as calmodulin. Changing magnetic field to calmodulin causes cyclotron resonance resulting in loosening of the bond between the calcium ion and the calmodulin. This ultimately causes metabolic disorder followed by cell death [11].

Dense Phase Carbon Dioxide (DPCD)

Carbon dioxide is used because of its safety, low cost, and high purity. Dense-phase carbon dioxide (DPCD) treatment has attracted great interest in the non-thermal treatment of liquid foods or liquid model solutions. DPCD has been shown to inactivate microorganisms as well as conventional heat pasteurization without the loss of nutrients or quality changes that may occur due to thermal effects. The temperature increase induced by the pressure build-up is negligible. The treatment times can range from about 3 to 9 min for continuous, or from 120 to 140 min for semi-continuous or batch DPCD processes.

A typical batch system has a CO2 gas cylinder, a pressure regulator, a vessel, a water bath or heater, and a CO2 release valve. The sample is placed into the vessel and the temperature is set to the desired value. Then CO2 is introduced into the vessel until the sample is saturated at the desired pressure and temperature. The sample is left in the vessel for a period of time and then the CO2 outlet valve is opened to release the gas. Some systems contain an agitator to decrease the time to saturate of the sample with CO2. A continuous high-pressure CO2 system has been developed to process 1 L/h of liquid at 40.0 MPa. The sample liquid was stored in two 5 liter high-density polyethylene containers, both connected to the pump. CO2 passed through an in-line 0.5 μm filter and a cooling system, then pumped to four mixing points. The pressurized CO2 was mixed with the liquid and the mixture went to a temperature-controlled holding tube.

Several valves along the holding tube allowed for sampling at different residence times. The treated liquid depressurized through a capillary tube inside a thermostatic bath. The liquid was degassed in two containers. The bacteriostatic action of pressurized CO2 compromises different steps such as solubilization of pressurized CO2 in the external liquid phase, cell membrane modification, intracellular pH decrease, key enzyme inactivation/cellular metabolism inhibition due to internal pH lowering, direct inhibitory effect of molecular CO2 and HCO3 on metabolism, disordering of the intracellular electrolyte balance, extraction of vital constituents from cells and cell membranes, physical disruption of cell membrane. Most of these steps occur consecutively and simultaneously in a complex and interrelated manner.

Another mechanism shows that carbon dioxide is having very high affinity for plasma consists in great part of lipid components. Due to the increased membrane permeability caused by the reaction of CO2 with water, which lowers the extracellular pH, pressurized CO2 may easily penetrate through the bacterial cell membrane and accumulate in the cytoplasmic interior of bacterial cells then structurally and functionally disrupt the cell membrane due to a loss of the order in the lipid chain If too much dissolved CO2 enters the cytoplasm, the cells may be unable to expel all the resulting protons and internal pH starts to decrease. If the internal pH is lowered too much, cell viability will be impaired leading to inhibition of cell metabolism or denature certain proteins and enzymes essential for metabolic and regulatory processes, such as glycolysis, amino acid and peptide transport. Finally internal damage of the metabolic processes induces microbial inactivation.

[16] investigated the effects of DPCD treatment of 8, 15, 22, 30 and 35 MPa for 5, 15, 30, 45, 60 min at 35°C, 45°C, 55°C, 65°C on vitamin C in Hami melon juice during storage at 4 0 C for 4 weeks. The authors found that vitamin C concentration decreased following DPCD processing, but percentage loss was lower than of the untreated samples. DPCD also appear to prevent losses of other potential bioactive compounds such as β-carotene. The study conducted by Chen J showed better retention of β-carotene in DPCD (55 0 C, 60 minutes, and 35 MPa) treated melon juice compared to conventional HTST pasteurization. Significant losses (57.87%) in β-carotene content was observed in heat pasteurized samples. It should be noted that exact mechanism for β- carotene stability is difficult to establish [17]. Many examples of the applications of the DPCD to juices demonstrated the protective nature of the process to antioxidants, phytochemicals, organoleptic attributes such as taste, color, and appearance. The relatively low process temperatures, the lack of oxygen in the environment, and for some nutrients, the lower pH, protect the vitamins such as vitamin C. Since the process can be made continuous, its control is easy. However this technology is facing some challenges such as lack of the commercially successful DPCD operation, higher cost of the operation, stringent environmental regulations regarding the release of CO2 into the atmosphere, both total capture and recycling of the gas needs to be designed into new systems, or a carbon-neutral source of CO2 needs to be used, limited data to satisfy the regulatory requirements.

Hurdle Technologies

Hurdle technologies employ several methods, some of which may include mild heat in synergy to preserve foods. Hurdle technologies include the use of MAP, active packaging, cryogenic cooling, antioxidants, ozonation and enzymes in conjunction with the aforementioned and other technologies. In MAP, the CO2 level is increased within the package, providing a shelf life markedly greater than that of traditional packaging. O2 is often reduced and other gases may also be added. The CO2 exhibits a microbistatic effect. When employing MAP, packaged food products should be stored at temperatures under 5°C.Active packaging is the addition of absorbing or emitting agents that limit product degradation or microbial growth by controlling oxygen, moisture, carbon dioxide and odours [14].Cryogenic cooling and freezing may be employed to rapidly chill a product, thus extending shelf life. Hurdle technologies may also employ combinations such as antimicrobials, moderately high temperatures (<55°C) and PEF to provide a synergistic effect, and are being studied to eliminate microorganisms in such products as apple cider, grape juice, mango juice and tomato juice. In Gauri Mittal's research, a hurdle approach was employed using a temperature of 44°C, acidity at pH 3.5, PEF of 80kV/cm and 100U nisin/ml. They achieved a 6 log reduction in orange juice that had a shelf life of 28 days without the use of aseptic packaging, and no significant differences were found in aromatic compounds analyzed by gas chromatography. Antioxidants as hurdles have demonstrated effectiveness in the minimization and retardation of lipid oxidation. Combinations of antioxidants from plant extracts and irradiation have been shown to reduce oxidation in chicken, and decrease warmed-over flavour in ground beef. Other forms of non-thermal food preservation methods include the use of enzymes to inactivate or inhibit the functions of other enzymes, due to their antimicrobial and antioxidant affect [6]. Pack- aging also plays a major role as a non-thermal preservation process in that it can extend shelf life and help preserve freshness in concert with other non-thermal processes. Thus, hurdle technologies appear to the best method to achieve results that the non-thermal technologies individually have not been able to accomplish.

High Voltage Arc Discharge (HVAD)

HVAD 'promotes the formation of an arc in the media (liquid food) while the pulse is applied [11]. HVAD is the application of electricity to pasteurize fluids by rapidly discharging through an electrode gap, generating intense waves and electrolysis, thereby inactivating the microorganisms. This chemical action is a complex effect and depends not only on the voltage applied, but also on the type of microorganism, initial concentration of cells, volume of the media used, distribution of chemical radicals and electrode material [23].

Fresh-squeezed grapefruit juice processed with HVAD was demonstrated to have a fresh flavour and a shelf life for more than 100 days. Research is also being conducted in the use of pulsed high- voltage arc discharge for surface contamination in food and beverages because it has been shown to be a highly efficient and effective method for microbial destruction. With indirect arc discharge, energy from the electric field can be converted to plasma, then to shock waves,

generating free radicals and oxidizing agents within the product. The plasma generation is a non-thermal process, thereby retaining the nutritional and organoleptic properties in foods, especially liquid products. High-voltage electrical pulses can be used as a means of non-thermal pasteurization and sterilization because it demonstrates no thermal effects, and because 90% of microorganisms are destroyed within 10 discharges. Charge-reversed electrical pulses are applied to food that is between two electrodes within a treatment chamber. Each electrical pulse has a pulse width from 1 to 5s, increasing the voltage to a peak. This is followed by a decrease in voltage, and continues until the voltage peaks at the opposite polarity. The vertical pulses are 0.1–25 J/pulse with field strengths of 15–120kV/cm. Inactivation of enzymes occurs with HVAD due to free radicals and oxidation reactions.

The major drawbacks of this electrical method, however, are contamination of the treated food by chemical products of electrolysis and disintegration of food particles by shock waves.

Conclusion

Non-thermal technologies are being investigated due to consumer demand for food products that are minimally processed, of high quality, and are convenient and safe. Non-thermal processes offer shelf life extension without the use of preservatives or additives, while still retaining colour, flavour, texture, nutritive and functional qualities. In order to file for a new or novel manufacturing process with the FDA, the following requirements must be met: first, communicate with the FDA during every step of the process design; second, have the FDA conduct a site visit at the pilot and production facilities; third, draft the proposed filing and submit a copy to the FDA; fourth, identify the resistant organisms that are of most concern for public health and commercial viability; and last, identify the least lethal treatment zone within the system. The problems associated with non-thermal methods include spore injury instead of death, and the rise in product temperature associated with the processing method. In HPP, spore injury can occur under decompression, thus skewing quality assurance results, and other issues may arise using PEF, HVAD, PL and OMF. Currently, non-thermal technologies can be employed for acidic foods, e.g. fruit juice, but more research is needed for the processing and packaging of shelf-stable low acid foods. High pressure processing is commercially used for entrees, guacamole, salsa and fruit juices, but this process will increase greatly in the future. Little food is irradiated in the USA. The other non-thermal processes discussed are still in development stages with considerable potential.

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