GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(**Published By**: Global Institute for Research & Education)
www.gifre.org

IMPACT OF NON-THERMAL PROCESSING ON THE MICROBIAL AND BIOACTIVE CONTENT OF FOODS

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Abstract

Over the past few decades, consumers have been increasingly demanding high-quality, minimally processed food. These requests, coupled with the inadequacy of traditional food processing technologies, have been the driving forces behind improvements in existing technologies and for the development of new food preservation technologies, such as high-intensity pulsed electric field, pulsed white light, UV-C light, ozone and ultraviolet irradiation. The majorities of these technologies are locally clean processes and therefore appear to be more environmentally friendly and have less environmental impact than the traditional ones. Non-thermal treatments have the potential to be an alternative to conventional techniques for food production. Several researchers have investigated how intense processing impacts the safety and shelf life of food. In addition, novel applications are under development, such as the improvement of mass transfer processes or the generation of bioactive compounds by using moderate field strengths. However, the impacts of non-thermal processes on the minor constituents of foods, such as bioactive compounds, have not been emphasized.

This review aims to summarize the current understanding of the impact of non-thermal processes, such as pulsed electric field, pulsed white light, UV-C light, ozone and ultraviolet irradiation, on the stability of inactivate microorganisms and spoilage enzymes and on the nutritional and quality parameters of food.

Keywords: *Pulsed electric fields, Ultraviolet irradiation, Ozone, Inactivate microorganisms.*

1. Introduction

Currently, most liquid foods are preserved commercially by ultra-high temperature (UHT) or high-temperature short-time (HTST) processes. Although heating inactivates enzymes and microorganisms, the organoleptic and nutritional properties of the food suffer because of protein denaturation and the loss of vitamins and volatile flavors (Mittal & Griffiths, 2005). Thus, extending the shelf life of food by heat treatment not only expends large amounts of energy but, in many cases, adversely affects the flavor, chemical composition and nutritional quality of the treated food (Martin *et al.*, 1997). There is a need for a non-thermal method that will destroy microorganisms and that is also economical, compact, energy efficient, safe, socially and environmentally acceptable and that does not adversely affect nutrition, texture or flavor of the treated food. Thermal processing can often lead to detrimental changes in the sensory and nutritional quality of food products (Farnworth *et al.*, 2001; Lee & Coates, 2003).

Patras *et al.* (2009), Patras *et al.* (2009), Patras *et al.* (2010) and Rawson *et al.* (2010) illustrated that heat processing can cause several chemical and physical changes that impair the organoleptic properties and may reduce the content or bioavailability of some bioactive compounds, particularly under severe conditions. Therefore, there is a demand for mild processing technologies, such as pulsed electric field processing, pulsed white light or UV-C light, ozone and ultraviolet irradiation. The aim of these technologies is not only to obtain high-quality food with “fresh-like” characteristics but also to provide food with improved functionalities. In addition to their potentially beneficial effects on nutritional and bioactive content, many of these novel technologies enable high-quality food to be produced in a more cost-effective and environmentally friendly manner, characteristics that have contributed to their commercialization (Butz & Tauscher, 2002; Piyasena *et al.*, 2003; Vikram *et al.*, 2005). Recently, considerable interest has been directed toward non-thermal technologies for the preservation of juice due to the increasing consumer demand for fresh, high-quality and nutritious food products (Patil *et al.*, 2009).

The regulations of the U.S. Food and Drug Administration (FAD, 2004) require all juice processors to meet the pathogen reduction regulations of the juice “Hazard Analysis and Critical Control Point (HACCP) Systems” (the juice HACCP regulation). These processors must ensure that the juice contains a maximum of 5-log of microorganisms of interest, and the treatment process must be validated. The term "novel non-thermal processing" is often used to designate technologies that have the ability to inactivate microorganisms, such as pulsed electric field, pulsed white light or UV-C light, ozone and ultraviolet irradiation (Butz & Tauscher, 2002).

Non-thermal processing technologies inactivate microorganisms and spoilage enzymes and enable the shelf life of foods to be extended while maintaining their organoleptic, nutritional and quality characteristics. This paper reviews non-thermal preservation techniques, including pulsed electric field, pulsed white light, UV-C light, ozone and ultraviolet irradiation.

2. Pulsed electric field

Pulsed electric field (PEF) technology has been used as an alternative to conventional methods for food pasteurization (Hoover, 1997; Yeom *et al.*, 2000). The pulsed electric field method is used to increase the shelf life of liquid food while maintaining its organoleptic characteristics. During the past five decades, substantial effort has been made to use PEF technologies on a commercial scale for pasteurization. Recently, industrial-scale pulsed electric field treatment systems have become available, involving both treatment chambers and power supply equipment (Butz & Tauscher, 2002). Salvia-Trujillo *et al.* (2011) reported that high-intensity pulsed electric field (HIPEF) processing may be a feasible method of obtaining shelf-stable and fresh fruit juice and milk beverages.

Pulsed electric field methods that use electropermeabilization are a valid technology for the safe production of beverage products. Additionally, this technology positively affects the texture of solid plant foods, leading to enhanced yields of metabolites as well as increased juice yields. High-intensity pulsed electric field (PEF) processing involves the application of high-voltage pulses (20–80 kV cm^{-1}) for short time periods (<1 s) on fluid foods between two electrodes (Senorans *et al.*, 2003). A study by Zulueta *et al.* (2010) found that effects of PEF vary with the intensity of the field used, as high-intensity fields (15–40 kV cm^{-1}, 5–100 pulses, 40 to 700 µs 1.1 to 100 Hz) are more effective in destroying microbes, whereas low- and medium-intensity fields (0.6–2.6 kV cm^{-1}, 5–100 pulses, short treatment time within 10^{-4}–10^{-2} s; 1 Hz) enhance mass transfer in solid foods (Corrales *et al.*, 2008). Barbosa-Cánovas *et al.* (2004) stated that the inactivation of microorganisms that are exposed to high-voltage PEF is caused by the electromechanical instability of the cell membrane, with irreversible pore formation (electroporation) occurring at trans membrane potentials in excess of 1 V. Giner-Segui *et al.* (2009) reported that the inactivation of microbes using pulsed electric field methods was greater when the treatment time and electric field intensity increased, and bipolar pulses more effectively inactivated PE than monopolar pulses.

Pulsed electric field treatment systems consist of a pulse generator, treatment chambers, a fluid-handing system and monitoring systems (Min *et al.*, 2007). A pulsed electric field treatment chamber is used to house electrodes and delivers high voltage to the food material. The chamber is generally composed of two electrodes held in position by insulating material, which forms an enclosure that contains the food material. Thus, the design of treatment chamber is one of the essential factors in the development of pulsed electric field treatment for non-thermal pasteurization technology (Alkhafaji & Farid, 2007); it should enable a uniform electric field to be applied to the food with a minimum increase in temperature, and the electrodes should be designed to minimize the effect of electrolysis (Butz & Tauscher, 2002). Pulsed electric field (PEF) processing of food consists of very short electric pulses (µs) at high electric field intensities and moderate temperatures. This treatment may be an alternative to traditional thermal processes because it is capable of destroying microorganisms and some enzymes while still maintaining the freshness of food products (Bendicho *et al.*, 2002).

The system described by Ho and Mittal (2000) consists of a 30 kV D.C. high-voltage pulse generator, a circular treatment chamber and devices for pumping and recording. The voltage of the 110 V A.C. is raised through a high-voltage transformer and then rectified, as shown in *Fig. 1*. The D.C. high-voltage supply then charges the 0.12 µF capacitor through a series of 6 MΩ resistors (the time constant = 0.72 s). The pulse generator emits a series of 5 V pulses, and the trigger circuit serves to convert them to 500 V pulses using a silicon control rectifier (SCR). The generation of high-voltage pulses relies on the discharge of the 0.12 µF capacitor through the thyratron. The batch unit can generate short-duration pulses (2 µs width, 0.5 Hz frequency) with a peak-to-peak electric field strength of up to 100 kV/cm. This pulsar is unique in that low-energy pulses (<25 J pulse^{-1}) and pulses of instant charge reversal shape are generated.

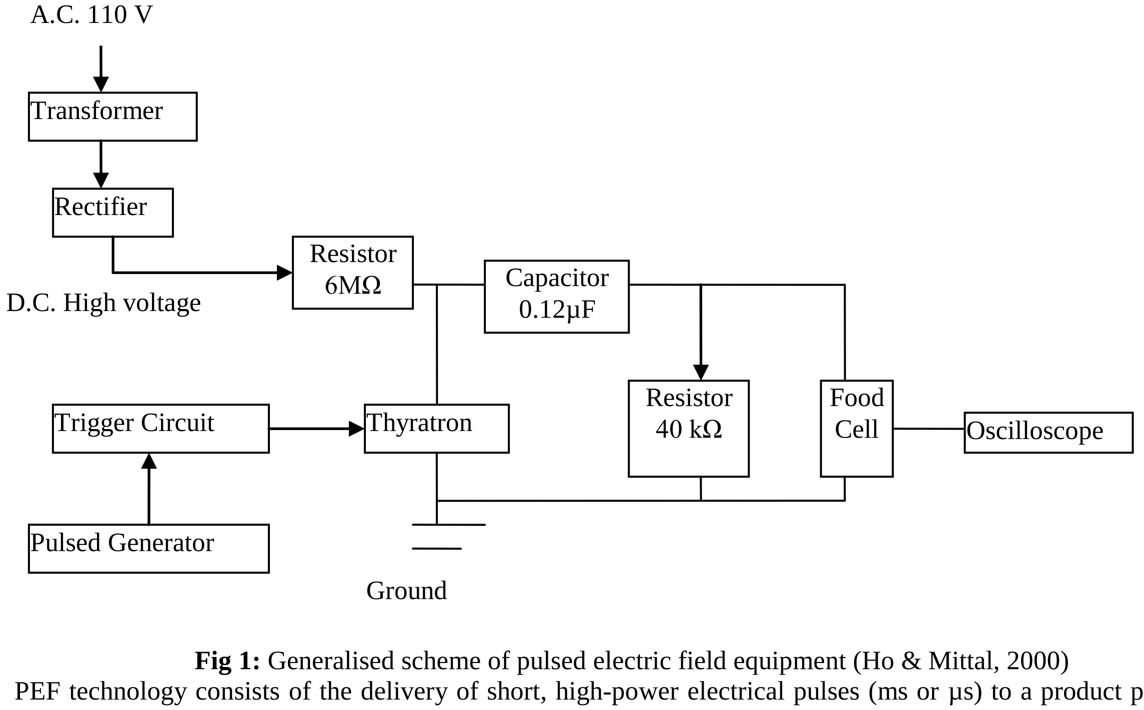

Fig 1: Generalised scheme of pulsed electric field equipment (Ho & Mittal, 2000)

PEF technology consists of the delivery of short, high-power electrical pulses (ms or µs) to a product placed in a treatment chamber between electrodes. Typical systems used for the treatment of pumpable fluids consist of a PEF generation unit, which is composed of a high-voltage generator and a pulse generator; a treatment chamber; a suitable product handling system; and a set of monitoring and controlling devices, as shown in *Fig. 2* (Soliva-Fortuny *et al.*, 2009).

The PEF treatment process may be either static or continuous. While in static processing, discrete portions of fluid foodstuffs are treated as a unit by adding all of the fluid to a PEF treatment chamber, in which uniform field strength is applied to all elements of the foodstuffs to be treated. In continuous processing, the treated foodstuffs are pumped into and out of the PEF treatment system in a steady stream (Dunn & Pearlman, 1987). The design of treatment chambers is moving from static systems to continuous treatment chambers. As reported by Huang and Wang (2009), the pulsed electric field process can provide consumers with microbiologically safe and minimally processed fresh products. The treatment chamber, which houses the electrodes and delivers high voltage to a food material, is one of the key components in the pulsed electric field pasteurization process.

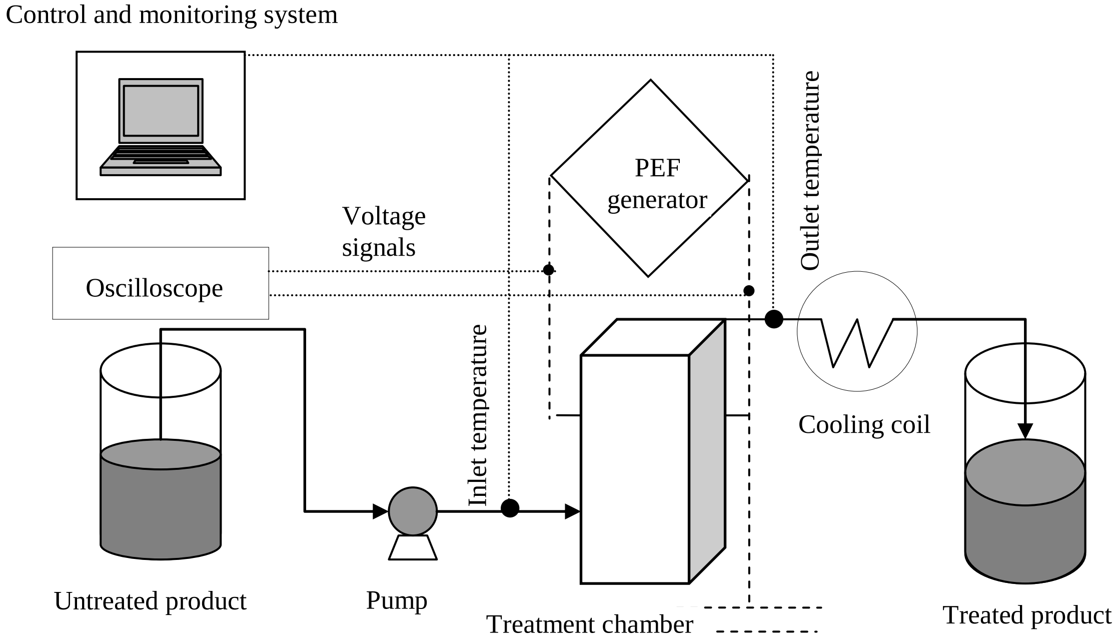

Fig. 2: Schematics of a PEF processing system for pumpable products.

Amiali *et al.* (2004) showed that the microbial inactivation rate increased with an increasing number of pulses, especially in egg yolk and whole egg products. The inactivation kinetics was exponential, with some tailing, and a new kinetic model for bacterial inactivation was proposed. Wesierska and Trziszka (2007) observed variations in the number of bacteria cells in suspension treated with pulsed electric field using 1-300 electrical impulses at 15, 20 and 25 kV when separated by a 1-s pulse period. A significant reduction in the bacterial population was noted using 200 pulses and an intensity of 25 kV. At the end of the process, the number of cells was reduced by more than 3–5 log cycles, depending on the type of bacteria. High inactivation levels of some microorganisms suggest that PEF can assure a safe, high-quality product with an extended shelf life, which is very important for egg products.

Pulsed electric field technology is effective against various pathogenic microorganisms and spoilage enzymes without an appreciable loss of flavor, color or bioactive compounds such as anthocyanins (Yeom *et al.*, 2000; Hodgins *et al.*, 2002; Cserhalmi *et al.*, 2006; Elez-Martinez *et al.*, 2006). High-intensity pulsed electric field (HIPEF) has been used to inactivate *Listeria spp.* in orange juice (McDonald *et al.*, 2000; McNamee *et al.*, 2010), melon juice and watermelon juice (Mosqueda-Melgar *et al.*, 2007) as well as milk (Reina *et al.*, 1998; Calderon-Miranda *et al.*, 1999; Dutreux *et al.*, 2000; Picart *et al.*, 2002; Fleischman *et al.*, 2004; Noci *et al.*, 2009; Guerrero-Beltran *et al.*, 2010), with encouraging results. Nevertheless, it has been suggested that *Listeria spp.* are among the most resistant bacteria to HIPEF processing (Fleischman *et al.*, 2004). Zhaoa *et al.* (2008) studied the effects of pulsed electric fields (PEF) on the inactivation of *Escherichia coli* and *Staphylococcus aureus* in green tea beverages. They found that the inactivation of *Escherichia coli* and *Staphylococcus aureus* by PEF treatment at 38.4 $kVcm^{-1}$ for 160 and 200 µs reached 5.6 and 4.9 log reductions, respectively. Storage tests at 4 °C showed that there was a synergistic effect of low-temperature storage and the antimicrobial functionality of green tea polyphenol (GTP) content, which resulted in a considerable reduction in the microorganisms of the PEF-treated tea beverage, extending its shelf-life to over 6 months at 4 °C.

The use of thermal processing may lead to a loss of bioactive compounds. If processors want to produce stable products with the maximum amounts of bioactive compounds, the use of existing thermal processing treatments should be re-evaluated in comparison to non-thermal techniques. This need has led to research efforts directed at novel non-thermal processes that will ensure product safety yet retain the desired bioactive compounds, as indicated in Table 1. These technologies involve preservation treatments that are effective at ambient or sub-lethal temperatures, thereby minimizing the negative thermal effects on the nutritional and quality parameters of the product.

Several models have been used to describe the microbial destruction and enzymatic inactivation in response to HIPEF, such as first-order, first-order fractional conversion, Weibull distribution and the Fermi and Hulsheger model. First-order kinetics, described in equation (1), is commonly used to describe the variation of health-related compounds in juices and nectars as a function of treatment time for heat processing (Vieira *et al.*, 2000; Vikram *et al.*, 2005; Wang & Xu, 2007).

$$RC = RC_o . \exp\left(- k_1 . t\right) \qquad (1)$$

where (RC) (%) is the relative content of health-related compounds or relative antioxidant capacity, (RC_0) (%) is the intercept of the curve, (k_1) is the first-order kinetic constant (μs^{-1}) and (t) is the treatment time (µs).

Table (1) Array of non-thermal technologies for processing exotic fruits and their products

Technology	*Description of technology*	*Fruit*	*Product type*	*References*
High-intensity pulsed electric fields (HIPEF)	High voltage pulses to foods between two electrodes (<1 s; 20–80 $kVcm^{-1}$; exponentially decaying, square wave, bipolar, or oscillatory pulses at ambient, sub-ambient, or above ambient temperature)	Orange, kiwi, pineapple	Fruit juice-soymilk	Morales-de la Pena *et al.*, (2010a,b)
		Orange, kiwi, pineapple	Fruit juice-soymilk	Morales-de la Pena *et al.*, (2010a,b)
		Cherry	Juice	Altuntas *et al.*, (2010)
		Watermelon	Juice	Oms-Oliu *et al.*, (2009)
		Strawberry	Juice	Odriozola-Serrano *et al.*, (2009)
Ozone processing	Ozone is a triatomic allotrope of oxygen and is characterized by a high oxidation potential that conveys bactericidal and viricidal properties	Kiwi	Cubes	Barboni *et al.*, (2010)
Ultraviolet (UV-C) light	UV radiant exposure, at least 400 Jm^{-2}, intense and short-duration pulses of broad spectrum (ultraviolet to the near infrared region)	Watermelon	Cubes	Fonseca and Rushing (2006)
		Pomegranate	Arils	Lopez-Rubira *et al.*, (2005)
		Mangoes	Cubes	González-Aguilar *et al.*, (2007)

The Weibull distribution (equation (2)) has been used to describe the destruction of microorganisms (Rodrigo *et al.*, 2001) and enzyme inactivation (Rodrigo *et al.*, 2003; Giner *et al.*, 2005; Soliva-Fortuny *et al.*, 2006) under high-intensity pulsed electric field (HIPEF). The use of the Weibull distribution function to describe the retention of health-related compounds and antioxidant capacity has not yet been reported.

$$RC = RC_o \exp\left(-\left(\frac{t}{\alpha}\right)^{\gamma}\right) \qquad (2)$$

where (α) is the scale factor (µs) and (γ) is the shape parameter that indicates the concavity (tail-forming) or convexity (shoulder-forming) of the curve when it takes values below or above 1, respectively. Derived from the Weibull distribution function parameters (α, γ), (t_m) is defined as the mean processing time to achieve complete destruction/inactivation of the health-related compound or antioxidant capacity. This parameter can be used to measure the resistance of these compounds to HIPEF treatments, as in equation (3):

$$t_m = \alpha \,.\, \gamma\left(1 + \frac{1}{\beta}\right) \qquad (3)$$

where (α) and (ß) are the parameters of the Weibull distribution and (γ) is the gamma function.

Because of the shape similarity between solid-liquid extraction curves in sorption processes and changes in lycopene retention, the fit of a model assessing the effect of HIPEF treatment time on lycopene content was evaluated (Peleg, 1988) (equation (4)). This model has been used to describe sorption processes in various foods (Turhan *et al.*, 2002; Palou *et al.*, 1994) and has been shown to properly fit the solid-liquid extraction kinetics of total polyphenols from grape seeds (Bucic-Kojic *et al.*, 2007).

$$RC = RC_o + \frac{t}{K_1 + K_2 \,.\, t} \qquad (4)$$

Where (*RC*) (%) is the relative lycopene content, (RC_0) (%) is the intercept of the curve, (t) is the treatment time (µs), (K_1) (µs/%) is the Peleg rate constant indicating the lycopene formation rate at initial treatment time ($t = t_0$) and (K_2) is the Peleg capacity constant. The Peleg capacity constant is related to the steady value reached for prolonged treatment times, as in equation (5):

$$RC_\infty = 100 + \frac{1}{K_2} \qquad (5)$$

3. Ultraviolet Irradiation

Ultraviolet (UV) treatment is a disinfection method that can be applied to inactivate harmful microbes in food. The treatment can be carried out at low temperatures and therefore can be grouped with other non-thermal methods. Each method has different mechanisms of inactivation (Tran, 2001). Ultraviolet irradiation is one of the non-thermal technologies used for the preservation of juices (Koutchma, 2009). The peak UV absorption efficiency for DNA lies between 250 and 280 nm. The UV rays at this germicidal wavelength alter the genetic material in the cells so that bacteria, viruses, mold and other microorganisms can no longer reproduce and are considered inactive (Billmeyer, 1997; Giese, 1997; Bolton, 2001). Ultraviolet (UV) treatment is based on the bactericidal action of short-wave UV light (UV-C, 200–280 nm), which can be absorbed by the DNA and impairs the reproductive processes of the cell (Lopez-Malo & Palou, 2004).

Irradiation treatment involves the exposure of food products (raw or processed) to ionizing or non-ionizing radiation for the purpose of food preservation. Ionizing radiation sources include high-energy electrons, X-rays (machine generated) and gamma rays (from cobalt-60 or cesium-137). Non-ionizing radiation, represented mainly by ultraviolet rays (UV-A, UV-B and UV-C), visible light, microwaves and infrared, is electromagnetic radiation that does not carry enough energy/quanta to ionize atoms or molecules. The irradiation of food products causes minimal modifications of the flavor, color, nutrients, taste and other quality attributes of food (Alothman *et al.*, 2009b). Depending on the radiation dose, foods may be pasteurized to reduce or eliminate food-borne pathogens. The inactivation of microorganisms by irradiation is achieved through DNA damage, which destroys the reproductive capabilities and other functions of the cell (DeRuiter & Dwyer, 2002). However, the levels of modification (in flavor, color nutrients, taste, etc.) vary depending on the basic raw material used, irradiation dose delivered and type of radiation source employed (gamma, X-ray, UV, electron beam) (Bhat *et al.,* 2007; Bhat & Sridhar, 2008).

The application of gamma radiation to pomegranate juice (Alighourchi *et al.*, 2008) and UV radiation to orange, guava and pineapple juice (Keyser *et al.*, 2008) has been reported for the inactivation of microorganisms. Irradiation induces negligible or minimal losses of bioactive compounds, as this method does not substantially raise the temperature of food during processing (Wood & Bruhn, 2000).

The radiant exposure (dosage), defined as the energy delivered per unit surface area of the UV reactor, was calculated using the following equation (6):

$$D = I \times t \qquad (6)$$

Where (*I*) is the measured irradiance of the lamp (W/cm^2) and (*t*) is the exposure time (s) (Caminiti *et al.*, 2011).

4. Ultraviolet Light

Pulsed light is also considered an emerging, non-thermal technology capable of reducing the microbial population on the surface of foods and food contact materials by using short and intense pulses of light in the ultraviolet near infrared (UV–NIR) range. PL systems have relatively low operation costs and do not have a significant negative impact on the environment, as these methods have the potential to eliminate microorganisms without the need for chemicals. Furthermore, PL systems do not produce volatile organic compounds (VOC) and generate minimal amounts of solids waste. The use of ultraviolet light at germicidal wavelengths has been approved to treat food surfaces and clear fruit juices (US-FDA, 2002). However, the efficiency of UV-C radiation depends on the UV-C absorption because increasing the amount of solid, large suspended particles or microbial populations will reduce the penetration of UV-C (Lopez-Malo & Palou, 2002; Guerrero-Beltran & Barbosa-Canovas, 2004; Koutchma *et al.*, 2007).

Since 1985, UV radiation has been used for water disinfection and has replaced conventional chlorination processes in some countries (Gibbs, 2000). Pereira and Vicente (2010) stated that ultraviolet (UV) radiation is an established disinfection alternative used to produce drinking water. More than 500 UV plants supplying drinking water operate in North America, and more than 2000 plants in Europe use this technology as a common disinfection technique for drinking water supplies. The benefits of UV treatment in comparison to other methods of disinfection are very clear: no chemicals are used; it is a non-heat-related process; changes in color, flavor, odor and pH are minimized; and no residuals are left in the fluid stream. However, a potential problem of using short-wave UV light is that it can damage human eyes, and prolonged exposure can cause burns and skin cancer in humans (Shama, 1999; Bintsis *et al.*, 2000), which is a concern for industry workers.

The common ultraviolet (UV) systems used to disinfect water, air and surfaces contain low-pressure vapor mercury lamps, which are characterized by a peak of emission in the germicidal region at 254 nm. The photochemical effect on DNA molecules is also thought to be the main microbial inactivation mode for high-intensity light pulse (HILP) technology. In this case, the broad-spectrum waves (200–1100 nm) are emitted by a Xenon lamp and are produced in very short (100–400 μs), intense pulses. The infrared region (800–1100 nm) may induce a photo-thermal effect, which may further destabilize the microorganisms by damaging the cell membrane, especially at high-fluency conditions (above 0.5 Jcm^{-2}) (Gomez-Lopez *et al.*, 2007).

UV-C light present in the 200–280-nm wavelength range has a germicidal effect on microorganisms such as bacteria, yeast, mold and viruses (Tran & Farid, 2004; Caminiti *et al.*, 2010). UV-C light is largely absorbed by the DNA of microorganisms and prevents both DNA transcription and translation through adjacent pyrimidine bases that are bonded to each other on the same strand of DNA (Franz *et al.*, 2009; Koutchma, 2009).

Qualls and Johnson (1983) developed alternative calibration methods to improve the accuracy of radiometers measuring the incident UV-C radiation near long tubular lamps. Thus, bench-scale equipment with collimated beams of light have been extremely useful as a standardized method in the study of the effects of UV on microorganisms (Bolton & Linden, 2003) and have been extensively applied to calibrate UV-C water disinfection systems. To improve their flexibility, complex irradiation geometries or larger sample amounts are required. In this case, the most accurate measure of incident photons is most likely the well-defined chemical actinometry (Linden & Darby, 1998; Kuhn *et al.*, 2004). Under collimated beams, however, the treatment surface and sample volume must be small due to the recommended tube dimensions. Furthermore, to enhance the UV germicide effects, industrial designs benefit from higher irradiances and highly reflective surfaces (Koutchma *et al.*, 2004; Keyser *et al.*, 2008).

When assessing liquid egg, Unluturk *et al.* (2007) used a flat, black collimated beam to evaluate the efficiency of UV-C as a non-thermal process for shelf-stable liquid egg product (LEP) fractions using the *Escherichia coli* strain ATCC 8739, among others. The best reduction (>2 log cycles) for this highly UV-resistant strain was achieved in liquid egg white (LEW) at 0.153 cm fluid depth and at a UV intensity of 1.314 mW cm^{-2}. However, under similar conditions, the maximum inactivation ranged from 0.675 $\log_{10}$ CFU ml^{-1} in liquid egg yolk (LEY) to 0.316 $\log_{10}$ CFU ml^{-1} in liquid whole egg (LWE). Ngadi *et al.* (2003) reported a reduction of 5 log cycles with 0.1 cm sample depth at 5 mW min cm^{-2}

on the inactivation of *E. coli* O157:H7 in LEW under a stainless steel tube. Furthermore, Geveke (2008) reported an effective UV treatment of *E. coli K12* (ATCC 23716) in LEW using a continuous process with a low-pressure mercury lamp surrounded by UV transparent tubing and a silicon rubber tape. In that work, the population of *E. coli* was reduced by 4.3 log cycles after being exposed to UV at 50 °C for 160 s. Souza and Fernandez (2011) confirmed that the UV-C process is a promising technology to reduce microbial loads without impairing the quality attributes of liquid egg products.

5. Ultraviolet C

UV treatment of juices is difficult due to the low UV transmittance through the juice, which contains high amounts of suspended solids. Hence, for the treatment to be effective, the juice must be exposed to UV as a thin film, unlike the conventional method used in water disinfecting systems. Tran and Farid (2004) reported that ultraviolet processing using a thin-film UV reactor was effective in reducing total aerobic plate count, yeast and mold in orange juice. The shelf life of fresh squeezed orange juice was extended from 2 days to more than 5 days after UV treatment, with a limited dose of 73.8 mJ cm^{-2}.

The USDA and US Food and Drug Administration (FDA) have approved UV-C irradiation as a safe method for juice pasteurization. Different types of UV-C reactors for fresh juice pasteurization have been evaluated (Koutchma, 2008). The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the United States Department of Agriculture (USDA) reported that non-thermal preservation technologies, including UV-C irradiation, ensure the scientific criteria for the pasteurization of juices (a 5 log reduction of the most resistant microorganisms of public health significance (NACMCF, 2006)).

The inactivation effect of UV-C irradiation on pathogens and spoilage microorganisms in apple juice (Ngadi *et al.*, 2003; Guerrero-Beltran & Barbosa-Canovas, 2005; Keyser *et al.*, 2008; Franz *et al.*, 2009; Caminiti *et al.*, 2010), orange juice (Tran & Farid, 2004; Keyser *et al.*, 2008), grape, cranberry and grapefruit juice (Guerrero-Beltran *et al.*, 2009), as well as strawberry and mango nectar (Keyser *et al.*, 2008), has been described. Additionally, the effects of UV-C treatment on some physicochemical properties and the sensory quality of various juices have been reported (Donahue *et al.*, 2004; Tran & Farid, 2004; Caminiti *et al.*, 2010; Falguera *et al.*, 2011). UV-C treatments with an electromagnetic spectrum from 200 to 280 nm have been used to preserve the quality of fresh-cut watermelon (Artes-Hernandez *et al.*, 2010) and can also inactivate the pectin methylesterase (PME) in tomatoes (Barka *et al.*, 2000), strawberries (Pombo *et al.*, 2009) and apples (Manzocco *et al.*, 2009).

Fresh orange juice (OJ) spoils over time due to the growth of microorganisms. Yeast and mold, *Lactobacillus*, *Leuconostoc* and thermophilic *Bacillus* (*Bacillus subtilis* and/or *Bacillus pumilus* spore formers) are common microorganisms that are found growing in orange juice (Kimball, 1991, 1996). In relation to retention quality, research has shown promising results from exposing apple juice to UV irradiation (Harrington & Hills, 1968). The recommendations from the Food and Drug Administration (2000) for juice manufacturing require fruit juice producers to apply a preservation method that is capable of reducing the most resistant pathogen that is likely to be present by a minimum of 5 log cycles. The FDA has approved UV irradiation as a standalone method suitable for fruit juice preservation provided that turbulent flow conditions occur throughout the process. However, these flow requirements would not be necessary where UV exposure is not the sole treatment used. Zhang *et al.* (2011) reported that ultraviolet-C treatments were a rapid and effective method to inactivate the pectin methylesterase (PME) of watermelon juice compared to the thermal and high-pressure treatments of the same duration and temperature.

Caminiti *et al.* (2011) studied the impact of a combination of ultraviolet (UV) light, pulsed electric field (PEF) and high-intensity light pulses (HILP) on the quality of an apple and cranberry juice blend. They found that there were no significant changes in non-enzymatic browning, total phenolics or the antioxidant activity of the juices. UV + PEF and HILP + PEF treatments did not affect the color of the product, and HILP + PEF processing retained more monomeric anthocyanins than any other combined treatment. Sensory analysis showed that the UV + PEF and HILP + PEF combinations did not impact the odor or flavor of the apple and cranberry juice blend.

5. 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).

A minimum ozonation time and concentration are required to achieve the inactivation of many pathogenic and spoilage microorganisms, which results in a lag-phase (Gujer & von Gunten, 2003). Rennecker *et al.* (1999) proposed a delayed Chicke Watson model to characterize the minimum ozonation time and concentration for the inactivation of *Cryptosporidium parvum* oocysts. According to the Chicke-Watson model, microbial inactivation follows the following equation (7):

$$\log_e \frac{N_t}{N_O} = \lambda C t \qquad (7)$$

where (N_t) is the number of microorganisms per unit volume of reactor, (N_o) is the initial number of microorganisms, (λ) is the coefficient of specific lethality (mg L^{-1} min), (t) is the reaction time and (C) is the dissolved ozone concentration (mg L^{-1})

Table (2) The effect of ozone on target microbial population, quality and nutritional parameters

Food product	*Phase or form*	*Target microbial population*	*Quality and nutritional attributes*	*Reference*
Fruit juice				
Apple cider	Ozone gas (pumped into juice)	*Escherichia coli* O157:H7 (0.9 LR)*;* *Salmonella* (1.0LR)		Williams *et al.*, (2005)
Orange juice	Ozone gas (pumped into juice)	*Escherichia coli* O157:H7 (0.4 LR)*;* *Salmonella* (1.8LR)		Williams *et al.*, (2005)
Orange juice	Ozone gas (pumped into juice)	Yeast (*S. cerevisiae*) (↓)	Ascorbic acid (↓), colour (×)	Angelino *et al.*, (2003)
Orange juice	Ozone gas (bubble column reactor)		Colour (↓), NEB(~), cloud value(~), pH (~), TA (~), AA (↓)	Tiwari *et al.*, (2008b)
Strawberry juice	Ozone gas (bubble column reactor)		Colour (↓), pH (~), TA (~), AA (↓), anthocyanins (↓)	Tiwari *et al.*, (2009a)
Blackberry juice	Ozone gas (bubble column reactor)		Colour (↓), pH (~), TA (~), ascorbic acid (↓), anthocyanins (↓)	Tiwari *et al.*, (2009b)
Apple cider	Ozone gas (pumped into juice)	Moulds (↓); yeast (↓)	Sediments (↑), colour (×)	Choi and Nielsen (2005)
Apple cider	Ozone gas (pumped into juice)	*Escherichia coli* O157:H7 (5.0 LR)		Steenstrup and Floros (2004)
Fruits and vegetable				
Lettuce	Ozonated water	*Shigella sonnei* (1.8 LR)		Gil *et al.*, (2006)
Tomatoes	Ozonated air	Mesophilic bacteria (1.07 LR); yeasts (0.5LR; moulds (0.5LR)	Appearance (~), taste (~), aroma (↓) and overall quality (~), texture (↑), TA (~), AA (↑), glucose (↑), fructose (↑)	Artes *et al.*, (2006)
Strawberry	Ozonated air	Fungal decay (delayed)	Sucrose (↓), glucose (↑), fructose (↑), vitamin C (↑);aroma quality (↑)	Perez *et al.*, (1999)
Strawberry	Continuous gaseous ozone Pressurised gaseous ozone	*Escherichia coli* O157:H7 (2.96 LR) *Salmonella enterica* (2.60LR)		Bialka and Demirci, (2007a)
Raspberry	Continuous gaseous ozone Pressurised gaseous ozone	*Escherichia coli* O157:H7 (3.75 LR) *Salmonella enterica* (3.55LR)		Bialka and Demirci, (2007a)
Strawberry	Aqueous ozone	*Escherichia coli* O157:H7 (2.90 LR); *Salmonella enterica* (3.3LR)		Bialka and Demirci, (2007b)
Raspberry	Aqueous ozone	*Escherichia coli* O157:H7 (5.6LR); *Salmonella enterica* (4.50LR)		Bialka and Demirci, (2007b)
Blueberries	Aqueous ozone Gaseous ozone	*Escherichia coli* O157:H7 (3.0LR) *Salmonella enterica* (2.60LR)	Colour (~)	Bialka and Demirci, (2007c)
Water melon	Ozone gas in cold water	APC (1– 1.5LR)	Colour (↓), overall quality (↓)	Fonseca and Rushing, (2006)
Celery	Ozonated water	Total bacteria (1.15LR) PPO (↓)	Total sugar (~), colour (~)	Zhang *et al.*, (2005)
Lettuce	Ozonated water	PPO (↓)	Antioxidants (~), vitamin C (↓), visual appearance (~)	Beltran *et al.*, (2005)

Where:
APC, aerobic plate count AA, ascorbic acid PPO, polyphenol oxidase
NEB, nonenzymatic browning TA, titratable acidity (×), significant difference
(~), no change LR, log reduction (↑), increases
(↓), decreasesrate. Predicted kinetic models should be able to establish appropriate treatment conditions

The ozone exposure (*OE*) in a batch reactor can be explained by a delayed Chicke-Watson model for inactivation in a batch process:

$$OE_{lag} = \frac{1}{k} \log_e \left(\frac{N_t}{N_O} \right) \tag{8}$$

If $OE \leq OE_{lag}$, then $N_t / N_O = 1$ or

$$\frac{N_t}{N_O} = \frac{N_C}{N_O} \exp\left(-k \times OE\right) \tag{9}$$

By rearrange equations (8) and (9),

$$\frac{N_t}{N_O} = \frac{N_C}{N_O} \exp\left(-k\left(OE - OE_{lag}\right)\right) \tag{10}$$

Where (OE_{lag}) is the ozone exposure in a lag-phase and (N_c) is the increased initial number of microorganisms to compensate for the initial lag-phase.

Several researchers have assessed the use of ozone as an antimicrobial agent in food processing (Kim *et al.*, 1999; Xu, 1999; Khadre *et al.*, 2001). The efficacy of ozone against Gram-positive bacteria, Gram-negative bacteria and fungi, in addition to its virucidal effects, have been reported (Restaino *et al.*, 1995). Manas and Pagan (2005) extensively reviewed the inactivation of microbes using novel food processing technologies, including the mechanisms of inactivation. The literature suggests that microbial inactivation by ozone is mainly due to the rupture of cellular membranes (EPRI, 1997) and the dispersion of the cytoplasm. The ozonation of food products requires a reliable model that describes the inactivation to achieve known levels of microbial inactivation, allowing for the production of stable and safe foods (Manas & Pagan, 2005).

Cullen *et al.* (2009) studied mathematical models incorporating various independent factors governing ozone processing that may be employed to describe the biochemical reactions and microbial inactivation of ozone, facilitating the enhanced control of both quality and safety parameters of ozonated foods. They reported that due to the complexity of food systems, the appropriate selection of models becomes critical. A detailed report of the influence of food ingredients on both the inactivation and quality degradation kinetics is required.

The mathematical models developed by Anderson *et al.* (1996), Peleg and Cole (1998), Augustin *et al*, (1998), Baranyi and Pin (2001), Peleg (2003), Geeraerd *et al.* (2005) and Valdramidis *et al.* (2006) describe non-log linear inactivation kinetics. The majority of curvilinear semi-logarithmic survival curves can be adequately described by the Weibull model, as shown in equation (11) (Bialka *et al.*, 2008), or by the biphasic inactivation model, as in equation (12) (Corradini & Peleg, 2007).

$$\log_e \left(\frac{N_t}{N_O} \right) = \exp\left(-\left(k\,t\right)^{\beta}\right) \tag{11}$$

where (*k*) is the inactivation rate constant and (ß) is the shape factor

$$\log_e \left(\frac{N_t}{N_O} \right) = a \exp\left(-k_1\,t\right) + \left(1-a\right) \exp\left(-k_2\,t\right) \tag{12}$$

where (*a*) is the proportion of ozone-sensitive microbes destroyed at rate (k_1) and (1 - *a*) is the proportion destroyed at rate (k_2), which can be described as the ozone-resistant fraction of the microbial population under study.

Selma *et al.* (2007) employed the Chicke-Watson (Haas *et al.*, 1995), modified Chicke (Kaymak, 2003), modified Chicke-Watson (Cho *et al.*, 2003) and modified multiple target (Kaymak, 2003) kinetic models to determine the influence of ozone concentration, reaction time and ozone demand for the inactivation of *Shigella sonnei* inoculated in ozonated water. The ozone demand (*OD*) factor allows for the comparison of the efficacy of different ozone treatments for which concentration and treatment times are different (Selma *et al.*, 2007). Ozone demand (*OD*) can be determined if instantaneous disinfectant demand (*D)* is known by using the following expression:

$$OD = \frac{OD_O - D}{k^*} \left[1 - \exp\left(-k^*\,t\right)\right] \tag{13}$$

The rate of ozone decomposition (*k**) can be determined by measuring the concentration of ozone applied (O_i) and the residual ozone (O_R) in the fluid after time (*t*) and using the following firs- order equation (Kaymak, 2003):

$$O_R = \left(O_i - D\right) \exp\left(-k^*\,t\right) \tag{14}$$

The instantaneous ozone demand can be defined as the minimum dose required to achieve the desired log reduction, which may be determined experimentally and is dependent on the microorganism under investigation.

6. Conclusions and Future Research Needs

Ensuring food safety while meeting the demand for nutritious food has led to increased interest in non-thermal preservation techniques. The mechanism by which bioactive compounds degrade are numerous, complex and in some cases unknown. The non-thermal technologies discussed in this review have the potential to meet the mandatory 5 log microbial reductions. Within the food industry, there is an increasing emphasis on and trend toward natural food preservation technologies in response to a growing consumer demand for environmentally friendly additives. Non-thermal processes represent rapid, efficient and reliable alternatives to improve the quality of food and also have the potential to develop new products with unique functionality. Although many innovative food-processing techniques have shown potential for improving the nutritive quality of all processed food, a significant proportion of these have not been applied to new food products.

Furthermore, because of the intrinsic characteristics of non-thermal technology, it is not easy to monitor in real time the processing conditions that determine the levels at which the primary treatment effects (caused by the electrical treatment, UV treatment and ozone treatment) outweigh the secondary effects (caused by heat).

Future studies should address these needs. Studies on the effect of non-thermal processing parameters on the bioactive content of treated foods should be conducted. In-depth research is needed to study the kinetics of the generation, retention and degradation of health-related compounds as affected by non-thermal treatment conditions and to elucidate the mechanisms underlying the induced changes.

Acknowledgements

This study has been carried out with financial support from Japan Society for the Promotion of Science, Overseas Fellowship Division, (Project No. L11560).

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