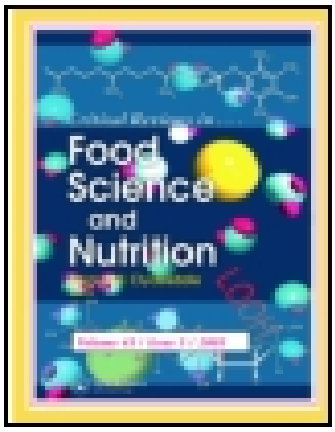

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Review: Alternatives to Conventional Thermal Treatments in Fruit-juice Processing. Part 1: Techniques and Applications

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Review: Alternatives to Conventional Thermal Treatments in Fruit-Juice Processing. Part 1: Techniques and Applications

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Abstract

This article provides an overview of alternatives to conventional thermal treatments and a review of the literature on fruit-juice processing for three key operations in fruit-juice production such as microbial inactivation, enzyme inactivation, and juice yield enhancement, these being radiation treatments (UV light, high-intensity light pulses, γ-irradiation), electrical treatments (pulsed electric fields, radiofrequency electric fields, ohmic heating), microwave heating, ultrasound, high hydrostatic pressure, inert gas treatments (supercritical carbon dioxide, ozonation), and flash-vacuum expansion. The non-thermal technologies discussed in this review have the potential to meet industry and consumer expectations. However, the lack of standardization in

operating conditions hampers comparisons among different studies, and consequently ambiguity arises within the literature. For the juice industry to advance, more detailed studies are needed on the scaling-up, process design, and optimization, as well as on the effect of such technologies on juice quality of juices in order to maximize their potential as alternative non-thermal technologies in fruit-juice processing.

Keywords

Microorganisms, enzyme, quality, technological, innovation

1. Introduction

Beverages, concentrated juices, and purees are vital food products due to the massive demand of the global market (Tumpanuvatr and Jittanit. 2012). Over the last few years, the consumption of fruit juices has been rapidly increasing (Duthie et al. 2000, Netzel et al. 2007, Tiwari et al. 2009c), making the fruit-juice industry among the largest agro-based industries worldwide (Ribeiro et al. 2010).

Fruit juices are finished products that have been subjected to a transformation process. According to the final product desired and the fruit used, the transformation has numerous variations (Jeantet et al. 2007). The initial steps in the juice extraction include washing, sorting, and crushing of fruits in a mill. All fruits designated for juice processing must be healthy, free of contamination and major bruises, especially free of mold or rot that would lead to defective juices (McLellan and Padilla-Zakour. 2004). In general, juice extraction should be done as rapidly as possible so as to minimize its oxidation by naturally present enzymes.

A variety of methods is used to open the fruit to release the juice, where pressure is applied to the mash the fruit or force it through a press. Several styles of separators are available for both batch and continuous production, such as the pack press (Taylor. 2005), the Bucher-Guyer horizontal rotary press (Downes. 1999), the belt press (Downes. 1999, Shaw. 1994), and the filter press, among others. Heating and addition of enzymes might also be included before the mash is transferred to the extraction stage.

The immediate turbidity in freshly pressed fruit juices is generally considered to be a result of suspended pectin particles from the plant-cell walls, but other disrupted cell-wall and cell materials may also contribute to juice cloudiness (Weiss. 1987, Binning, R. and Possmann, P.

1993). A common problem arising mostly in cloudy fruit juices is the spontaneous clarification during storage. This, usually referred to as haze formation, is assumed to be caused by interactions between haze-active proteins and polyphenols that form insoluble multi-molecular structures (Siebert et al. 1996, Siebert. 2006, Pinelo et al. 2010). Industrial juice clarification typically involves enzyme-catalyzed depectinization and fining by the addition of pectinases, gelatin, silica sol, and/or bentonite, respectively, to encourage pectin degradation and subsequent physico-chemical precipitation of sediments and haze-active components (Konja, G. and Lovric, T. 1993, Grassin, C. and Fauquembergue, F. 1996). These treatments are followed by filtration and/or centrifugation (Weiss. 1987, Grassin, C. and Fauquembergue, F. 1996, Pinelo et al. 2010). Juices are pasteurized immediately after pressing so as to denature any residual enzymes. Centrifugation then removes large pieces of debris, leaving most of the small particles in suspension (Kashyap et al. 2001). The final step is usually a heat treatment or equivalent non-thermal process to achieve a safe and stable juice. For a concentrate, the juice is transferred to an evaporator to remove water to the desired concentration level. Other processes used for water removal include reverse osmosis and freeze concentration, which are best suited for heat-sensitive juices. The concentrate is then ready for final processing, packaging, and storage. (McLellan and Padilla-Zakour. 2004, Downing. 1996).

Thermal processing is the most commonly used processing technique (Suh et al. 2003). However, it may degrade organoleptic, physical, and physicochemical properties, and the nutritional quality of juices (Kubo et al. 2013, Qin et al. 1995, Charles-Rodríguez et al. 2007, Ibarz et al. 1999, Suh et al. 2003, Chen et al. 2013).

Therefore, recent consumer demands for safe and minimally processed foods with high-quality attributes have encouraged the food industry and scientific researchers to find innovative food-processing techniques to produce foods with a minimum of changes induced by the technologies themselves (Enomoto et al. 1997, Hong and Pyun. 2001, Liao et al. 2007, Esteve and Frígola. 2007). Intense investigation has evaluated alternative and complementary processes to thermal treatments (Heinz et al. 2003, Stewart et al. 2002). Innovations in fruit-juice processing have focused in recent years on microbial and enzymatic inactivation and on increasing juice yield. Therefore, the present paper provides an overview of alternatives to conventional thermal treatments and the published literature in fruit-juice processing for these three key operations in fruit-juice production, such as UV light, high-intensity light pulses, γ-irradiation, pulsed electric fields, radiofrequency electric fields, ohmic heating, microwave heating, ultrasound, high hydrostatic pressure, supercritical carbon dioxide, ozonation, and flash-vacuum expansion.

2. Alternative technologies to conventional thermal treatments in fruit-juice processing for microbial inactivation

Fruit-juice producers have traditionally relied on the acidity of their products to ensure microbiological safety. Nevertheless, several incidents of food-borne disease have been associated with juices. Recent regulations by the Food and Drug Administration (FDA) have required processors to achieve a 5-log reduction in the numbers of the most resistant pathogens in their finished products. The ruling has accelerated the search for novel non-thermal processes that can ensure product safety yet maintain the desired nutritional and sensory characteristics (Tiwari et al. 2009c). In this review, radiation, electrical, ultrasound, high-hydrostatic-pressure, and inert-gas treatments are examined as alternatives to conventional thermal methods for the

preservation of fruit juices. Table 1 presents the results achieved during the last decade in terms of microbial inactivation in fruit juices.

2.1. Radiation treatment

2.1.1. Ultraviolet light

Fresh food products may be processed using ultraviolet (UV) light as a germicidal medium to reduce the food-borne microbial load. The radiation absorbed by DNA may stop cell growth and lead to cell death (Liltved and Landfald. 2000), the most lethal effect taking place at 254 nm (Murakami et al. 2006, Oteiza et al. 2005, Ibarz et al. 2005). Compared to thermal pasteurization, UV-treated juice may have the added benefit of having a more fresh-like quality in addition to a simpler process with lower operating costs. In addition, photoreactivation may occur when the UV-C injured cells are exposed to wavelengths higher than 330nm (Liltved and Landfald. 2000). In this ca se, the damage occurring at the DNA level could be repaired by protein factors (DNA repair genes)(Yajima et al. 1995). However, a dark environment might avoid photoreactivation of irradiated products (Stevens et al. 1998, Guerrero-Beltrán and Barbosa-Cánovas. 2004).

UV treatment of juices is difficult due to their low UV transmittance through the juice containing dense suspended solids, where microorganisms continue to survive despite continued exposure to high amounts of UV-light energy. Juices with high amounts of suspended matter need a stronger UV dose than do clear juices, i.e. apple juice needs a lower UV dose to achieve effective reduction as expected, whereas orange juice and tropical juices require higher UV dosages to achieve the reductions needed (Keyser et al. 2008). In recent years, to treat juices having high amounts of suspended solids and low UV transmittance, different approaches have been employed, such as extremely thin film UV reactors (Oteiza et al. 2005, Tran and Farid. 2004) to

decrease the path length of UV light and thus avoid problems associated with lack of penetration and reactors used to increase the turbulence within a UV reactor to bring all material into close exposure to the UV light (Koutchma et al. 2006). Flow rates and mixing in the turbulent flow also affected microbial inactivation, i.e. the higher the flow rates the higher inactivation rates in a turbulent flow UV reactor (Koutchma et al. 2004).

UV radiation has been reported to be effective in inactivating *Escherichia coli* ATCC 35218 in apple juice, *Escherichia coli* ATCC 35218 and its cocktail were more sensitive than *Saccharomyces cerevisiae* KE162 and the cocktail of yeasts (Char et al. 2010). In addition, Oteiza et al. (2010) reported that the presence of native yeast cells in orange juice weakens the UV inactivation of *Escherichia coli*. UV-absorption coefficients in the juice increase at greater yeast concentrations, and higher UV doses are necessary to inactivate bacteria.

UV radiation has also been combined with sonication, enhancing the inactivation effect in orange juice, and being more effective simultaneously rather than in a series of ultrasound-UV-C approach (Char et al. 2010). The disadvantages are the possible flavor and color changes in some juice products (Murakami et al. 2006). Furan has also been reported to be formed during UV treatments (Bule et al. 2010).

2.1.2. High-intensity light pulses

High-Intensity Light Pulses (HILP), also known as pulsed light, is an emerging non-thermal technology which uses light pulses of short duration (100-400 μs) ranging from ultraviolet to infrared wavelengths (200-1100 nm), for microbial inactivation. The lethal effect of HILP on microorganisms is attributed mostly to the photochemical action of the UV part of the spectrum emitted by the flash lamp.

Several critical parameters should be considered when designing experiments to assess the suitability of HIPL, such as transparency of the medium, type of microorganism, energy dose supplied, the number of pulses, and the depth of the samples (Palgan et al. 2011b). As expected, and as in UV treatments, microbial inactivation declines with decreasing transparency of the medium (Pataro et al. 2011). Indeed, treatment at total energy doses of 7-28 J/cm^2 during 2-8 s has been demonstrated to be efficient to inactivate *Escherichia coli* in apple juice, but not in orange juice, due to its lower transparency (Palgan et al. 2011b).

Results also depend on the type of microorganism examined. *Escherichia coli* cells showed greater susceptibility to the HILP treatment than did *Listeria innocua* cells in both apple and orange juices. Furthermore, it should be noted that the HILP sensitivity of the various groups of microorganisms may be diverse since each organism has a different requirement in terms of lethal dose (Pataro et al. 2011). Although no clear pattern can be established regarding the differences in HILP sensitivity of the different microorganisms investigated (Gómez-López et al. 2005), it has been generally observed that Gram-positive bacteria are more resistant than Gram-negative ones (Anderson et al. 2000, MacGregor et al. 1998, Rowan et al. 1999, Sharifi-Yazdi and Darghahi. 2006, Pataro et al. 2011).

Concerning lethal doses, results have highlighted that the lethal effect of HILP depended on the energy dose supplied, i.e. the higher the quantity of the energy delivered to the juice stream, the greater the inactivation level in a range of energy dosages from 1.8 to 5.5 J/cm^2 (Pataro et al. 2011). By contrast, other findings suggest that the application of a higher energy dose (5.1 J/cm^2) did not significantly raise levels of *Escherichia coli* inactivation in comparison to a lower dose

(4.03 J/cm^2) (Muñoz et al. 2011). Similarly, energy doses of 5.1 J/cm^2 or 4.0 J/cm^2 gave no significant differences regarding microbial reduction (Caminiti et al. 2009).

However, the combination of HILP as an initial hurdle followed by thermosonication, led to significant inactivation of 3.37 log CFU/mL and 3.46 log CFU/mL when applied at either the lower and higher energy settings for each hurdle, respectively, giving significantly greater reductions than any of the treatments applied individually (Muñoz et al. 2011); and also followed by manothermosonication, performed to reduce *Escherichia coli* and *Pichia fermentans* in a blend of fresh apple and cranberry juice, inactivation levels of approximately 6 log have been achieved (Palgan et al. 2011a).

2.1.3. γ-Irradiation

For the γ-Irradiation of food, the product is exposed to a source of gamma rays (Cobalt-60 (Mahapatra et al. 2005). X-rays, or electrons may also be used, although few fruit-juice applications are available in the literature. Microorganisms are inactivated by γ-irradiation primarily due to DNA damage, which destroys the reproductive capabilities and other functions of the cell (Tiwari et al. 2009c, De Ruiter and Dwyer. 2002).

Several factors such as composition of the medium, the moisture content, presence or absence of oxygen, influence radiation resistance, particularly in case of vegetative cells reportedly influence the process (Farkas. 2006). However, some evidence calls into question the link between the microbial-inactivation efficiency and the composition of the medium (Niemira. 2001); as the dose required to achieve 90% destruction of salmonella Enteritidis in orange versus orange-tangerine blend varied only slightly (0.35 to 0.37 kGy), with no significant differences among the juices. Other authors reported that *Salmonella Enteritidis* sensitivity to γ-irradiation is

not strongly affected by the composition of formulated commercial orange juices, and in further studies they asserted that neither the resistance of each *Salmonella* isolate inoculated in juice preparations of varying turbidity nor the pattern of relative resistance among isolates was altered in juice preparations of reduced turbidity, therefore proposing the variable resistance of *Salmonella* isolates to irradiation as a more significant factor than turbidity in designing antimicrobial juice-irradiation protocols (Niemira et al. 2001).

Improvement in microbiological quality by radiation processing was evidenced by the dose-dependent reduction in total viable counts, yeasts, and molds (Chervin and Boisseau. 1994, Niemira et al. 2003, Kim et al. 2007, Lee et al. 2009, Alighourchi et al. 2008). Irradiation doses of more than 2 kGy were sufficient to completely inactivate the total bacteria and fungi counts and to retard microbial growth during storage in pomegranate juices (Alighourchi et al. 2008). Similarly, γ- irradiation of 5 kGy at 15°C for carrot juice (Jo and Lee. 2012) and ashitaba and kale juices (Jo et al. 2012) showed 99% or higher sanitation compared to control. They reported that γ-irradiation was superior to UV treatment in terms of energy efficiency and post-treatment effect on microorganism growth (Jo and Lee. 2012).

On the other hand, resistance to γ-irradiation depends on the microbial type examined. A study of microorganism survival indicated that *Escherichia coli* was sensitive to irradiation and can be reduced by 7 log at 1 kGy, whereas the total colony and *Bacillus subtilis* spore bacteria endured stronger irradiation (Wang et al. 2006). Irradiation at 3 kGy showed no viable cell growth of *Salmonella typhimurium* and *Escherichia coli* in carrot or kale juices, *Escherichia coli* being more sensitive than *Salmonella typhimurium* to radiation (Song et al. 2006). However, other studies have reported the presence of two typical radiation-resistant bacteria, *Bacillus*

megaterium and *Exiguobacterium acetylicum* in 5 kGy-irradiated kale juices (Kim et al. 2007). Differences in radiation sensitivities among the microorganisms are related to differences in their chemical and physical structure, and in their ability to recover from radiation injury. The amount of radiation energy required to control microorganisms in food, therefore, varies according to the resistance of the particular species and according to the number of organisms present.

Nonetheless, evidence suggests off-odor formation in γ-irradiated juices. Strong off-odor at 2 kGy and higher was found in melon juice (Wang et al. 2006), which agrees with findings of other authors (Fan et al. 2002, Foley et al. 2002, Yoo et al. 2003), who found that total volatile off-odor compounds increased in a dose-dependent manner.

Combinations of γ-irradiation with heat sterilization at 85-95°C have been reported to lower the doses required for elimination of the *Alicyclobacillus acidoterrestris* spores (Nakauma et al. 2004). Similarly, the combination of frozen storage (-20°C) plus irradiation at doses from 0.5-2.0 kGy has resulted in greater overall reductions than either process alone (Niemira et al. 2003). Other approaches such as the addition of preservatives, including citric acid (0.3%), sodium benzoate (0.015%), potassium sorbate (0.025%), and sucrose (10%), have been used to extend the shelf life of sugarcane juice (Mishra et al. 2011).

2.2. Electrical treatment

2.2.1. Pulsed electric fields

Pulsed electric field (PEF) technology involves the application of high-voltage pulses to liquid or semi-solid foods placed between two electrodes. Although a temperature might rise due to the electric current flowing through the liquid food (ohmic heating) (Vega-Mercado et al. 1997, Lindgren et al. 2002, Barsotti et al. 1999), PEF is intended to be a non-thermal technique.

The pulse caused by the discharge of electrical energy is extremely short (with nanoseconds to microseconds), while the interval between discharges is comparatively long (1 millisecond to seconds). The strength of the electric field that passes through the food is directly proportional to the voltage supplied across the electrodes and inversely proportional to the gap or distance between the electrodes. PEF technology utilizes electric field strengths of 10-80 kV/cm (Deeth et al. 2008).

To establish a typical PEF system, a pulse generator is needed. A typical system comprises a high-voltage power supply, one or more (energy storage) capacitors, a high-voltage switch and a treatment chamber. The chamber design exhibits a significant influence on the effectiveness of the process by affecting treatment uniformity, peak electrical field strength, and product throughput (Buckow et al. 2011, Qiu et al. 1998, Buckow et al. 2013). One challenge is to design a treatment chamber capable of operating at high and uniform electric field intensities and which prevents dielectrical breakdowns (Qiu et al. 1998), which may occur when the applied electrical field strength exceeds the dielectric strength of the treated food product in the chamber (Zhang et al. 1995, Buckow et al. 2013). Dielectrical breakdowns can also be caused by local field enhancement and impurities (gas bubbles or solids) in liquid foods. Batch chambers with parallel plate electrode configurations provide relatively low throughputs but high-treatment uniformity. Treatment chambers with colinear configurations of electrodes allow continuous operation at high throughputs, but often exhibit poor treatment uniformity. Nonuniform electric field and flow velocity distributions can result in under- (often in central regions or dead spaces) or overtreated (often in boundary regions) volume elements, which lead to an increased chance of electrical sparks and system breakdown, as well as degradation of the quality of the treated product. The

majority of pilot and industrial-scale PEF systems comprise treatment chambers (flow cells) with cofield and colinear configurations of electrodes. This configuration provides a very nonuniform electrical field which may result in insufficient treatment, dead spaces and, thus, possibly recontamination of the treated medium with microorganisms. To reduce the risk of under- or overprocessing, multiple PEF treatment chambers can be placed in series. This numbering up of treatment zones also means that the required processing time can be broken up into smaller fractions allowing intermediate cooling of the product. This can slightly reduce the effectiveness of the treatment but will preserve the quality of the product (Buckow et al. 2013).

The application of PEF pulses leads to the permeabilization of biological membranes. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field; permeation then causes swelling and the eventual rupture of the cell membrane (Vega-Mercado et al. 1997), reducing or eliminating the microbial load. Indeed, several authors have also reported total inactivation (<1 log CFU/mL) of mesophilic bacteria, mold, and yeast in different fruit juices, after applying different PEF procedures (Li and Zhang. 2004, Mosqueda-Melgar et al. 2008, Morales-de la Peña et al. 2010). This phenomenon depends on parameters such as fruit maturity, the degree of material fragmentation (particle size), and oxidation-preventing methods, among others (Turk et al. 2010). Intrinsic characteristics of the juice such as its conductivity can influence the microbial inactivation rate. Chen et al., studying *Staphylococcus aureus* inactivation in apple juice of different conductivities (1.5, 2.0 and 2.5 mS/cm), achieved inactivation with a 75 μs treatment at electric-field strengths of 25, 30, and 35 kV/cm, respectively (Chen et al. 2010).

Optimization of electrical parameters (including pulse profile, pulse polarity, pulse duration, number of pulses, pulse frequency, and electric-field strength) and expression parameters (pressure, type of equipment, length of treatment) is crucial to achieve the desired output (Bi et al. 2013). It has been reported that as the field strength was increased during stand-alone PEF treatment from 24 to 34 kV/cm, a greater number of *Escherichia coli* cells were inactivated (2.8 compared with 4.2 log CFU/mL), in a tropical fruit smoothie (Walkling-Ribeiro et al. 2008a), and the same effect was found in carrot juice (Akin and Evrendilek. 2009).

Increasing the treatment time also had an impact on microbial reduction, although according to Akin and Evrendilek (2009), not as pronounced as offered by field strength. Morales-de la Peña et al. (2010), reported that by prolonging PEF treatment from 800 to 1400 µs, microbial shelf-life of juice and soymilk juices could be extended from 31 to 56 days.

Other authors have reported that the pulse with shorter rise time (200ns) had a better effect of inactivation on *Staphylococcus aureus* incubated into apple juice compared to a longer one (2 µs) (Chen et al. 2010).

Some researchers even recommend that PEF should be used together with moderate temperatures, i.e. approximately 45-55 °C for increasing the microbicidal effect (Lindgren et al. 2002, Walkling-Ribeiro et al. 2008a, Dunn and Pearlman. 1985). Walkling-Ribeiro et al. (2008a), investigated the effect of PEF-treatment combined with moderate heat from 25°C to either 45 or 55°C over 60 s in a tropical fruit smoothie inoculated with *Escherichia coli* K12. The higher temperature during the PEF treatment induced a greater inactivation, these results being comparable (6.9 log CFU/mL) with those for thermal pasteurization (6.3 log CFU/mL; 72°C, 15 s). These results are in agreement with other authors (Evrendilek et al. 2000, Yeom et al. 2000).

However, when a PEF treatment at 32 kV/cm for 90 s was compared to heat treatment at 100°C for 1 min, PEF treatment proved to be efficient in yeast inactivation and moderate in *Escherichia coli* inactivation, although thermal pasteurization was effective for *Escherichia coli* and yeast inactivation (Zhang et al. 2010).

Other studies have demonstrated the efficiency of the addition of antimicrobials to the PEF treatment. When tomato juice was treated alone at mild heat and PEF at field strength of 80 kV/cm, 20 pulses and 50 C, microbial reduction proved minor. However, with the addition of small amount of nisin (100 U/mL) the microbial reduction became significantly greater, about 4.4-log reductions in microbial counts (Nguyen and Mittal. 2007). Positive results have also been achieved by combining PEF with citric acid (0.5-2.0%, w/v) or cinnamon-bark oil (0.05-0.30%, w/v) against populations of *Escherichia coli* O157:H7, *Salmonella Enteritidis* and *Listeria monocytogenes* in melon and watermelon juices. However, the taste and odor in those PEF-treated melon and watermelon juices containing antimicrobials were significantly affected (Mosqueda-Melgar et al. 2008).

Synergistic effects have been reported for combinations of PEF treatment with UV light (Noci et al. 2008, Walkling-Ribeiro et al. 2008b, Caminiti et al. 2011a), and HILP (Caminiti et al. 2009a, Caminiti et al. 2011b).

2.2.2. Radiofrequency electric fields

Radiofrequency electric fields (RFEF) or electric currents that oscillate at radio frequencies in the range of about 3 Hz to 300 GHz could be used as a non-thermal pasteurization for the inactivation of bacteria in foods. The RFEF process is similar to the PEF process. The difference is that in PEF processing, the high voltage is applied in pulses using a pulse generator, whereas

in RFEF processing, the voltage is applied continuously using an alternating current generator (Geveke et al. 2007). Figure 1 shows a schematic diagram of a continuous radio frequency electric fields process including two treatment chambers in series. It has been reported that inactivation of bacteria is by disruption of the bacterial surface structure, leading to the damage and leakage of intracellular biological active compounds (Ukuku et al. 2012, Ukuku et al. 2008).

It seems clear that increasing the number of treatments and temperature improved inactivation (Geveke et al. 2007, Geveke et al. 2002, Geveke and Brunkhorst. 2003, Geveke and Brunkhorst. 2004). However, the electric-field strength and frequency involves certain complications. It has been stated that increasing the field strength strengthened inactivation within a range of 20-60 kHz (Geveke and Brunkhorst. 2003). However, when experiments were made at lower field strengths (15-20 kV/cm), varying the electric field strength had no effect on the inactivation (Geveke et al. 2007). Frequencies in the range of 20-60 kHz were at first reported to have no effect on microbial inactivation (Geveke and Brunkhorst. 2003). However, it was subsequently shown that lower frequencies of 15 and 20 kHz inactivated *Escherichia coli* better than did frequencies of 30 to 70 kHz (Geveke and Brunkhorst. 2004).

It has been stated that the RFEF application of moderate heat provides a much higher inactivation than when used alone. Increasing the temperature to 55°C enhanced the inactivation of the bacteria, leading to 99.99% reduction (Ukuku et al. 2008).

RFEF has been studied alone and in combination to UV light for inactivating *Escherichia coli* K-12 in apple juice. At 40 °C, UV-light treatment alone caused a 5.8-log reduction of *Escherichia coli* in apple juice while RFEF caused only a 2.8-log reduction. A combination of the two processing treatments did not increase cell injury or leakage of intracellular bacterial UV-

substances more than that from the UV-light treatment (Ukuku and Geveke. 2010). In further studies, RFEF at 25 kV/cm, 75°C and 3.4 ms was used in combination to mild heat at 75°C and the viability loss for *Escherichia coli* averaged 7 log CFU/mL (Ukuku et al. 2012).

2.2.3. Ohmic heating

Ohmic heating (OH) of food products involves the passage of alternating current through them, thus generating internal heat as a result of electrical resistance of the food components (Reznick. 1996, Valero et al. 2010). Figure 2 shows a schematic diagram of an ohmic heating circuit. This technique differs from PEF, as the latter involves the application of a short burst of high voltage to a food placed between two electrodes, destroying the cell membrane by mechanical effect with no intended heating (Barbosa-Cánovas et al. 1999).

This may be considered a thermal treatment although it has some advantages over the conventional heating system because the heat is instantly generated within the food with the passing of the electrical current (Icier et al. 2006, Icier and Tavman. 2006) whereas the conventional method must rely on the heat-transfer mechanisms, especially heat conduction and convection, which are usually limited due to the thermo-physical properties of food and the fouling on the heat contact surface (Tumpanuvatr and Jittanit. 2012, Lalande. 1985, Bansal and Chen. 2006). Therefore, ohmic heating has been attributed to a high heating rate, high energy-conversion efficiency, uniform volumetric heating (Assawarachan. 2010, Castro et al. 2004, de Alwis and Fryer. 1990) and therefore inflicts less thermal damage on the product (Leizerson and Shimoni. 2005a, Leizerson and Shimoni. 2005b).

The heating rate is directly proportional to the square of the electric field strength, and the electrical conductivity of the product. Thus, foods having lower electrical conductivities heat

more slowly than do those of higher electrical conductivities if the same electrical-field strength is applied. The electrical conductivities of food products normally depend on their temperatures and constituents, especially salt, acid, and fat (Tumpanuvatr and Jittanit. 2012, Shiksat et al. 2004, Sarang et al. 2008). Many published works have demonstrated that the electrical conductivities of foods would increase if their temperatures were raised (Shiksat et al. 2004, Sarang et al. 2008, Icier and Ilicali. 2005b, Icier. 2009, Assawarachan. 2010, Lee et al. 2012, Darvishi et al. 2011, Singh et al. 2008, Kong et al. 2008). If the product has more than one phase, such as in juices containing a mixture of liquid and particulates, the electrical conductivity of all the phases should be considered (Ruan et al. 2001, Zhou et al. 2011) concluded that the conductivity of apple juice with granules increases linearly as temperature increases and particle size and mass fraction decreases. However, other authors found that the electrical conductivities of liquid food matrices might instantaneously drop after the occurrence of bubbling (Icier and Ilicali. 2005a, Icier and Ilicali. 2005b). The bubbles are characterized as electrical insulators; therefore, they will interfere the flow of electrical current and lessen the electrical conductivity of food matrices as a whole (Tumpanuvatr and Jittanit. 2012).

Increases in electric-field strength will enhance OH treatment efficiency. (Lee et al. 2012) reported that as the electric-field strength increased from 25 to 40 V/cm, surviving populations of foodborne pathogens decreased more effectively for lower electric field strength, longer treatments were needed to achieve the same microorganism reduction (Sagong et al. 2011, Baysal and Icier. 2010).

Not all microbial species are equally resistant to the OH treatment, and thus (Onwnka et al. 2008) found significant differences in bacterial survival according to the pathogen type, with

Salmonella spp. being the most resistant one to a treatment at 100°C and *Clostridium perfringens* at 70°C, while *Escherichia coli* proved to be the least resistant, the total destruction of the pathogens being achieved at 20 min of treatment. Sagong et al. reported in 2011 that *Escherichia coli* O157:H7 was more resistant than other species such as *Salmonella Typhimurium* or *Listeria monocytogenes* to a treatment at 10-20 V/cm for up to 540 s. On the other hand, significantly higher lethality for of *Alicyclobacillus acidoterrestris* spores treated with OH was achieved than for spores treated with conventional heating in orange juice (Baysal and Icier. 2010). However, other authors have reported that the type of thermal treatment applied did not significantly affect the shelf life in terms of microbial counts (Leizerson and Shimoni. 2005b). Also it has been found that the significant parameter in the inactivation of microorganisms is the thermal effect, regardless of the kind of thermal treatment (ohmic or conventional) (Leizerson and Shimoni. 2005a).

Moreover, conditions such as pressure at 121°C in combination with OH have been used to raise the boiling point and thus reduce the viable *Bacillus subtilis* spores in orange juice four logarithmic orders in less than 1 s of treatment (Uemura and Isobe. 2003).

The type of electrode is also a factor to be considered. Onwuka and Ejikeme (2005) suggested that heavy electrolysis could occur with the use of both Cu/Cu and Cu/Al electrodes, resulting in reactions on the metals, which could enter the juice solution, thereby making the product unsafe to consume. They advise that the enhancement of juice extraction by OH should therefore use electrodes devoid of electrolysis, such as platinum coated with titanium.

2.3. Ultrasound treatment

Power ultrasound (US) may also be employed as an alternative processing option to conventional thermal approaches for pasteurization and sterilization of food products.

The propagation of high power US in a liquid induces bubble cavitation due to pressure changes. These resulting micro-bubbles collapse violently in the succeeding compression cycles of propagated ultrasonic waves. These results were found with the use of localized high temperatures and pressures, and high shearing effects. Consequently, the intense local energy and high pressure bring about a localized pasteurization effect without causing a significant rise in macro-temperature (Tiwari et al. 2009c). Figure 3 shows a schematic diagram of an ultrasound exposure system for fruit juice.

It has been reported that viscosity of the juice influence the degree of cavitation (Patil et al. 2009b), as the composition of each juice could provide some protective effect on the cells against the effect of cavitation. It has also been reported that grape juice showed the highest inactivation of *Saccharomyces cerevisiae*, followed by pineapple and cranberry juice (Bermúdez-Aguirre and Barbosa-Cánovas. 2012).

Other authors, modeling the inactivation kinetics of yeasts in tomato juice, found that parameters such as amplitude level and processing time had a significant effect on increasing the inactivation of yeasts, but the effect was relatively weak at lower amplitude levels and processing times (Adekunte et al. 2010a). These authors also observed that yeast inactivation followed the Weibull model, as in the work of (Bermúdez-Aguirre and Barbosa-Cánovas. 2012), the yeast-survival curves being concave downward. Further studies found an increase in temperature with a higher amplitude level (Adekunte et al. 2010b). Other authors, increasing temperature to between 40°C and 60°C, achieved higher *Saccharomyces cerevisiae* inactivation rates during

ultrasonic treatment at 24 kHz and 120 μm for 10 min. Moreover, a continuous mode was found to be more effective in inactivating *Saccharomyces cerevisiae* than the pulsed mode (Bermúdez-Aguirre and Barbosa-Cánovas. 2012).

It has been stated that US treatment is more effective than the treatment alone for microbial inactivation when combined with moderate heat, as previously mentioned, and other conditions such as pressure (manosonication) (Arroyo et al. 2011), mild heat and pressure (manothermosonication) (Palgan et al. 2011a, Caminiti et al. 2011a), osmosonication which combines US with non-thermal concentration (Wong et al. 2010,Wong et al. 2012), or antimicrobials (Ferrante et al. 2007,Bevilacqua et al. 2012). Nevertheless, other combinations such as sonication coupled with carbonation were found to be of little value in inactivating microorganism at room temperature (Cheng et al. 2007).

The US-assisted process reportedly required a higher total energy input than conventional heating due to the high amount of electrical energy required for US generation (Zenker et al. 2003). The choice of implementation of this technology should be based on the determination of whether the product quality improvements resulting from US-assisted thermal treatment may justify its increased energy requirement (Valero et al. 2007). Although the possibility of deactivating micro-organisms by ultrasonic processing has been demonstrated under laboratory conditions, industrial adoption of this technology is limited, due to the significant challenges encountered in industrial scaling-up (O'Donnell et al. 2010).

2.4. High-hydrostatic-pressure treatment

In the high-hydrostatic-pressure (HHP) process, the juice is subjected to pressures from 100 MPa to 900 MPa. The pressurization is applied for the duration of the treatment and then released.

Pressure is applied isostatically, i.e. equally applied in all directions. The pressure applied and the holding time depends on the type of product treated and the expected final result. HHP has also been used for enzyme inactivation, but microbial inactivation generally requires lower pressures than enzyme inactivation (San Martín et al. 2002). Although HHP in intended to be a non thermal technique, an inherent mild increase in temperature may occur. The combined pressure and mild heat might improve the application of HPH treatment for liquid food pasteurization {{767 Belloch,C. 2012}}.

The efficacy for inactivating microorganisms in fruit juices is strongly affected by the juice matrix, with the concentration and pH being determinant factors (Wang et al. 2012). The effectiveness of the treatment may change with the juice concentration (Basak et al. 2002). Some authors hold that differences in water availability explain the greater resistance of *Alicyclobacillus acidoterrestris* spores to high-pressure inactivation in the juice with high soluble-solid content (Sokolowska et al. 2013a, Sokolowska et al. 2013b). Other authors reported that during the 45 min pressurization (200 MPa, 50°C) of *Alicyclobacillus acidoterrestris* spores in concentrated apple juice (71.1°Brix), no significant changes were detected in their number. However, in the juices with a lower soluble-solid content of 35.7, 23.6, and 11.2°Brix, the reduction in spores was 1.3-2.4 log, 2.6-3.3 log, and 2.8-4.0 log, respectively (Lee et al. 2006). Aerobic bacteria are more pressure resistant in a neutral system than in an acid system (Patterson. 2005, Zhao et al. 2013).

In general, microorganisms showed pressure sensitivity that was more pronounced at higher pressure levels (Basak et al. 2002). Research has revealed that the microbial-inactivation curve,

at pressures of 120-400 MPa and temperatures ranging from -5 to 45°C showed a biphasic trend of pressure destruction, i.e. an initial rapid drop in the survival curves described by first-order kinetics followed by a long tailing (Erkmen. 2009, Reyns et al. 2000). Erkmen attributes the tailing phenomena to a smaller portion of the microorganism population being more pressure-resistant or adapted to the pressure stress that made the remaining cells more resistant.

Other authors, investigating the use of pulsed HHP in apple juice using pressure of 150 to 300 MPa, 25 to50°C, 1 to 10 pulses of 60 s, concluded that the efficiency of pulsed HHP processes depends on the combination of the pulse holding time and number of pulses (Donsì et al. 2010). They asserted that the efficacy of the single pulses diminishes with the greater the pulse number and pressure level, and therefore the first pulse cycle is more effective than the following ones. Other authors (Buzrul et al. 2008) suggest that multiple pulses for a total holding time of 5 min at 300 MPa instead of continuous (single pulse) treatment had no significant effect on the microbial inactivation in kiwifruit juice; however, it did in pineapple juice for *Escherichia coli* and *Listeria innocua*. Similarly, other findings suggest that repeating the high-pressure treatment at least 4 times at 300 MPa for a total of 40 min would inactivate more *Escherichia coli* strains in tomato juice than using continuous pressure for the same amount of time (Bari et al. 2007).

The effect of decompression has also been tested. The results found in the literature agree that faster compression rates have a greater impact on *Escherichia coli* inactivation in orange and apple juices treated at 0.1-600 MPa at 4 and 25°C than at slower compression rates (Abbas Syed et al. 2013, Noma et al. 2004). However, when it comes to *Bacillus subtilis* spores, slow

compression combined with slow decompression had a greater impact on their inactivation than did any combination of fast compression and fast decompression at 600 MPa at 60°C and 70°C (Syed et al. 2012).

The choice of an optimal temperature is crucial in the HHP process. Normally, a synergic effect between pressure and temperature is reported in literature (Lee et al. 2006, Donsì et al. 2010, Briñez et al. 2006, Suárez-Jacobo et al. 2010, Mert et al. 2013). It bears noting that when moderate heating is coupled with high pressure, thermal degradation of the products may be detected, and therefore the optimization of the process conditions results in a compromise between the reduction of the pressure value, due to the synergetic temperature action, and the achievement of quality of the final production (Donsì et al. 2010). Conversely, Buzrul et al. (2008) reported that using low (0°C) or sub-zero (−10°C) temperatures instead of an ambient temperature (20°C) during pressurization did not change the effectiveness of HHP treatment on *Escherichia coli* or *Listeria innocua* in kiwifruit and pineapple juices treated at 300 MPa for 5 min. Other authors, instead, reported higher *Escherichia coli* inactivation at 4°C than at 25°C in orange and apple juices treated at 0.1-250 MPa for 20 min (Noma et al. 2004). Different processing conditions, matrix, as well as differences in the equipment may explain these differences (Suárez-Jacobo et al. 2010, Vachon et al. 2002). It is also worth noting that the temperature might rise due to the HHP treatment. Carreño et al. (2011) worked at 200 MPa at 30°C for 10 s, achieving a final temperature of 45°C and at 400 MPa at 45°C for 1 min, achieving a final temperature of 60 °C. Likewise, it has been reported a surge in temperature at 600 MPa, from 25 to 43°C, which is calculated as 3°C/100 MPa (Cao et al. 2012).

The type of microorganism to be inactivated strongly affects the efficacy of the process (Briñez et al. 2006), determining the optimum combination of pressure, time, and temperature. For example, all *Salmonella* serovars tested in orange juice treated at 550 MPa for 2 min at 6°C and held for 24 h showed a >5-log decrease, while *Escherichia coli* O157:H7 strains required greater pressure, a higher temperature, longer pressurization, or a chemical additive to achieve a 5-log decrease (Whitney et al. 2007). Similarly, *Saccharomyces cerevisiae* was more resistant than *Escherichia coli* and *Listeria innocua* when orange juice was processed in the high-pressure range of 103 to 241 MPa. However, at pressures higher than 241 Mpa, a rapid inactivation was observed for the three types of microorganisms (Guerrero-Beltran et al. 2011).

There is abundant research assessing the performance of HHP regarding microbial inactivation. For example, Sokolowska et al. (2012, 2013a) achieved a 3.5-log reduction of *Saccharomyces cerevisiae* NCFB 3191 using high hydrostatic pressure of 300 MPa at 20°C with a holding time of 0, 1, 5, and 10 min in beet juice, the inoculum being 5.4 log CFU/mL. Total inactivation (5 and more than 7 log for red and white grape juices, respectively) were observed at 250 MPa, 40°C for 10 and 15 min and 200 MPa, 40°C for 5, 10, and 15 min for red and white grape juices, respectively (Mert et al. 2013). Moreover, reduced microbial counts have been maintained under storage at 4°C up for 22 days (Patterson et al. 2012), for more than 35 days (Varela-Santos et al. 2012), and at room temperature up to 90 days (Mert et al. 2013).

Other combinations such as added dissolved CO_2 (Wang et al. 2012) have been reported to inactivate bacteria effectively in high-acid fruit juice. Other authors have added chitosan,

(Kumar et al. 2009), nisin (Zhao et al. 2013) or essential oils (Espina et al. 2013) which act synergistically with the pressure to give higher microbial inactivation.

2.5. Inert-gas treatment

2.5.1. Supercritical carbon dioxide

Supercritical carbon dioxide (SC-CO_2) inactivation technology represents a non-thermal processing method for the inactivation of microorganisms. The mechanism of microbial inactivation by SC-CO_2 has not yet been fully elucidated. SC-CO_2 combines the solvent capacity of liquids with the mobility of gases. This is because, in supercritical state (31.1°C; 7.38 MPa), the density and viscosity of CO_2 lie midway between a gas and a liquid (Berna et al. 2000), so it may quickly penetrate complex structures and porous materials. Several theories explaining the inactivation mechanism of SC-CO_2 involve the diffusion of CO_2 into the cells (Ortuño et al. 2012, Ortuño et al. 2013). SC-CO_2 is reported to have significant lethal effects on microorganisms in liquid foods (Park et al. 2002).

The most important parameter in SC-CO_2 is the adequate combination of pressure and temperature. Liao et al. concluded that when using SC-CO_2 at 32°C and 20 MPa, 75 min were required to reduce the population of *Escherichia coli* by a 5-log reduction, whereas 42°C and 30 MPa were needed to achieve a reduction of 7 log in the same time (Liao et al. 2008). Other authors achieved 4.7 log reduction of *Saccharomyces cerevisiae* at 10 MPa at 36°C in apple juice (Spilimbergo et al. 2007), and also in peach and kiwi juices (Spilimbergo and Ciola. 2010). Researchers have suggested that as the CO_2-to-juice concentration (0, 85, and 170 g/kg), temperature (25 and 35°C), and pressure (6.9, 27.6, and 48.3 MPa) increase, the yeast-inactivation rate increases, and they have also reported that CO_2 was more effective in the

supercritical state than in the subcritical state for inactivating yeast (Gunes et al. 2005). In subsequent studies, the same authors suggested that *Escherichia coli* was very sensitive to dense CO_2 treatment, with a more than 6-log reduction in treatments containing 70 and 140 g/kg CO_2, irrespective of temperature and pressure. The CO_2:product ratio was the most important factor affecting the inactivation rate of *Escherichia coli*. No effect of temperature or pressure was detected, given the high sensitivity of the cells to dense CO_2 (Gunes et al. 2006).

Other combinations such as PEF (Spilimbergo et al. 2003) or USs (Ortuño et al. 2012, Ortuño et al. 2013) have been shown to have additive or synergistic effects on SC-CO_2 microbial inactivation, reducing the SC-CO_2 processing requirements (time, temperature, and pressure).

2.5.2. Ozonation

The FDA approval of ozone as a direct food additive for the treatment, storage, and processing of foods in 2001 (Khadre et al. 2001) has resulted in food scientists and the juice-processing industries employing ozone for pasteurization of fruit juices. (Cullen et al. 2010).

Ozone is a powerful broad-spectrum agent active against bacteria and fungi, and their spores, as well as viruses and protozoa. The wide antimicrobial spectrum, combined with a high oxidation potential make it an attractive processing option for the food industry. Ozone processing within the food industry has been used for fresh fruits and vegetables either by gaseous treatment or washing with ozonated water (Tiwari et al. 2009b, Tiwari et al. 2009c, Tiwari et al. 2009d, Tiwari et al. 2009e). However, liquid phases are most frequently ozonated by injecting ozone gas (mixtures of air/ozone or oxygen/ozone) through a sparger into a liquid. Figure 4 shows the schematics of ozone processing equipment for fruit juice. Residual ozone is the concentration of ozone that may be detected in the medium after application to the target surface. Both the

instability of ozone under certain conditions and the presence of ozone-consuming materials affect the level of residual ozone available in the medium. Therefore, it is important to distinguish between the concentration of applied ozone and residual ozone necessary for effective disinfection (Cullen et al. 2010).

Reviewed modeling approaches employed for describing the kinetics of microbial inactivation include non-linearities. These non-linearities could include shoulder and tailing effects of the microbial kinetics, the Weibull model frequently being adopted (Choi et al. 2012, Patil et al. 2010). The formation of a shoulder or a tailing effect on the microbial kinetics during ozone treatment could also be due to rapid reactivity between ozone and microorganisms, and environmental factors, such as the presence of organic material (Restaino et al. 1995, Hunt and Mariñas. 1997). Patil et al. (2011) developed a new model that described the growth of a *Saccharomyces cerevisiae* population in unprocessed and ozone-processed apple juice under dynamic conditions that simulated a storage-temperature abuse. At the lower temperatures (4 and 8°C), the longer lag phase indicates that the yeast population needed a longer time to adapt to the environment. However, at higher storage temperatures (12 and 16°C), this effect was not evident, indicating the ability of yeasts to grow at these temperatures with a reduced or seemingly absent lag time.

Process parameters that highly influence the efficacy of ozone treatment include flow rate, ozone concentration, and temperature. At high flow rates a small number of large bubbles are produced, which rise to the liquid surface quickly and result in poor gas dissolution. These large bubbles escape the medium quickly, thereby reducing the contact time, leading to a lower inactivation rate. At low flow rates, small bubbles are produced, but, as the amount of ozone applied is low,

inactivation is slow (Cullen et al. 2010). Patil et al. (2009c) explored the effect of the ozone flow rate on inactivation of *Escherichia coli* in liquid food, regardless of the lag time; after 25 min, complete inactivation was achieved using flow rates ranging from 0.03-0.5 L/min. However, complete inactivation was not achieved with flow rates of 0.3 and 0.75 L/min after a 30-min treatment. The optimum flow rate was 0.12 L/min, being the time required to achieve a 5-log reduction of 20 min.

Effect of ozone concentration on *Escherichia coli* ATCC 25922 has also been explored. Concentrations ranging from 17 to 75 µg/mL for a flow rate of 0.12 L/min, and from 28 to 120 µg/mL in the case of 0.6 L/min were assessed. They reported that for both flow rates, the highest concentration was the most effective to inactivate *Escherichia coli* (Patil et al. 2009a, Patil et al. 2009b, Patil et al. 2009c).

Same authors have assayed the effect of temperature on the efficacy of ozone inactivation *Escherichia coli* ATCC 25922 at an optimum inactivation flow rate of 0.12 L/min. Four different temperatures have been investigated: ambient temperature (12–15°C), 20, 25, and 30°C, ambient temperature giving the best inactivation levels (Patil et al. 2009c). Williams et al. reported that in orange-juice samples, *Salmonella* and *Escherichia coli* O157:H7 populations were undetectable after 15 and 75 min of ozonation process at 50°C, respectively. Ozonation at 4°C reduced *Salmonella* and *Escherichia coli* O157:H7 by4.2 log CFU/mL and 4.8 log CFU/mL in orange juice, respectively. Treatment at ambient temperature resulted in population reductions of less than 5.0 log CFU/ml (Williams et al. 2004). There is no consensus on the effect that temperature exerts on the biocidal efficacy of ozone. For example, a drop in the temperature of the aqueous medium increases ozone solubility and stability, augmenting its availability in the

medium and consequently boosting its efficacy. The simultaneous contribution of these two factors (solubility/stability and reactivity) to ozone efficacy may vary with experimental conditions, making it difficult to predict the influence of temperature on a particular application (Cullen et al. 2010, Pascual et al. 2007).

The effect of ozone in combination with dimethyl dicarbonate and hydrogen peroxide for orange-juice preservation has been explored by (Williams et al. 2005). These researchers reported that a 5-log reduction of *Escherichia coli* O157:H7 was achieved using ozone in combination with mentioned antimicrobials followed by refrigerated storage.

Ozone has also been used routinely for washing and storing fruits and vegetables (Karaca and Velioglu. 2007, Wu et al. 2013, Bataller et al. 2012). Technologies that use ozonated water could result in an improvement of the quality characteristics of intact fruits by aiming for example at reducing fruit damage, excessive softening as well as the decontamination of product surfaces, resulting in higher quality juices (Rodoni et al. 2010).

3. Enzymatic inactivation

The use of enzymes in the food industry has expanded significantly, generating more added value to the final product and production rise while lowering costs. The juice industry has been using enzymes, especially in extraction for yield increase, juice clarification, filtration and stability (Ribeiro et al. 2010).

Pectinases were among the first enzymes used for processing juices. Studies suggest that the use of these enzymes, whether isolated or associated with other enzymes may enhance yield and help in the clarification of a wide range of juices, as they are able to degrade pectin. Although there are several types of pectinases, the most widely studied are polygalacturonases and pectin methyl

esterases. Polygalacturonases catalyze the hydrolysis of glycosidic linkages in polygalacturonic acid, producing D-galacturonate, while pectin methyl esterases catalyze deesterification of the methoxyl group of pectin, forming pectic acid and methanol (Pedrolli et al. 2009). These enzymes are normally present in plant tissues. However, the technological effect produced by these endogenous enzymes, is reportedly unsubstantial, and exogenous pectinases are added to produce the desired technological effect (Mahfuzur Rahman and Rakshit. 2004).

Other enzymes of interest in fruit-juice processing naturally present in fruits are polyphenoloxidases, peroxidases, lipooxigenases and ascorbate peroxidases. Polyphenoloxidase oxidizes *o*-diphenols into *o*-quinones, which condense, via a non-enzymatic pathway, with amino acids, proteins or other compounds to form brown pigments (Coseteng, W.Y. and Lee C.Y. 1987). These browning products decrease both the acceptability and nutritional quality of the fresh juice (de la Rosa et al. 2011). Peroxidase may oxidize not only various substrates in the presence of hydrogen peroxide, but can also produce reactive oxygen species (Mohamed et al. 2011, Falguera et al. 2012), which are found in most raw and unblanched fruit and vegetables, and are associated with the development of off-flavors and browning pigments (O'Donnell et al. 2010). Lipoxygenase activity in fruit and fruit products is reported to be related to oxidation of fatty acids and pigments. Lipoxygenase catalyzes the oxidation of polyunsaturated fatty acids containing a cis, cis-1, 4-pentadiene system, which produces 9- or 13-cis, trans-hydroperoxides. Lipooxigenase has been associated with quality deterioration because of its negative effects on pigments such as carotenes during storage, and its role in off-flavor and odor production (Aguiló-Aguayo et al. 2008, O'Donnell et al. 2010, King and Klein. 1987). Ascorbate peroxidase

detoxifies peroxides such as hydrogen peroxide using ascorbate as a substrate (Raven. 2000). This would lead to a detriment of ascorbate in fruit juices, lowering their nutritional quality.

Therefore, inactivation of these and other enzymes is required during fruit-juice processing to produce high-quality products. Heat treatment of fruits can be critical in decreasing the activity of these enzymes. However, high-temperature heating may also degrade other important components (Threlfall et al. 2005) and trigger chemical reactions that lower the quality of juices (Garde-Cerdán et al. 2007). For this reason, numerous alternatives to heat treatments have been developed over the years, including UV light, γ-irradiation, pulsed electric fields, ohmic heating, ultrasound, and high hydrostatic pressure, which are reviewed in this section. Table 2 gathers the results achieved during the last decade in terms of enzymatic inactivation in fruit juices.

3.1. Radiation treatment

In this section, UV light and γ-irradiation are reviewed. It is generally accepted that the enzymes in raw food materials and non-thermally treated foods are stable against low-dose radiation. Nevertheless, there few works examine UV influence on some enzymatic activities. Falguera et al. (2011) reported that pectin methyl esterase was inactivated after 40 min of irradiation at an incident energy of $3.88 \cdot 10^{-7}$ E/min, while polyphenoloxidase was completely inactivated after 100 min of treatment, and peroxidase after only 15 min, regardless its initial activities. High-intensity treatments, being either in the visible or UV-C spectral ranges, have been associated with non-reversible structural changes which result in enzyme inactivation (Manzocco et al. 2009).

Regarding γ-irradiation, it has been reported that in kale juice a dose of 5 kGy gamma irradiation did not affect polyphenoloxidase (Kim et al. 2007). By contrast, other authors achieved 53.34%,

34.31%, and 45.54% decreases in peroxidase, polyphenoloxidase, and lipooxygenase activities, respectively, at 1 kGy, and 56, 32, and 85%, respectively, at 5 kGy in cantaloupe juice. Enzyme-activity determination indicated that lipooxygenase was the easiest one to be inactivated, followed by polyphenoloxidase and peroxidase, but the 3 enzymes still remained active even at 5 kGy. These authors observed that at doses higher than 1 kGy a slight irradiation off-odor was produced, and a strong off-odor at 2 kGy and higher. Therefore, they indicate that γ-irradiation may not completely inactivate the enzymes on the premise of acceptable off-odor (Wang et al. 2006).

3.2. Electric treatment

3.2.1. Pulsed electric fields (PEF)

PEF has been also used to inactivate enzymes. Some authors have achieved residual activity of pectolitic enzymes involved in viscosity changes of watermelon juices processed at pulse frequencies from 50 to 250 Hz and pulse widths ranging from 1.0 to 7.0 μs in monopolar or bipolar mode (Aguiló-Aguayo et al. 2010). When PEF treatment was compared to conventional heat treatments, a decrease in pectin methyl esterase activity of 88% was achieved in orange juice with a PEF treatment at 35 kV/cm for 59 μs while heat pasteurization at 94.6 °C for 30 s inactivated 98% of pectin methyl esterase activity, which was not restored during storage at 4 and 22°C for 112 days, in both cases (Yeom et al. 2000). According to Elez-Martínez et al. (2006), 100% inactivation of the pectin methyl esterase when processing orange juice at 90°C for 1 min was reached while PEF treatment inactivated 81.6% activity in the juice without exceeding 40°C. These researchers suggest that enzyme inactivation was probably due entirely to the PEF treatment itself, as temperature was not high enough to inactivate pectin methyl esterase.

Similarly, PEF treatment at 80 kV/cm 2 μs reduced pectin methyl esterase activity up to 55% in tomato juice (Nguyen and Mittal. 2007). Given that a 90-100% reduction of the pectin methyl esterase activity is normal in commercial heat-pasteurized orange juice (Yeom et al. 2000, Irwe and Olsson. 1994), a better optimization of the variables or a complementary heat treatment would be necessary in cases that did not achieve this reduction. However, PEF treatment at 80 kV/cm 2 μs did not affect polygalacturonase activity, while conventional heating to 50°C its activity was reduced significantly (Nguyen and Mittal. 2007).

Bi et al. (2013) analyzed the effectiveness of PEF as a method of inactivating enzymes to prevent enzymatic discoloration. It was concluded that with increasing the electric-field strength and pulse rise time, the residual activity of polyphenoloxidase and peroxidase decreased, almost completely inactivating both enzymes at 35 kV/cm-electric-field strength and 2 μ-pulse rise time. Other authors found the greatest decrease in enzymatic activity by using a combination of preheating to 50°C, and a PEF treatment time of 100 μs at 40 kV/cm, this being significantly higher than that recorded in juice processed by conventional mild pasteurization (Riener et al. 2008).

3.2.2. Ohmic heating (OH)

OH proved to be promising in enzyme-inactivation experiments. Some experiments have achieved additional inactivation of food enzymes with OH when compared to conventional heating in juices and other foods (Icier et al. 2006, Demirdöven and Baysal. 2009, Jakób et al. 2010). Leizerson and Shimoni (2005a, 2005b) found no significant effect for the type of thermal treatment on pectin methyl esterase inactivation in orange juice. According to other authors, pectinase in lemon juice was inactivated with a very short time length, 0.63 sec at 75°C (Inoue et

al. 2007). The effects of voltage gradient, temperature, and holding time on the polyphenoloxidase activity were investigated for grape juice, and it was found that the critical deactivation temperatures were 60°C or lower for 40 V/cm, and 70°C for 20 and 30 V/cm (Içier et al. 2008). In other experiments, OH heating of polyphenoloxidase caused their inactivation to be faster than during conventional heating (Castro et al. 2004). Moreover, OH has been attributed to cause less browning than conventional heating (Leizerson and Shimoni. 2005a, Leizerson and Shimoni. 2005b, Jakób et al. 2010, Yildiz et al. 2009).

3.3. Ultrasound (US) treatment

Studies on US treatment for enzyme inactivation in fruit juices include Tiwari et al. (2009a, 2009b), who assayed different ultrasonic acoustic energy-density levels of 0.42, 0.47, 0.61, 0.79, and 1.05 W/mL with treatment times of 0, 2, 4, 6, 8, and 10 min, concluding that ultrasonic acoustic energy density decreased pectin methyl esterase activity in orange juice by 62% at the highest level. Results indicate that sonication alone cannot completely inactivate pectin methyl esterase under the experimental conditions employed. However, it has been reported that the particle suspension stability of juice depends not only on pectin methyl esterase activity but also on alterations of the pectin. Changes in chord-length distributions as a function of US intensity have been reported, this size reduction contributing to improved cloudiness stability (Tiwari et al. 2009a). The sonication in combination with heat (thermosonication) has been reported to inactivate pectin methyl esterase following first-order kinetics (Terefe et al. 2009). The results of these authors showed that thermosonication (20 kHz, 75 μm) processing of tomato juice at 75°C caused almost complete inactivation of pectin methyl esterase and about 72% inactivation of polygalaturonase after 4 min. These researchers asserted that under these conditions, the residual

polygalacturonase activity is unlikely to cause substantial pectin degradation as the preferred substrate for polygalacturonase is demethylated pectin, which would be less available because pectin methyl esterase is almost completely inactivated.

The minimal residual activity (23.29%) resulted from the most drastic treatment (376 W/cm^2 for 10 min), achieving 23% residual activity for peroxidase, 70% for polyphenoloxidase and 2% for ascorbate peroxidase, being a function of US power intensity and processing time (Fonteles et al. 2012), which is consistent with the results of Costa et al. (2013).

3.4. High-hydrostatic-pressure treatment

HHP has also been used for enzymatic inactivation; however, less information is available than for microbial inactivation. Concerning pectin methyl esterase inactivation, the literature reports that it is rather heat-labile but pressure-stable (Jolie et al. 2009). More concretely, Stoforos et al. explored the inactivation of endogenous pectin methyl esterase in tomato juice during combined HHP (ambient to 800 MPa) and moderate-temperature (60 to 75°C) treatments under isobaric and isothermal processing conditions. Their findings suggest that pressure and temperature did not act synergistically with respect to tomato pectin methyl esterase inactivation, in the whole range of pressure and temperature investigated, but they had mainly counteracting effects. Consequently, in the cases where a certain level of residual pectin methyl esterase inactivation activity is desired, high pressure could be used to inactivate other, undesired, enzymes while reserving this one, leading to a desired viscosity/texture, in some products (Stoforos et al. 2002). This agrees with Hsu et al., who reported pectin methyl esterase inactivation reduction of 27.8% using the treatment of 200 MPa at 25°C, 10 min, this being the

most efficient when compared to the cases of higher-pressure treatments (beyond 300 MPa) at all temperatures in this study (4, 25 and 50°C) (Hsu et al. 2008, Hsu. 2008). Therefore the highest efficiency was found with low-pressure/mild-temperature treatments. This fact has been attributed to reversible configuration and/or conformation changes of the enzyme and/or substrate molecules (Hsu et al. 2008, Ogawa et al. 1999).

Concerning polygalacturonase activity, pressures beyond 400 MPa at ambient and low temperatures (25 and 4°C) strongly reduce its activity up to 90% while pressure from 100 to 300 MPa has slight or insignificant effects on the inactivation of polygalacturonase (up to 14%), demonstrating a certain pressure resistance of polygalacturonase (Hsu et al. 2008, Hsu. 2008).

Regarding polyphenoloxidase, studies in cloudy apple juice performed by Buckow et al. (2009) showed synergistic effects of pressure and temperature on the inactivation of apple polyphenoloxidase at pressures above 300 MPa and antagonistic effects at lower pressures. Compared to ambient pressure conditions, temperatures required to inactivate polyphenoloxidase in apple juice were increased 10-15°C at 100-300 MPa. Other authors did not inactivate polyphenoloxidase with treatment for 1 min at 500 and 600 MPa at 20°C in apple juices, which turned brown during storage (McKay et al. 2011).

4. Yield improvement

Since juice extraction is a slow, laborious and highly energy-consuming step in fruit-juice production, various methods have been tested to improve efficiency and augment yield (Wang and Sastry. 2002). Although enzymatic inactivation would also boost juice yield, there are some other methods that directly offer improvements such as pulsed electric fields, ohmic heating,

microwave heating, ultrasound, and flash-vacuum expansion, which are reviewed in this section.

4.1. Electric treatment

4.1.1. Pulsed electric fields

Efficiency of hydraulic pressing can be increased by raw material plasmolysis, cellular damage or permeabilization prior to expression. This can be achieved by combined pulsed-electric-field application and pressure, found to be useful as a pretreatment to enhance juice expression and solute extraction (Flaumenbaum. 1968, Jemai and Vorobiev. 2002). Pressure damages cells exposed to PEF, which have developed pores in the cell membrane, allowing diffusion migration of moisture and depressing the cell-resealing processes, thereby increasing juice yield (Grimi et al. 2009, Schilling et al. 2007). The effectiveness of the PEF treatment depends on the uniform and tight packing of raw material between electrodes. The excessive quantity of extraparticle high-conductive liquid (from cells destroyed by cutting) increases electrical energy losses because of great current flow through the system. Moreover, the low values of external moisture content also may restrict the effect of the PEF because of the absence of contact between solid particles. Thus, we would expect greater PEF treatment efficiency after pre-compression of the raw material and removal of excess liquid from the extracellular volume at the initial steps of compression. PEF application at the moment when the press-cake's specific electrical conductivity reaches a minimum and the pressure achieves its constant value seems to be the most optimal (Bazhal et al. 2001).

This technology has been previously used on a laboratory scale, with improvements in juice yields in apple juice production after adding ascorbic acid to prevent oxidation (Schilling et al.

2007). Similarly, other authors augmented juice yield from 49-54% to 76-78% at 45 min of pressing in grapes, the energy input on the order of 20 kJ/kg at electric-field strength 750 V/cm being sufficient for optimum PEF-assisted expression. These authors also found that PEF pre-treatment application before pressurization exerted the most pronounced effects on the expression kinetics and on the juice yield and quality, though it was accompanied by the highest electric-energy consumption (Praporscic et al. 2007).

When PEF are combined with heating at moderate temperatures (~40° C), juice extraction from apples is notably enhanced (Schilling et al. 2008, Lebovka et al. 2004), resulting in noticeable softening of the tissue, resulting in more damage of apple tissue than with the PEF treatment alone.

In further studies, same authors (Praporscic et al. 2007) tested the effect of the size of slices of apples and carrots on juice yield. They reached the conclusion that PEF treatment at moderate electric field strength (250–400 V/cm) resulted in more pronounced additional expression of juice for the larger slices. However, at the end of the treatment at 1000s, little difference was detected between small and large slices. Similarly, (Turk et al. 2010) found scant difference between small and large mash using 10 pulses delivered continuously at the electric field strengths of 450 V/cm. Other authors (Grimi et al. 2011) compared the effect of PEF treatment on whole and sliced apples, sliced apples resulting in higher juice yield and faster extraction kinetics compared to whole ones.

PEF experiments have also been extrapolated to a continuous pilot-plant scale using a single-belt press, increasing cider and apple-juice yield by 4.1% (Turk et al. 2012a) and by 5.2% on an industrial scale (Turk et al. 2012b). In further studies, Jaeger et al. made a systematic study on an

industrial scale with various de-juicing systems, achieving substantial improvements in juice yield for apple, 77.5% (belt press) and 86.1% (pack press), and for carrots, up to 76% (belt press and filter press) (Jaeger et al. 2012).

4.1.2. Ohmic heating (OH)

OH is believed to have an additional electopermeabilization effect leading to extraction improvement even at moderate temperatures that do not exceed 50°C (Wang and Sastry. 2002, Praporscic et al. 2005). A 13.76% increase in juice recovery over control from carrot was demonstrated using 2-stage pressing with OH heating, achieving maximum juice recovery of 98.9% with a first pressing of 2.72 min., OH up to a final temperature of 65.6°C under a voltage gradient of 15 V/cm followed by a second pressing of 10 min (Ranmode and Kulshreshtha. 2011). Similarly, other authors achieved greater juice yield in ohmically treated samples than in non-treated, the former showing less mechanical resistance than the raw material. These researchers also studied the effect of frequency and concluded that lower frequency caused a greater effect on increasing moisture diffusion, which resulted in high juice yield Wang and Sastry. 2002). This agrees with other authors (Lima and Sastry. 1999, Lima et al. 1999), who also had greater yields at lower frequencies.

Moreover, satisfactory results were achieved when OH and PEF at electric field strength less than 100 V/cm were combined, promoting a high level of membrane destruction and mechanical softening of tissues even at a moderate temperature under 50°C of apples, potatoes, and sugar beets. Both treatments might have led to some synergetic effect, related to electroporation of cell membranes and thermal softening of tissue (Praporscic et al. 2005, Praporscic et al. 2006).

4.2. Microwave heating treatment

Microwave heating (MH) is caused by the ability of the materials to absorb microwave energy and convert it into heat. The presence of moisture or water causes dielectric heating due to the dipolar nature of water (Datta and Davidson. 2000). There are many factors which affect MH and its heat distribution and the most important of them are the dielectric properties and penetration depth (Chandrasekaran et al. 2013).

Cendres et al. (2011) implemented a system for extraction of fruit juice using a microwave heater with a multimode 2450 MHz microwave oven with a maximum delivered power of 1000 W at 10 W increments. These authors concluded that the process of microwave extraction of fruit juice could thus be divided in four stages. Stage 1 corresponds to the time necessary for heating the matrix from room temperature to the boiling point of water; it is also the time needed for the first fruit juice droplets to get outside the microwave cavity. Stage 2 corresponds to an intensive extraction of fruit juice at an increasing flow rate; it is also the phase of extraction of easily exchangeable water in the fruits. Stage 3 corresponds to a decrease in the flow rate of extraction, when the remaining water is more linked to plant structure and becomes increasingly difficult to extract. Finally, stage 4 is the end of extraction process, when the fruit is almost dry and the temperature increases rapidly due to the absence of water which regulated the temperature at its boiling point (100°C) in the preceding stages. They also observed different diffusion behavior during extraction according to the type of fruit used. The best yields were consistently obtained for grapes, followed by plums and apricots. These authors attribute this fact to the different availability of water in fruit as well as the

thickness and texture of the layer of pulp, the viscosity of the juice, the porosity of the wall, etc.. For all three fruits, increasing power densities from 0.5 W/g to 1.5 W/g markedly shortened the duration of extraction but also of the yields. Higher yields were obtained with initially frozen fruits, in which ice might interfere in the process by acting as a buffer, a power sink, as some heat is consumed for melting.

Other authors reported that the thermal effect of MH heating on increasing juice yield decreased with the pretreatment temperature from 40 to 50°C, which might be due to moisture loss at a higher temperature. Pretreated samples also showed less mechanical resistance than did the raw material, suggesting that less input work was required for expression, possibly due to a breakdown of cell membrane and wall constituents, not only increasing juice yield but also saving energy. However, when it was compared to ohmic heating, less effect in increasing juice yield and increasing input work was found (Wang and Sastry. 2002).

Other researches developed a tunable microwave applicator for extracting date juice and similar products consisting of a microwave unit and a press/filter unit, showing good performance characteristics with power conversion efficiency of more than 70% (Ali. 2000).

4.3. Ultrasound treatment (US)

US treatment may also been used to enhance juice yield. However, there is little evidence to support this statement. Lieu and Le. (2010) have assayed the effect of ultrasound together with temperature at 74 °C. They reached the conclusion that US treatment increased extraction yield 3.4% and shortened treatment time by more than three-fold; meanwhile, a combined US and enzyme treatment increased extraction yield only 2%, but shortened treatment time by more than four-fold. After US treatment, enzymatic treatment (0.05% pectinase) boosted extraction yield

7.3% and the total treatment time of this method was still shorter than that of the traditional enzymatic treatment method.

4.4. Flash-vacuum expansion treatment (FVE)

FVE is another technology developed to improve yield and also the nutritional quality of juice. In this technique, plant material at 60-90°C is rapidly placed in a vacuum chamber at 1-10 kPa absolute pressure. Figure 5 shows a FVE squeme. At this reduced pressure (high vacuum), the boiling point of water in the tissues is much lower than the temperature of the plant material (60-90°C). The plant material expands or disintegrates due to instantaneous evaporation of constituent water and forms micro-channels inside the tissues (Paranjpe et al. 2012). Steam heating induces a thermal denaturation of endogenous oxidases, while the whole process, performed with the absence of oxygen, prevents oxidation and subsequent browning of the products (Brat et al. 2001b).

Pressure and temperature are significant factors in this procedure. Some researchers have assayed temperatures of 60, 75, and 90°C and pressure of the vacuum chamber of 1, 5 and 10 kPa absolute in extracting grapes. It is concluded that higher temperatures and lower pressures of flash-vacuum expansion improve juice yield with pressure being a more significant factor (Paranjpe et al. 2012, Anonymous 2007).

Brat et al. compared passion fruit puree obtained by flash-vacuum expansion with steam-heated puree and cold-pressed juice, all passed through the same screen. It is stated that FVE gave about two-fold higher yield compared to the steam treatment and produced a significantly thicker puree, which is related to its content in de-starched alcohol-insoluble residue (Brat et al.

2001b). Other studies have improved grape-juice yield by 5-20% as compared to heat treatment (Anonymous 2007).

After vacuum-expansion, the puree is impoverished in volatile components because of instant evaporation of some water when steam-heated fruits are placed in the vacuum vessel. However, most of these volatiles can be recovered in aromatic liquors generated by the vacuum-expansion step, and they could be added back to the vacuum-expanded puree (Brat et al. 2001a, 2001b).

FVE has also been used to recover essential oils from citrus peels, allowing yields comparable to the Food Machinery Corporation Process (Brat et al. 2001a).

5. Conclusion

In this paper, non-thermal alternatives to conventional heating in fruit-juice processing are reviewed. The literature reveals that research is still underway, and many aspects remain to be fully studied and understood.

Ensuring food safety and at the same time meeting the demand for nutritious foods, has intensified interest in non-thermal preservation techniques. The non-thermal technologies discussed in this review have the potential to meet industry and consumer expectations. However, the lack of standardization in operating conditions makes comparisons between different studies difficult. Consequently ambiguity arises within the literature, as these control conditions may not be reported in detail or are reported differently. More complex considerations arise for combinations of technologies, particularly with respect to optimization of practical applications. In many cases, combination with mild heat has been used, which does not reach

usual heating temperatures, and achieve a synergistic effect. A fundamental understanding of these phenomena is essential for optimum process design.

Thus, in order to advance in the juice industry, more studies need to be carried out in detail on the scaling-up, process design, and optimization, as well as the effect of such technologies in the overall quality of fruit juices in order to maximize their potential as alternative non-thermal technologies in fruit juice processing.

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Table 1. Alternative technologies to conventional thermal treatments in fruit-juice processing for microbial inactivation: summary.

Technique	Scale	Microorganisms inactivated	Parameters assayed	Combinations with enhanced efficiency	References
UV light	Laboratory	Aerobic plate count Total yeasts Total moulds *Saccharomyces cerevisiae* *Escherichia coli*	UV dose: 0.087-6 J/cm^2	Ultrasound: 20 kHz, 95 µm	Oteiza et al. 2005, Tran and Farid. 2004, Koutchma et al. 2004, Koutchma et al. 2006, Char et al. 2010, Oteiza et al. 2010
HILP	Laboratory	*Escherichia coli* *Listeria innocua*	Light dose: 1.15-1.2 J/cm^2/pulse Pulse width: 360 µs Treatment length: 1-8 s	Thermosonication: 24 kHz, 100 µm, 2.8-5 min, 40-53°C PEF 24-34kV/cm, 93µs, 92 s	Palgan et al. 2011b, Pataro et al. 2011, Muñoz et al. 2011, Caminiti et al. 2011, Caminiti et al. 2009b
γ-irradiation	Laboratory	Total aerobic counts Coliforms Total moulds *Escherichia coli*	Irradiation dose: 1-5 kGy	Preservative addition: Citric acid (0.3%), sodium benzoate (0.015%), potassium sorbate (0.025%), and sucrose (10%)	Jo and Lee. 2012, Jo et al. 2012, Mishra et al. 2011, Alighourchi et al. 2008, Kim et al 2007, Wang.et al. 2006, Song et al. 2006
PEF	Laborato	Total aerobic	Electric field: 15-35	Heating: 60-72°C, 15-30 s	Mosqueda-Melgar et

	ry, pilot plant and industrial	mesophilic bacteria Total moulds Total yeasts Total enterobacteriaceae *Escherichia coli* *Salmonella enteritidis* *Listeria monocytogenes* *Staphylococcus aureus*	kV/cm Pulse rise time: 200 ns-4 µs Treatment length: 2 ms-250 s Bipolar mode	Preservative addition: citric acid (1.5-2%); bark oil (0.2 %)	al.2008, Morales-de la Peña et al. 2010, Chen et al. 2010, Evrendilek et al. 2000, Akin et al. 2009, Elez-Martínez et al. 2006, Yeom et al. 2000, Zhang et al. 2010, Walkling-Ribeiro et al. 2008, Nguyen 2007
RFEF	Laboratory	*Escherichia coli*	Electric field: 15-18 kV/cm Frequency: 20-40 Hz Treatment length: 190-270 µs	-	Geveke et al. 2004, Geveke et al. 2007, Ukuku et al. 2008, Ukuku and Geveke 2010
OH	Laboratory	*Escherichia coli* *Salmonella typhimuriums*	Electric field: 10-40 kV/cm Treatment length. 300 s-30 min	-	Leizerson et al. 2005b, Baysal et al. 2010, Onwnka et al. 2008, Sagong et al. 2011, Lee et al. 2012

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		Listeria monocytogenes *Clostridium perfringens* *Alicyclobacillus acidoterrestris*	Temperature. 70-150°C		
Ultrasound	Laboratory	Total lactic acid bacterium Total yeasts *Escherichia coli* *Fusarium oxysporum* *Saccharomyces cerevisiae* *Salmonella spp.* *Shigella sp.*	Wave amplitude: 23-120 µm Frequency: 20-24 kHz Treatment length: 26 s-20.4 min	Heating: 40-72°C Pressure: 400 kPa Osmotic pressure: 12.6 MPa Additive addition: benzoate (100 ppm) and citrus extract (1800 ppm)	Adekunte et al 2010a, Adekunte et al. 2010, Bermúdez-Aguirre and Barbosa-Cánovas. 2012, Palgan et al. 2011, Bevilacqua et al. 2012, Wong et al. 2010
HHP	Laboratory	Total aerobic count Total mesophilic bacteria count Total psicotrophs	Pressure: 207-700 MPa Treatment length: 2-60 min	Heating: 45-71°C High pressure CO_2: 4.9 MPa Additive addition: sucrose laurate (1%), chitosan (0.01-0.1%),	Alpas et al. 2000, Slifko et al. 2000, Shearer et al. 2000, Park et al. 2002, Lee et al. 2002, Alpas et al. 2003, Doğan and Erkmen. 2003, Briñez et al. 2006, Bayındırlı et al. 2006, Lee et

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Total lactic acid bacteria	nisin (100IU/mL)	al. 2006, Bari et al. 2007, Dede et al. 2007, Hsu et al. 2008, Buzrul et al. 2008, Lavinas et al. 2008, Schlesser et al. 2009, Kumar,S. et al. 2009, Xu et al. 2009, Suárez-Jacobo et al. 2010, Ferrari et al. 2010, Carreño et al. 2011, Varela-Santos et al. 2012, Patterson et al. 2012, Zhao et al. 2013, Mert et al. 2013, Sokolowska et al. 2013a, Sokolowska et al. 2013b
Total enterobacteriaceae		
Total moulds and yeasts		
Total coliforms		
Staphylococcus aureus		
Cryptosporidium parvum oocysts		
Bacillus coagulans		
Lactobacillus plantarum		
Alicyclobacillus sp.		
Escherichia coli		
Salmonella enteritidis		
Listeria innocua		
Listeria monocytogenes		
Yersinia pseudotuberculosis		

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		Fracisella tubularensis *Saccharomyces cerevisiae*			
SC-CO_2	Laboratory	Total yeasts *Escherichia coli*	Pressure: 6.9-48.3 MPa CO_2 concentration: 70-170 g/kg Temperature: 25-45°C	-	Gunes et al. 2005, Gunes et al. 2006
Ozonation	Laboratory	Total aerobic count *Listeria innocua* *Listeria monocytogenes* *Escherichia coli* *Salmonella spp.*	Flow rate: 0.12-2.4 L/min Ozone concentration:0.048-0.098 mg/mL/min Treatment length: 5-240 min	Additive addition: dimethyl dicarbonate (250-500 ppm), hydrogen peroxide (300-600 ppm)	Patil,S. et al. 2010b, 534 Patil,S. 2009b Williams et al. 2005, Williams et al. 2004

Abbrevations

UV Ultraviolet

HILP High Intensity Light Pulses

PEF .. Pulsed Electric Fields

RFEF Radiofrequency Electric Fields

OH … .Ohmic Heating

HHP High Hydrostatic Pressure

SC-CO_2 Supercritical CO_2

Table 2. Alternative technologies to conventional thermal treatments in fruit-juice processing for enzymatic inactivation: summary.

Technique	Scale	Enzymes inactivated	Parameters assayed	Combinations with enhanced efficiency	References
UV light	Laboratory	Pectin methyl esterase Polyphenoloxidase Peroxidase	UV dose: $3.88 \cdot 10^{-7}$ E/min Treatment length: 15-100 min	-	(Manzocco et al. 2009)(Oteiza et al. 2005,Tran and Farid. 2004,Koutchma et al. 2004,Koutchma et al. 2006,Char et al. 2010,Oteiza et al. 2010) Oteiza et al. 2005, Tran and Farid. 2004, Koutchma et al. 2004, Koutchma et al. 2006, Char et al. 2010, Oteiza et al. 2010
γ-irradiation	Laboratory	Polyphenoloxidase Peroxidase Lipooxigenase	Irradiation dose: 1-5 kGy Irradiation rate. 0.5 kGy/h	-	Kim et al. 2007, Wang et al. 2006
PEF	Laboratory	Pectin methyl esterase Polygalacturonase Polyphenoloxidase	Electric field: 35-80 kV/cm Pulse rise time: 1-7 µs Treatment length: 59-	-	Aguiló-Aguayo et al. 2010, Elez-Martínez et al. 2006, Nguyen and Mittal. 2007, Irwe and Olsson. 1994, Yeom et al. 2000

			100 µs Bipolar mode		
OH	Laboratory	Pectin methyl esterase Polyphenoloxidase Peroxidase	Electric field: 20-40 kV/cm Treatment length. 0.63 s-120 min Temperature: 54-150°C	-	Jakób et al. 2010, Demirdöven and Baysal. 2012, Icier et al. 2006, Içier et al. 2008, Leizerson and Shimoni. 2005a, Leizerson and Shimoni. 2005b, Castro et al. 2004, Yildiz et al. 2009
US	Laboratory	Pectin methyl esterase Polygalacturonase Polyphenoloxidase Peroxidase Ascorbate peroxidase	Wave amplitude: 65-75 µm Frequency: 19-20 kHz Acoustic energy density: 0.42-376 W/cm^2 Treatment length: 2-10 min	-	Tiwari et al. 2009g, Terefe et al. 2009, Fonteles et al. 2012, Costa et al. 2013
HHP	Laboratory	Pectin methyl esterase Polygalacturonase	Pressure: 300-800 MPa Treatment length: 5-10 min	High pressure carbon dioxide	Jolie et al. 2009, Stoforos et al. 2002, Hsu. 2008, McKay et al.

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Abbrevations

UV Ultraviolet

PEF .. Pulsed Electric Fields

OH Ohmic Heating

US Ultrasound

HHP High Hydrostatic Pressure

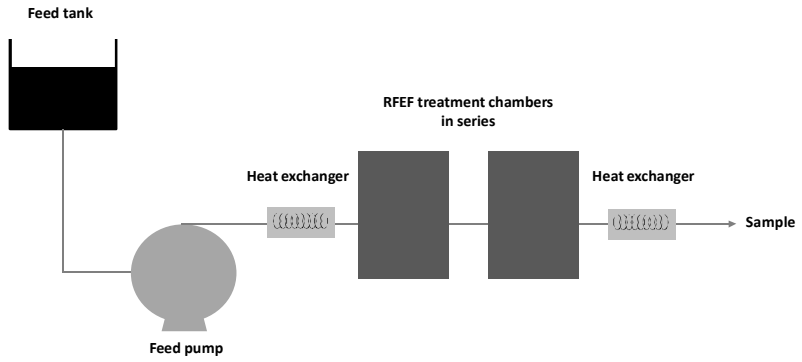

Figure 1. Schematic diagram of continuous radio frequency electric fields process including two treatment chambers in series (Geveke et al. 2007).

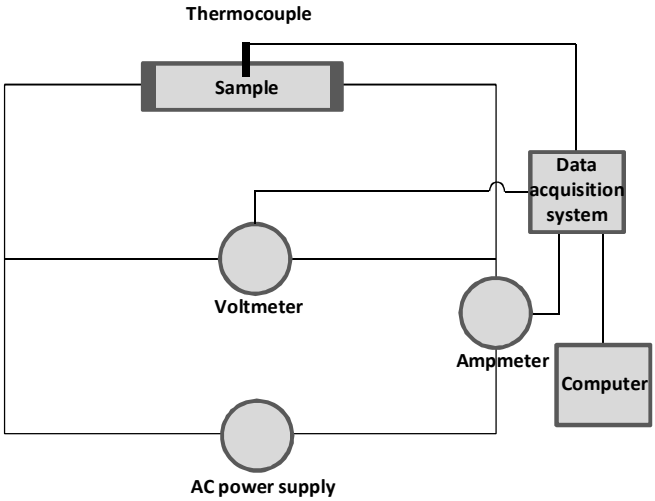

Figure 2. Schematic diagram of an ohmic heating circuit (Tumpanuvatr and Jittanit. 2012).

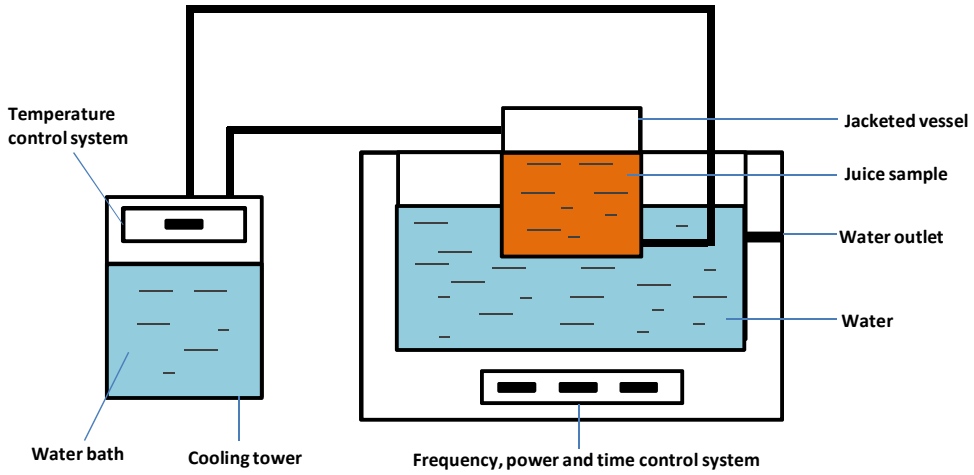

Figure 3. Schematic diagram of an ultrasound exposure system (Abid et al. 2013).

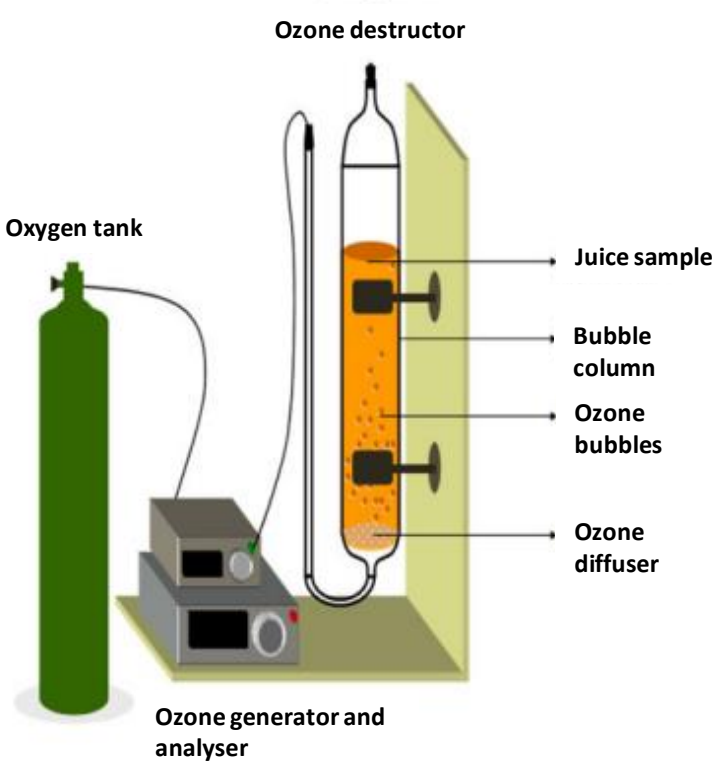

Figure 4. Schematics of ozone processing equipment (Patil et al. 2011).

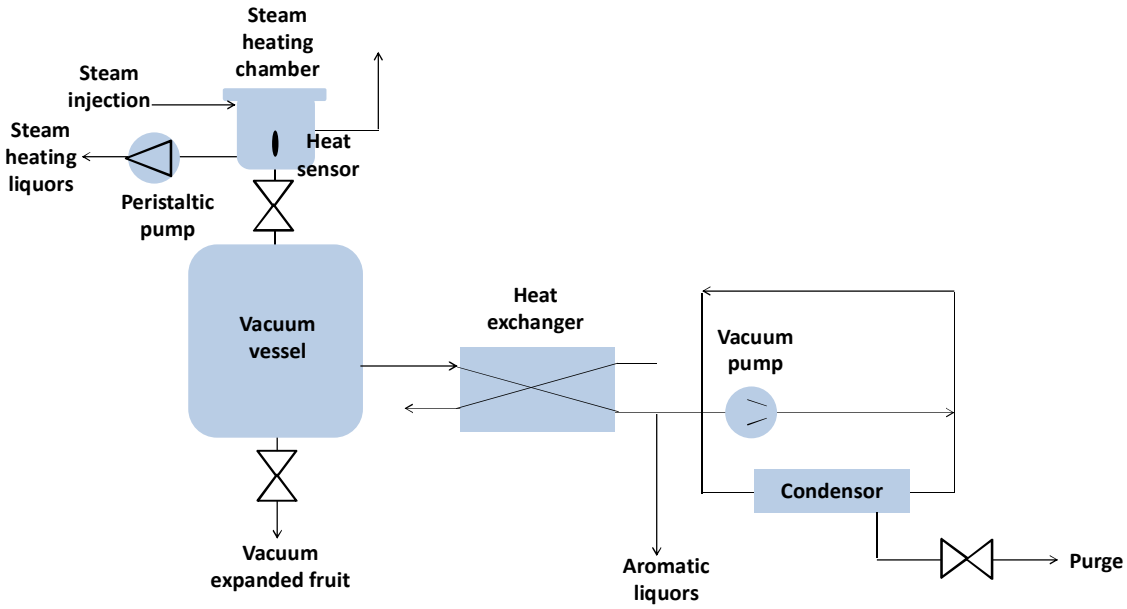

Figure 5. Flash-vacuum expansion scheme. Adapted from Brat et al. 2002.