Chapter 5
The Hurdle Concept in Fruit Processing

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5.1 Introduction

Fruits and vegetables (F&V) are important elements of nutrition in most world cultures (Schwentesius and Gómez 2000). Today, and increasingly more often, humans tend to eat healthy diets that include an increase in the intake of fruits and vegetables as their beneficial effects on health have been evidenced. Comparisons made between populations who consume small amounts of F&V and those that eat larger amounts as part of a balanced diet, show that the latter have a reduced risk of chronic diseases, including some types of cardiovascular disorders (Dauchet et al. 2004), type II diabetes (Montonen et al. 2005; Venn and Mann 2004) and certain types of cancer (Chang et al. 2005; Nkondjock et al. 2005; Rashidkhani et al. 2005; Ray 2005; Zhang et al. 2005; De Stefani et al. 2005). Based on these facts it is not surprising that authorities of most countries in the world advice to increase the intake of F&V as part of a healthy diet. The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) recommend consumption of at least 400 g of fruit and vegetable (five servings per day) and this has been included into dietary guidelines such as is the case of the United States (WHO/FAO 2003; USDA 2005).

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Another aspect that affects consumption positively is the variety and availability of products in the current scenario of globalized trade that allows a significant flow of fruits considered exotic. This has resulted in major advances in technologies that have improved postharvest management and control of the cold chain, a condition for long-distance transportation of highly perishable commodities. One of the main problems of developing countries is the absence of an efficient integration between production and consumption, which is reflected in the lack of modern distribution channels. Moreover, the seasonality of production imposes an accelerated time for processing the fruit, and an accumulation of high-price stocks during the harvest months. Because of this, it is necessary to develop expedite, simple, low-cost, preservation techniques that may act as a regulatory factor of the offer. These techniques in their majority have to be used in combination so as to be alternatives to concentration, freezing, and other techniques intensive in energy expenditure, even refrigeration, with the enormous challenge of not compromising safety and quality during prolonged storage, and ideally be simple enough to be applied in the production sites in small agro-industries, which may in turn generate an important social and economic impact. The incorporation of a hurdle approach that utilizes individual sublethal physical, chemical, and biological stressors in a sequential application with a cumulative inhibitory effect on the microbiological load present and quality loss reactions in a food matrix is very adequate for the fruit industry, especially for the minimal processing sector with all its variants. This approach allows tailoring practices to individual operations considering process and final products (Tapia and Welti-Chanes 2012).

The produce industry is faced with an ever-increasing demand for fresh-likeness, convenience, and "health" in foods with the minimally processed fruit sector becoming one of its fastest growing segments. To harmonize these trends without compromising safety, a number of milder fruit preservation factors (some already used commercially) had been explored (i.e., "nonthermal" physical agents such as high hydrostatic pressure, pulsed electric fields, ultrasound, pulsed light, ultraviolet light; chemical agents, such as natural antimicrobials, ozone, hydrogen peroxide, among others, and minimal heating) (Gómez et al. 2011c). The alternative "nonthermal" physical agents, intensely investigated in the last two decades, can cause inactivation of microorganisms at ambient or sublethal temperatures avoiding the deleterious effects that severe heating has on quality, but most preservation systems would still require refrigeration to assure safety and keeping properties. The trend for mildly preserved fruits comes with the combined preservation/hurdle technology as the principle in designing the overall treatment. In general, the processes are less robust and need to be well controlled through adequate product and process design and proper implementation and monitoring through HACCP (Havelaar et al. 2010).

Assuring safety is essential to accessing the market, and global recognition of standardized protocols to eliminate risk at every step from "farm to fork" has translated into food safety policy for many countries. Although the Hazard Analysis and Critical Control Points (HACCP) system is not yet mandatory and not currently required by law for an important sector of the minimal processed industry like fresh-cut produce, the whole and fresh and industry itself has been encouraging their

fresh-cut processors to voluntarily implement HACCP programs in their facilities, as well as to demand safe practices by partners throughout the supply chain. Many segments of the fresh-cut produce industry have adopted HACCP principles. In produce operations, however, HACCP systems have limited application since specific critical limits cannot be established and monitored to ensure that the hazard is reduced to acceptable levels. Instead, Good Agricultural Practices (GAPs)—voluntary—, Good Manufacturing Practices (GMPs)—mandatory—, and Sanitation Standard Operating Procedures (SSOPs)—voluntary—, provide the primary levels of risk management for operations in the field that will directly impact the fresh-cut processing plant (Tapia et al. 2009).

Most fruits contain between 10 % and 25 % carbohydrate, less than 1 % protein and less than 0.5 % lipid (USDA 2004). There are fruits that are exception in terms of lipid content, such as avocados and olives which may contain up to 20 % or 30 % of total fat. Carbohydrates include cellulose, hemicelluloses, lignin, and pectic substances that contribute as a source of dietary fiber, and sugars that give them a sweet taste. The relative acidity of the fruit is due to the presence of acids; mainly malic and citric. The acidity of the fruits is also influenced by the crop, the soil conditions, and the degree of fruit maturity, but in general is always within a certain range. Fruits may differ in their composition and structure, which determines the kind of deterioration and how easily it can be attacked by microorganisms. The more acidic pH of fruits and the presence of carbohydrates promote the deterioration due to the growth of molds, yeasts, and some acid-tolerant bacteria in a greater extent. Water is the major component of fruits and fruit–water activity (a_w) is determined by the nature and concentration of dissolved naturally occurring chemicals, such as sugars, organic acids, inorganic salts, and other soluble substances. As the concentration of solutes (nonionic or ionizable) naturally present in the aqueous phase of fresh fruits is relatively small, a_w is close to unity (Chirife and Ferro Fontán 1982). This high value facilitates the growth of microbial populations that have access to these foods, as is evident by observing the natural occurrence of numerous deteriorative genera of bacteria, molds, and yeasts as well as occasional pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7, *Clostridium botulinum,* and others. Fruits have also become increasingly important identified vehicles for microorganisms capable of causing disease, which is found in the many documented outbreaks associated with fresh fruits and fresh juices in recent years (Lee et al. 2001; Sewell and Farber 2001; Sivapalasingam et al. 2004).

In the case of fruits, commercial quality and storage life depends on certain conditions that occur before, during, and after harvest. During growth, fruit diseases can be produced by: molds, yeasts, bacteria, mycoplasma, and viruses (Messiaen et al. 1994). Levels of disease vary greatly from season to season. Crop losses can be the result of the attack by more than one pathogen to the plant. The microbial load of fruits grown in the field reflects the land on which they are grown. Therefore, during preharvest, important factors are the land, irrigation water, the presence of human or animal fecal material, the type of fertilizer, air and people who care for the crops (Fernández Escartín 2000; Jay 2002). These elements may constitute a source of various microorganisms that are normally deposited on the surface of

fruits and vegetables, which remain in place while maintaining the structural integrity of the first layers of cells. Fruit spoilage usually occurs during storage and transport and/or during postharvest handling and waiting time for processing. As soon as fruits are collected in boxes, baskets, or trucks during the harvest, are exposed to contamination with organisms from spoiled fruits (collected earlier) and/or containers. Mechanical damage can increase susceptibility to decay and microbial growth. When the epicarp is broken, the microorganisms on the surface may enter and find the right conditions to cause deterioration. The microbial load expected in plants will be greater in those crops that have more contact with the soil and its irregular surface also allows for greater adhesion of contamination such as strawberries. Commodities that are not directly in contact with the soil and have smooth surfaces will have lower microbial loads. In postharvest handling major contamination sources are machinery and equipment, containers, pets and wildlife, workers, vehicles and the atmosphere (Beuchat 2000; Bracket 2001; Jay 2002). Fruit sold in markets are primarily contaminated during handling. Another mechanism of contamination that occurs when losing the integrity of the cuticles, shells, or tests covering fruits, is that insects can be deposited in these wounds with microorganisms attached and can even lay their own eggs (Jones and Widemo 2005).

Internalization is another process of contamination of fruits. Microorganisms can enter into plant tissue through structures such as stomata, lenticels, and stem vascular tissues. Although washing is a primary step to reduce microbial load, it can be a source of contamination and a vehicle for dissemination of microorganisms. The temperature differential between the fruit and the wash water favors the entry of microorganisms. The extent of infiltration of water in fruits generally depends on factors such as time of exposure, magnitude of the temperature differential, depth of immersion, agitation, viscosity of the external environment, and the size and number of points of entry that allow access to the inner space, but also on the adherence of microorganisms to the porous structure of the tissue. This phenomenon reinforces the need for proper management to change the temperature of the fruit after harvest and to use water with good microbiological quality in the washing step (Richards and Beuchat 2004; Bartz 1982; Penteado et al. 2004). The way by which microorganisms penetrate plant tissues have not been clearly established. Products in the field can carry heavy microbial loads on their surfaces, but sometimes the internal tissues contain certain bacteria too, which by unknown mechanisms penetrate the interior. In addition, procedures for decontamination, reducing the initial microbial load, and methods to prevent post-process contamination, change the original microflora ecosystem and therefore affect the microbial associations. The microbial association is specific to each type of food and is affected by intrinsic and extrinsic factors that play an important ecological role in the establishment of the saprophytic microflora and colonization of the food by pathogens (Martínez et al. 2000). All operations undergone by the precut fresh products may damage plant tissues and can increase microbial populations. The use of equipment improperly sanitized may allow microorganisms transferred by contact, as in the case of *Geotrichum candidum*, which can accumulate in the equipment and contaminate fruits (Bracket 2001). In general, the specific effects of pre- and postharvest and

storage on the microflora of fresh produce is difficult to predict, given the complexity of ecosystems and the variety of products.

In an attempt to satisfy consumers and industry, a number of combinations of several antimicrobial factors in a multifactorial fruit preservation approach ("hurdle" technology) have been developed in the past 20 years. Targeted application of the hurdle concept has aimed to improving quality and safety of fruit products at the farm level, and in the whole and fresh-cut minimally processed fruits industry (Alzamora et al. 2000; Leistner and Gould 2002; Raso and Barbosa-Cánovas 2003; Ross et al. 2003; Raso et al. 2005; Allende et al. 2006; Rico et al. 2007; Raybaudi-Massilia et al. 2009; Gómez et al. 2011c; Tapia and Welti-Chanes 2012).

The objective of this chapter was to present a panoramic view of recent promising hurdle combinations explored for the conservation of tropical and subtropical fruits and their subproducts, and point out some areas of study to fully exploit the potential of the hurdle concept in the design and optimization of preservation techniques.

5.2 The Hurdle Concept

5.2.1 Basic Aspects

Microorganisms have evolved different mechanisms to resist the adverse effects of the stresses provided by preservation factors. As internal media stability (composition and volume of fluids) is vital for survival and growth, these mechanisms, called "homeostatic mechanisms", act to ensure that key physiological activities and parameters in the cells remain relatively unchanged, even when the environment around the cell is different and greatly perturbed (Leistner and Gould 2002; Gould 1995). When a stress is sensed by the microorganism, signals that induce mechanisms to cope with the stressor are developed. These mechanisms involve modifications in gene expression and protein activities (Capozzi et al. 2009). Homeostatic mechanisms that vegetative cells have evolved in order to survive extreme environmental stresses are energy dependent and allow microorganisms to keep functioning. In contrast, homeostasis in spores is passive, and adaptive mechanisms are built into the bacterial spore prior to the environmental stress being imposed (Leistner and Gould 2002). They act to keep the central protoplast in a constant low water level environment, this being the prime reason for the extreme metabolic inertness or dormancy and resistance of these cells to high temperature, high hydrostatic pressure, ultrasonication, and other hostile environments. For instance, one of the most mechanisms studied in more depth is osmoregulation. When a microorganism is put into an environment of reduced a_w, water is extracted from the cytoplasm of the cell (in a passive way or possibly mediated by water channels) and membrane turgor is lost. The homeostasis (or internal equilibrium) is disturbed and the organism will not multiply but will remain in the lag-phase until the equilibrium is reestablished. Although the specific details of how each organism responds to an hyperosmotic

shock are different and the organisms differ widely in the range of osmolarity over which they will grow, several common features, both physiologically and genetic, have arisen (Tapia et al. 2007; O'Byrne and Booth 2002). Bacterial response to hyperosmolarity encloses two aspects. The first one has to do with the ability of bacteria to accumulate osmoprotective low molecular weight compounds (by synthesis and/or by active transport) in their cytoplasm at concentrations sufficient to just exceed the osmolality of the external medium for turgor and growth restoration. The second one refers to the osmotic regulation of the expression of a number of genes to optimize growth under the stress condition, allowing cells to modulate the rate of acquisition of compatible solutes. Many of these genes are under the control of alternative stress and stationary-phase sigma factors, σ^S in the Gram-negative and σ^B in the Gram-positive species (Pichereau et al. 2000). For example, the growth of *E. coli* in absence of other compatible solutes from the growth medium occurs by the accumulation via its synthesis of trehalose. Trehalose synthetic enzymes are under the control of the *rpoS* sigma factor, which accumulates when cells are grown at high osmolarity (O'Byrne and Booth 2002). In *E. coli* the σ^S regulon includes over 50 different genes and the products of these genes confer resistance to wide range of stress conditions.

The second one concerns the osmotic induction of general stress systems, with the consequent development of multitolerances towards other environmental stresses when subjected to hypertonic environments (Pichereau et al. 2000; O'Byrne and Booth 2002).

As presently known, there is a general response mechanism (the so-called "global response") underlying many of the apparent distinct responses of microorganisms to different stresses imposed on them in foods (e.g., low a_w, low pH, low or high temperature, oxidative stress, starvation, etc.). This global response is mediated by the stationary-phase regulator RpoS, that regulates the expression of many important stationary-phase stress resistance genes linked to survival under starvation conditions and to survival in the stationary-phase. As Gould (2000) stated, this overlapping stress responses would explain the cross-resistances to different stresses that have usually been found to occur in response to a single stress. Reduced a_w causes an increase in maintenance metabolism and a reduction in yield and growth rate because the solute accumulation process is energy-dependent. If the osmoregulatory capacity of the cell is exceeded (by a severe reduction in a_w), the cell ceases growth.

Other example is acid stress response. The maintenance of intracellular pH within a narrow range is essential for microorganism growth. Lowering the external pH by strong acids causes denaturation of enzymes present on cell surface and lowering of the cytoplasmic pH due to proton permeation through membrane when the pH gradient is very large. When weak acids are used, undissociated acids act as "proton ionophores" and permeate through the membrane, increasing the rate at which protons enter the cytoplasm, but also the acid anion may have specific effects on metabolism amplifying the action of the low pH. Proton influx could lead to a complete dissipation of the proton motive force (Capozzi et al. 2009). Major adaptive mechanism to regulate the cytoplasmic pH is the energy-dependent proton extrusion, which acts to keep the cytoplasmic pH higher than that of the environment, and,

sometimes to extrude the organic acid (Booth and Kroll 1989; Leistner and Gould 2002). When the stress severity increases and the microorganisms' capacity for generating energy is not enough to prevent the net proton influx, the cytoplasmic pH falls, growth ceases, and cells may die.

Oxidative stress by reactive oxygen species (ozone, chlorine dioxide, hydrogen peroxide, electrolyzed water, peroxyacetic acid), and nitrogen species caused an imbalance between intracellular oxidant concentration, cellular antioxidant protection, and oxidative change of lipids of membrane, proteins, and DNA repair enzymes. A number of protection systems (catalases, peroxidases, superoxide dismutases, superoxide reductases, etc.) acts in cellular defense against oxidative stresses (Capozzi et al. 2009).

Temperature downshift decreases membrane fluidity and stabilizes secondary structures of nucleic acids (reducing the efficiency of mRNA translation and transcription). Homeoviscous adaptation involves among others the incorporation of fatty acids with lower melting points into lipids to reestablish the optimal fluidity of the membrane and the production of cold shock inducible proteins to prevent mRNA secondary structures (Capozzi et al. 2009).

Preservation procedures are effective when they overcome, temporally or permanently, the various homeostatic reactions that microorganisms have evolved in order to resist stresses. The degree of change in environmental conditions will determine whether the microorganism lose their viability, become injured, or express adaptive mechanisms that would allow them to survive or even to growth during stress (Capozzi et al. 2009).

In foods preserved by combined methods, the active homeostasis of vegetative microorganisms and the passive refractory homeostasis of spores are disturbed by a combination of gentle antimicrobial factors at a number of sites ("targets") or in a cooperative manner (Gould and Jones 1989). Low levels of different stresses are employed rather a single intensive stress. Moreover, a more effective preservation (i.e., synergistic effects of hurdles or preservation factors) is obtained if small stresses with different targets ("multitarget preservation") are selected to inhibit microorganisms' growth instead of small stresses with the same target (i.e., additive effect of hurdles). For example, for vegetative cells (where homeostasis is energy dependent), the goal is to reduce the availability of energy (for instance by limiting the amount of oxygen available for facultative organisms) and/or to increase the demand for energy (by imposing some other stresses). Placing a number of sublethal stresses (i.e., hurdles or preserving factors) and/or increasing the intensity of a particular sublethal hurdle on a vegetative microbial cell increases the expenditure of energy, and energy is completely used up for repairing the homeostasis. The microorganism becomes metabolically exhausted and dies (Leistner and Gould 2002). On the contrary, when preservation factors are used at high intensity, metabolic exhaustion does not occur because the initiation of homeostatic mechanisms is prevented, and survival of cells is actually enhanced. The metabolic exhaustion is of enormous practical significance in hurdle-preserved fruits, since the microbiological status of such fruits improves with time of storage (Alzamora et al. 1995; Tapia de Daza et al. 1996). Sublethal treatments also may result in an increased sensitivity to adverse environmental factors, such as so longer lag-phase of sublethally damaged cells,

when the cell resumes growth after treatment (Smelt et al. 2002). For spores (where homeostasis is nonenergetic and depends on the structure of the organism), the goal is to damage key structures (i.e., by chemical, enzymatic, or physical attack on coats, cortex, etc.) or to release spores from dormancy (i.e., initiating germination with natural germinants or with false triggers, or applying high pressures).

The hurdle concept exploits synergistic and/or additive interactions between sublethal stressors, helping to reduce the detrimental effects on product quality, the energy input, and the treatment intensities required while guarantying safety.

5.2.2 Most Commonly Used Hurdle Combinations

The combination of physical and chemical methods to decontaminate whole and fresh-cut F&V produce is the way forward to follow for the industry and different approaches have been explored for obtaining stability and fresh-likeness. One of these approaches involved the use of combinations of traditional stressors in simple and inexpensive processing methods. Over the past decades, Alzamora et al. (1995, 2000) developed innovative technologies for obtaining shelf-stable "high-moisture fruit products" with shelf-life of 3–8 months without need of refrigeration. Thus, for various fruit products (pieces, purées), the stress factors employed were: blanching and/or a mild heat treatment—applied without affecting the sensory and nutritional characteristics—, a_w and pH reductions, and antimicrobial agents added to prevent potential microbial spoilage. The a_w reduction (a_w 0.94–0.98, usually adjusted with glucose, sucrose, fructose, maltodextrins, corn syrups, and/or some polyols), control of pH (pH 3.0–4.1, usually adjusted with citric or phosphoric acid), addition of antimicrobials (in doses legally approved and/or sensory compatible, usually weak acids), and depending on the fruit type, the addition of antibrowning agents, were the factors selected to formulate fruit preservation procedures (Alzamora et al. 1989, 1993, 1995; Guerrero et al. 1994; Cerrutti et al. 1997; Argaiz et al. 1995; Tapia de Daza et al. 1995). Analyzing the role of each hurdle in the combined-technique system, the blanching step applied with saturated vapor is a critical operation in the decontamination of fruits. Although its primary objective is enzymes inactivation, heating during blanching also inactivates yeasts, most molds, and aerobic natural flora and sensitizes remaining microorganisms to other hurdles. Reductions in the microbial load from 60 % to 99 % have been reported after blanching of papaya, mango, pineapple, and strawberry (Alzamora et al. 1995, 2000; Tapia de Daza et al. 1995, 1996). The a_w factor was selected in the range 0.93–0.98, accomplishing emergent interest for "fresh-likeness" and low-sugar in foods. The pH was maintained equal or near the pH value of fresh fruit. In less acidic fruits, pH was adjusted to the lower value that was sensory compatible with the natural flavor of the fruit. Foods with high a_w are suitable for the growth of bacteria, molds, and yeasts, but high acidity determines an unsuitable environment for the growth of most bacteria. So, the low pH determines a potential type of spoilage by fungi and acid-tolerant bacteria. Considering that a slight reduction of pH increases the lower limit of a_w for bacterial growth and, vice versa, a slight reduction

of a_w diminishes the range of pH that permits growth, it is expected that interaction pH—a_w in those ranges will be enough to suppress the growth of most bacteria of concern in fruit preservation (Alzamora et al. 1993, 2003). Ability of fungi to tolerate reduced a_w and pH, on the contrary, demands the incorporation of antifungal (e.g., sorbic or benzoic acid) in moderate amounts (400–1000 ppm potassium sorbate or sodium benzoate). The major goal for the design of these combined techniques was the development of simple and inexpensive techniques for bulk storage without refrigeration that were energy efficient and suitable to preserve fruits "in situ", that helped overcoming seasonal production constraints and reduce postharvest losses (Alzamora et al. 1995; Argaiz et al. 1995; Tapia de Daza et al. 1995). To optimize the level of the stress factors at high-moisture contents, the microbial response to stress factors was addressed using different approaches: studies in laboratory media, studies of evolution of native flora in fruit products and microbial challenge tests with microorganisms of concern. The results of such studies have been reported (Alzamora et al. 1989, 2000) and demonstrated that the appropriate selection of stress factors and their levels, can lead to fruit products with longer shelf-life, stable at room temperature. The use of antimicrobials of natural origin as replacement (total or partial) of sorbates, benzoates, and other synthetic additives to meet consumer's expectations about chemicals, was the other aspect considered to improve combined techniques (Alzamora et al. 2003).

A second approach for improving control of foodborne and spoilage microorganisms with promising results involves combinations of emerging "nonthermal" factors or combinations of these stressors with traditional ones. Table 5.1 presents some selected hurdles or stressors (already used industrially or still in development or testing) along with their mode of action, their advantages and disadvantages and the combined processes in which they had been applied to preserve fruits.

Emerging nonthermal factors reported herein have not broad-spectrum inactivation processes like thermal treatment, but represent pasteurization techniques that allow minimizing the disadvantages of severe thermal processing. High hydrostatic pressure, pulsed electric field, ultraviolet radiation, pulsed light, and ultrasound are gaining commercial uses most quickly with fruit-derived products, probably due the low pH that naturally exists in this type of food materials, and are considered "hurdles" that cooperate well in an overall preservation strategy. On the other hand, it should be mentioned that it is probable that the acid adaptation of contaminant flora could adversely affect the microorganism resistance to these technologies, fact that promotes the intelligent combination with other hurdles.

5.3 Research and Commercial Application: Examples of Combined Traditional and Novel Stressors

Targeted application of the hurdle concept has been used/suggested in fruit preservation in different arrangements: (a) using two or more stressors simultaneously to prevent growth or inactivation of spoilage and pathogenic microorganisms; (b) using one or more stressors to inactivate/injure or physically remove some microorganisms, and

Table 5.1 Selected emerging stressors applied in fruit preservation

Factor and mechanism of action	Advantages	Limitations and drawbacks	Potential application/products on the market	Hurdles investigated in combination
High hydrostatic pressure (HHP)				
Application of 100–800 MPa, below 0–100 °C, from seconds to about 20 min, instantaneously and uniformly throughout food, independent of size, shape and food composition Mechanism: Multitarget and dependent on pressure level: membrane damage, protein denaturation, leakage of cell contents, and dissociation of ribosomes	Inactivation of some enzymes according to HHP dose Little change in vitamins, pigments, flavor and antioxidant activity, although effects depend on fruit matrix, pressure, and temperature	High cost of equipment, increased metal fatigue, long cycle times High resistance of browning enzymes and PME to HHP Undesirable sensory changes at high doses (color, appearance, skin loss, structural/texture changes)	Jam, jellies, fruit juices and purées, guacamole, fruit yogurts, dairy-based fruit smoothie, sauces (in use since 1990)	Low pH Natural and synthetic antimicrobials Temperatures below or above room temperature Vacuum packaging and refrigerated storage Mild heating
Short-wave ultraviolet light (UV-C)				
Radiation from the short-wave ultraviolet region of the electromagnetic spectrum (200–280 nm) Mechanism: Damage to DNA, membranes and enzyme activity induced by UV-C light absorption. Hormetic effects in agricultural produce	Moderate to low cost of equipments Little effect on color, vitamin C and taste of fruit juices Little changes in tissue darkening, color, texture, and visual quality of cut fruits at low doses	Low penetration into solids and opaque juices, long treatment times in solids Enzymatic browning of cut fruit surfaces at high doses, more notorious as storage time increase	Pasteurization of apple cider and clear juices (in use since 2000). Surface decontamination of whole and cut fruit surfaces. Reduction of fruit decay and softening	Refrigerated storage MAP Mild thermal treatment US Sanitizers

Pulse light (PL)				
Few flashes applied in a fraction of a second of intense pulses of broad spectrum light (ultraviolet to the near-infrared region) Mechanism: Damage to DNA and destruction of cellular components by the high peak power and the photothermal effects of visible and near-infrared portions of the flash spectrum	Very short treatment times (≤60 s) Little effect on color, texture, antioxidant, and sensory properties at low doses	Low penetration into solids and opaque juices Engineering solutions needed for juice treatment Thermal damage of product at high doses Browning and dehydration of cut fruit surfaces, more notorious as storage time and PL dose increase	Reduction of microbial load on surfaces of whole and cut fruits and in clear juices	Refrigerated storage UV-C Mild thermal treatment
Pulsed electric fields (PEF)				
Application of oscillating, bipolar, exponentially decaying or square wave electric pulses of high voltage (20–70 kv/cm, pulse duration 1–30 s) Mechanism: perturbation of cell membrane and loss of membrane permeability	Little effect on food quality attributes Very short treatment times (≤30 s)	Restricted to foods that can sustain high electric fields, have low electrical conductivity and do not have/produced bubbles	Limited to pasteurization of fruit juices (in use since 2005) and fruit smoothies	Bio-preservatives, essential oils, other antimicrobials Moderate temperatures UV-C PL
High power ultrasound (US)				
Energy generated by sound waves of 20 kHz or more and intensities higher than 1 W/cm^2 Mechanism: Disruption of cellular structures (wall, membranes, organelles, DNA) and cell lysis attributed to cavitation	Inactivation of enzymes when US is combined with heat, and pressure Little change in color of juices and cut fruits	High energy consumption, intensity of industrial-scale equipments limited, long treatment times Heating of the product. Undesirable sensory changes and rupture of skin in berries at high doses	No commercial fruit products; suggested for juice pasteurization. Actual applications limited to product modification and process efficiency improvements (enhancement of mass and heat transfer, degassing of liquids, cleaning of surfaces)	Moderate temperature, pressure Sanitizers Natural antimicrobials UV-C PEFs

then, in sequential mode, one or more stressors to prevent survival/proliferation of remaining refractory or sublethally damaged cells (these last with greater sensitivity to adverse agents); (c) using two or more stressors in sequence for inactivating microorganisms. The order in which they are applied may affect the effectiveness of inactivation. Type, number, and intensity of hurdles had been proposed according to the type of fruit, the impact of the hurdles on quality, the shelf-life required, and/or the available processing/storage infrastructure. These processes have their own set of limitations and advantages. It must be emphasized that no single process will allow obtaining high quality and safety for every fruit product. Some applications to exemplify the different possibilities are addressed next.

5.3.1 Cut and Whole Fruits

UV-C (short-wave ultraviolet) radiation can be considered a promising tool for keeping overall quality of fresh-cut fruits. Direct inactivation by UV-C of microorganisms is limited solely to those associated to the surface of the fruit as UV-C has extremely low penetration into solids, but inactivation can occur in the entire fruit at the dose levels used to induced hormesis (0.5–9 kJ/m^2 for optimal effects according to the type of fruit), with production of antifungal enzymes and phytoalexins (Shama and Anderson 2005). However, semilogarithmic survival curves of inoculated microorganisms on tomatoes, pears, and apple surfaces showed upward concavity and pronounced tailing effect attributed not only to the heterogeneity in the resistances of the population to UV-C irradiation, but to the shielding or physical protection of microorganisms on the solid surface from incident UV-C (effect of surface topography) and/or the internalization of microorganisms into fruit porous tissues (Schenk et al. 2008; Yaun et al. 2004; Gómez et al. 2010). This inactivation pattern reinforced the need for a hurdle approach to reach microbiological stability.

UV-C disinfection has been extensively studied as a postharvest treatment for reducing the number of microorganisms on the surface of fresh and cut fruits (Shama 2006; Allende and Artes 2003; Yaun et al. 2004; Fonseca and Rushing 2006), combined with posterior chilling or MAP to preserve quality. Doses of UV-C light up to 6.9 kJ/m^2 were a satisfactory sanitizing treatment (≈1–1.5 log reduction) for fresh-cut watermelon without causing deterioration of quality in terms of juice leakage, flesh darkening, visual quality, and color values compared to controls after 7 days of storage at 3 °C (Fonseca and Rushing 2006). Gómez et al. (2010, 2011a) examined the effect of UV-C irradiation at different doses on native flora and inoculated microorganisms, surface color, and rheological characteristics of cut-apple disks stored in refrigeration for 7 days. They also explored the use of some pretreatments (hot water blanching, dipping into a solution containing ascorbic acid and calcium chloride) to minimize browning of UV-C irradiated apple slices. Color and compression parameters were found to be dependent on UV-C dose, storage time, and type of pretreatment. Changes in structural features, color, and viscoelastic parameters were mainly evidenced after refrigerated storage. At the end of storage, samples exposed to only UV-C light turned darker (lower L^* values) and less green (higher a^* value) when

compared to fresh-cut apple slices or to samples on day 0 and this effect was more pronounced at the greatest UV-C dose. Light microscopic images showed breakage of cellular membranes in UV-C treated samples which may explain the increase in browning of irradiated apples. Both pretreatments helped in maintaining the original color of apple slices after UV-C light exposure. Natural microflora counts were higher in untreated UV-C than in UV-C treated samples along the whole storage. All samples showed a viscoelastic solid behavior with the storage modulus (G') dominating the viscoelastic response. Overall, both dynamic moduli decreased, and creep instantaneous compliance (J_0), decay compliances (J_1 and J_2), and fluidity significantly increased after treatments and storage at 5 °C. However, a texture-trained panel only significantly differentiated stored untreated apple from the other samples regarding fracturability and juiciness, showing the potential of this technology to maintain texture characteristics in preserved fruit.

Recently, Gómez et al. (2011b) performed a similar study but using pulsed light (PL) instead of continuous UV-C. They examined the dose effect of PL irradiation on surface color, microstructure, and microbial stability of cut-apples stored under refrigeration. An increase in surface browning was noticed when increasing PL doses were applied. Again, light microscopy observations indicated that the modifications on color of treated apples could be at least partially ascribed to the breakage of cellular membranes, which would cause a loss of functional cell compartmentalization, increasing enzyme-substrate contact with the consequent increase in tissue browning. But increases in temperature during PL irradiation at high doses could also cause non-enzymatic browning.

The combination of UV-C with mild heat treatment (sequential hurdles) had been suggested by Marquenie et al. (2002) and Pan et al. (2004) for controlling postharvest decay of berries (strawberries and sweet cherries) stored at room temperature. Previous irradiation with UV-C (4.1 kJ/m^2) enhanced the benefits of heat treatment (45 °C, 3 h in air) and further reduced decay, softening, and reddening of the strawberry fruit (Pan et al. 2004).

An important group of combined treatments recently developed were oriented to hurdle strategies that consider the use of traditional or novel sanitizers (ozone, acidified sodium chlorite, hydrogen peroxide, electrolyzed water–acidic, or neutral, peroxiacetic acid, organic acids, etc.) combined among themselves and/or combined with physical inactivation stressors (ultrasound; light-based techniques; mild heat, low temperature, etc.). In many cases, the result is the on-site destruction of even refractory organisms without the generation of residues. Contemporary strategies based on sanitizers to provide the whole and fresh-cut produce industry have been deeply discussed in the comprehensive review by Tapia and Welti-Chanes (2012). One of these approaches is the employment of UV-C in combination with hydrogen peroxide for decontaminating fresh fruits. Schenk et al. (2012) investigated the simultaneous or serial combined use of UV-C light (7.5 min; 3.7 kJ/m^2) and H_2O_2 treatment (3 % w/v; pH 3.0; 25 °C) to preserve fresh-cut pear discs under 10-day refrigerated storage (5 °C). This combination was successful preventing the proliferation of the residual native flora population during refrigerated storage, being the browning effect less pronounced compared with the individual treatments. Additionally, the product processed by the combined H_2O_2/UV-C treatment was well accepted by the consumers.

Among ultrasound-based approaches, Jang and Moon (2011) proposed the combined use of ultrasound (40 kHz) and ascorbic acid (1 %) to extend the shelf-life of refrigerated (10 °C) fresh-cut "Fuji" apples by inhibiting enzymes related to enzymatic browning (polyphenol oxidase and peroxidase). These authors observed that the simultaneous treatment has synergistic inhibitory effects on PPO activity without deformation of enzyme protein. They inferred that the combined treatment allowed the ascorbic acid to act inside the cell disrupted by ultrasound treatment.

Chen and Zhu (2011) designed a combined preservation method for plum fruit (*Prunus salicina* L.) storage based on the simultaneous application of the hurdles ClO_2 (40 mg/L; 10 min) and ultrasound (100 W, 10 min) with the purpose of reducing respiration rates and maintaining firmness of the fruits, which were packaged after the treatment into aseptic polyethylene bags and stored at 4 °C for 60 days. The ClO_2 and ultrasound-treated fruit showed significantly higher flesh firmness than the untreated plum. The activities of ripening-related enzymes in plum fruit might be retarded by the ClO_2 and ultrasonic treatments and thus softening inhibited. The effect of combined treatments on maintaining contents of total flavonoids, ascorbic acid, reducing sugars, and titratable acids were similar but were more beneficial than the individual treatments and the untreated control. This confirmed that ascorbic acid is insufficient in controlling browning and maintaining the commercial value of fresh-cut products (its inhibitory enzyme effect is reversible and temporary). The simultaneous mode was effective in reducing the initial microflora, leaving no detectable chemical residues and retaining sensory qualities of plum fruit, and fruit shelf-life could be extended to 60 days compared to 35 days for the control.

HHP is gaining popularity in the fruit industry because its ability to destroy microorganisms and to significantly reduce the enzymatic activity on acid fruit juices and fresh fruits without greatly affecting vitamins, pigments, and flavor and antioxidant activity, probably due to the stability of covalent unions to high pressure. Because of inherent low pH, most fruits can be easily stabilized by HHP, since yeasts, molds, and vegetative cells of bacteria can be inactivated by pressures in the range of 200–700 MPa near room temperature. But the presence of HHP-resistant enzymes requires a careful selection of the operative/storage conditions for best quality retention. HHP affects enzymes and there is an optimum temperature range at which proteins are more resistant to pressure. As an example, good storage stability of strawberries was obtained over at least 3 months when HHP at temperatures between 20 and 40 °C was combined with vacuum packaging and refrigerated storage since polyphenol oxidase was highly resistant to high-pressure inactivation (Terefe et al. 2009).

The combination of HHP and plant-essential oils had been suggested as an alternative control for fruit diseases. *Colletotrichum gloeosporioides* spores, which cause anthracnose in papaya, were efficiently inhibited by a 350 MPa-30 min treatment or by a combination of 150 MPa-30 min and 0.75 mg/ml of citral or lemongrass oil. An explanation for the enhanced effect of pressure plus lemongrass essential oil is that pressure facilitates the uptake of the oil constituents into the spore, increasing the number of targets affected (Palhano et al. 2004).

Krebbers et al. (2003) analyzed the effects of combined HHP—thermal treatments on consistency, viscosity, color, lycopene content, enzyme activity, and

microorganisms of tomato purée as a model of tomato-based products. Single HHP (700 MPa, 2 min, 20 °C) resulted in inactivation of natural flora to a level below the detection limit and partial inactivation of galacturonase but activation of pectin methylesterase. The product appeared stable during storage at 4 °C at least 8 weeks. After high-pressure sterilization treatments combined with elevated starting temperatures (≥80 °C, one or two pulses), an ambient stable product was obtained, with more than 99 % inactivation of polygalacturonase and pectin methylesterase, with better color, greater lycopene content, and improved water-binding capacity than the conventional sterilized tomato product.

Several chemical compounds have been used in combination to reduce bacterial populations on fruit and they are still the most widely used treatments, either before processing or during pre- and post-cutting operations. Many efforts are being made to replace chlorine-based chemicals used as fruit sanitizers since in many European countries their use is prohibited due to their potential toxicity (Gil et al. 2009). Electrolyzed oxidizing water (EOW) has been shown to be a promising alternative decontamination technique with a strong bactericidal effect. This technique has been suggested as a valuable disinfection tool for wash water sanitation in the minimally processed fruit industry (Gil et al. 2009). An evidence is provided by Wang et al. (2007), who proposed a sequential washing treatment (5 min) of acidic EOW (pH 2.7; oxidation-reduction potential 1150 mV; free chlorine 45 mg/L) followed by calcium ascorbate solution (5 %; pH 5.0) to effectively delay bacterial growth, browning, and firmness loss of fresh-cut apple wedges under passive modified atmosphere storage conditions (polypropylene bags; 4 °C; 11 days).

An interesting and more recent approach was proposed by Silveira et al. (2011), who examined the effect of hot water immersion dipping (60 °C; 90 and 120 s) followed by immersion in peroxiacetic acid (80 mg/L; 60 s) on metabolic activity and microbial and sensory quality changes of fresh-cut Galia melon pieces packaged into oriented polypropylene heat-sealed trays (generation of a passive modified atmosphere packaging, 7.4 kPa O_2 and 7.4 kPa CO_2) and stored for 10 days at 5 °C. This combined treatment, using an eco-friendly sanitizer, had positive effect on melon overall quality and reduced its metabolism helping to maintain fruit firmness.

5.3.2 Juices

The nonthermal factors applied to fruit juices that have been mostly investigated were pulsed electric field (PEF), high hydrostatic pressure (HHP), short-wave ultraviolet irradiation (UV-C), and ultrasound (US) (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Keenan et al. 2010; Liang et al. 2006).

Table 5.2 resumed some investigations on PEF combined with many other hurdles in PEF-based strategies. Several studies, mainly in apple juice, reported that microbial PEF inactivation enhanced by its combination with UV-C irradiation. Walkling-Ribeiro et al. (2008) observed an *Staphylococcus aureus* reduction of 9.5 $\log_{10}$ cycles in apple juice reconstituted from concentrate processed by batch UV-C treatment (30 min;

Table 5.2 Efficacy of PEF-based hurdle strategies for fruit juice preservation

PEF-based strategy/storage conditions	Evaluated conditions	Parameters of evaluation	Product	Conclusions on hurdle strategy	Reference
Continuous flow PEF system (PEF; 30 kV; 1 μs; 200 pulses/s)/moderate temperature (*T*)/antimicrobials	PEF (27 and 33 kV/cm; 3, 6, and 10 L/h); *T* (45 and 50 °C); (2) Antimicrobials: nisin (27.5 U/ml)/lysozyme (690 U/ml), clove oil (3 ml/100 ml)	Natural microflora; PPO	Apple cider	PEF: 3.1 $\log_{10}$ red (3 L/h; 50 °C) PEF/T/nisin/lisozyme: Less than additive PEF/T/clove oil: No significant additional effect 33 % PPO activity decrease (PEF/T)	Liang et al. (2006)
Continuous flow lab scale PEF system (40 kV/cm, 100 μs; 15.75 ml/min)/ Batch UV-C system (254 nm, 30 min, 30 W)	(1) UV-C; (2) PEF; (3) UV-C + PEF; (4) PEF + UV-C; (5) Heat processing (26 s, 94 °C) and (6) Heat processing (26 s, 72 °C)	Natural microflora; NEBI; color; pH; Antioxidant activity; polyphenol content; PPO; POD	Fresh apple juice	(3) UV-C + PEF or (4) PEF + UV-C: Less than additive antimicrobial effect; similar to (5) No significant differences in chemical parameters with respect to (1) and (2) No significant differences in % enzyme activity with respect to (2): 47.2 % and 49.5 % POD activity; 42.8 % and 41.3 % PPO activity	Noci et al. (2008)
Continuous flow lab scale PEF system (3.5 μs)/continuous flow UV-C system (254 nm; 25 W)	(1) UV-C (10, 20, 30, 40 and 50 cm length tube; 8–20 ml/min); (2) PEF (40, 50 and 60 kV/cm; 8, 14 and 20 ml/min); (3) UV-C + PEF and (4) PEF + UV	*E. coli* ATCC 23472	Pasteurized apple juice	UV-C + PEF or PEF + UV-C: additive effect (5.3 $\log_{10}$ red.; 60 kV/cm; 8 ml/min; 30 cm UV-C length)	Gachovska et al. (2008)

Batch UV-C system (254 nm; 26 cm distance; 30 W; 30 min; 20 °C)/ continuous mild preheating (60 s)/ continuous flow lab scale PEF system (1.0 µs pulse width; 15 Hz)	(1) Combined treatment, orthogonal design: UV-C/ Preheating (T_{inlet} 35, 43, 46 and 50 °C)/PEF (28, 32, 36 and 40 kV/cm; 25, 50, 75 and 100 µs); (2) HTST pasteurization (94 °C, 26 s)	*Staphylococcus aureus* SST 2.4; color; pH; conductivity; NEBI; polyphenol content; AA content	Apple juice reconstituted from concentrate	(1) UV-C/preheating/PEF: 9.5 $\log_{10}$ red. (30 min-UV-C/46 °C preheating/40 kV-75 µs PEF). No significant changes in physical and chemical properties (2) HTST: 8.2 $\log_{10}$ red.	Walkling-Ribeiro et al. (2008)
Continuous flow lab scale pulsed light system (PL; 360 µs pulse width; 200–1100 nm; 3 Hz; 1.213 J/cm^2/ pulse)/continuous flow lab scale PEF system (PEF; 1.0 µs pulse width; 18 Hz; 20.8 ml/min; T_{inlet}:20 °C; 34 kV/cm; 93 µs) Continuous flow lab scale UV system (UV; 254 nm; 30 W; 176 ml/min; 5.3 J/cm^2/30 s)	(1) UV-C; (2) PL; (3) PL + PEF; (4) UV-C + PEF; (5) Heat processing (26 s, 72 °C) (H72)	*E. coli* K12 DSM 1607 *Pichia fermentans* DSM 70090; Shelf-life (natural microflora at 4 °C)	90:10 Fresh apple and cranberry juice blend	*E. coli* and *P. fermentans*: (1) and (2): less than 5 $\log_{10}$ red. (3) and (4): more than 6 $\log_{10}$ red. (5): 6 $\log_{10}$ red. Shelf-life (3): 21–28 days Shelf-life (4): 14–21 days	Palgan et al. (2011)
Continuous flow lab scale PL system (360 µs pulse width; 200–1100 nm; 3 Hz; 17 ml/min)/continuous flow lab scale PEF system (1.0 µs pulse width; T_{inlet}: 20 °C; 89 µs)	(1) PL (4.0 and 5.1 J/cm^2); (2) PEF (24 and 34 kV/cm; 13.4 and 17.0 ml/min; 15 and 12 Hz); (3) PEF + PL; (4) PL + PEF	*E. coli* K12 DSM 1607; color; pH; polyphenol content; NEBI; sensory consumer tests	Apple juice reconstituted from concentrate (1:7.8 v/ water v)	(1) PL: 3.3–3.9 $\log_{10}$ red.; (2) 1.8–3.5 $\log_{10}$ red. (3) Additive or synergistic effect (more than 6.5 $\log_{10}$ red.) (4) 4.5–6.2 $\log_{10}$ red. (~additive effect) No significant changes in physical; sensory; and chemical properties (4) Most acceptable treatment	Caminiti et al. (2011)

(continued)

Table 5.2 (continued)

PEF-based strategy/storage conditions	Evaluated conditions	Parameters of evaluation	Product	Conclusions on hurdle strategy	Reference
Continuous flow lab scale PEF system (40 kV/cm; 1.0 μs pulse width; 15 Hz; 100 μs; max T_{outlet}: 56 °C)/bio-preservatives (BP; 2.5 ppm nisin; 500 ppm lactic acid; 100 ppm benzoic acid or 10 ppm natamycin)	(1) PEF; (2) BP; (3) BP + PEF	*E. coli* K12 HB101; *Listeria innocua* IMD 11288; *P. fermentans* CBS	Pulp-free orange juice	(1) PEF: 4.8 $\log_{10}$ red. *P. fermentans*; 6.0 $\log_{10}$ red. *E. coli* and 4.2 $\log_{10}$ red. *L. innocua*. (2) BP: 0.5–2.0 $\log_{10}$ red. (3) Synergistic effect (7.8 $\log_{10}$ red.; *P. fermentans*; lactic acid); additive effect (5.5 $\log_{10}$ red.; *P. fermentans*; benzoic acid); without effect (4.2 $\log_{10}$ red. *P. fermentans*; natamycin); synergistic effect (5.6 and 7.9 $\log_{10}$ red.; *L. innocua* and *E. coli*; nisin); synergistic effect (6.1 $\log_{10}$ red.; *L. innocua*; lactic acid); without effect (5.8 $\log_{10}$ red.; *E. coli*; lactic acid)	McNamee et al. (2010)
Continuous flow bench-scale PEF system (35 kV/cm; 4 μs pulse width; $T < 38$ °C)/citric acid (CA) or cinnamon bark oil (CO)	(1) PEF (200, 600 and 1000 μs; 100, 150 and 200 Hz); (2) CA (0.5 %, 1.0 %, 1.5 % and 2.0 % w/v); (3) CO (0.05 %, 0.10 %; 0.20 % and 0.30 % v/v); (4) CA (1 h)/PEF (1000 μs; 100 Hz); (5) CO (1 h)/PEF (1000 μs; 100 Hz)	*Salmonella enterica* Ser. Enteritidis 1.82	Tomato juice	(1) PEF (4.184 $\log_{10}$ red., 1000 us, 100 Hz) (2) CA: little effect (<1.0 $\log_{10}$ red.) (3) CO: 1.09–4.11 $\log_{10}$ red. (CO: 0.1–0.3 %) (4) CA/PEF: synergistic effect (CA > 1 %); 5 $\log_{10}$ red. (CA: 2 %) (5) CO/PEF: synergistic effect (CO > 0.1 %); total inactivation (CO > 0.2 %)	Mosqueda-Melgar et al. (2008a)

Continuous flow bench-scale PEF system (35 kV/cm; 4 µs pulse width; $T<38$ °C)/citric acid (TA) or cinnamon bark oil (TO)	(1) PEF (500, 1250 and 2000 µs; 100, 1750 and 250 Hz); (2) CA (0.5 %, 1.0 %, 1.5 % and 2.0 % w/v); (3) CO (0.05 %, 0.10 %; 0.20 % and 0.30 % v/v); (4) CA (1 h)/PEF (1575–1700 µs; 100–235 Hz); (5) CO (1 h)/PEF (1575–1700 µs; 100–235 Hz)	*Salmonella enterica* Ser. Enteritidis 1.82; *E. coli* O157:H7	Apple, pear, orange, and strawberry juices	(1) PEF: 4.34 (apple)–5.15 (orange) $\log_{10}$ red. *S.* Enteritidis; 4.28 (apple)–5.46 (strawberry) $\log_{10}$ red. *E. coli* (4) CA/PEF: synergistic effect (CA: 1.0–1.5 % *S.* Enteritidis; orange and strawberry); (CA: 1.5–2.0 %, *E. coli*, orange and strawberry); (CA: 1.5 %, *S.* Enteritidis and *E. coli* above 5 $\log_{10}$ red., apple and pear) (5) CO/PEF: additive effect (CO > 0.1 % *S.* Enteritidis, apple, pear and orange; CO > 0.05 % *E. coli*, strawberry)	Mosqueda-Melgar et al. (2008b)
Continuous flow PEF system (35 kV/cm; 4 µs pulse width; 150 Hz; 90 ml/min)/bacteriocin (B; enterocin AS-48; 3.5 AU/µg protein)	(1) PEF (100–1000 µs; 20 and 40 °C); (2) B (30; 45 and 60 µg/ml) (3) PEF/B	*Salmonella enterica* CECT 915	Apple juice	(1) PEF: 3.04 $\log_{10}$ red. (2) B: no effect (3) PEF (1000 µs; 40 °C)/B (60 µg/ml): synergistic effect, 4.5 $\log_{10}$ red.	Martinez Viedma et al. (2008)

AA ascorbic acid, *NEBI* non-enzymatic browning index, *POD* peroxidase, *PPO* polyphenoloxidase, *red.* reductions

20 °C) followed by mild preheating (35–50 °C) and PEF treatment (25–100 μs; 28–40 kV/cm). This approach introduced an advantage with respect to the sole PEF treatment from the point of view of energy consumption, requiring shorter treatment time to reach a desired inactivation. In a subsequent investigation, these researchers studied the influence of this hurdle strategy on selected quality parameters (color, pH; Brix; non-enzymatic browning index; antioxidant capacity) and enzymatic activity (polyphenoloxidase and peroxidase) of freshly squeezed apple juice. They applied these hurdles as stand-alone treatments (UV-C or PEF) or using two types of combinations (UV-C+PEF or PEF+UV-C). Additionally, they processed apple juice by using traditional thermal treatments in a heat exchanger (72 or 94 °C) (Noci et al. 2008). The application of the combination UV-C+PEF to freshly squeezed apple juice resulted in a similar total microbial reduction compared to the severe heat treatment (7.1 and 6.7 log cycle reduction, respectively), being the inactivation effect for both combinations less than additive. However, the quality attributes measured in juice processed by PEF or by the combined approach were similar to those observed in juice treated by the milder heat process (72 °C) and consistently superior when compared to the severe heat treatment (94 °C). With regard to the effects on enzyme activity, juices processed by PEF; UV-C+PEF or PEF+UV-C did not show any significant difference (47.5 %; 47.2 %; or 49.5 % respectively). Gachovska et al. (2008) found an additive effect on the inactivation of *E. coli* ATCC 23472 in pasteurized commercial apple juice processed in a continuous flow chamber using a combination of UV-C and PEF applied sequentially. Regardless of the order of treatments, a maximum *E. coli* reduction of 5.35 log CFU/ml was achieved using PEF (60 kV/cm, 11.3 pulses) and UV-C (length of 50 cm, treatment time of 2.94 s, and flow rate of 8 ml/min). Palgan et al. (2011) studied the potential of the nonthermal hurdles UV-C (5.3 J/cm^2), high-intensity light pulses (PL) (3.3 J/cm^2), PEF (34 kV/cm, 18 Hz, 93 μs) or manothermosonication (MTS) (4 bar, 43 °C, 750 W, 20 kHz) applied individually or in paired sequences, to inactivate *E. coli* and *Pichia fermentans* inoculated in a fresh blend of apple and cranberry juice. Selected sequential combined treatments (PL+PEF; PL+MTS; UV-C+PEF; UV-C+MTS) gave comparable reductions ($p \geq 0.05$) for each of the organisms examined to those observed in thermally pasteurized samples (approx. 6 log CFU/ml) while none of the individual hurdles was capable of achieving the 5 log reduction required by FDA for fruit juices. As all combinations led to reductions below detection levels, authors could not determine the presence or absence of synergistic or additive effects. This work showed the potential for combinations of a light-based technology (UV-C or PL) followed by either PEF or MTS; however more investigation would be needed since the shelf-life (4 °C) of UV-C+PEF and PL+PEF-treated samples was 14 and 21 days respectively, shorter than that corresponding to thermally treated samples (35 days). In a recent study, Caminiti et al. (2011) also proposed the PEF (24 or 34 kV/cm; 89 μs)/PL (360 μs, 3 Hz; 4.0 or 5.1 J/cm^2) hurdle combination and the reverse sequence as an alternative of thermal pasteurization for bacterial control in apple juice. They reported a synergistic interaction on *E. coli* K12 (more than 6 log reductions) in reconstituted apple juice but did not find any difference in pH, °Brix, total phenolics and sensory parameters between apple juice processed by the PEF/PL combination and the pasteurized product.

Other authors reported an increase in the killing effect of PEF on microorganisms in various fruit juices (apple, pear, tomato, orange, and strawberry) or apple cider in

combination with natural antimicrobials such us bacteriocins (McNamee et al. 2010; Martinez Viedma et al. 2008); essential oils and organic acids (McNamee et al. 2010; Mosqueda-Melgar et al. 2008a, b; Liang et al. 2006). While the mechanism of synergy is not fully understood, the additional stress of PEF probably facilitates the income of antimicrobials to the cytoplasmic membrane, improving the efficacy of the antimicrobial compounds, which allows proposing very low doses of antimicrobials.

As it can be seen, although the combination of different nonthermal technologies have shown a significant microorganism inactivation effect, strong differences were found between the different nonthermal hurdle strategies depending on type of microorganism; operation mode (continuous or batch) and equipment; order of sequence; matrix; etc. Thermal treatment may still be required to achieve the desired level of inactivation for practical uses, but the combination of different hurdles with heating allows the use of significantly reduced temperature in fruit juices with the consequent less detrimental effect on quality parameters (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Liang et al. 2006). Aronsson and Rönner (2001) gave a detailed discussion on thermally influenced PEF inactivation and drew conclusions about synergy of PEF and thermal treatment.

UV-C radiation can be effectively used for pasteurization of different kinds of fruit juices, without in general affecting in a severe way color profiles, vitamin C content, and taste (Tran and Farid 2004; Keyser et al. 2008). The combination of UV-C treatment and low temperature storage allowed a shelf-life extension from ≈2 days to more than 5 days. However, the use of UV-C is still limited due to the low UV transmittance of fruit juices. The penetration of UV-C radiation depends on the type of liquid, its absorptivity, soluble solids, and suspended matter. Thus, different UV-C reactors (thin film, turbulent, laminar Taylor-Couette, Dean flow reactors) are being studied to ensure effective radiation penetration (Koutchma 2009).

HHP can induce changes in fruit structure and texture which, along with taste, are the most important sensory attributes for consumer acceptability. For this reason, most of the commercialized and/or investigated HP processed products of plant origin are in the form of purée or juice (e. g. guacamole, fruit jams, and juices). In juices, control of PME is crucial for assure cloud stability, since demethylation of pectin results in the separation of a clear serum and a sediment constituted by complexes of low methoxyl pectin and calcium ions. In general quality-related enzymes are rather pressure-stable and pressure treatments were usually combined with mild heating to obtain juices or fruits of high quality. For example, a synergistic effect of HHP and moderate temperature on orange PME inactivation was found by Polydera et al. (2004) except in the high-temperature–low-pressure region where an antagonistic interaction was noted. Buchow et al. (2009) also reported a synergism between pressure and temperature on the inactivation of apple polyphenol oxidase (PPO) above 300 MPa where an antagonistic effect was found at lower pressures. The stability interfaces of apple juice in order to optimize a HHP treatment when considering different types of quality targets (PPO, PME, and vitamin C) were determined by Valdramidis et al. (2009) for a given number of design variables (level of pressure, treatment time/temperature, storage time/temperature). Previously, apple juice spoilage constraints of *Issatchenkia orientalis* and other vegetative microorganisms

were defined. The two enzymes appeared to be much more resistant than vegetative microorganisms with respect to pressure–temperature treatments. The application of 750 MPa and 50 °C or higher was required to ensure both microbial stability and quality of apple juice, while only 350 MPa for 10 min would be necessary to minimize the probability of spoilage during storage at 8 °C.

Sonication applied alone (at room temperature and atmospheric pressure) is not very effective inactivating microorganisms in juices. However, the combination of ultrasound with other preservation factors and/or the selection of operative conditions that enhance the per se effect of high-power sonication shows considerable promise (Piyasena et al. 2003; Knorr et al. 2004; Alzamora et al. 2011). López-Malo et al. (2006) analyzed the response of *L. monocytogenes* and *S. cerevisiae* to the single and combined effects of high-intensity ultrasound (20 kHz, 400 W, 95.2 μm, *T*: 35 °C) and UV-C light (continuous flow system; 90 cm long glass tube with a 100 W Hg lamp, 1100 μW/cm^2) in clarified apple juice. The effect of the US/UV-C combination was additive and led to a great inactivation (≈4–5 log cycles reduction after 5 min treatment), with the majority of the population dead in the first minutes of treatment. Char et al. (2010) studied the use of continuous flow (0.2 L/min) US treatment (20 kHz, 95 μm, 40 °C) combined with UV-C light (100 W) to inhibit *E. coli* ATCC 35218 in orange juice (pH 3.5; 9° Brix). The poor single effect of UV-C light in orange juice (1.7 log reduction), probably due to the opaque nature of matrix, was enhanced by the combination with US achieving approximately 4 log reductions (additive effect). Combined treatment was more effective in simultaneous rather than in a series of US–UV-C arrangement.

Thermoultrasonic treatment caused a higher killing effect than only sonication treatment. Raising the temperature and hence membrane fluidity (i.e., weakening the intermolecular forces) would enhance the disruption by US (Russell 2002). However, as temperature increased toward lethal values, the benefits of ultrasound application are reduced probably as a result of an increased thermal effect and a reduced intensity of cavitation (López-Malo et al. 1999). Inactivation studies of *Listeria monocytogenes* 10403S, an ultrasound resistant strain, were conducted at sublethal (20–40 °C) and lethal (50–60 °C) temperatures in apple cider (pH 3.4) with and without application of US (20 kHz, 750 W, 99 ml sample) (Baumann et al. 2005). Ultrasound increased the inactivation rate at both lethal and sublethal temperatures. The bactericidal effect of the combined process was additive. After a 5-min of thermoultrasonic treatment at 60 °C, cells of *L. monocytogenes* 10403S died during a 6 h period at room temperature. This treatment conditions could provide a solution for apple cider industries to achieve the required 5 log reduction in pathogenic populations.

Ferrante et al. (2007) applied a ultrasound-based hurdle strategy (20 kHz; 95 μm) for *Listeria monocytogenes* inactivation in fresh-squeezed orange juice which included the use of moderate temperature (45 °C) and addition of naturally occurring antimicrobials vanillin (1000 or 1500 ppm) and citral (100 ppm). The bacteria inactivation was total in 10 min treatment of 1500 ppm vanillin and 100 ppm citral containing orange juice, being the product pleasant for the consumers according to a sensory study. Phenolic compounds have lipophilic nature and could accumulate

in the lipid bilayer of the cell, disturbing and sensitizing the membrane to ultrasound (Brul and Coote 1999). In a more recent study using pineapple, grape, and cranberry juices, under 24 kHz, 400 W, 120 μm and 60 °C, between 5 and 7 log-reduction of *S. cerevisiae* were achieved (depending on the juice) after 10 min of continuous ultrasonic treatment but significant changes in color and pH of juices were detected after processing. The use of lower temperatures (40 or 50 °C) or pulsed mode delayed yeast inactivation (Bermúdez-Aguirre and Barbosa-Cánovas 2012). Gómez-López et al. (2010) reported a non-negligible effect of ultrasound -and refrigeration- on shelf-life in orange juice. They applied ultrasonic treatments at a frequency of 20 kHz and three-wave amplitudes for 2, 4, 6, 8, and 10 min, to orange juice with added calcium. Wave amplitude of 89.25 mm for 8 min was selected for final treatment of the juice, and storage studies were performed at 4 and 10 °C. The treatments decreased mesophilic counts by 1.38 log CFU/ml, and yeast and molds counts by 0.56 log CFU/ml. The sensory quality of the juice was slightly deteriorated after treatment, but during storage, control samples degraded earlier than sonicated samples. Controls were rejected by the sensory panel after 6 days storage at 4 °C due to off-flavor, while ultrasonicated juice was rejected after 10 days due to off-odor. Consequently, a shelf-life extension of 4 days was achieved. Sonication also affected color and decreased ascorbic acid content. This study shows that ultrasonication may be useful to extend the shelf-life of orange juice and could be further enhanced by the use of other hurdles.

5.4 Recommendations

Examples described above present the promising use of combined processes for the production of mildly preserved fruit products. Knowledge of the fundamentals of traditional and emerging factors and the number of reports on preservation of foods in general, and fruits in particular, have dramatically increased during the last 15 years. But more research is needed to fully exploit the potential of the hurdle concept in the design and optimization of fruit preservation techniques and facilitate/increase their adoption by the industry. Vital areas for further studies include the following points.

5.4.1 Microbial Behavior in Response to Stressors

New food preservation strategies can be developed on a sound scientific base if combination of hurdles is thought taking into account their different modes of action over microbial cells and the mechanisms mediating microbial adaptive responses (Ross et al. 2003; Gould 2000). Our knowledge of these subjects is far from complete. Nowadays, the advent of new methodic developments may provide a significant conceptual advance in the understanding of responses in microorganisms to a variety of environmental stresses.

Microbial cells react upon stress by producing specific stress transcriptors, proteins, and metabolites, to bring cellular metabolism to homeostasis (Brul et al. 2002, 2006). Booming "genomics" technologies (genomics, transcriptomics, proteomics, and metabolomics) contribute to the understanding of cellular behavior by a simultaneous approach in which the whole set of cellular biomolecules is studied in a given experimental setup. Cellular response at molecular level can then be used to study cellular physiology and/or to generate databases of cellular reactions to environmental conditions, supporting the development of effective food preservation processes.

Changes in plasma membrane potential and/or ion flux modulation are some of the earliest cellular modifications occurring when the environments become hostile, and are linked to cell viability and physiological processes. The noninvasive microelectrode ion flux estimation technique allows calculating net fluxes of H^+, Ca^{2+}, K^+, Na^+, Cl^-, Mg^{2+}, and other ions in real time from the measured voltage gradient at the surface from molds, yeasts, and biofilms (Shabala et al. 2006). The study of the kinetics of membrane-transport processes across cellular membranes has shown to be a valuable tool to investigate various aspects of adaptive responses to temperature, osmolality, and acid stress. For instance, evaluation of temperature-induced changes in net H^+ fluxes in *Listeria monocytogenes* allowed quantifying the critical temperature at which membrane-transport activity undergoes dramatic changes, thus enabling to assess the chilling resistance of the bacteria (Shabala et al. 2006). Shabala et al. (2002) used in combination noninvasive ion flux measurements with fluorescence microscopy to quantify intracellular pH when studying *L. monocytogenes* response to acidic stress. The kinetics of pH_i change and net H^+ fluxes indicated that plasma membrane H^+ transporters play a central role in the bacteria pH homeostasis and the acid tolerance response.

Multiparameter flow cytometry (FC) is another modern tool for monitoring the stress response of microorganisms. By means of both scattering and fluorescence signal measurements, information on cell parameters (physiological state, size, surface roughness, and granularity) at single cell level and their distribution within cell populations is provided with a relatively high degree of statistical resolution (≅50,000 cells in minute) enabling assessment of population heterogeneity (Hewitt and Nebe-Von-Caron 2004; Díaz et al. 2010). Cell viability evaluation is an issue of particular concern in preservation technologies design. Resistance to different stressors has been attributed to an entry into viable but non-culturable (VBNC) states. VBNC, dormant, or damaged cells are not able to grow on culture media but generally maintain their metabolic machinery active and remain alive, provoking undesirable effects such as food spoilage, accumulation of toxins, or transfer of genes. FC allows identifying functionally homogeneous subpopulations including VBNC. FC was used to monitor pressure-induced changes in *Lactobacillus rhamnosus* and *Listeria monocytogenes*, and the studies showed that pressurized inactivated bacteria were still in possession of enzyme activity and not completely membrane compromised (Ananta et al. 2004; Ritz et al. 2001). Ananta et al. (2005) showed that high-intensity ultrasound provoked the rupture of the lipopolysaccharide layer of the outer membrane of

Gram-negative bacteria, suggesting the ultrasound-assisted physical disruption of the outer membrane to facilitate the entry of bacteriocins. Based on FC studies, Mathys et al. (2007) suggested a three-step inactivation model of pressure–heat-treated spores of *Bacillus licheniformis* involving a germination step following hydrolysis of spore cortex, an unknown step and finally an inactivation step with physical compromise of the spore's inner membrane. Schenk et al. (2011) demonstrated that mechanisms of UV-C induced cellular damage differed according to exposure time and the organism tested: a significant damage in the cytoplasmic membrane integrity and in the enzyme activity was found in *E. coli* and *S. cerevisiae* in the first minutes of UV-C treatment while a VBNC subpopulation of *L. innocua* was detected at near all UV-C doses assayed.

The introduction of any alternative technology as well as its optimization requires quantitative data about microbial response (Alzamora et al. 2010). In particular, kinetic parameters and models are essential to develop food preservation processes that ensure safety (McMeekin 2007; Alzamora and López-Malo 2002; Alzamora et al. 2010). The parameters also allow comparison of the ability of different process technologies to reduce microbial populations. Accurate model prediction of survival curves would be beneficial to the F&V industry in selecting the optimum combinations of lethal agents and environmental factors as well as exposure times to obtain desired levels of inactivation while minimizing production costs and maintaining a maximum degree of sensory and nutrient quality. Potential combinations of preservation factors are numerous, but until now not much quantitative microbiological information is available about combination of alternative processes with traditional constraints. Predictive microbiology provides the tools to compare the impact of different factors on reduction of microbial population (Alzamora et al. 2010). For instance, when *L. monocytogenes* and *S. cerevisiae* undergone US, UV-C and US/UV-C treatments and inactivation data were fitted using the cumulative Weibull distribution function, the correspondent frequency distributions of resistances showed that the combined action of US/UV-C light not only increased the microbicidal effect of sonication but changed the distribution of inactivation times. When both physical inactivation agents were applied together, narrowest frequency shapes, skewed to the right, with low dead time means and a very substantial decrease in its overall spread, were in general obtained, indicating a more homogeneous response of the microbial population to the combined treatment (López-Malo et al. 2006).

5.4.2 Engineering Solutions

Process uniformity is an important factor that affects the effectiveness of the treatment and attempts with its successful commercialization (Heldman et al. 2008). Engineering solutions are required to design new equipments which assure that all product pieces or volume elements receive the same stressor dose For example, in continuous UV-C and pulsed light processes, the distance and the relative position

of the sample with respect to the Hg and Xenon lamps significantly influence the received dose or fluence as well as the increase in temperature with the PL dose (Gómez et al. 2010, 2011b).

5.4.3 *Support Studies for the Design of Preservation Techniques*

While much relevant information is available in the scientific literature concerning stressors/interaction of stressors that influence microbial activities in fruits, it is not often practical in formulating combined preservation techniques. In many cases the information provided involves only data from traditional challenge testing with pathogenic microorganisms in particular conditions, microbial presence or absence tests. These isolated results do not allow us to compare quantitatively what happens when the levels of the independent preservation factors are changed. Neither can the sensitivity of the key microorganisms to the different factors be inferred (Alzamora et al. 2010). There are no systematic in vivo studies that analyze the response of microorganisms to different doses of a stressor employed alone or in combination and/or the influence of critical extrinsic and intrinsic parameters. In the case of novel inactivation technologies, it has also been found that the principle of equi-effectiveness of the product of stressor intensity and exposure time is not always valid and the germicidal efficiency would depend on stressor intensity at similar doses (Gómez-López et al. 2007; Raffellini et al. 2011).

Studies that consider the effect that combined antimicrobial strategies inflict on the population and type of native microorganisms, and their dynamic and influence on pathogenic organisms along storage are rarely found in scientific literature. According to reported findings, mild processing combined stressors could extend the shelf-life, increase the destruction of inoculated pathogens without increasing the shelf-life, or increase both the safety and the shelf-life of the fruit product (Ferrario et al. 2011). Therefore, survival studies and spoilage studies during different storage conditions are of high interest for evaluating the shelf-life of these products.

Studies documenting the impact of mild preservation techniques on structure and quality attributes of fruits generally only consider the effect of processing per se, but major changes in quality attributes occur during storage (Gómez et al. 2010, 2011b; Welti-Chanes et al. 2009). It is also important to mark that interventions for sensory and functional stability used along with combined stressors in the preservation technologies could also impair microbial inactivation. Gómez et al. (2010, 2011b) reported that microbial inactivation by UV-C or PL decreased when cut-apples were previously immersed into an antibrowning calcium chloride–ascorbic acid aqueous solution.

Finally, physiology and kinetics studies to select preservation factors have focused on microbial cells in aqueous planktonic phase or in fruit pieces just inoculated, but association of microorganisms with surface forming biofilms is the pre-

vailing microbial lifestyle and planktonic cell studies constitute a biased view of microbial life (Lindsay and von Holy 2006). Biofilm cells in fruit surfaces or microorganism aggregates in juices are immensely heterogeneous and much more resistant to stressors than the planktonic, freely suspended cells. This fact highlights the importance of performing experiments with attached cells for evaluating the effectiveness of the combined hurdles.

5.5 Future Trends

Hurdle concept-based techniques offer many advantages in meeting consumer and industry demands of freshness, safety, and/or convenience. However, the cumulative knowledge base should be increased. Key points for their design and commercialization include a more deep knowledge on mechanisms of stressors mode of action, the availability and interpretation of systematic kinetic data on microbial and quality attributes behavior (with special relevance to dose response and the influence of critical parameters), and the optimization of equipment. Only in this way it would be possible to address the scientific bases for: (a) comparing the relative level of protection afforded by different combinations of stressors/critical process parameters; (b) selecting the best combined strategy that ensures high quality and safe fruit products; and (c) establishing food safety objectives and performance and process criteria that expedite the commercialization of the fruit products (Stewart et al. 2002).

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