# Hurdle Technology in Fruit Processing

Paula Luisina Gómez,[1] Jorge Welti-Chanes,[2] and Stella Maris Alzamora[3]

[1]CONICET-Universidad de Buenos Aires, Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina; email: gzpaula@gmail.com

[2]Instituto Tecnológico y de Estudios Superiores de Monterrey, División de Biotecnología y Alimentos, Monterey, México; email: jwelti@itesm.mx

[3]Universidad de Buenos Aires, Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Buenos Aires, Argentina; email: smalzamora@gmail.com

Annu. Rev. Food Sci. Technol. 2011. 2:447–65

First published online as a Review in Advance on January 3, 2011

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi:
10.1146/annurev-food-022510-133619



1941-1413/11/0410-0447$20.00

## Keywords

combined methods, emerging factors, fruit, juice, minimally processed

## Abstract

Conventional preservation technologies such as thermal processing ensure the safety and shelf life of fruit-derived products but can result in the loss of physicochemical and nutritional quality attributes. This review examines innovative hurdle techniques to obtain novel fruit products with fresh-like characteristics. The multifactorial processes were based on emerging preservation factors in combination or combining emerging factors with traditional ones. Selected practical examples of fruit processing using UV light, pulsed light (PL), ultrasound (US), and high hydrostatic pressure (HHP) are presented. Some issues of key importance for the design of combination processes are also addressed.

## INTRODUCTION

**MPF:** minimally processed fruits

**HHP:** high hydrostatic pressure

**PEFs:** pulsed electric fields

**US:** ultrasound

**PL:** pulsed light

**Hurdle:** preservation factor or stress parameter

In response to consumer expectations, researchers in the food industry, the academy, and government institutions have explored in the past two decades milder fruit-preservation techniques with better retention of product flavor, texture, color, and nutrient content than comparable conventional treatments. Consumer trends toward fresh food on one side and convenience on the other side often conflict. In most cases, fresh quality is negatively affected by the processing procedure. The most crucial challenge is to retain the natural functional properties and the sensory and nutritional quality of fruits with the appropriate shelf life and safety. In addition, the potential for microbiological contamination of fresh fruits is high because of the wide variety of conditions to which produce is exposed during growth, harvest, and distribution. Over the past several years, the detection of outbreaks of foodborne illnesses associated with fresh fruits and fruit juices has increased, and produce has been identified as an area of food safety concern. Recent outbreaks of *Escherichia coli* 0157:H7 and *Salmonella* spp. in apple and orange juices have challenged the belief that high acid foods cannot harbor viable pathogenic bacteria. For this reason, raw fruits cannot be excluded from the application of any of the modern tools that prevail in today's food industry for ensuring safety of produce. Sanitation of whole fruit is conducted generally with an initial washing in tap water to eliminate pesticide residues, dirt, and plant debris, followed by a dip in chlorinated water to effectively reduce the microbial loads on the fruit surface. However, alternative decontamination methods are being investigated because of the association of chlorine with the formation of carcinogenic chlorinated compounds and its low effectiveness in reducing microorganism population in surface of fruits as well as the growing consumer refusal of chemical additives (Beuchat 2000).

The trends for minimally processed fruits (MPF) come with three approaches being investigated. The first one is the optimization of traditional preservation methods to enhance sensorial, nutritional, and microbiological quality of fruits, yield, and energy efficiency (i.e., radiofrequency heating, cryogenic freezing, vacuum dehydration). The second approach refers to the development of mild processes by novel combinations of traditional physical and chemical preservation factors, each one applied at low intensity, to obtain products with quality attributes reminiscent of the fresh or native state of a given fruit but with a longer shelf life (i.e., modified/controlled atmosphere packaging, active packaging techniques). Finally, the last approach places the interest on the development of innovative techniques to obtain novel fruit products with fresh-like quality attributes by using emerging preservation factors [e.g., nonthermal physical agents such as high hydrostatic pressure (HHP), pulsed electric fields (PEFs), ultrasound (US), pulsed light (PL), and UV light, and natural antimicrobials, among others] in combination or otherwise combining emerging factors with traditional ones, all of them applied at low doses. In fact, the novel alternative physical agents, intensely investigated in the past two decades, can cause inactivation of microorganisms at ambient or sublethal temperatures, avoiding the deleterious effects that severe heating has on quality.These approaches to obtaining fruit products of higher perceived quality face different limitations and possibilities for the design, optimization, and experimental assessment as well as for validation of process conditions. In particular, the classical hurdle concept introduced by Leitsner and colleagues (Leistner & Rödel 1976) and also deeply investigated by Gould (Gould & Jones 1989, Gould 1995, Gould et al. 1995) is the basis of the mild processes for fruit preservation found in the last two approaches. In the second group of techniques, an important sector of the MPF comprises chilled products that rely either exclusively or primarily on cold storage for preservation (i.e. raw fruits; low-risk raw and uncooked ingredients such as fruit salads). A major concern over the microbiological safety of these foods is improper refrigeration (accidental due to mechanical failure or intentional to save energy costs) during manufacture,

distribution, retail sale, and at home. On the other side, many of the emerging inactivation agents are effective in destroying vegetative cells of bacteria, yeasts, and filamentous fungi, but spores of bacteria and molds are resistant to these factors. Thus, chilling and emerging preservation procedures have to be included as components or hurdles in combined preservation systems to assure food safety.

Microorganisms have evolved different mechanisms to resist the adverse effects of environmental stresses. As internal media stability (composition and volume of fluids) is vital for the 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 & Gould 2002, Gould 1995). For instance, when the water activity ($a_w$) of the medium is reduced, vegetative microorganisms lose water to come rapidly into osmotic equilibrium with the surroundings. Depending on water loss extent, metabolism is reduced or prevented and growth ceases. A universal and major response of vegetative cells to osmotic stress, often referred to as osmoregulation or osmoadaptation, is the accumulation, by synthesis and/or by active transport, of low molecular weight solutes in their cytoplasm at concentrations sufficient to just exceed the osmolality of the external medium. In this way, the cells regain water by osmosis and maintain the turgor in the membrane that is essential for its proper functioning. Reduced $a_w$ causes an increase in maintenance metabolism and a reduction in yield and growth rate because of the energy input necessary for the accumulation process. If the osmoregulatory capacity of the cell is exceeded (by a severe reduction in $a_w$), the cell ceases growth. Another example is pH homeostasis. 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 the 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. Major adaptive mechanisms to regulate the cytoplasmic pH are the energy-dependent proton extrusion, which acts to keep the cytoplasmic pH higher than that of the environment, and the extrusion of the organic acid (Booth & Kroll 1989, Leistner & Gould 2002). When the microorganisms' capacity for generating energy is not enough to prevent the net proton influx, the cytoplasmic pH falls, growth ceases, and the cells may die. Also, vegetative microorganisms react homeostatically to lowered and raised growth temperatures (by altering the composition of membrane lipids) and to ultraviolet (UV) radiation (by repairing the damaged DNA).

Homeostatic mechanisms that vegetative cells have evolved in order to survive extreme environmental stresses are energy dependent and allow microorganisms to continue functioning. In contrast, homeostasis in spores is passive, acting 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, HHP, ultrasonication, and other hostile factors. Preservation procedures are effective when they overcome, temporarily 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 is killed, ceases growth, or grows at a reduced rate. 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 & 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) is obtained if small stresses with different targets (multitarget preservation), instead of small

**Stress:** an applied state caused by deviation from normal conditions in the environment that generates changes in normal patterns of metabolism, imposing either reduced growth or survival potential

**Homeostasis:** tendency to uniformity or stability in the internal environment of the organisms

**UV:** ultraviolet

**Multitarget preservation:** disturbance of microorganism homeostasis by exposure to various sublethal stresses (simultaneous or sequential) that act on different cell targets

stresses with the same target (i.e., additive effect of hurdles), are selected to inhibit microorganisms' growth. For example, for vegetative cells (where homeostasis is energy dependent), the goal is to reduce the availability of energy (e.g., 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 preservation factors) and/or increasing the intensity of a particular sublethal hurdle on a microbial cell increases the expenditure of energy, and so more energy is diverted from the normal biosynthetic activities of growing cells, resulting in metabolic exhaustion and death (Leistner & 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 because the microbiological status of such fruits improves with storage time. Examples of this phenomenon have been reported by Latin American researches in studies of autostable high-moisture fruit products preserved by a combination of factors (slight thermal treatment, pH, $a_w$, sorbate, sulphite): The population of bacteria, yeasts, and molds that survive the mild thermal treatment decreased quickly during unrefrigerated storage (Alzamora et al. 1995, Tapia de Daza et al. 1996). Sublethal treatment also results in an increased sensitivity to adverse environmental factors, such as the 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 structures of the organism), the goal is to damage key structures (by chemical, enzymic, or physical attack on coats, cortex, etc.) or to release spores from dormancy (initiating germination with natural germinants or with false triggers, or applying high pressures) (Gould & Jones 1989).

**Metabolic exhaustion:** autosterilization, the vegetative microorganisms completely use up their energy for repairing their homeostasis, become metabolically exhausted, and die

**Sublethally damaged cells:** surviving cells that have been injured by a mild (nonlethal) stress and show an increased sensibility to adverse environmental conditions

In the past 20 years, the popularity of the hurdle concept has dramatically increased, and numerous publications have now indicated its potential for the development of MPF and/or the improvement of fresh fruit safety. Hurdle technology can be applied in many ways: (*a*) at various stages of the fruit distribution chain, in storage, in processing, and/or in packaging as a back-up measure in existing MPF products with short shelf lives, in order to diminish the risks and/or increase their shelf lives (26); (*b*) as an important tool for improving quality of long shelf life fruit products without sacrificing their microbiological stability; or (*c*) as a new preservation procedure deliberately intended for obtaining novel MPF.

The present review focuses on the application of combined technologies (already used industrially or still in development or testing) based on the third approach for obtaining MPF or improving the microbiological safety of fruit raw materials and final products. Examples of application involving inactivation of microorganisms by UV light, PL, US, and HHP are given. Some issues that should be addressed in setting criteria for a successful design of the MPF process are also reviewed.

This review does not pretend to cover the enormous amount of work conducted during the past few years in hurdle technologies for processing fruits and fruit juices (for further references, see Alzamora et al. 2000, Leistner & Gould 2002, Raso & Barbosa-Cánovas 2003, Ross et al. 2003, Raso et al. 2005, Allende et al. 2006, Rico et al. 2007, and Raybaudi-Massilia et al. 2009).

## NONTHERMAL EMERGING PRESERVATION FACTORS AND HURDLE TECHNOLOGIES

**Table 1** summarizes the mode of action, the critical parameters (variables of control) of the process, and the advantages and disadvantages of HHP, UV light, PL, and US, as well as their application for fruit preservation and some hurdles considered for the design of combined techniques.

## Short-Wave UV Light

**UV-C:** short-wave UV light

The maximum lethal effect of short-wave UV light (UV-C) has been reported in the range of 250–260 nm, inactivating bacteria, virus, protozoa, fungi, and algae (Shama 2006). Although UV-C radiation can be strongly absorbed by different cellular components, the most severe cell damage occurs when nucleic acids absorb UV-C light, crossing DNA pyrimidine bases of cytosine and thymine to form crosslinks and impairing formation of hydrogen bonds with a purine base pair on the complementary strand of DNA (Shama 2006). Cellular death occurs after the threshold of crosslinked DNA molecules is exceeded. The mutation can be reverted by dark and/or enzymatic mechanisms, and this depends on the repair systems of each microorganism. However, flow cytometry analysis demonstrated that targets other than DNA could account for UV-C inactivation. UV-C radiation also produces significant damage in the cytoplasmic membrane integrity and cellular enzyme activity (Schenk et al. 2011). Exposure to low doses of UV-C light has also been shown to elicit a range of chemical responses in fresh produce ranging from antifungal enzymes to phytoalexins (Shama 2007). This beneficial plant response of agricultural produce, called hormesis, results from the application of a low dose of a stressor (in this case UV-C irradiation). Hormetic effects to inhibit fungal pathogens and delay ripening occur after UV-C irradiation at periods of time ranging from hours to days. Hormesis is quite distinct from surface disinfection, occurs throughout the entire fruit, and may even be considered as an additive to it (Shama 2006). Direct inactivation by UV-C of surface-associated microorganisms is limited solely to the surface of the fruit, as UV-C has extremely low penetration into solids but inactivation of this kind can occur at the dose levels used to induce hormesis (0.5 to 9 kJ $m^{-2}$ for optimal effects according to the type of fruit) (Shama & Alderson 2005). Both direct and induced inactivation effects are not easily distinguished in the literature.

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 & Artés 2003, Yaun et al. 2004, Fonseca & Rushing 2006) combined with posterior chilling or modified atmosphere packaging (MAP) to preserve quality.

Doses of UV-C radiation 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 with controls after 7 d of storage at 3°C (Fonseca & Rushing 2006).

UV-C was investigated by Schenk et al. (2008) for its microbicidal effects on pear slices with and without peel. Semilogarithmic survival curves of inoculated *Listeria monocytogenes*, *Listeria innocua*, *Zygosaccharomyces bailii*, and *Debaryomices hansenii* showed upward concavity and pronounced tailing effect, indicating that the majority of the organisms were destroyed in a short time during UV-C exposure, whereas a fraction of the population survived after the treatment. Similar inactivation patterns were reported for other microorganisms inoculated on the surface of different fresh fruits and vegetables (e.g., tomatoes, apples, and lettuce) (Yaun et al. 2004, Gómez et al. 2010). This well-defined tail to the inactivation data was attributed to the heterogeneity in the resistances of the population to UV-C irradiation and the shielding or physical protection of microorganisms on the solid surface from incident UV-C (effect of surface topography).

Gómez et al. (2010) analyzed the effect of UV-C radiaton at different doses (with or without dipping into an antibrowning solution) followed by storage at 5°C on native flora of cut apple as well as of surface-inoculated microorganisms (*L. innocua*, *Escherichia coli*, *and Saccharomyces cerevisiae*). The log reduction ranges of inoculated population varied between 1.0 and 1.9 log cycles depending on the UV-C dose (5–14 kJ $m^{-2}$), the type of microorganism, and the apple pretreatment. During subsequent storage at 5°C, counts of inoculated bacteria maintained nearly

**Table 1 Selected nonthermal emerging preservation factors**

| Factor and mechanism of inactivation | Critical parameters | Advantages | Limitations and drawbacks | Potential application/ products on the market | Hurdles investigated in combination |
|---|---|---|---|---|---|
| **High hydrostatic pressure** | | | | | |
| Application of 100–800 MPa, below 0°C to 100°C, from seconds to about 20 min, instantaneously and uniformly throughout food, independent of size, shape, and food composition. Mechanism: membrane damage, protein denaturation, leakage of cell contents, dissociation of ribosomes. | Temperature, pressure magnitude, rate of compression and decompression, holding time at pressure, time to achieve treatment pressure, composition, pH and $a_w$ of the food, product initial temperature, and critical parameters of procedures used in combination. | Inactivation of some enzymes according to HHP dose. Little change on 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 smoothies, 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** | | | | | |
| 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 | Transmissivity of the material, homogeneity of the flow pattern and the radiation field, UV wavelength, thickness of the radiation path through the food (geometric configuration of the system); product composition, solids content, and critical parameters of procedures used in combination. | 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 increases. | 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, modified atmosphere packaging (MAP), mild thermal treatment, ultrasound (US). |

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|---|---|---|---|---|---|
| **Pulsed light** | | | | | |
| Few flashes applied in a fraction of a second of intense pulses of broad spectrum light (ultraviolet to the near infrared region).<br>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. | Light characteristics (spectrum, intensity, duration, and number of pulses), homogeneity of the flow pattern and the radiation field, packaging and type, transparency and color of food, and critical parameters of procedures used in combination. | Very short treatment times (≤60s).<br>Little effect on color, texture, antioxidant, and sensory properties at low doses, although reports on the subject are few. | Low penetration into solids and opaque juices.<br>Engineering solutions needed for juice treatment.<br>Thermal damage of product at high doses.<br>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, Ultraviolet (UV), Mild thermal treatment. |
| **Ultrasound** | | | | | |
| Energy generated by sound waves of 20 kHz or more.<br>Mechanism: disruption of cellular structures (wall, membranes, organelles) and cell lysis attributed to cavitation. | Power and amplitude of ultrasonic waves, exposure time, volume, and composition of the food to be processed, temperature of treatment and critical parameters of procedures used in combination. | Inactivation of enzymes when US is combined with heat and pressure.<br>Little change in color of juices and cut fruits. | High energy consumption, intensity of industrial-scale equipments limited, long treatment times.<br>Heating of the product.<br>Undesirable sensory changes and rupture of skin in berries at high doses. | No commercial fruit products; suggested for juice pasteurization.<br>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, pulsed electric fields (PEFs). |

constant levels in apples treated by UV-C, with or without dipping pretreatment, and were lower than those of untreated controls. Microflora counts were higher in UV-C untreated samples than in UV-C treated samples along the whole storage, meaning the shelf life of the product would be prolonged from the microbiological point of view.

Combination of UV-C with mild heat treatment (sequential hurdles) has also been suggested for controlling postharvest decay of berries (strawberries and sweet cherries) stored at room temperature (Marquenie et al. 2002, Pan et al. 2004). For instance, previous irradiation with UV-C (4.1kJ $m^{-2}$) 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).

UV-C illumination has also been focused on the treatment of liquid foods and beverages (Koutchma 2009) since the U.S. Food and Drug Administration (FDA) approved in 2000 the use of UV-C light as a novel technology for pasteurization of fruit juices. UV-C can be effectively used to reduce the number of spoilage and pathogenic bacteria, yeasts, and molds in different kinds of fruit juices, without affecting in a severe way color profiles, vitamin C content, and taste (Tran & Farid 2004, Keyser et al. 2008). The combination of UV-C treatment and low temperature storage allowed a shelf life extension from approximately two days to more than five days. However, the use of UV-C is still limited because of 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. Major researches efforts are now concentrated in UV-C reactor design to ensure effective radiation penetration (e.g., thin film, turbulent, laminar Taylor-Couette, Dean flow reactors) (Koutchma 2009).

## Pulsed Light

PL involves the use of intense and short-duration (1 μs to 0.1 s) pulses of broad spectrum light of a wavelength ranging from UV to near-infrared (200 to 1,100 nm). The mechanisms responsible for microbial inactivation are still in debate. A major contribution to inactivation appears to be provided by the rich UV content from 220 to 290 nm in the UV spectrum. The primary mode of action is identical to that of UV-C radiation, but in addition to UV-C-induced photochemical changes, the high peak power and the photothermal effects caused by visible and near-infrared portions of PL spectrum seem to be involved (FDA 2000, Woodling and Moraru 2007, Gómez-López et al. 2007). Proteinaceous or fatty foods have been reported to be inappropriate for decontamination by PL because these components reduce the killing efficiency of PL. Foods poor in fats and proteins, such as fruits and vegetables, appear to be very suitable for it (Gómez-López et al. 2005).

The literature on hurdles in combination with PL is scarce. Combined methods have been devoted mainly to decontamination of fruit surfaces using PL alone or in combination with other inactivation factors, and then the produce was immediately packaged and stored in refrigeration to decrease/inhibit the growth rate of sublethally injured cells and other surviving microorganisms (simultaneous and sequential hurdles), focusing on an extension of shelf life and/or on increased fruit safety.

The application of PL (30 μs, 15 Hz, 40–250 s) alone or in combination with UV-C (0.5 or 1.0 kJ $m^{-2}$) or a thermal treatment (40 or 45°C, 3 or 15 min) to reduce the fungal development on strawberries inoculated with conidia of *Botrytis cinerea* and stored at 12°C for 10 d was investigated by Marquenie et al. (2003). When PL was used alone, no delay in fungal development was observed. Combining PL with UV-C illumination had no effect on fungal development at the end of the 10 d storage period, but the most severe conditions assayed (1.0 kJ $m^{-2}$, 120 s PL) delayed the appearance of mycelium on the fruit for one day. A combination of 120 s PL and 15 min-40°C thermal treatment resulted in a two day delay of fungal spoilage. The use of mild thermal treatments is important because external damage and softening of the fruit were found at 45°C.

Regarding fruit juices, Sauer & Moraru (2009) analyzed the effect of LP treatment (up to 12 pulses, 3 pulses per s, 360 μs, 0–13 J $cm^{-2}$) on the inactivation of *E. coli* inoculated in liquids with different levels of clarity [Buttersfield's phosphate buffer (BPB), tryptic soy broth (TSB) apple juice, and apple cider]. The different levels of PL effectiveness (for static treatment >8 log in BPB, ~3.5 log in TSB, ~2.6 log in apple juice, and 2.3–3.2 log in apple cider) were attributed to the absorption, reflection, and scattering of light by the substrate. However, it is possible to decrease the shading effects (reflection and scattering) caused by the particulates in the no-clear liquid substrates (TSB, apple juice, and apple cider) by performing the inactivation treatment under turbulence. Turbulent treatments resulted in 5.8- and 7.1-log reduction in cider and juice, respectively. This result is relevant for the potential commercial application of PL for pasteurization of apple juice or apple cider according to FDA requirements of a 5-log reduction in the numbers of the most resistant pathogen.



## High-Power Ultrasound

Injury or disrupting microorganisms by high-energy US (i.e., intensities higher than 1 W $cm^{-2}$ and frequencies between 18 and 100 kHz) is widely attributed to cavitation, i.e., the rupture of liquids when applying high-intensity US and the effects produced by the motion of the cavities or bubbles thus generated (Lauterborn et al. 1999). In the so-called stable cavitation, the bubbles can undergo relatively stable, low energy oscillations, provoking the liquid in the vicinity of the bubble flows or streams (microstreaming effect). This microstreaming could shear and disrupt cellular membranes or break cells. In the transient or inertial cavitation, small bubbles expand rapidly, often to many times their original size, and on the positive pressure half-cycle, collapse violently, breaking up into many smaller bubbles, resulting in shock waves with very high energy densities and short flashes of light that shear and break cell walls and membrane structures and also depolymerize large molecules. Recent transmission electron microscopy and flow cytometry studies of yeast, and Gram-negative and Gram-positive bacteria have demonstrated that (*a*) microbial cells contain several targets for the disruptive action of US (at least the cell wall, the cytoplasmic membrane, the DNA, the internal cell structure, and the outer membrane); (*b*) cytoplasmic membranes do not appear to be the primary target of US for *S. cerevisiae*, *E. coli*, and *Lactobacillus* spp.; and (*c*) the primary target depends on the specific microorganism (for instance, the outer membrane in *E. coli*) (Ananta et al. 2005, Alzamora et al. 2010).

Several reports have indicated that sonication applied alone (at room temperature and atmospheric pressure) is not very effective in inactivating microorganisms. However, the combination of US 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). The efficiency of US has been demonstrated to be improved when applied in combination with heat and/or pressure, chemicals often used for sanitation and disinfection (e.g., synthetic or natural antimicrobials), and other physical preservation factors (e.g., UV-C light, PEFs) (Alzamora et al. 2010).

Thermo-ultrasonic treatment caused a higher killing effect than sonication treatment alone. If pressure changes that occur during cavitation are responsible for the inactivation effect of US, then raising the temperature and hence membrane fluidity (i.e., weakening the intermolecular forces) would enhance the disruption (Russell 2002). However, as temperature increases toward lethal values, the benefits of US 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 with *L. monocytogenes* 10403S, an US 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,

**TS:** thermosonication

**CIELAB:** color space specified by the International Commission on Illumination

750 W, 99 ml sample) (Baumann et al. 2005). US increased the inactivation rate at both lethal and sublethal temperatures. The bactericidal effect of the combined process was additive. After a 5-min thermo-ultrasonic treatment at 60°C, cells of *L. monocytogenes* 10403S died during a 6-h period at room temperature. These treatment conditions could provide a solution for apple cider industries to achieve the required 5-log reduction in pathogenic populations.

Phenolic compounds have lipophilic nature and could accumulate in the lipid bilayer of the cell, disturbing and sensitizing the membrane to US (Brul & Coote 1999). In addition, ultrasonic waves improve the antimicrobial action by weakening the cell wall. Improved efficiency achieved by the combination of vanillin, citral, US, and moderate temperature was demonstrated by Ferrante et al. (2007). The addition of 1,000 ppm vanillin followed by sonication (600 W, 20kHz, 95 μm, 45°C) slightly improved *L. monocytogenes* inactivation observed in orange juice when single treatments were applied, achieving 1.8-log cycle reduction after 15-min exposure. The addition of a small proportion of citral (75 ppm) notoriously increased *L. monocytogenes* inactivation (>5 log reductions after 10-min treatment). Both vanillin and citral would alter microbial membrane permeability, facilitating the loss of specific ions from the interior affecting proton motive force and reducing the intracellular ATP content and the overall activity of microbial cells (Conner & Beuchat 1984). Ternary combination (citral plus vanillin plus sonication) at moderate temperature highly reduced times of exposure to US in order to reach a determined inactivation.

The effect of thermosonication (TS) followed by PEF on inactivation of *Staphylococcus aureus* and selected quality aspects in orange juice was investigated by Walkling-Ribeiro et al. (2009). TS for 10 min at 55°C with PEF at 40 kV $cm^{-1}$ for 150 μs resulted in an overall bacterial reduction of 6.8 log cycles, similar to the cycle reduction obtained by conventional thermal pasteurization (94°C for 26 s). An additive effect on microbial inactivation between TS (1.8 log) and PEF (5.5 log) was detected. TS/PEF did not affect the pH, conductivity, or °Brix and had a milder impact on juice color than thermal treatment. Thermal treatment caused an overall darkening of orange juice. By contrast, when components of the CIELAB space were recorded,TS showed a significant increase in $L^*$ (lightness or luminance), a minor decrease in $a^*$ (chromaticity on a green (-) to red (+) axis), and increase in $b^*$ (chromaticity on a blue (-) to yellow (+) axis). In conclusion, the TS/PEF hurdle approach showed great potential for improving the quality and the safety of orange juice.

Lopez-Malo et al. (2006) analyzed the response of *L. monocytogenes* and *S. cerevisiae* to the single and combined effects of high-intensity US (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 inactivation pattern of individual and combined treatments was highly dependent on the type of microorganism. In spite of the presence of organic compounds and colored compounds that reduced the efficiency in UV-C disinfection, the effect of the US/UV-C combination was additive and led to sizeable inactivation (~4–5 log cycles reduction after 5-min treatment), with the majority of the population dead in the first minutes of treatment. The different survival patterns could be described in terms of Weibull distributions, considering that there was a spectrum of resistances to the treatments in the population (Peleg & Cole 1998). **Figure 1** illustrates survival data of *L. monocytogenes* and *S. cerevisiae* after having undergone US, UV-C, and US/UV-C treatments using the cumulative Weibull distribution function. The correspondent frequency distributions of resistances (not shown) show that the combined action of US/UV-C light not only increases the microbicidal effect of sonication but changes 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 usually obtained.

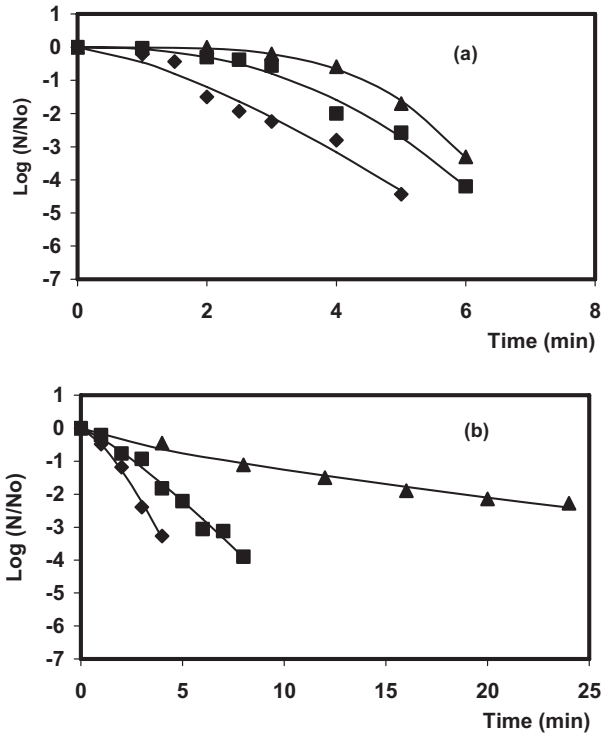


**Figure 1**

(*a*) Semilogarithmic survival curves of *Listeria monocytogenes* and (*b*) *Saccharomyces cerevisiae* in clarified apple juice. Experimental values: ▲, US treatment; ■, UV-C light treatment; ♦, combined UV-C/US treatment. Lines: fitted values by Weibullian model. No, initial number of microorganisms; N, number of microorganisms at time t, CFU $ml^{-1}$.

## High Hydrostatic Pressure

The effects of pressure are multitargeted and depend on pressure level. HHP causes a perturbation of the membrane, inhibits the synthesis of some membrane proteins, dissociates ribosomes, and brings changes in the quaternary structure of proteins (Smelt et al. 2002). HHP affects enzymes, and there is an optimum temperature range at which proteins are more resistant to pressure. Yeasts, molds, and vegetative cells of bacteria can be inactivated by pressures in the range of 200–700 MPa near room temperature. Vegetative cells of bacteria become more susceptible to pressure at low pH, and bacteria surviving pressure treatment become more sensitive to suboptimal pH after processing. According to this mechanistic background, stress factors such as temperatures below or above room temperature during processing, natural and synthetic antimicrobials, and pH have been mainly considered in combined strategies.

HHP is gaining popularity in the fruit industry because of its ability to destroy microorganisms and to reduce significantly 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, but the presence of HHP-resistant browning enzymes requires an antibrowning treatment (Cano & de Anco 2005). As an example, best quality retention of strawberries was obtained when HHP was combined with

vacuum packaging and refrigerated storage because polyphenol oxidase (PPO) is highly resistant to high-pressure inactivation (Terefe et al. 2009).

**PPO:** polyphenol oxidase

**PME:** pectinmethylesterase

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 150 MPa-30 min and 0.75 mg $ml^{-1}$ 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).

In juice, control of pectinmethylesterase (PME) is crucial to assure cloud stability because 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 pressure-stable and pressure treatments are usually combined with mild heating to obtain high quality juice or fruit (Balog et al. 2004). For example, a synergistic effect of HHP and 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. Buckow et al. (2009) also reported a synergism between pressure and temperature on the inactivation of apple PPO above 300 MPa and antagonism at lower pressures.

Inactivation of enzymes was reported to be dependent on medium. Balog et al. (2004) studied the high-pressure inactivation of PME in carrot juice and carrot pieces. PME added to carrot juice at 700 MPa and 10°C was inactivated at a similar rate as in situ PME at 750 MPa and 40°C. This remarks on the importance of making resistance studies in whole cells and not in isolation.

## ISSUES OF CONCERN FOR DESIGNING HURDLE PRESERVATION TECHNIQUES

### Microbial Response

One important issue is the knowledge of the mode-of-action of the preservation factor(s) and the microorganisms' response. Microorganisms sometimes react or adapt to mild stress factors by evolving signal transduction systems, which in response to environmental stresses control the coordinated expression of genes involved in cellular defense mechanisms, repairing the damages and becoming even more resistant, surviving more severe homologous or heterologous stresses (global stress response) (Gould et al. 1995, Abee & Wouters 1999). After adaptation to mild stresses, cells behave differently from unadapted ones and can grow at values outside the traditionally known ranges of temperature, water activity, and pH determined under optimal conditions, or show greater resistance to inactivation agents. When developing hurdle technologies, it is crucial that resistance development is avoided. Microbiological challenge testing to assess the risk of food poisoning or to establish MP product stability needs careful design, and stressed known or potential pathogens would also be selected to validate the process.

To come to a knowledge-based rather than a mainly empirical combination of appropriate hurdles, modern tools in biology, such as genomics, protein expression data, and metabolic pattern recognition, can bring additional insights in mode-of-action of preservation factors (Brul & Coote 1999, Brul et al. 2002). The integration of these data can result in a clear understanding of the total response of cells toward their environment, allowing specific targets to be identified and collaborating in the development of extrapolable mechanistic models on microbial behavior. For example, construction of the *S. cerevisiae* cell wall is a tightly regulated process involving approximately 1,200 genes. The cell continually adapts its wall organization, as well as the proteins

at the cell surface, to changing environmental stimuli. Thus, cell wall–assembling enzymes and enzymes involved in remodeling of cell wall polymers could be potential targets for new antifungal compounds (Klis et al. 2002).

**Food preservation:** temporary or permanent disturbance of microorganism homeostasis by preservation factor(s) or hurdle(s)

## Characteristics of the Hurdle Interaction

The rational selection of hurdles in terms of type and intensity should result in synergistic or at least additive interaction. It is not easy to anticipate synergistic, additive, or antagonistic activity. When combining antimicrobials, for example, the effects had been reported to depend not only on the type of stress factors, but on the composition of the food matrix and the storage period (Alzamora et al. 2003). At pH 3.5, combinations of vanillin and potassium sorbate used to inhibit *Aspergillus flavus* growth initially appeared as synergistic and evolved to additive as incubation time increased. At pH 4.5, however, the interaction was antagonistic for all incubation times analyzed. Quantification of the interaction must be realized in the real fruit and should consider the desired shelf life.

## Attached Versus Planktonic Cells

A biofilm is a community of microorganisms attached to a surface, biotic or abiotic, producing extracellular polymeric substance (EPS) and interacting with each other. Association of microorganisms with the surface is the prevailing microbial lifestyle, and planktonic cell studies constitute a biased view of microbial life (Lindsay & von Holy 2006). Biofilm cells in food surfaces or product contact surfaces are heterogeneous and much more resistant to preservation factors than the planktonic, freely suspended cells. Various mechanisms, such as the diffusion barrier to penetration of antimicrobial agents by the EPS, induction of resistant phenotypes, slow growth, and general stress response, have been proposed to account for this greater overall antimicrobial resistance.

Traditionally, physiology and kinetics studies to select preservation factors have focused on microbial cells in aqueous planktonic phase. However, such studies only have value as a preliminary screening, and experiments with attached cells should be carried out to evaluate the effectiveness of the combined techniques.

## Quantification of Microbial Behavior

Kinetic data of inactivation and growth are essential to select preservation factors and levels with a statistical sense to develop food preservation processes that ensure safety (McMeekin & Ross 2002). Predictive microbiology provides the tools to compare the impact of different environmental stress factors/levels on reduction or growth inhibition of microbial population and is an aid to understanding biological system behavior. Thus, if we can predict with accuracy the decay or growth kinetics for an identified target microorganism under multiple factors in combination, the selection of such factors can be made on a sound basis, and the selected preservation factors can be kept at their minimum doses. Sensory selection of hurdles and their levels may be done between several safe equivalent combinations of interactive effects determined by the models. The experimental design for obtaining quantitative data must involve a wide range of the factors in combination to obtain a comprehensive picture of the microbial response to the dose.

As an example, Raffellini (2009) studied and modeled the influence of sanitizer concentration (0 to 3.00% w/v), pH (3.0–7.2), temperature (12.5–50°C), and exposure time on the antimicrobial activity of $H_2O_2$ against planktonic cells of *E. coli* ATCC 35218. In general, more *E. coli* was

inactivated as the exposure temperature and the $H_2O_2$ concentration increased and the pH decreased. Traditionally, microbial inactivation has been considered a process that follows first-order kinetics. It has been implicitly assumed that all cells or spores have identical resistance to a lethal agent, and each microorganism has the same probability of dying. However, as in this case, the decrease of the population does not usually follow first-order kinetics (Peleg & Cole 1998). On the assumption that the individual microorganisms in a population do not have identical resistances and that microbial sensitivity to lethal agents is distributed, curvilinear (concave and convex), semilogarithmic survival curves can be modeled using the Weibull distribution. As microbial mortality is increased by lowering the organism's resistance to the treatment (e.g., when additive or synergistic combination of lethal agents are used and/or the severity of a lethal agent is increased), the mode and the mean of the distribution are lowered. From the point of view of preservation system design, the combinations of factors/levels to choose are those that also decrease the spread or variance of the distribution. **Figure 2*a*,*b*** illustrates the survival curves obtained at different



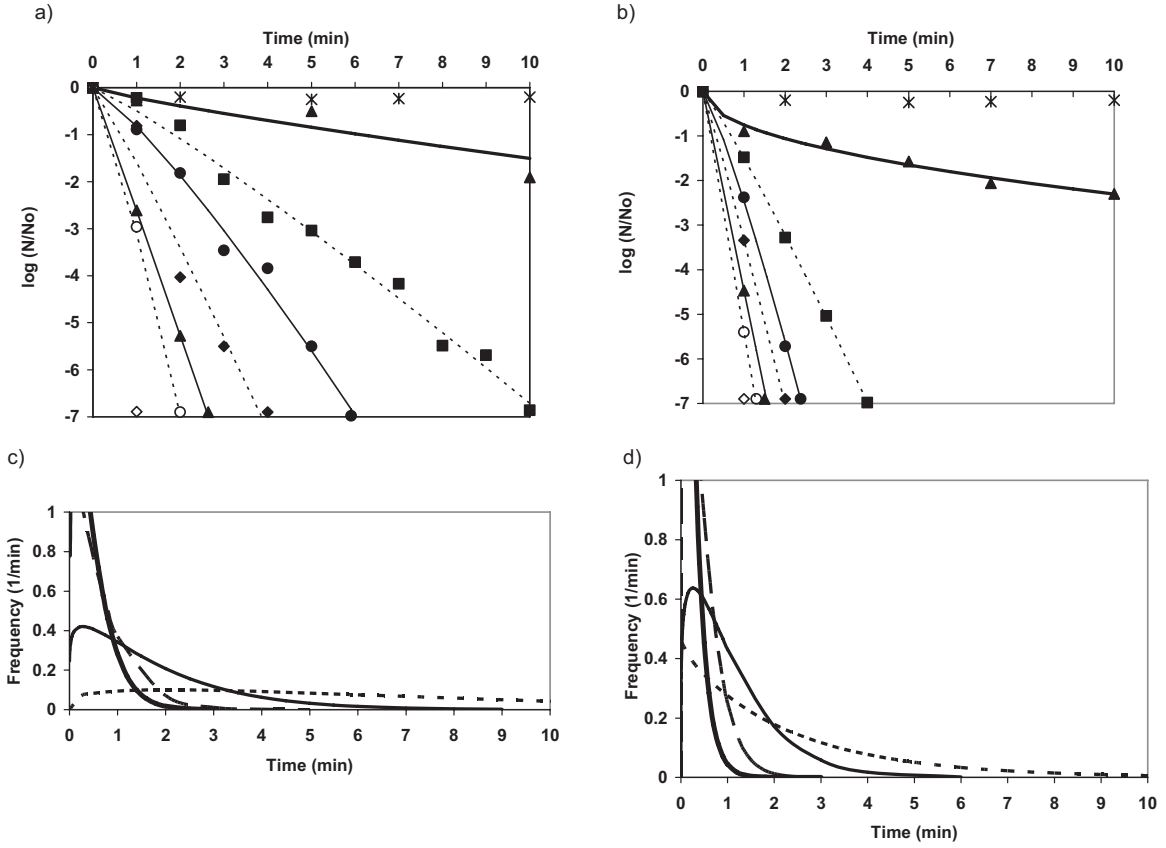


**Figure 2**

(*a*,*b*) Effect of $H_2O_2$ concentration and temperature on semilogarithmic survival curves of *Escherichia coli* at pH 5.8. Experimental (points) and fitted values derived from the Weibullian model (lines). Control (∗); 0.10% w/v $H_2O_2$ (▲); 0.50% w/v $H_2O_2$ (■);1.00% w/v $H_2O_2$ (●); 1.50% w/v $H_2O_2$ (♦); 2.00% w/v $H_2O_2$ (▲); 2.50% w/v $H_2O_2$ (O); 3.00% w/v $H_2O_2$ (◇); (*a*) 25.0°C; (*b*) 37.5°C. No, initial number of microorganisms; N, number of microorganisms at time t, CFU/ml). (*c*,*d*) Frequency distributions of resistances of *E. coli* obtained at different temperatures and $H_2O_2$ concentrations at pH 5.8: (- -) 12.5°C; (—) 25.0°C; (— — —) 37.5°C; (——) 50.0°C. (*c*) 0.50% w/v; (*d*) 1.00% w/v

sanitizer concentrations and temperatures (25.0°C and 37.5°C) at pH 5.8, as well as the frequency distributions of resistances at the four temperatures assayed for 0.50% w/v and 1.00% w/v $H_2O_2$. Survival patterns and frequency distribution profiles markedly changed with $H_2O_2$ concentration and temperature. At the less drastic combinations, frequency shapes with a considerable spread of data, heavy tails, and large mode, mean, and variance values meant that an important fraction of the microorganism population survived after these treatments (**Figure 2*c*,*d***). The greater the $H_2O_2$ concentration and the greater the temperature, the narrower the distribution and the lower the mean, the mode, and the variance values. Population was not only more sensitive on average, but it had a more uniform sensitivity to the treatment.

## Uniformity of the Process and Accessibility of the Preservation Agent to Microorganisms

The efficacy of a decontamination treatment is influenced by the accessibility of chemical and some physical agents to microorganisms. Many of the emerging techniques result in survival curves with a tailing effect. In surface decontamination, this shape of inactivation curves would indicate not only that a subpopulation of cells is more resistant to the treatment but probably irregularities of surface and internalization in fruit protect microorganisms from decontamination treatments (Gómez-López et al. 2008).

Process uniformity is another important factor that affects the effectiveness of the process and attempts with its successful commercialization (Heldman et al. 2008). For example, in continuous UV-C and PL processes, the distance and the relative position of the sample with respect to the Hg and Xe lamps influence significantly the received dose or fluence, as well as the increase in temperature with the PL dose (Gómez 2010).

## Impact on Structure, Quality, and Functionality of the Product

Combinations of preservation factors should allow the required level of protection against pathogenic or spoilage microorganisms to be achieved while at the same time retaining organoleptic quality and functional and nutritive value. Systematic studies documenting the impact of combination techniques on structure and quality attributes of fruits are scarce. Because of this, it is crucial to investigate not only the effect of the preservation factors at different doses after processing but also the influence along storage. For instance, an increase in surface browning was observed in UV-C-irradiated cut apples over 7 d of storage at 5°C as compared with nonirradiated fruit or fruit just after irradiation, mainly at the greatest UV-C dose assayed (Gómez et al. 2010). This color modification was attributed to increased enzymatic activity caused by UV-C-induced membrane breakage, with consequent loss of compartmentalization. These results indicate that UV-C light must be combined with a suitable antibrowning pretreatment to be used as a tool by the minimally processed fruit industry to reduce surface microbial load and avoid color deterioration. Welti-Chanes et al. (2009) treated just-squeezed orange juice by high-pressure homogeneization (50 to 250 MPa) at three initial temperatures (22°C, 35°C, and 45°C) to inactivate PME to avoid the loss of cloudy appearance. The higher the pressure and the higher the initial temperature, the higher was the PME inactivation. However, the PME activity increased throughout 12 d storage at 4°C, probably due to the rise of isoenzymes. Lower PME activation was observed in orange juice previously heated at 45°C and treated at 250 MPa. This combination of temperature and high pressure maintained cloudy appearance for 12 d after refrigeration.

## FUTURE ISSUES

1. Combining emerging technologies with conventional preservation technologies or with other novel techniques to interfere with the homeostatic mechanisms of microorganisms in fruits has been successfully explored in the last years. However, a more deep understanding of the combined techniques will be critical in obtaining safe and high quality fruit products.
2. New strategies and targets can arise from fundamental knowledge (e.g., physiological studies, omics research, multiparametric flow cytometry analysis) about the mechanisms of action of individual and combined factors and its integration with model development and process design. Relevant information is available in the scientific literature concerning factors/interaction of factors that influence microbial activities in foods, but it is seldom usable in formulating combined techniques and/or has low practical relevance.
3. Systematic studies documenting and modeling the dose response of native flora, inoculated microorganisms of concern, and quality attributes to single/combined factors need to be developed in the fruit matrix to support hurdles selection and their levels.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

The authors are grateful for the support from Universidad de Buenos Aires, CONICET and ANPCyT-BID from Argentina and from the Instituto Tecnológico y de Estudios Superiores de Monterrey and CONACyT from Mexico.

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Annual Review
of Food Science
and Technology

Volume 2, 2011

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**Errata**

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