Technology, Science, and Culture: A Global Vision, Volume II

Universidad de las Américas Puebla

IntechOpen

Chapter

Mild Intervention Technologies for Increasing Shelf Life and/or Safety of Fresh Fruits: Opportunity and Challenge

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Abstract

Postharvest diseases and senescence changes represent the most severe sources of loss of fruit production. Fruits are perishable products with active metabolism during postharvest period, which plays a major role in the senescence and affects commercial life. Many different species of fungi and bacteria are associated with fruits and contamination may occur during growing, harvesting, handling, and distribution, and while waiting to be processed. Fruits are also vehicles for transmission of infectious microorganisms. Foodborne illness outbreaks and cases associated with fresh and minimally processed fruits have been rising in the last two decades, both in developing countries as well as in the developed world. These issues lead to major economic losses and the industry is constantly seeking postharvest treatments to extend fruit shelf life while retaining its quality. This presentation is aimed to explore the application of some mild and environmental-friendly techniques (ozone, pulsed light, and ultraviolet light, among others), applied alone or in a hurdle approach, for improving the shelf life and safety of fruits and fruit products. Examples about the application of some tools to hurdle technology design for berries and other fruits are also given, evidencing opportunities and future challenges.

Keywords: fruit, mild preservation technologies, design, microorganisms

1. Introduction

Current scientific evidence shows that a high intake of fruit and vegetables reduces the risk of cardiovascular, ophthalmological, and gastrointestinal diseases, neurodegenerative disorders, some types of cancer, chronic obstructive pulmonary disease, and hypertension, among others. Concomitantly, consumption of fresh produce has largely increased in recent years in the world. Moreover, the consumption of at least 400 g of fruit and vegetables (five servings per day) has been recommended by the World Health Organization.

Fruits have a very limited postharvest life due to high metabolic rates and vulnerability to decay, traduced in rapid dehydration, loss of firmness, tissue degradation, and susceptibility to mechanical injury and color degradation. Fruits may differ in their composition and structure, which determine the kind of deterioration and how easily they can be attacked by microorganisms. The more acidic pH of most fruits and the presence of carbohydrates promote the deterioration due to the growth of molds, yeasts, and some acid-tolerant bacteria to a greater extent. Water is the major component of fruits, and fruit water activity (a_w) is determined by the nature and concentration of the 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. 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 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 [1]. Fruit contamination can occur either pre- (soil, feces, irrigation water, dust, insects, wild or domestic animals, reconstituted fungicides and insecticides, and manure and human handling) or postharvest (human handling, harvesting equipment, rinse water, dust, ice, transport vehicles, and processing equipment) [2, 3]. Moreover, pathogens have been shown to enter plant tissues through both natural apertures (stomata, flowers, and cracks of the cuticle) and damaged (wounds and cut surfaces) tissues, or they can be entrapped in crevices [4]. Common unit operations such as peeling, cutting, and slicing may damage tissues, which release nutrients and facilitate microorganisms' growth. In particular, postharvest internalization of pathogens via cut surfaces, which appeared to have long-persistence, and decontaminating agents used during minimal processing are unlikely to reach them. Most fresh fruits receive minimal processing and often are eaten raw without a pathogen "kill" step before consumption. Therefore, minimal processing is expected to result in an increased risk. In general, mild preservation treatments for obtaining fresh-like fruit products are less robust and need to be well controlled through adequate product and process design, as well as proper implementation and monitoring through the hazard analysis and critical control point (HACCP) [5].

These issues lead to major economic losses, and the industry is constantly seeking postharvest treatments to extend fruit shelf life while retaining its quality.

Current crop protection methods rely on horticultural practices, good agricultural practices (GAP), and synthetic conventional fungicide applications. However, these chemicals may not be the best solution because of the development of fungicide resistance, the risk to humans and environmental health, and the restrictions of governmental regulatory agencies, as well as the commercial requirements imposed by marketing chains for commodities with low number of residual pesticides [6]. Consequently, a number of alternative technologies rose up to replace historically proven synthetic fungicides. Research efforts have been focused on the following groups of treatments: microbial biocontrol agents [7], natural antimicrobials [6], disinfecting agents [8], and physical means [9, 10], as well as their combinations [11].

There is a wide range of modern agents that cause physical or chemical inactivation of microorganisms at ambient or sublethal temperatures. Some of these inactivation agents that are under research include high electric field pulses (PEF), high hydrostatic pressure (HHP), ultrasound (US), pulsed light (PL), shortwave ultraviolet light (UV-C), and ozone and hydrogen peroxide. These nonthermal factors are being encouraged for fruit preservation because, without the need for severe heating, they cause minimal damage to flavor, texture, and nutritional quality of some foods. Most of them are effective in inactivating vegetative cells of most microorganisms, but spores are far more tolerant. Thus, their applications are analogous to thermal pasteurization. It is likely that combining nonthermal agents or nonthermal agents with traditional preservation factors in a multi-hurdle preservation approach will control spoilage and foodborne microorganisms while reducing treatment intensities, detrimental effects on product quality, and energy input [12–14]. Combined preservation systems including emerging nonthermal agents are gaining commercial uses most quickly with fruit-derived products, probably due the low pH that naturally exists in this type of food materials, a hurdle that cooperates in an overall preservation strategy. On the other hand, acid adaptation of contaminant flora could adversely affect the microorganism resistance to these technologies, a fact that promotes the intelligent combination of them with other stressors or hurdles. Preservation procedures are effective when they overcome, temporally or permanently, the various homeostatic reactions that microorganisms have evolved in order to resist stresses, and 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 grow during stress [15]. When 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 [15]. 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, 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. In foods preserved by combined methods ("hurdle" technologies), the active homeostasis of vegetative microorganisms and the passive refractory homeostasis of spores are disturbed by a combination of sublethal antimicrobial factors or stressors at a number of sites ("targets") or in a cooperative manner [16]. That is, low levels of different stresses are employed rather a single intensive stress allowing less severe preservation procedures and higher quality. Overall, multiple disturbance of microbial homeostasis has been used/suggested in fruit preservation in different arrangements: (a) using two or more stressors simultaneously to prevent growth of spoilage and pathogenic microorganisms and (b) using one or more stressors (in simultaneous or in sequence) to inactivate/injure or physically remove some microorganisms and 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).

The targeted application of the hurdle concept has aimed to improve quality and safety of fruit products at the farm level and in the whole and fresh-cut minimally processed fruit industry [17–25].

This presentation will discuss the application of some mild stressors (ozone, PL, and UV-C, among others), used in a hurdle approach, for improving the shelf life and safety of fruits and fruit products. The impact on microbiota, structure, and quality factors will be analyzed. Some tools for preservation technology design will be also highlighted, evidencing opportunities and future challenges.

2. Selected nonthermal preservation factors

Table 1 presents some selected hurdles or stressors (already used industrially) 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 are not broad-spectrum inactivation processes like thermal treatment but represent pasteurization techniques that allow minimizing the disadvantages of severe thermal processing. Most of these factors do not affect one specific cell target but individual constituents, structures, molecules, and reactions, killing cells through multiple mechanisms.

2.1 Ozone, hydrogen peroxide, and other oxidants

Oxidative stress by reactive oxygen species (ozone, chlorine dioxide, hydrogen peroxide, electrolyzed water, and 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 [15].

Application of ozone (in gaseous or aqueous forms) as a potential sanitizer against plant and human pathogens in easy-to-damage soft fruit such as blueberries, strawberries, and raspberries had been widely investigated. Ozone was approved by the US Food and Drug Administration for the decontamination of raw commodities in 2001. It is one of the most potent disinfectant agents due to its powerful oxidizing action, being effective against a broad spectrum of microorganisms [26]. It is very unstable mainly in water state. Its degradation product is oxygen, leaving no undesirable by-products on produce surface [26, 27]. Its effectiveness largely depends on its concentration, pH, temperature, and organic material.

2.2 UV-C

A maximum lethal effect of shortwave ultraviolet light (UV-C) has been reported in the range of 250–260 nm, inactivating bacteria, virus, protozoa, fungi, and algae [28]. While UV-C radiation can be strongly absorbed by different cellular components, the most severe cell damage has been reported to occur when nucleic acids absorb UV-C light, which crosses the DNA pyrimidine bases of cytosine and thymine to form cross-links, impairing the formation of hydrogen bonds with the purine base pair on the complementary strand of DNA [28]. Cellular death occurs after the threshold of cross-linked 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 other targets than DNA could be accounted for UV-C inactivation. UV-C radiation also produces significant damage in the cytoplasmic membrane integrity and cellular enzyme activity [29]. Exposure to low doses of UV-C light has been also shown to elicit a range of chemical responses in fresh produce ranging from antifungal enzymes to phytoalexins [30]. This beneficial plant response of agricultural produce or hormesis to inhibit fungal pathogens and delay ripening occurs 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 additive to it [28]. 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 (0.5–9 kJ m^{-2} for optimal effects according to the type of fruit) [31]. Both inactivation effects, direct and induced, are not easy to be distinguished in the literature information.

2.3 Pulsed light

PL involves the use of intense and short-duration (1 μs to 0.1 s) pulses of broad-spectrum light of wavelength ranging from UV to near-infrared (200–1100 nm).

In addition to UV-C-induced photochemical changes, photophysical effects and photothermal effects caused by the high peak power and the visible and near-infrared portions of pulsed light spectrum, respectively, seem to be involved [32].

2.4 Ultrasound

Injury or disrupting microorganisms by high-energy ultrasound (US) (i.e., intensities higher than 1 W/cm^2; frequencies between 18 and 100 kHz) are widely attributed to cavitation, that is, the rupture of liquids when applying high-intensity ultrasound and the effects produced by the motion of the cavities or bubbles thus generated 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) that could shear and disrupt cellular membranes or break cells. In the "transient 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 density 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 ultrasound (at least the cell wall, the cytoplasmic membrane, the DNA, the internal cell structure, the outer membrane); (b) cytoplasmic membranes do not appear to be the primary target of ultrasound at least for *S. cerevisiae*, *E. coli*, and *Lactobacillus* spp.; and (c) primary target would depend on the specific microorganism (for instance, the outer membrane in *E. coli*) [33, 34].

3. Design of preservation techniques: points to be addressed

Challenges associated with research and commercial adoptions of these technologies are still numerous. Ten years ago, Heldman et al. [35] indicated different aspects to be taken into account:

a. Understanding and appropriate monitoring of processes to ensure uniform application of the stressors on the product.

b. Fundamental knowledge about inactivation of spores, vegetative microorganisms, and enzymes to improve process effectiveness.

c. Fundamental knowledge about the changes in food structure and functionality to evaluate the impact of the process.

d. Identifying effective combinations of stressors to achieve acceptable safety and shelf life.

However, nowadays, a lack of systematic studies about the effect of the stressor/dose on the safety and quality of food products is still detected in the literature.

The key points for their design and commercialization of these technologies should include not only a deeper understanding on the mode of action of combined stressors and microbial response but also the availability and interpretation of systematic kinetic data on microbial and quality attribute behavior (with special relevance to dose-response and the influence of critical process parameters) and the optimization of equipment.

The design of hurdle techniques to obtain high-quality and safe food products needs a multidisciplinary perspective (**Figure 1**) [19]. The Food Safety Technology and Food Quality Technology approach, connecting science with engineering components, will provide a systematized knowledge, and a consistent design of hurdle strategy is more likely to emerge. Moreover, the complexity of the phenomena and its practical importance to food safety and quality requires qualification and quantification of these responses. This integration of the appropriate disciplines and the new and exciting tools that they offer will undoubtedly result in a reduction not only of pathogen risk and spoilage microorganism incidence but also of uncertainty.

3.1 Microbial aspects

Booming "genomic" 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 of cellular reactions to environmental conditions, supporting the development of effective food preservation processes.

The so-called predictive microbiology not only allows comparing the impact of different environmental stress factors/levels on reduction or growth inhibition of microbial population but also allows understanding microbial behavior in a systematic way [36, 37]. The model prediction of survival curves would be beneficial to the fruit industry in selecting the optimum combinations/doses of preservation agents to obtain desired levels of impact on microbial (pathogenic and spoilage organisms) behavior with minimal effects on costs and quality [19]. Sensory selection of preservation factors and their levels may be done between several "safe" equivalent combinations of interactive effects determined by the models.

The microorganisms may die, survive, adapt, or grow when mild preservation factors or stressors are applied. Sublethal damage and subsequent recovery present a big problem to manufacturing industry and catering service in terms of safety and spoilage. Microbial populations are heterogeneous. Different cells may exhibit chemical differences (they can be in different reproductive phases or in different physiological states due to differences in nutrient availability and/or environmental conditions). Also, sharing of genetic material results in the existence of genetically different individuals [38]. Using methods of multiparameter flow cytometry (FC), it is now possible to characterize the physiology of individual microorganisms. By means of both scattering and fluorescence signal measurements, information on cell parameters (physiological state, such as metabolic activity, internal pH, or integrity of cytoplasmic membrane—size, surface roughness, and granularity) at single-cell level and their distribution within cell population is provided with a relatively high degree of statistical resolution (≈5000–50,000 cells in minute), enabling assessment of population heterogeneity [39].

Evaluation of the response of microorganisms and the changes in quality during a period of storage similar to the shelf life required is essential since the major changes in quality attributes due to these techniques generally occur not after processing but during storage. Regarding microorganisms, different patterns of microbial growth in nondecontaminated and decontaminated minimally processed vegetables reported in the literature were identified by Gómez-López et al. [40], evidencing the difficulties to control microbial loads of these products during storage at low temperatures:

- No decontamination occurred, but the growth rate of microorganisms in treated samples was slower than that in untreated samples.

- No decontamination occurred, but microorganisms in treated samples exhibited a longer lag phase than that in untreated samples.

- Decontamination occurred, and growth rate of microorganisms in treated samples was slower (or counts decreased) than that in untreated samples.

- Decontamination occurred, and growth rate of microorganisms in treated samples was equal than that in untreated samples.

- Decontamination occurred, and microorganisms in treated samples did not grow or exhibit lag phase.

- Decontamination occurred, and the growth rate of microorganisms in treated samples was faster than that in untreated samples.

4. Application of food safety and food quality approaches to fruit preservation

Different examples in the literature or from the studies made in our research group illustrate the use of these concepts and will discuss the following during the presentation:

- Evaluation of the combination ozone refrigeration for increasing the postharvest shelf life of strawberries and blueberries [41, 42].

- Evaluation of the combination PL refrigeration for increasing the postharvest shelf life of strawberries [43].

- Evaluation of the combination PL refrigeration for preserving fresh-cut apples [20].

- Mathematical modeling and flow cytometry studies of different microorganisms subjected to PL, US, and ozone [29, 33, 44].

5. Future trends

- The major challenges and opportunities in the future state of mild preservation techniques will arrive with a more in-depth knowledge of microbial behavior at molecular and physiological levels, as well as of the impact on quality attributes.

- Besides, the key points for their design and commercialization of these technologies include the availability and interpretation of systematic kinetic data on microbial and quality attribute behavior (with special relevance to dose-response and the influence of critical process parameters) and the optimization of equipment.

- The selection of the stressors and their levels is fruit-specific and depends on the required shelf life.

Appendix

See **Figure 1** and **Table 1**.

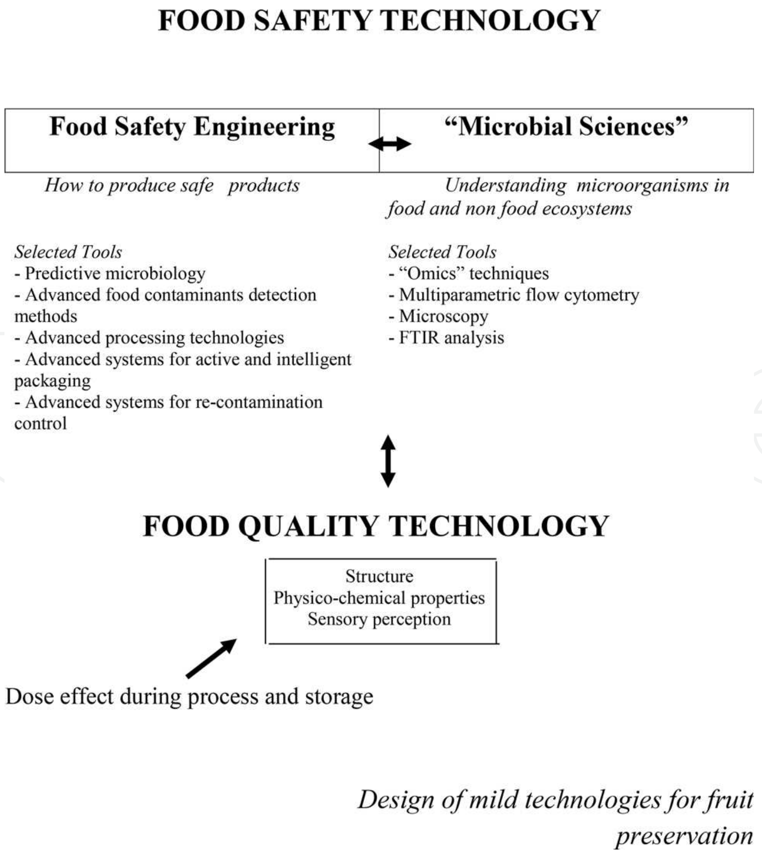
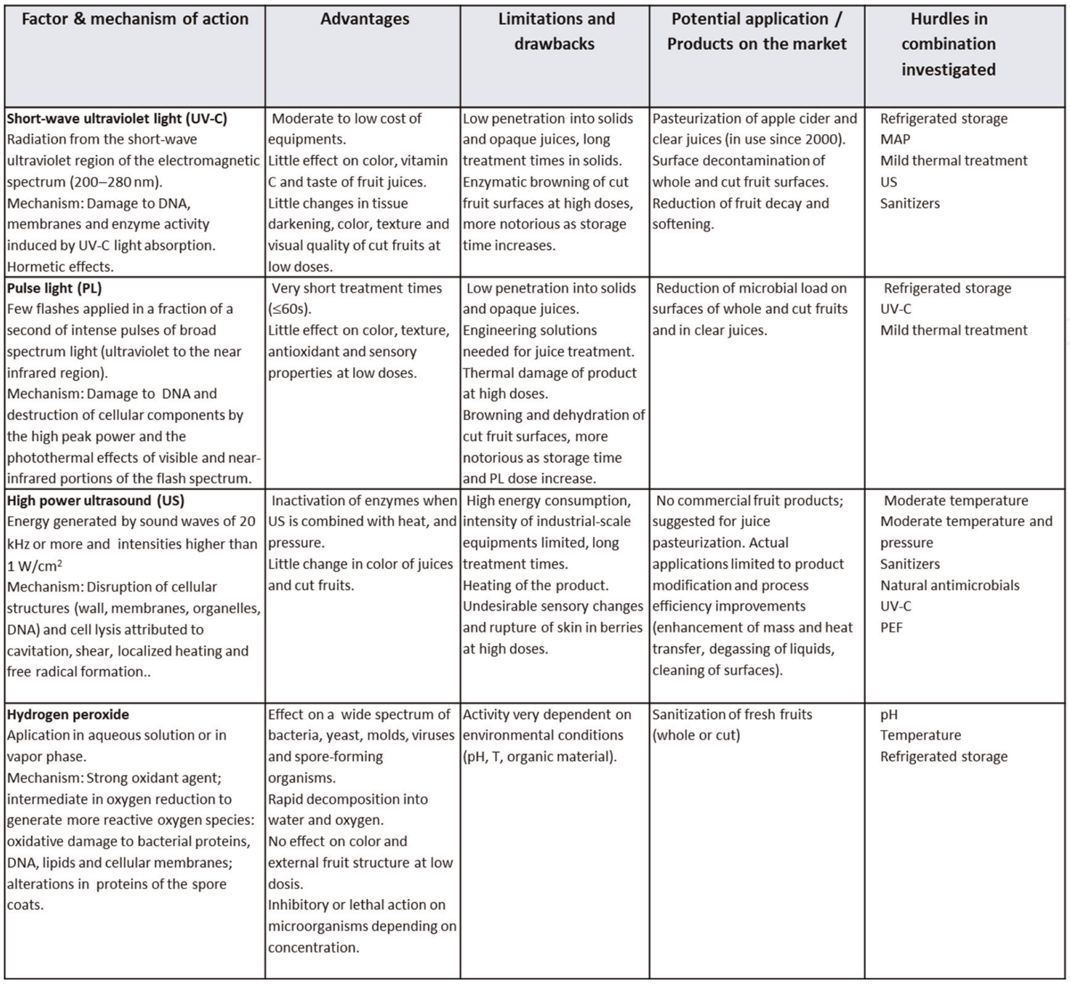
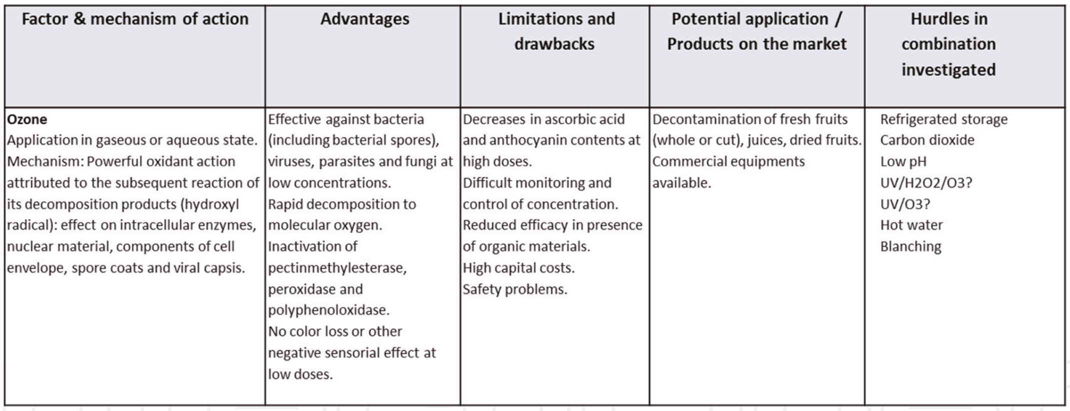


Figure 1.
Food safety technology and food quality technology approach in the design of mild techniques for fruit preservation (adapted from [19]).



Factor & mechanism of action	Advantages	Limitations and drawbacks	Potential application / Products on the market	Hurdles in combination investigated
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.	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 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 (≤60s). 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
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, shear, localized heating and free radical formation..	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 Moderate temperature and pressure Sanitizers Natural antimicrobials UV-C PEF
Hydrogen peroxide Aplication in aqueous solution or in vapor phase. Mechanism: Strong oxidant agent; intermediate in oxygen reduction to generate more reactive oxygen species: oxidative damage to bacterial proteins, DNA, lipids and cellular membranes; alterations in proteins of the spore coats.	Effect on a wide spectrum of bacteria, yeast, molds, viruses and spore-forming organisms. Rapid decomposition into water and oxygen. No effect on color and external fruit structure at low dosis. Inhibitory or lethal action on microorganisms depending on concentration.	Activity very dependent on environmental conditions (pH, T, organic material).	Sanitization of fresh fruits (whole or cut)	pH Temperature Refrigerated storage



Factor & mechanism of action	Advantages	Limitations and drawbacks	Potential application / Products on the market	Hurdles in combination investigated
Ozone Application in gaseous or aqueous state. Mechanism: Powerful oxidant action attributed to the subsequent reaction of its decomposition products (hydroxyl radical): effect on intracellular enzymes, nuclear material, components of cell envelope, spore coats and viral capsis.	Effective against bacteria (including bacterial spores), viruses, parasites and fungi at low concentrations. Rapid decomposition to molecular oxygen. Inactivation of pectinmethylesterase, peroxidase and polyphenoloxidase. No color loss or other negative sensorial effect at low doses.	Decreases in ascorbic acid and anthocyanin contents at high doses. Difficult monitoring and control of concentration. Reduced efficacy in presence of organic materials. High capital costs. Safety problems.	Decontamination of fresh fruits (whole or cut), juices, dried fruits. Commercial equipments available.	Refrigerated storage Carbon dioxide Low pH UV/H2O2/O3? UV/O3? Hot water Blanching

Table 1.
Selected nonthermal stressors for obtaining fresh-like fruit products (from various sources).

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