Three Pillars of Novel Nonthermal Food Technologies: Food Safety, Quality, and Environment

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Review Article

Three Pillars of Novel Nonthermal Food Technologies: Food Safety, Quality, and Environment

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This review gives an overview of the impact of novel nonthermal food technologies on food safety, on quality, and on the environment. It confirms that research in this field is mainly focused on analyzing microbial and/or chemical aspects of food safety. However, recent research shows that in spite of various food safety benefits, some negative (quality oriented) features occur. Finally, this paper shows the necessity of analyzing the environmental dimension of using these technologies.

1. Introduction

Nonthermal technologies are used in interdisciplinary sciences, in biotechnology, and in many other research and applied areas. In food processing, they are used mainly for preservation in treating of food and wastewaters. Consumer demands for minimally processed foods in addition to the negative effect of heat on nutritional properties of foods are making nonthermal processing popular in the food industry. The main task of nonthermal processing is to assure food safety [1], and research effort is focused on microbial inactivation, food safety, and preservation while retaining the quality of obtained products. This advantage gives nonthermal processing the potential to replace classical thermal processing. Besides the food safety and quality dimensions, these processing technologies have the possibility to shorten treatment time, lower energy consumption, and lower carbon footprint [2].

Nonthermal technologies have different types of action, depending on the source of energy transfer. They are used in inactivation of microorganisms in radical formation (plasma, ultrasound, ozonation, UV light, etc.); mechanical action through hydrodynamic effects, shock waves (ultrasound and plasma), electric and magnetic fields (pulsed electric fields, cold plasma, radiofrequency and oscillating magnetic fields, electrohydrodynamic processing, and electron beam processing); or extremely high pressures that are causing rupturing and bursting of microorganisms [3–6]. These treatments may be used alone or in combination, within the so-called "hurdle" concept [7–9]. The most researched techniques with proven scientific results in the food industry are high-pressure processing (HPP), supercritical fluid extraction (scCO$_2$), and pulsed electric fields (PEFs).

There are many research projects dealing with microbial inactivation [10–12], enzyme inactivation [11, 13], and nutritional improvements [14–16] when using nonthermal technologies. All of these techniques have been successfully applied in assuring food safety [17–20]. However, besides assuring food safety, more attention is needed to maintain or improve food quality. Quality of food after nonthermal processing has shown both positive [21, 22] and negative [23–25] effects depending on the technique and processing
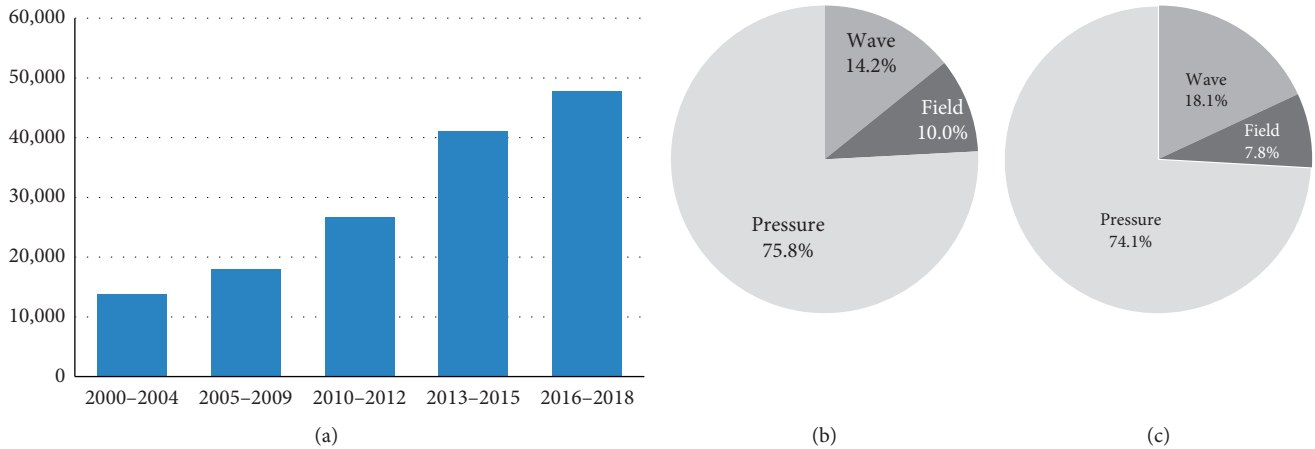
Figure 1: Presentation of publications related to the application of nonthermal technologies in the food industry: (a) number of publications; (b) share of publications; (c) share of patents. Wave: ultraviolet, ultrasound, and plasma; field: electric and magnetic fields' technologies; pressure: high-pressure processing/homogenization.

parameters. This raises the first challenge in succeeding inactivation of microorganisms while impairing quality and sensory parameters of treated samples and opens a research gap of unresearched areas like negative aspects of application of novel nonthermal processing on food quality, stability of food during shelf life after nonthermal processing, negative sensory properties of food treated by novel nonthermal techniques, life cycle assessment, and sustainability of nonthermal processing techniques. Advantages of novel nonthermal processing in terms of energy consumption can be considered as "green" techniques for "green" extraction. In order to gain better output products using nonthermal processing, it is important to overview processing in terms of safety, quality, and environmental aspects.

The objective of this review paper was to present the three main pillars related to the use of novel food technologies—food safety, quality, and environmental impacts on the one side stressing advantages and constraints and on the other side revealing future synergic research perspectives.

2. Materials and Methods

Online literature on the use of nonthermal technologies in the food industry is dispersed in a heterogeneous way in the form of scientific manuscripts, book chapters, conference proceedings as well as patents, legislation, and even company reports. According to our goal, we carried out a search in scientific literature spanning the research for the period 2000–2018. The authors mainly focused the attention on the international journals to assure a more scientific content mainly caused by a rigorous revision process. Therefore, the selection of scientific manuscripts was based on the journals impact factor, matching to the scope of the journal and preferring those indexed by international repositories such as the Scopus index and publishers (Elsevier, Springer, Wiley, Taylor and Francis, and EBSCO). This research identified relevant articles, both review and research papers, published in the domains of nonthermal technologies split

into two subsections: specific nonthermal technologies (HPP, scCO2, PEF, etc.) and its application on the specific type of food (beverages, fruit, vegetables, etc.). There were no geographical restrictions applied.

This type of literature review identified that there are over 300,000 publications related to the application of nonthermal technology in the food industry. In this millennium, the number is increasing as presented in Figure 1(a) where the period before 2010 was divided into two five-year periods: 2000–2004 and 2005–2009. The period starting from 2010 was analysed in three-year periods. Although there are papers published in journals that are not strictly in the "food science and technology" scope, the top five journals that have at least 500 publications are Food Chemistry, LWT Food Science and Technology, Journal of Food Engineering, Innovative Food Science and Emerging Technologies, and Food Research International. The journal covering the environmental impact of these technologies is published in the Journal of Cleaner Production and Bioresource Technology. Depending on the type of technology, the share of publications and patents was analysed and is presented in Figures 1(b) and 1(c). It is important to note that high-pressure processing and homogenization are the most analysed technologies in around 75% of all research/review publications. The same applies to publication of patents.

Deeper analysis of patents reveals that the majority of patents were published in journals up to 2010 and covered patents of new nonthermal technologies, food substitution with novel food derived from new technologies, and aspects of food preservation using these technologies. Majority of patents came from the developed countries (EU, USA, China, Japan, Australia, etc.), and no other patterns were observed.

A literature review revealed that these technologies were evaluated separately either from a food technology/food safety perspective or from an environmental perspective. Combination of two types of criteria—environmental and quality/food safety—has not been a focus of research, and

this has been identified as a research gap by the authors of this paper.

3. Safety of Food Processed with Nonthermal Technologies

Nonthermally processed food presents some kind of a risk due to incomplete preservation of food. At the beginning of an extensive research and application of nonthermal food technologies, the US Food and Drug Administration (FDA) requested the Institute of Food Technologists (IFT) to give a report on the effectiveness of microbial inactivation of alternative food-processing technologies. Back in 2000, the IFT reported general guidance for future research on novel techniques based on microbiological demands like the evaluation of the adequate linear first-order survivor curve model and launching experimental protocol, identifying inactivation action/mechanism(s) among alternative technologies, and determining the synergism or antagonism of one alternative processes [26]. The IFT also emphasized the importance to determine potential formation of indigestible and toxic by-products of processing as well to develop methods for measuring and monitoring physical-chemical changes during treatments [26].

As a result, from the year 2004, the definition of pasteurization changed and now, according to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the USDA, includes any process, treatment, or combination, which is applied to food to assure microbial safety [27]. In order to evaluate alternative pasteurization methods, there are several steps to pay attention on, like the properties and composition of the treated food product, microbial effects, and commercial, economic, and environmental aspects [28]. Each of these technologies has specific critical process parameters that must be monitored and controlled (critical control points).

The Novel Food Regulation by the EU lacks a joint classification of new technologies across all member states which slows down their widespread commercialization in Europe. In order to proceed with the technology readiness levels of novel food technologies, one of the necessary prerequisites is to validate them in relevant environment [29]. Novel food technologies may be used for different purposes in the food industry [30], such as (1) preservation/ decontamination and shelf life extension, (2) food modification (i.e., gelatinization), (3) stress induction (i.e., increase in biosynthetic activities), (4) mass transfer modification (i.e., extraction), and so on.

In parallel with developing nonthermal technologies, it is of vital importance that equipment is hygienically designed [31]. Hygienic design is defined as "design and engineering of equipment and premises assuring that food is safe and suitable for human consumption" [32]. It is not widely understood, and there is still little awareness of possible consequences of equipment that is not hygienically designed [33]. Public health and economic aspects of microbial contamination in foods may cause financial and public concerns, particularly if these result in product recalls. The hygienic design of equipment plays an important role not only in controlling the microbiological safety and quality of the products made but also in prevention of residues of chemicals used for cleaning and disinfection. Also, the hygienic design should prevent food from being contaminated with other contaminants. This becomes more important with novel food-processing technologies where new designs may result in new (re)contamination pathways, while minimized food processing may not achieve heat-standard inactivation [9].

Legislation covering the hygienic design of food-processing equipment is vague [34]. In the EU, some legislation mentions the importance of the hygienic design such as the regulation of hygiene of foodstuffs [35]. On the contrary, there are a number of different types of standards related to the hygienic design with different approaches in highlighting similar hygiene issues [33]. Most used standards that outline requirements related to the hygienic design are industry-tailored guidelines or sanitary standards [36–39].

3.1. Microbial Food Safety. All aspects of microbial food safety need to be monitored, and this includes assuring FDA regulative for 5 log reduction using preservation processes. High-pressure processing (HPP) and pulsed electric fields (PEFs) have been greatly researched and proved to be successful in assuring food safety, and by that, they were successfully commercialized [4, 40, 41] dealing with nonthermal inactivation of microorganisms. PEF provides minimal changes in food attributes while assuring optimum safety. Pulsed light is used in decontamination of various (transparent) liquids. However, limitation of this technology leads to undesirable results, such as decomposition of nutrients and changes in sensory quality. Food safety is one of the important components that force the development of novel technologies to reduce, control, or eliminate food-borne pathogens from food products and contact surfaces. State of the art of assuring and demonstrating 5 log reduction was described in terms of applying optimized nonthermal treatment [1, 20, 42]. Nonthermal techniques can be combined [10, 13, 43–46] or be used with antimicrobial agents [47–49] or in combination with mild heating [1, 22, 50]. It is necessary to follow critical control processes of nonthermal processing [51–53] in order to have no recovery or revitalization of microorganisms after processing [54, 55]. There are laboratories in the United States that validate food process for all nonthermal techniques, and processing needs to be evaluated and overviewed for microbial stability, toxicology, interactions between the product and packaging, chemistry, and so on. Foods derived from these technologies are also subject to this kind of validation.

High pressures result in extreme mixing and high-intensity shear forces moving throughout the medium. This release can result in examples of aggregate disruption, polymer chain fractures, and chain length degradation causing permanent changes in molecules [56]. This mechanism works particularly well at low frequencies such as 20 and 40 kHz. Although ultrasound has shown some

benefits to processing of foods, for example, in extraction, crystallization, and microbial inactivation, there are also some concerns as expressed by Pingret et al. [25] who presented a review on the possible degradative effects of sonication on food with high lipid contents and indicated the possible degradation of some compounds and changes to physiochemical qualities of some food products.

The extent of microbial inactivation by HPP in foods depends on multiple factors related to the processing conditions and the food matrix. Additionally, it was broadly observed that different microorganisms express a wide range of sensitivity to HPP [57, 58].

In particular, prokaryotic cells are observed to be more pressure resistant than eukaryotes [57], yeasts and moulds are relatively more HPP sensitive than bacteria, and Gram-positive bacteria are more resistant to pressure than Gram-negative bacteria, likely because the higher complexity of the cell or of the cell membrane might increase HPP susceptibility [59]. Additionally, cocci are more resistant than rod-shaped bacteria [59].

However, the most resistant species are the endospores, which are capable of withstanding pressures >1,000 MPa [59]. HPP is reported to induce the germination of bacterial spores, at an extent depending on the food matrix and the microorganism [59, 60].

In general, the efficiency of high-pressure homogenization (HPH) for microbial inactivation depends on the properties of the process fluid (viscosity, temperature, suspended solids, or fats), the specific resistance of the microbial strains, and the operating conditions, such as the operating pressure, the number of HPH passes, the operating temperature, and the homogenizing valve geometry [61]. The pressure limit separating HPH (high-pressure homogenization) from UHPH (ultrahigh-pressure homogenization) is not clearly defined, whereas pressures above 200 MPa are often named UHPH [62, 63].

The inactivation kinetics for most microorganisms appear to be first order with respect to the applied pressure, in the range of HPH and UHPH pressure levels (100–350 MPa) [64]. In contrast, for repeated HPH passes, an asymptotic behavior is generally observed, which can be attributed to the natural distribution of individual cell resistance to pressure [65]. Moreover, the homogenizing valve geometry also appears to be determining factors for microbial inactivation. In fact, the microbial inactivation is a direct consequence of the physical cell disruption due to the fluid-mechanical stresses generated in the valve, such as shear and elongational stresses, turbulence, cavitation, and impact on the valve surfaces, which depend on the specific valve design [66, 67].

Due to the temperature rise in the homogenization valve and due to the frictional heating associated with the pressure energy dissipation, the thermal inactivation of the microorganisms is likely to occur during HPH treatments, if the inlet and outlet temperatures are not carefully controlled.

If a purely nonthermal treatment is desired, to preserve the thermosensitive food components, the inlet temperature should be adjusted as a function of the operating pressure, taking into account the inherent heating of the system

(generally comprised between 0.15 and 0.22°C/MPa) [68]. Moreover, also a heat exchanger should be placed immediately downstream of the homogenizing valve, which is desirable to minimize thermal damage to the product.

Both HPH and UHPH treatments primarily kill the vegetative bacteria, through the mechanical destruction of the cell integrity [61, 67]. Gram-positive bacteria are reported to be more resistant than Gram-negative bacteria, which have thinner cell walls, formed by 1–5 layers of peptidoglycan chains, in comparison with the 40 layers of peptidoglycan chains of the Gram-positive bacteria [69, 70].

Yeasts and fungi exhibit an HPH resistance, which is intermediate between Gram-negative and Gram-positive bacteria, because of their wall structure, which is thicker than that in Gram-positive bacteria, but more complex than that in Gram-positive bacteria due to the larger size and a different cell wall structure, with glucans, mannans, and proteins as basic structural components [71].

There are different actions of nonthermal processing, but in some use of one technology *per se* is not enough to assure inactivation of microorganisms in a significant way. Efficiency in inactivation of microorganisms by those treatments differs depending on treatment parameters like treatment time, power, strength, dosage, frequency, and so on. On the other hand, by working at lower temperatures, there are possibilities that applied treatment is not enough in prolonging the shelf life of the product and there are significant hazards like revitalization and recovery of microorganisms (sublethal injuries, stress, viable but nonculturable state, etc.). When nonthermal treatments achieve food safety, there is possible deterioration of food quality. One example is treated wine which was treated by ultrasound. Ultrasound caused the formation of negative oxidative smell and the formation of aromas which are described by panellists as burns or smoke [72]. It is explained by the formation of oxidized aroma (acetaldehyde) in young red wines, that is, the reaction of wine polyphenols (initiated by the ultrasound treatment) to form peroxide which oxidizes ethanol to acetaldehyde. This is often observed in high oxidative techniques (plasma, ultrasound, etc.). Through formation of free radical and high reactive oxygen or nitrogen species, the nonthermal processing can be efficiently introduced in wastewater treatments and recovery of agro and food waste.

Another area of safety concerns within food processing is sterilization and reduction of contamination by bacteria and other microbes. Ultrasound has been shown to be very effective in treating the rate of bacterial growth and increasing the kill rate of microbes at a range of frequencies, the most effective being 850 kHz due to the short life span of the cavities at this frequency. It is thought to affect microbial inactivation via the weakening or disruption of bacterial cells through a number of different processes which include mechanical and chemical effects. Mechanical effects are induced by sonication at lower frequencies of 20 kHz, as a result of increased pressure gradients formed during the collapse of cavitation bubbles within or near the bacteria, which result in enhanced shear forces, microstreaming, and high levels of mixing resulting in disruption of the bacteria. Evidence continues to grow for the use of ultrasound in the

Table 1: Ultrasonic parameters and effects on various bacteria and fungi.

Author(s)	Type of food	Bacteria and fungi	Experimental setup parameters	Effects of ultrasound treatment
[73]	Skimmed milk	Enterobacter aerogenes, Bacillus subtilis, Staphylococcus epidermidis, S. epidermidis, Staphylococcus pseudintermedius	$\nu = 20$ kHz; $P = 13$ W; $t = 20$ min; $T = 30°C$	Up to 4.5 log reduction for E. aerogenes and B. subtilis. Staphylococcus spp. not affected
[74]	Skim milk powder	Geobacillus stearothermophilus	$\nu = 20$ kHz, $T = 45°C$, $t = 30$ s for cells; $\nu = 20$ kHz, $T = 67.5°C$, $t = 17.5$ s for spores	Cell reduction (4.8 log) at 19.75% total solids and spore reduction (0.45 log) at 31.5% total solids
[75]	Pomegranate juice	Escherichia coli and Saccharomyces cerevisiae	$\nu = 20$ kHz; amplitude levels of 50, 75, and 100%; $t = 0$, 3, 6, 9, 12, 15 min; $T = 25 \pm 1°C$	100% amplitude level for 15 min, reduced levels by 3.47 and 1.86 log·cfu/mL, respectively
[76, 77]	Strawberry, orange, apple, pineapple, and red fruit juice	Saccharomyces cerevisiae, Pichia membranifaciens, Wickerhamomyces anomalus, Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Candida norvegica	$\nu = 20$ kHz; $P = 130$ W; amplitude levels 20% to 60%; pulse = 2, 6 s; $t = 2$–6 min	Reduction of spoilage organisms
[78]	Cactus pear juice	Escherichia coli	$\nu = 20$ kHz; $P = 1500$ W; amplitude levels of 60%, 70%, 80%, and 90%; $t = 1$, 3, 5 min	Total inactivation in both fruit juices after 5 min of ultrasound treatment at most amplitude levels
[79]	Ayran, an acidic milk drink	Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus	$\nu = 35$ kHz; $T = 60$, 70, 80°C; $t = 1$, 3, 5 min	Counts decreased as the temperature and time increased
[80]	Orange juice	Alicyclobacillus acidoterrestris spores	$\nu = 24$ kHz; AI = 460 W/cm², $P = 33$ W; AI = 105 W/cm², $P = 162$ W	Thermosonication required at least 8°C lower temperatures than thermal treatments to achieve the same spore inactivation
[81, 82]	Natural squeezed apple juices	Alicyclobacillus acidoterrestris spores and Saccharomyces cerevisiae	$\nu = 20$ kHz; $P = 600$ W and 95.2 μm wave amplitude; $t = 10$ or 30 min; $T = 20$, 30, or $44 \pm 1°C$ and pulsed light (PL) (xenon 1 and 3 pulses/s; 0.1 m distance; 2.4–71.6 Jcm⁻²; initial $T = 20$, 30, $44 \pm 1°C$)	Combination of these technologies led up to 3.0 log of spore reduction in commercial apple juice and 2.0 log in natural juice; for S. cerevisiae, 6.4 log reduction and 5.8 log reduction were achieved
[82]	Apple juices	Escherichia coli ATCC 35218, Salmonella Enteritidis MA44 and Saccharomyces cerevisiae	$\nu = 20$ kHz; $P = 600$ W and 95.2 μm wave amplitude; pulsed light 0.73 Jcm⁻², 155 mL/min	Combined ultrasound and pulsed light led up to 3.7–6.3 log reductions of inoculated microorganisms
[83]	Milk	Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus, and Debaryomyces hansenii	$\nu = 24$ kHz; amplitude levels 70 and 100%; $t = 50$, 100, 200, 300 s	Population reduction. Milk deterioration
[75, 84]	Pomegranate juice	Escherichia coli and Saccharomyces cerevisiae	$\nu = 20$ kHz; amplitudes levels 50, 75, and 100%; $t = 0$, 6, 12, 18, 24, 30 min	More than a 5 log inactivation of E. coli and a 1.36 log inactivation of S. cerevisiae
[85]	Orange juice	Saccharomyces cerevisiae	$\nu = 20$ kHz, $P = 778.2$ W ultrasonic power, $t = 11$ min; $P = 350$ W microwave power, $T = 35°C$	Complete inactivation

TABLE 1: Continued.

Author(s)	Type of food	Bacteria and fungi	Experimental setup parameters	Effects of ultrasound treatment
[86]	Fresh produce	Gram-positive bacterial strain *Listeria innocua* and Gram-negative bacterial strains *Escherichia coli* O157:H7 and *Pseudomonas fluorescens*	$t < 10$ min	0.5 log·CFU/cm^2 reduction of all strains when washed with ultrasound
[87]	Chicken	*Campylobacter jejuni* and spoilage bacteria	$T = 4°C, 25°C, 54°C$; $t = 1, 2, 3$ min	Treatments significantly reduced total viable counts
[88]	Salads	*Listeria monocytogenes*	$t = 5$ min in water inoculated with herbs/spices	Samples exhibited up to 4 log reduction 1 day after treatment

ν, frequency (kHz); T, treatment temperature (°C); t, treatment time (min or s); P, treatment power (W); AI, acoustic intensity (W/cm^2).

deactivation and sterilization of many different bacterial strains. This can be achieved within short treatment times with higher frequencies of sonication, thus resulting in minimal disruption to the food material itself. An overview of ultrasound effects on microbial safety is presented in Table 1.

Liu et al. [89] investigated the inactivation of *Saccharomyces cerevisiae* under varying conditions such as bacterial load concentration, pH, and treatment temperature and determined that ultrasound had the most significant role in the inactivation of the bacteria. Kang et al. [90] examined the effects of ultrasound on the number of *Escherichia coli* O157:H7 and vegetative cells of *Bacillus cereus* in brining and beef during the curing processing. After 30 min of treatment, *E. coli* appeared to be more affected by sonication than the *B cereus*. This was thought to be due to the formation of hydrogen peroxide acting as a sterilization agent as a result of recombination reactions of OH radicals during the sonication process. Sienkiewicz et al. [91] examined the growth of the strain of *Salmonella enterica* subsp. *typhimurium* during sonication. Total inactivation of *Salmonella* spp. occurred with low bacterial populations after sonication at 20 and 40 kHz for 30 min and with high bacterial population at 20 kHz for 30 min with reductions observed after only 15 min of treatment. Bacterial inactivation, after sonication, lasted for up to 48 h in storage at 21°C.

The levels of *Campylobacter jejuni* and spoilage organisms in raw chicken were examined by Kassem et al. [87] who employed sonication alone or in combination with different solutions containing either lactic acid, sodium decanoate, or trisodium phosphate at a range of temperatures and treatment times. While all the solutions exhibited some reduced bacteria levels as compared to the control, combination treatments fared far better with only sonication in conjunction with 3% sodium decanoate solution showing any significant improvements and much reduced total viable counts.

Khandpur and Gogate [48] investigated microbial growth in a range of fruit and vegetable juices via the application of sonication in the presence and absence of crude orange oil and compared these to thermal controls alongside other quality parameters such as pH, acidity, Brix, and yeast content. The optimized ultrasound parameters for juice sterilization were ultrasound frequency and power of 20 kHz and 100 W with a 15 min treatment time, and more than 5 log reduction was achieved with lower microbial growth and improved quality characteristics as compared to the thermally processed juice.

3.2. Chemical Food Safety.

Milne et al. [92] examined OH* radical formation employing ultrasonic frequencies. Comeskey et al. [93] also employed a range of ultrasonic frequencies to determine levels of hydrogen peroxide formed in sonicated aqueous systems. Using a range of ultrasonic frequencies, they determined that the highest levels of hydrogen peroxide occurred at 850 kHz with 380 and 512 kHz also exhibiting some oxidative effects however not to the same extent.

Kang et al. [94] investigated treatment time versus ultrasonic power in an attempt to examine the oxidation of beef proteins. They determined that sonicating beef under varying treatment conditions greatly increased the amount of lipid oxidation compared to static brining. Protein oxidation was determined by examination of carbonyl levels and levels of disulphide cross-linking, which indicated a decrease in total sulfhydryl, as a result of free radicals contributing to protein oxidation. Continuing their work, Kang et al. [90, 95] sonicated beef at 150 and 300 W for 30 and 120 min and found that this increased the water holding capacity and tenderness of the beef as compared to salt brining. This was in this case attributed to induced oxidation of myosin causing polymerization of the muscle fibres, thus increasing the water holding capacity of the meat.

Sun et al. [96] examined the link between anthocyanin degradation and ultrasonically formed hydroxyl radicals. They discovered that the absorbance of the antioxidant cyanidin-3-glucosylrutinoside at 282 and 518 nm decreased significantly on increased sonication which was confirmed by 1,1-diphenyl-2-picrylhydrazyl and ferric-reducing antioxidant assays, thus indicating a negative effect on antioxidant levels as a direct result of extended sonication. Yao et al. [97] also observed a similar effect when examining the effect of sonication on antioxidant levels in blueberries and discovered that sonication significantly increases the degradation of cyanidin-3-glucoside as compared to thermal treatments.

The inactivation of horseradish peroxidase was investigated by Tsikrika et al. [98] who determined that sonication for 60 min using 20, 378, 583, 862, 995, 1144, and 1175 Hz ultrasound at power levels (acoustic energy) between 2.1 and 64 W was very effective at inactivating the enzymes with little effect observed at the 20 kHz lower frequency. The fact that the greatest levels of inactivation were observed at 378 and 583 nm suggests that some radical effect may be the cause. There is much evidence presented to suggest that it is the higher frequencies of sonication, above 370 kHz to 850 kHz, which result in high levels of oxidative radical formation. It is therefore suggested that, in order to avoid radical interference with food materials, lower frequencies for treatment should be employed with shorter sonication times to limit any oxidative effects.

Food allergies have posed a severe risk in the last decade. According to [99], allergic reactions are caused mainly due to "epitopes," a small linear stretch of amino acids or a specific three-dimensional structure which is a part of a much larger protein. During food processing (both conventional and novel), the epitopes that are present within the food matrix may be destroyed or new epitopes may be formed. Also, these technologies can result in producing conformational changes in the protein structure and formation of epitope centres, but also few of them can be used for the future development of the hypoallergenic foods by reduction or by mitigation of the reactivity on processing. Scientists are still trying to explain and understand the

conformational changes in the protein which can affect the allergenicity.

4. Quality of Food Processed with Nonthermal Technologies

Stakeholders, such as legislators, retailers, and manufacturers, care about consumers' opinion regarding food processed with novel technologies [100]. Giving the possibility to evaluate and to taste, a novel product seems to influence consumer acceptance for new technologies. This is still largely an unexplored area, but it has been suggested that including consumers in the process of evaluation, that is, by pairing the new technology with a positive sensory experience of the product, can lead to the positive consumers' reaction [101].

High-pressure processing (HPP) might significantly impact the quality and functionality of food, affecting the color, flavor, and texture, with a relevant impact on sensory perception and consumer acceptance [61]. Generally, HPP, by slowing down some biological reactions, such as Maillard's reactions, contributes also indirectly to a better preservation of the quality attributes and nutritional value over their shelf life [102]. In addition, HPP is reported to have only a limited effect on the covalent bonds of low-molecular-mass compounds, such as those responsible for color, flavor, and health-beneficial attributes. For example, HPP at low and moderate temperatures did not cause any significant alterations of the pigments, such as chlorophyll, carotenoids, and anthocyanins, responsible for the color of fruit juices [103]. The color compounds can, however, change during the storage of HPP-treated products more rapidly than in thermally treated ones, due to the incomplete inactivation of enzymes and microorganisms by high pressure [61].

Several studies clearly showed that the flavor of fruit juices is not affected by HPP because the structure of small molecular flavor compounds is only marginally affected by high pressure [104–107]. However, similar to pigments, the flavor of fruits and vegetables subjected to HPP might be indirectly altered, through enhancing or delaying of some enzymatic reactions, which might alter the balance of flavor composition [105, 108, 109]. HPP is also reported to affect the rheological behavior of the juices and their cloud stability, as both these parameters are controlled by the composition of the soluble pectins. Pectin breakdown or retention, induced by HPP also through enzymatic reactions, enables the control of the rheological behavior of the juice. For example, the residual activity of pectin methylesterase, not completely inactivated by HPP, caused a decrease in orange juice viscosity during its shelf life [109]. In contrast, the viscosity of tomato juice was observed to increase linearly with pressure, in the high pressure range (200–500 MPa), whereas at low pressures (100–200 MPa), a decrease in viscosity was observed because of enzymatic degradation of pectins [110].

One of the main quality indicators upon food processing is the preservation of the content of bioactive molecules, which might contribute to the health-beneficial properties of the food products. Remarkably, nonthermal technologies not only are reported to better preserve bioactives than thermal treatments but also in some cases can stimulate their release from the intact cells contained in the product, which translates in the increase of instrumentally detectable bioactive concentration and, often, of their bioaccessibility. In fact, HPP of vegetable cells at 250 MPa for 10 min was reported to induce 99% of the pigment to be released [111].

Figure 2(a) summarizes the effect of HPP on different bioactive compounds, in comparison with the corresponding values in untreated products.

Vitamin A was reported to increase slightly in orange juice, treated between 100 and 400 MPa for 1–5 min at temperatures between 30 and 60°C [112], and significantly in apple juice treated for 5 min at 400 and 500 MPa and 25 and 45°C [61].

In contrast, different authors reported that vitamin C decreased upon high-pressure homogenization (HPH) and ultrahigh-pressure homogenization (UHPH) treatment, as observed in orange juice [112], in blueberry juice treated at HPP pressures between 200 and 600 MPa and at temperatures of 42°C for 5–15 min [113], and in melon pieces treated for 10 min at HPP 600 MPa and ambient temperature [114]. In the case of vitamin E, a slight decrease was observed in rosehip puree, HPP treated at 200–600 MPa and 20°C for 5 or 10 min [115], whereas a measurable increase was reported by the same authors for spinach leaves [115] and in sliced ham, HPP treated at 400–900 MPa and 12°C for 10 min [116].

In the case of total carotenoids and of their main components, such as β-carotene, lutein, and zeaxanthin, it is generally reported that they are well preserved during HPP. In rosehip puree, similar to what reported for vitamin E, a decrease in total carotenoids, and in particular in lutein and zeaxanthin, was observed, while in spinach leaves, the total carotenoids were reported to increase [115].

In melon pieces treated by HPP, β-carotene was observed to increase, differently from what was observed for vitamin C [114]. In orange juice treated between 100 and 400 MPa for 1–5 min at temperatures between 30 and 60°C, β-carotene, lutein, and zeaxanthin significantly increased [112]. Remarkably, in bee pollen paste treated at 200–400 MPa and 20°C for 5–15 min, a substantial increase in total carotenoids was observed [117].

The anthocyanins slightly decreased in blood orange juice treated at 400–600 MPa and 20°C for 15 min [118], while significantly increased in must obtained from grapes treated at 400–550 MPa and 20°C for 10 min [119].

HPP at 400–600 MPa and 25–50°C for 5 or 10 min caused a decrease of the total polyphenols in pomegranate juice [106], whereas in blueberry juice [113] and in bee pollen paste [117], total polyphenols significantly increased. In the preservation of anthocyanins and polyphenols, a significant role is also played by the inactivation by HPP of the enzymes responsible for their degradation [120].

HPH and UHPH treatments are reported to better preserve the natural functional compounds of the juices, such as vitamins C and A, flavonoids, and polyphenols, while reducing the microbial load to the desired value [121–123].
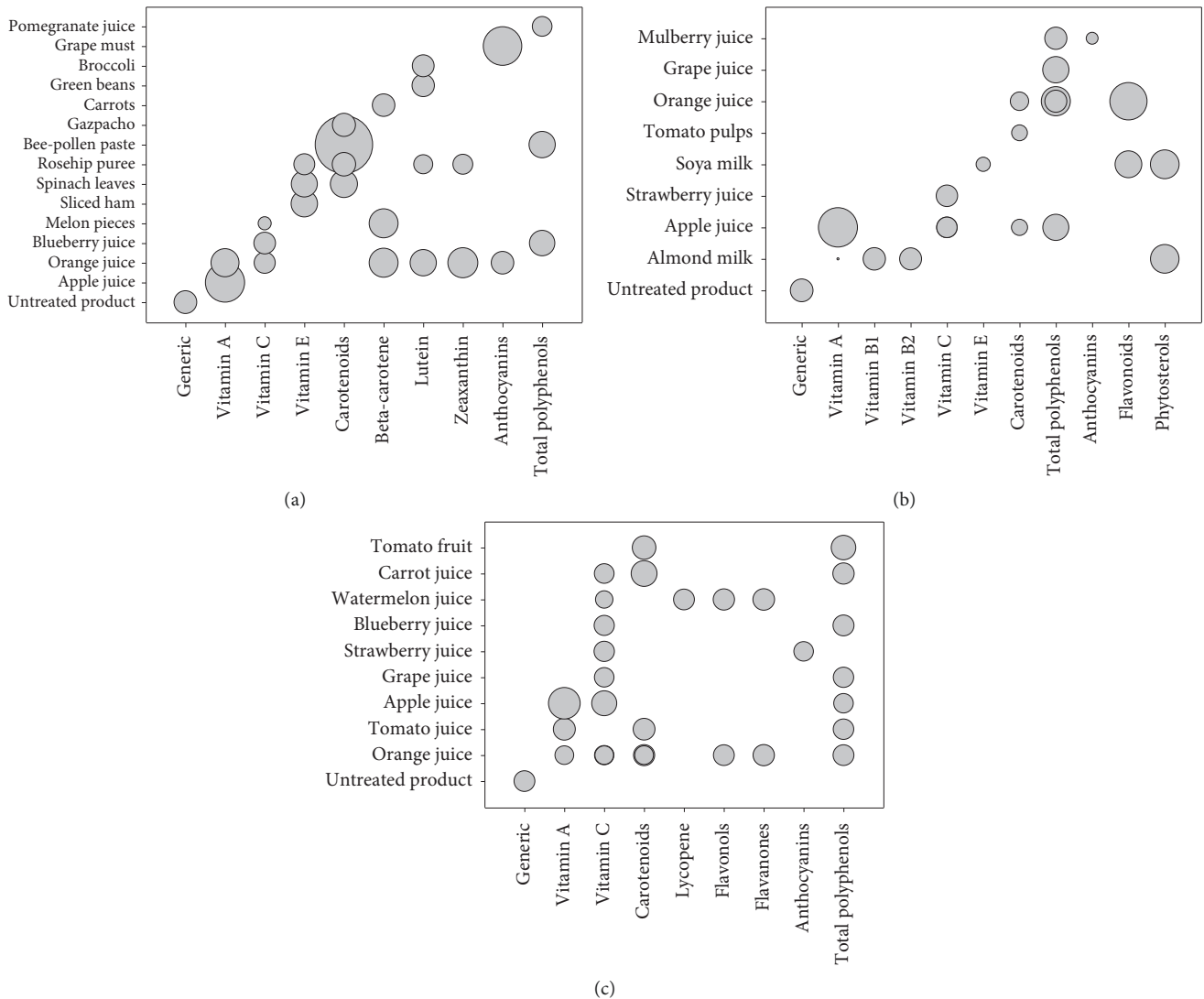
(a)

(b)

(c)

Figure 2: Effect of various treatments of different food products on the concentration of different bioactive compounds, with respect to a generic compound in an untreated product. (a) High-pressure processing; (b) high-pressure homogenization; (c) pulsed electric fields. Size of each bubble is related to the concentration of the bioactive compounds after the nonthermal treatment, with respect to the initial concentration.

In the dairy industry, homogenization has been extensively used for the stabilization of food emulsions and the disruption of fat globules. The higher operating pressures of HPH/UHPH treatments enable also the direct microbial inactivation, the disruption of smaller particles, and the modification of proteins or other food constituents [124]. Figure 2(b) depicts the effects of HPH/UHPH treatment of different liquid food products on the concentration of different bioactive compounds.

For example, both HPH and UHPH treatments reported to induce a significant reduction of the suspended particle size distribution in juices [121, 125, 126] and the juice viscosity [125, 127, 128]. Additionally, the color attributes of HPH-treated juices are not significantly altered, in comparison with the untreated product [121, 122, 125], whereas the cloudiness and opalescence stability are significantly improved [123, 126].

However, during pressure homogenization treatments, due to the significant temperature rise occurring in the homogenization valve, most of thermosensitive compounds, such as vitamins, carotenoids, and anthocyanins, are degraded to a higher extent than that by HPP. For example, when almond milk was treated at 200 or 300 MPa and very high inlet temperature (55–75°C), in order to obtain a microbiologically stable product, vitamin A was almost completely degraded [129]. However, in cloudy apple juice, a treatment, carried out at 100–175 MPa for 3–5 passes and an inlet temperature of 10–35°C, caused a significant increase in the content of vitamin A in the juice, with respect to the untreated product, due to the disruption effect on suspended vegetable cells.

Vitamins B1 and B2 were slightly reduced in almond milk treated for a single UHPH pass at 350 MPa and an inlet temperature of 40°C [130]. UHPH treatment caused a slight

reduction in vitamin C in strawberry juice treated at higher pressure (205 MPa) for 3 passes at an inlet temperature of 20°C [131]. The vitamin E content in soya milk was significantly affected by an UHPH treatment carried out at 200 or 300 MPa and very high inlet temperature (55–75°C), required to obtain a microbiologically stable product [132].

The total carotenoid content also exhibited a significant decrease upon HPH/UHPH processing, independently on the food matrix. In apple juice, treated for a single pass at pressures between 100 and 300 MPa and inlet temperatures of 10–20°C, a reduction in carotenoids of 30% was observed [133]. In orange juice, treated at the same conditions, the carotenoids decreased about 20% [134]. In tomato pulp, processed, because of its higher viscosity, at lower HPH pressures (20–100 MPa) for 1 pass and an inlet temperature of 4°C, a decrease in the total carotenoids of about 30% was observed [135].

In contrast, the total polyphenols were well preserved by pressure homogenization treatments, independently on the food matrix. For example, in apple juice, treated for a single pass between 100 and 300 MPa and an inlet temperature of 10–20°C, the total polyphenols slightly increased [133]. In orange juice, treated under the same conditions, the total polyphenols remained constant [134]. In orange juice and grape juice, treated at 250 MPa and room temperature, the total polyphenols slightly increased, with respect to untreated juices [136]. Similarly, mulberry juice, treated by UHPH at 200 MPa for 3 passes and an inlet temperature of 4°C, the total polyphenols remained constant [137]. Conversely, the same treatment had a detrimental effect on the anthocyanins of mulberry juice, with an observed reduction of almost 50% [137].

Remarkably, the content of flavonoids exhibited a significant increase upon UHPH processing. For example, in soya milk, despite the treatment at high inlet temperatures (55–75°C, with a single pass at 200 or 300 MPa), the flavonoids increased of about 20% [132]. In orange juice, treated in the pressure range (100–300 MPa) but at significantly lower inlet temperatures (10–20°C), an even more significant increase in the content of flavonoids was observed, in comparison with untreated juice [134].

Pulsed electric field (PEF) technology is recognized to be a technique able to cause a significant microbial inactivation in beverages, while causing only a minimal impact both on the quality properties and on the content of health-beneficial compounds. This is mainly due to the low treatment temperature: although the intensity of PEF treatments might reach electric fields intensities up to 40 kV/cm and a total energy delivered to the product of 40–100 kJ/L [61], the product temperature can be maintained below 40°C [138, 139].

Figure 2(c) clearly shows that, in different fruit and vegetable juices, no significant decreases are observed among the main health-beneficial compounds, including vitamins, carotenoids, and phenolic compounds. In contrast, in some cases, a significant increase is observed, which can be attributed to the enhanced extraction of bioactives from the vegetable cells [140].

In particular, vitamin A is observed to only slightly decrease of less than 10% in orange juice [141], but to significantly increase in apple [61] and in tomato juice [138].

Similarly, the vitamin C retention was always very high, comprised between 90 and 100%, independently on the food matrix [139, 142–145]. In the case of carotenoids, the PEF treatments did not cause any significant decrease in concentration in orange juice [141, 146], tomato juice [138], and watermelon juice [144] and a measurable increase (+25%) in carrot juice [147].

In addition, no significant variation was observed for flavonols in orange [148] or watermelon juice [144], for flavanones in orange juice [146, 148] or watermelon juice [144], for anthocyanins in strawberry juice [139], for total polyphenols in apple [145], or in orange [148], in grape [145], in blueberry [142], in tomato [139], and in carrot juices [147].

Ultrasound is thought to enhance the destabilization of casein micelles in milk [149]. This can be used to advantage in the coagulation of various milk sources, for example, goats' milk and reconstituted milk. Goats' milk is known for its weaker coagulation abilities as compared to cows' milk. The use of ultrasound treatment prior to addition of rennet resulted in smaller and more uniform particle sizes in the coagulant formed [150, 151]. Ultrasonically treated milk and yogurt samples also showed an increase in the gel firmness, coagulum strength, final storage modulus, cohesiveness, and water holding capacity [152]. These factors were stated to be due to a decrease in the soluble proteins and an increase in insoluble high-molecular-weight coaggregates formed as a result of protein denaturization on sonication [153, 154].

Monteiro et al. [155] noticed that ultrasound energy affects the physical properties of chocolate milk and the subsequent size distribution of fat globules and resultant rheological behavior of the treated sample; however, the bioactive compounds present and the nutritional quality of the product were still maintained. Changes to food materials is dependent in the first case on the frequency employed for sonication whether physical or mechanical changes are required, thus employing lower frequencies, or whether chemical changes are required where higher frequencies would be of more benefit.

Supercritical drying with the use of supercritical fluids (CO_2) is used as an alternative process to conventional drying techniques [156]. Carbon dioxide (CO_2) at high pressures (7.0 to 30.0 MPa) or in supercritical phase (above 31°C, 7.3 MPa) is considered as a novel nonthermal technology [157]. This preservation technology achieves inactivation of microorganisms and also meets consumers' demands for a product with high nutritional and sensory qualities [158]. Its main advantage is operation at relatively low temperature that avoids the thermal effects of traditional heat preservation, retaining the food freshness [159].

In food application field, nonthermal plasma (NTP) comes in various ways, from food surface application to direct in liquid food application. NTP is effective and causes minor harm to the exposed materials, such as biological samples or processed foods or packaging materials [160]. Researches have been mostly exploring plasma effect on inactivation of microorganisms [161, 162], but lately, plasma effect on food ingredients becomes focus topic due to combined physicochemical effects and complexed food
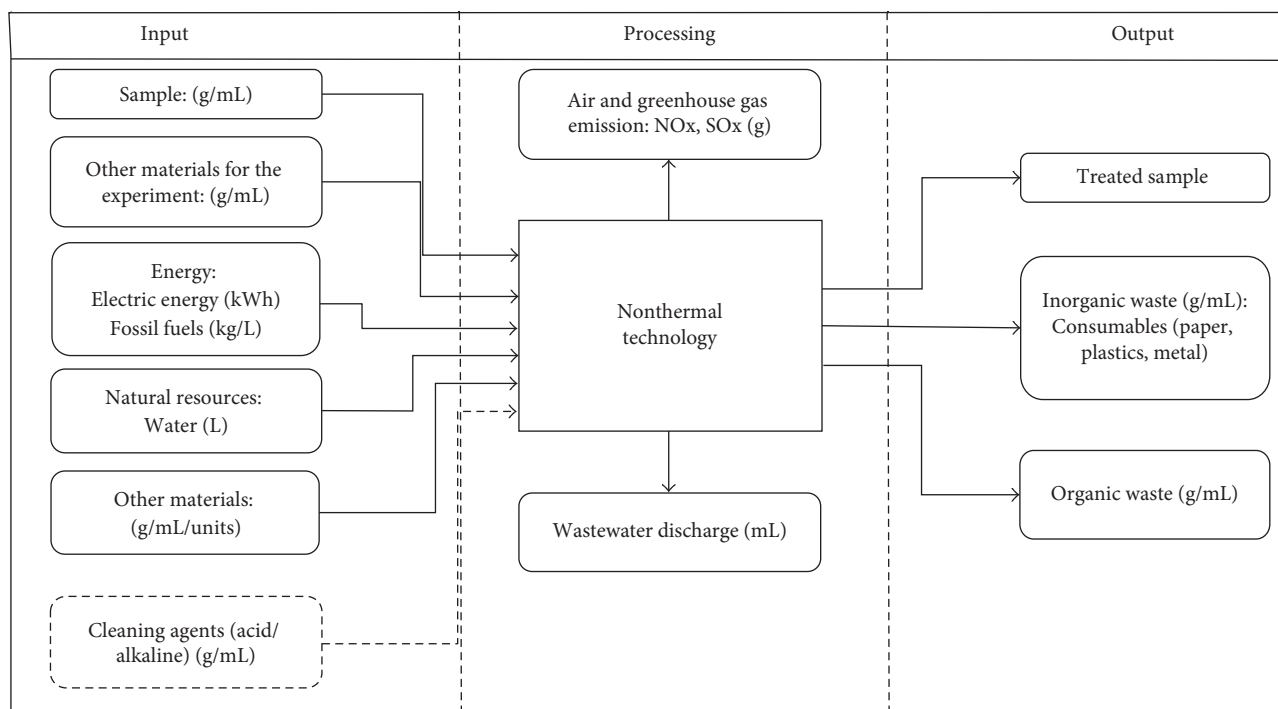
FIGURE 3: Generic model of using nonthermal technologies. Rounded rectangles outlined in solid lines present direct inputs and outputs; rounded rectangles outlined in dashed lines present inputs/outputs not directly related to nonthermal technologies.

structure. Plasma application in/on food brings another perspective of possible negative effects on phenolic compounds due to production of oxidative species.

Grzegorzewski et al. [163] have noted a degradation of phenolic compounds in lamb's lettuce after NTP treatment, but Misra et al. [164] have reported that cold plasma treatments had no significant effect on anthocyanins in strawberries and that, at the same time, phenolic acids have remained unchanged. Regarding liquid food, like fruit juices, an increase in total phenolic content [165] and anthocyanin content [166] in pomegranate juice after plasma treatment has been observed, as well as an increase in anthocyanin and phenolic acid contents in sour cherry Marasca juice [167] has been observed. Lukić et al. [168] report effects of plasma treatment on wines (red wine Cabernet Sauvignon and white wine Graševina) which have resulted in slight changes of chromatic characteristics and in reduction of phenolic compounds in both red and white wines, including total phenolics, total anthocyanins, total tannins, and certain free anthocyanins, while the concentrations of the most individual phenolic acids and flavan-3-ols slightly increased. These results led to new opening field of plasma treatment as a new extraction method due to improvement in the extraction of phenolic and other compounds.

5. Importance of Analyzing Environmental Impacts of Nonthermal Food Technologies

Besides obtaining safe products with high quality with nonthermal technologies, food processors are increasing their interest in reducing the environmental footprint of the products and the processing cost [169]. However, analysis and comparison of environmental impacts of nonthermal technologies pose a challenge mainly because of the differences in the scale of the facilities and food processed (meat, egg, fruit, vegetables, liquid food, etc.). In most of the cases, these techniques are not implemented in large-scale industrial facilities and are often studied on lab scale or pilot level without deep analysis of the complete process [170].

Technologies, such as pulsed electric field treatment or high-pressure treatment, not only achieve microbial inactivation under mild conditions or inactivate certain enzymes and prevent undesired changes in food but also decrease processing time and decrease energy consuming [6, 171].

In analyzing nonthermal technologies, it is common to use the life cycle assessment (LCA) approach. It is a scientific method that includes mapping the process, setting the scope and boundaries, collecting data, calculating, evaluating, and interpreting the results with the aim to propose environmental improvements [172]. Hospido et al. [170] stress the difficulties in evaluating environmental impact of these technologies in terms of (i) the lack of real data for the inventory phase, which is often based on lab-scale information or theoretical data; (ii) the definition of the functional unit for comparative studies since new products or processes might have unique properties; and (iii) that manufacture of products or processes can be expected to start several years ahead and assumptions on surrounding systems will be required. Once the technologies are transferred from labs to real production plants, novel processing technologies can be compared with existing commercial alternatives and environmental hotspots can be identified [173].

Evaluation of environmental impact of novel technologies is usually performed using a partial life cycle assessment (LCA) approach. It included mapping the process of novel food treatments, setting scope and boundaries as lab scale, collecting and calculating data, and evaluating the results [172]. Functional unit (FU) as an output reference may be set as 1 kg/1 L of treated food product. Figure 3 depicts the generic system boundaries.

There are some comparative LCA studies of conventional and novel technologies. Pardo and Zufía [174] evaluated the environmental impacts of some traditional and novel food preservation technologies with the aim to contribute to the development of more sustainable food products. Some general improvements were identified, and environmental criteria were provided in order to select the more adequate preservation method when designing new food products. Valsasina et al. [175] compared ultrahigh-pressure homogenization with common thermal treatment for milk. The upscaling showed a decrease in carbon footprint up to 88% achievable with improvements in efficiency.

Aganovic et al. [176] studied the energy balance and LCA of pulsed electric fields and high-pressure processing technologies in comparison with conventional thermal processing applied to the preservation of tomato and watermelon juices. However, at a pilot scale, both pulsed electric field and high-pressure processing technologies presented higher energy consumption expressed per liter of juice, indicating the necessity for further optimization of the process.

6. Future Challenges of Novel Nonthermal Technologies and Conclusion

Legislation on hygienic design of food-processing equipment is rather vague [34]. Considering that there are a large number of different types of standards and regulations related to hygienic design and due to the redundancy of many requirements, a compact tool for evaluating novel technologies is more than needed [33].

In the future, studies related to comparison of environmental impacts of novel and conventional techniques will need to go in two directions: (i) improving the environmental performance of nonthermal technologies *per se*, and (ii) comparing environmental aspects of nonthermal and conventional technologies, along with weighting other factors such as quality of the final product or investment costs [2].

Conflicts of Interest

The authors confirm that there are no conflicts of interest associated with this publication.

Acknowledgments

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