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Review:

Ultrasound decontamination of pesticides and microorganisms in fruits and vegetables: a review

Übersichtsarbeit:

Ultraschalldekontamination von Pestiziden und Mikroorganismen in Obst und Gemüse: ein Review

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Summary

Improper agricultural practices; poor hygiene at all stages of the food chain; lack of preventive controls in food production, processing and preparation; misuse of agricultural chemicals; contaminated raw materials, soil and water are just a few of the factors contributing to potential hazards in foods. In this review, topical advances and trends in ultrasonic decontamination of pesticides and microorganisms associated with fruits and vegetable are presented. Contaminated fruits and vegetables have continually become vehicles of foodborne illnesses. These xenobiotics present in fresh produce cause rot, deleterious contamination and subsequently impacting negatively on people's health. Ultrasonic irradiation as a possible advanced oxidation process has received growing considerations for the degradation of assorted organic pollutants and inactivation of microbes that commonly contaminate pre and post-harvest food produce. Sonolytic effects of ultrasound occur as a consequence of cavitation, which is incessant creation and collapse of bubbles on a split second basis alongside the formation of free radicals and hot spots, characterized by extremely high temperatures and pressure. A variety of studies consistently portrayed ultrasound as an effective tool capable of inhibiting the incidence of deterioration and preserving the quality of post-harvest fruits and vegetables. Combining ultrasound and other decontamination treatments has also produced synergistic benefits of effectively removing residual pesticides and reducing microbial loads in fruits and vegetables.

Keywords: pesticides, microorganisms, fruits and vegetables, ultrasound, cavitation, decontamination

Zusammenfassung

Unsachgemäße landwirtschaftliche Praktiken; schlechte Hygiene in allen Phasen der Lebensmittelkette; Mangel an präventiven Kontrollen bei der Herstellung, Verarbeitung und Zubereitung von Lebensmitteln; Missbrauch von landwirtschaftlichen Chemikalien; Kontaminierte Rohstoffe, Böden und Wasser sind nur einige der Faktoren, die zu möglichen Gefahren in Lebensmitteln beitragen. In dieser Übersichtsarbeit werden aktuelle Fortschritte und Trends bei der Ultraschalldekontamination von Pestiziden und Mikroorganismen, die mit Obst und Gemüse assoziiert sind, vorgestellt. Kontaminiertes Obst und Gemüse ist immer wieder ursächlich für lebensmittelbedingte Erkrankungen. Diese Xenobiotika, die in frischen Produkten vorhanden sind, verursachen Fäulnis, schädliche Kontaminationen und wirken sich negativ auf die Gesundheit der Menschen aus. Die Ultraschallbestrahlung als ein möglicher fortschrittlicher Oxidationsprozess hat wachsende Überlegungen für den Abbau von verschiedenen organischen Schadstoffen und die Inaktivierung von Mikroben, die üblicherweise Nahrungsmittelprodukte vor und nach der Ernte kontaminieren, erhalten. Sonolytische Effekte von Ultraschall treten als Folge von Kavitation auf, die unaufhörliche Entstehung und Kollaps von Blasen im Bruchteil einer Sekunde neben der Bildung von freien Radikalen und Hotspots ist, gekennzeichnet durch extrem hohe Temperaturen und Druck. Eine Vielzahl von Studien stellte Ultraschall als ein wirksames Werkzeug dar, das in der Lage ist, die Verschlechterung zu hemmen und die Qualität von Obst und Gemüse nach der Ernte zu erhalten. Die Kombination von Ultraschall und anderen Dekontaminationsbehandlungen hat auch synergistische Vorteile bei der effektiven Entfernung von Restpestiziden und der Verringerung der mikrobiellen Belastungen in Obst und Gemüse gebracht.

Schlüsselwörter: Pestizide, Mikroorganismen, Früchte und Gemüse, Ultraschall, Kavitation, Dekontamination

Die Inhalte sind urheberrechtlich geschützt. Eine Weitergabe an unberechtigte Dritte ist untersagt.

Introduction

Fruits and vegetables as vital components of the human diet are required mostly to complete a balanced diet. They provide essential nutrients that are required to maintain a healthy body through the prevention or reduction of diseases. These produce similar to other crops, during pre and postharvest stages are mostly attacked by pests and (Keikotlhaile and Spanoghe, 2011). Pesticides the likely solution to pest damage and possible disease infections are mainly used in agriculture to enhance productivity by preventing or reducing losses from insect pests (Ibitomi and Mohammed, 2016) that can noticeably decrease the yield of harvestable produce (Zhang, 2009). Nevertheless, organic pesticides have negative consequences on living organisms and the environment (Keikotlhaile and Spanoghe, 2011). They are characteristically persistent against degradation and removal, semi-volatile, bio-accumulative and even highly toxic to humans and their surroundings at significantly lower concentrations (Yu et al., 2005; Liu et al., 2009). Long term, lower dose exposures are increasingly linked to immunosuppression, hormone disorder, diminished brainpower, reproductive abnormalities, cancer, asthma and heart related ailments (Kitamura et al., 2003; Neishabouri et al., 2004; Gilden et al., 2010).

Rigorous research has been devoted to understanding and accepting the complex interactions of human pathogens with plants and how advances in microbiological safety issues concerning fresh produce can be accomplished (Warriner et al., 2009). Fruits and vegetables which are mostly consumed raw or minimally processed are considered likely carriers and transmission vehicles of pathogens (Berger et al., 2010). Recent public health campaigns for healthier living styles, has led to an exponential increase in demand and consumption of fruits and vegetables, hence an increase in possible cases of foodborne illnesses. Several investigations directed towards the potential sources of fruits and vegetable contaminations by a microorganism in the food supply chain have settled on the pre-harvest and post-harvest stages of production (Berger et al., 2010). Possible sources of vegetable contamination include irrigation and postharvest processing water (Sivapalasingam et al., 2003; Hamilton et al., 2006), poorly composted or raw animal manures or sewage (Santamaria and Toranzos, 2003), droppings of wild animals (Ackers et al., 1998) and insects such as flies (Talley et al., 2009).

Recently, cost effective and efficient methods have been developed that could eliminate residual pesticides and microbial activities while partially or completely reducing or excluding thermal requirements. Emerging technologies including high hydrostatic pressure (Iizuka et al., 2013; Iizuka and Shimizu, 2014), pulsed electric field (Zhang et al., 2012), non-thermal plasma (Bai et al., 2009; Misra et al., 2014), irradiation (Trebse and Arcon, 2003), ozonation (Wu et al., 2007; Ikeura et al., 2011; Chen et al., 2013) and ultrasonication (Knorr et al., 2004; Matouq et al., 2008; Cameron et al., 2009; Yao et al., 2010), have been extensively investigated for numerous decontamination applications in food and water research. Ultrasound has been one promising technology developed to lessen processing, maximize quality and ensure the safety of food products. Through its cavitational effects on matrices, ultrasound has been applied effectively in the inactivation of microorganisms (Knorr et al., 2004; Cameron et al., 2009; Birmpa et al., 2013; Ferrario et al., 2015; Cruz-Casino et al., 2016), removal and degradation of pesticides (Tianli et al., 2009; Zhang et al., 2010a, 2010b; Lozowicka et al., 2016) in fruits and vegetables. High-power ultrasound possesses the potential to be developed into an advance oxidation process for food safety enhancement, preservation and shelf-life improvement devoid of quality and organoleptic property losses (Knorr et al., 2004; Chemat et al., 2011; Gao et al., 2014). As an alternative technique to pasteurization and sterilization, ultrasound is attaining significance owed to the increasing level of awareness and consumer demand for new, improved methods of food processing that do not impact negatively on overall food quality (Yuting et al., 2013; Gao et al., 2014). Factors that may turn to be influential to ultrasound efficiency as a decontamination technology are as follows: (1) type of fruit or vegetable; (2) target pesticide or microorganism; (3) initial pesticide concentration or microbial load; (4) chemical composition of the pesticide or physiological state of the bacterial cells; and (5) ultrasonic operating conditions and variables.

In this review, ultrasound applications in pesticide removal and degradation, and microorganism inactivation in fruits and vegetables were highlighted. Routes of contamination and scientific literature supporting the mechanisms, levels and possible inefficiencies of ultrasonic decontamination, and quality attributes of sonicated products were presented.

Principles of Ultrasound

Ultrasound is described as sound waves having frequencies that surpass the hearing limit of the human ear (Awad et al., 2012) and commences at 16 kHz which is closer to the upper limit of the human hearing (Elmehdi et al., 2003). The sound band is divided into three main categories and designated as infrasound ($v < 20$ Hz), acoustic (20 Hz $< v <$ 20 kHz) and ultrasound ($v >$ 20 kHz). The ultrasound band is further divided into power or low frequency ultrasound (20–100 kHz), high frequency ultrasound (100 kHz–1 MHz) and diagnostic ultrasound (1–500 MHz) (Zhou et al., 2013). Engineering applications of ultrasound are categorised into two important distinct groups: non-destructive ultrasound (low intensity or high frequency; >100 kHz, <1 Wcm^{-2}) and power ultrasound (high intensity or low frequency; <100 kHz; >10 Wcm^{-2}). The former is applied in the detection of imperfections or location of hidden substances, medical and industrial imaging (Dolatowski et al., 2011) while the latter basically effects physical and mechanical changes in materials or initiates reactions chemically (Golmohamadi et al., 2013).

Generation of power ultrasound

Modern ultrasonic equipments which come as either a bath or a probe (horn) type consist basically of a power generator, a transducer and an emitter (Bermudez-Aguirre and Barbosa-Canovas, 2012).

The electrical generator serves to provide energy for the operation of the ultrasound system. It creates electrical current with a definite power rating, and instantaneously allows the power to be fixed indirectly through voltage and current setups which respectively represents stored energy in electrons and net charge of electrons (São José et al., 2014a). Most generators which operate ultrasound at lower frequency ranges are designed and produced precisely for purposes of decontamination (disinfection and cleaning) (Bermudez-Aguirre and Barbosa-Canovas, 2012).

Transducers in ultrasonic devices convert electrical energy into sound energy via mechanical vibrations. Transducers are the components within the ultrasound system that generates sound waves with frequencies exceeding the human hearing limit. Liquid-driven, magnetostrictive and piezoelectric transducers are the three main types of transducers in ultrasound devices. Whilst piezoelectric and magnetostrictive transducers convert electrical and magnetic energy into ultrasound, liquid-driven transducers solely transform mechanical energy into ultrasound (Bermudez-Aguirre and Barbosa-Canovas, 2012).

The emitter, also known as the coupler or reactor directs and radiates ultrasound waves from the transducer into the treatment medium for the desired effect. Emitters are either of bath or horn types (São José et al., 2014a).

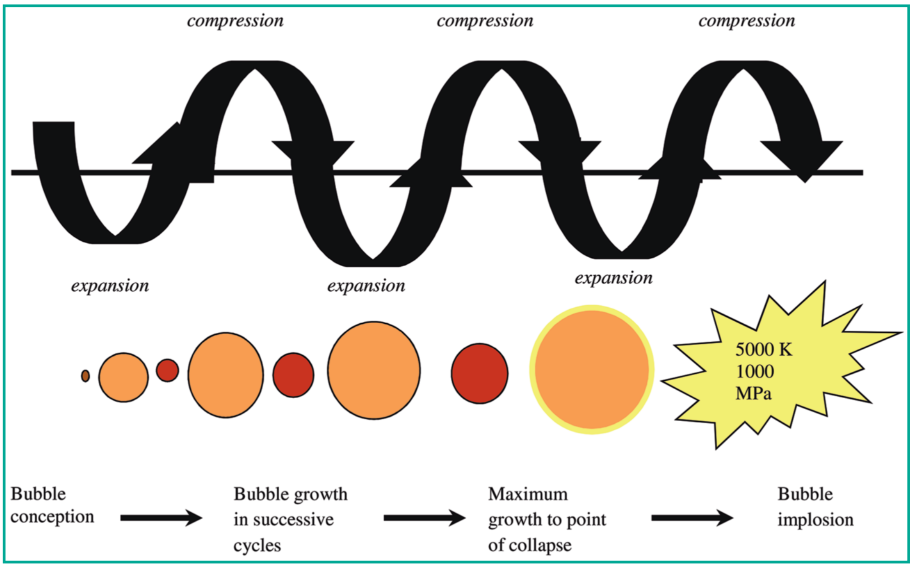

FIGURE 1: *Conception, growth and implosion of the cavitation bubble.*

Power Ultrasound

Power ultrasound has in recent years being used in processing of food products. Its use in the food industry dates back to 1927 when a preliminary survey was published regarding the chemical effects of the waves (Dolatowski et al., 2007). Power ultrasound is an effective technique in the degradation of contaminants without the addition of chemicals (Serpone and Colarusso, 1994) and especially in the degradation of organic pesticides (Matouq et al. 2008; Yao et al., 2010) and inactivation of microbes (Knorr et al., 2004; Sagong et al., 2011; Birmpa et al., 2013; Cruz-Casino et al., 2016). It is characterized by power intensities generally ranging between 10 and 10^3 W/cm^2 at frequencies between 20 and 100 kHz (São José & Vanetti, 2012). High intensity or low frequency ultrasonic appliances usually depend on multifaceted vibration induced effects in the transmitting intermediates, which produce cavitations in matrices. Table 1 presents a summary of studies on ultrasound degradation of pesticides and inactivation of microbes in fruits and vegetables.

Ultrasonic Cavitation

Cavitation is the mechanism that promotes the desired effects of power ultrasound to occur in food materials. This occurrence is responsible for removal of pesticides, the extermination of pathogenic cells and inactivation of enzymes and their activities (Povey and Mason, 1998). Alternating compressions and expansions are created when excited waves come into contact with aqueous matrix during sonication while a squeezing and releasing phenomenon (sponge effect) is the reaction developed when sound waves acts on a solid material (Piyasena et al., 2003). Eventually, the pressure from the various internal activities creates bubbles that expand in size within microseconds and finally collapse viciously, creating a cavitational condition as shown in Figure 1 (Gogate and Kabadi, 2009; González-García et al., 2010; Chemat et al., 2011). These bubbles have outsized surface areas during the expansion cycle, which increases the diffusion of gas, causing the bubble to expand. Throughout the sonication process, multitudes of such cavitation generating bubbles are formed and based on structural properties are grouped in two forms namely transient (non-stable, small) and stable (non-linear, large) bubbles of cavitaion (Awad et al., 2012). The effects of cavitation can produce a number of desirable changes in foods. Decline in reaction time, raise in reaction yield and operating under reduced conditions of temperature and pressure are some of the benefits derived from cavitation compared to some conventional ways.

Ultrasound frequency

Ultrasonic frequency (10–100 kHz) depends on the power and the emitter dimensions. Power is inversely proportional to the square of the frequency as shown in Equation (1) (Povey and Mason, 1998).

$$P \propto \frac{1}{f^2} \qquad (1)$$

Where P is desired power and f the frequency

Irradiation at higher frequencies (≥100 kHz) augments reaction rates than that at lower frequencies (≤20 kHz) (González-García et al., 2010) due to the fact that the extent of cavitation taking place in a solution depends on the frequency-bubble size relationship. Frequency is the single most essential operational variable in a sonication process despite the fact that bubble radius is somewhat subjective to the characteristics of the dissolved gases. Soaring pressures from sound vibrations initiates pulsating and subsequent rapid increase in size of bubbles in liquids (Povey and Mason, 1998). Ultrasonic frequency forces the bubbles into resonance and affects the reaction location by shaping the resonant bubble radius, since the efficient absorption of energy by bubbles depends on the excitation level between bubble nuclei and resonant radius. Variations in bubble radii will result in different bubble implosion periods with diverse surface area to volume ratios (González-García et al., 2010).

Ultrasound power intensity

In general, power intensity (Pi) is defined as the amount of power (P) transmitted over a given surface area (A) as depicted in Equation (2) (Hecht, 1996). Ultrasonic power intensity influences both pyrolysis and radical attack. High power intensities augment bubble numbers and boosts reaction rates, thus increasing the final pressure and temperature within the bubbles as sound intensity is directly related to sound pressure (Equation 3) (Lim et al., 2007). An increase in ultrasound intensity causes temperature and pressure to increase within the bubble core (Equation 4 & 5) (González-García et al., 2010). This leads to an enhancement in the general removal or inactivation rate of the target biological or chemical entity and accelerates the formation of free radicals (Lim et al., 2007). High temperature initiates the production free radicals while the release of pressure increases the chemical reaction.

$$Pi = \frac{P}{A} \quad (2)$$

$$Is = \frac{Ps^2}{2pc} \quad (3)$$

$$Tmax = T\left[\frac{Pn(X-1)}{Po}\right] \quad (4)$$

$$Pmax = Po\left[\frac{Pn(X-1)}{Po}\right] \quad (5)$$

where, *Is* denotes the sound intensity, *Ps* is the sound pressure amplitude, *p* is the density of the solution, *c* is the acoustic speed within the solution, *Tmax* and *Pmax* are the respective maximum temperature and pressure at implosion, *T* is the ambient temperature; *X* is the specific heat ratio; *Po* is the pressure in the bubble at its maximum size and mostly said to be equal to the vapour pressure of the solution; and *Pn* is the utmost pressure within the bubble at the time of implosion (Lim et al., 2007; González-García et al., 2010).

Pesticide contamination of fruits and vegetables

Synthetic pesticide use became widespread in the 1950s as part of the scientific world's effort to ending post Second World War hunger (Zhang, 2009). The use of pesticides in agriculture brought about a decline in pest abundance and damage to levels that were not previously attainable. Pesticides are applied per cropping period to about 30 % of agricultural produce and without which will result in an estimated combined fruit, vegetable and cereal loss of between 32 and 78 % to pest damage (Liu et al., 2002; Cai, 2008). Lewis and Jamie (2005) attributed doubling in wild blueberry production and ensuing increase in its consumption predominantly to herbicide use that enhanced weed control. Despite their positive role in protecting crops and subsequently impacting yields, pesticides can be exceptionally perilous to the human body and other living organisms considering their persistent nature over time. Due to limited agricultural lands and increasing population, most crop farmers make it a necessity to maximise production in order to ensure food security, hence the global rise in pesticide use (Zhang, 2009). Collectively, pesticides of high agricultural importance are insecticides, fungicides and herbicides (Ibitomi and Mohammed, 2016). Fruits and vegetables that are produced and marketed following the use of pesticides in crop protection may contain trace pesticides. These pesticides which happen to infiltrate the tissues of fruits and vegetables and remain as residues may result in contamination and a possible threat to human health (Abdulra'uf et al., 2012). Their incidence in the food production chain (Figure 2) is of high concern for consumers with regards to their potential harmful effects on non-targeted organisms. Pesticides of agricultural importance can be classified according to the target pest, environmental stability, formulation, chemical structure, toxicity and mode of action on target organism. They can be categorised into inorganic (borates, silicates and sulfur) and organic compounds (organochlorine, organophosphorus, organonitrogen (carbamates) and pyrethroid) (Fenik et al., 2011). The acute and subacute health implications of these compounds have been well recognised and documented (EPA, 2003).

Mechanisms of pesticide degradation by sonication

Power or material-altering (Povey and Mason, 1998) ultrasound is associated with a strong acoustic wave action that can introduce and effect extreme pressures inside aqueous matrices, generating streams of vigorously moving microbubbles and causing these bubbles to implode forcefully generating exceedingly elevated temperatures (5000 K) and pressure (1000 atm) (Mann and Krull, 2004; Mukhopadhyay and Ramaswamy, 2012) in a process known as acoustic cavitation. This phenomenon subsequently generates three different zones for chemical reaction to occur (Joseph et al., 2009; Yao et al., 2010):

(1) The core of the imploding bubble cavity where unstable and hydrophobic molecules undergoes pyrolysis due to elevated temperature (5000K) and hydroxyl radicals formed in the process through sonolysis. In contrast, hydrophilic or stable molecules tend to remain in the mass solution during irradiation such that the main reaction location becomes the solution medium at low concentration and/or the bubble-solution interface at higher concentration (González-García et al., 2010).

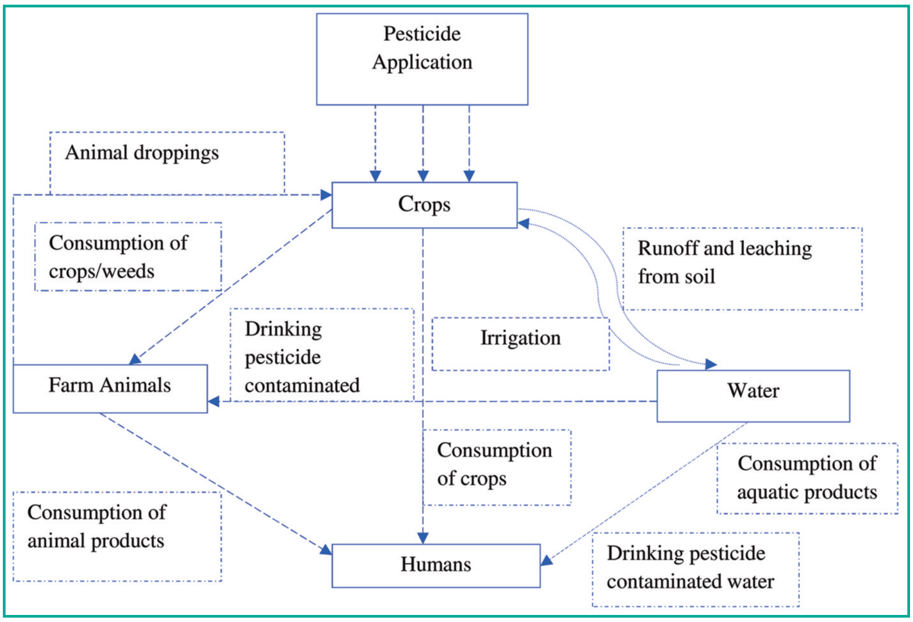

FIGURE 2: *Contamination routes of pesticides in the food chain.*

$$H_2O \rightarrow OH^{\bullet} + H^{\bullet} \quad (6)$$

$$OH^{\bullet} + \textit{Pesticide} \rightarrow H_2O + CO_2 \quad (7)$$

(2) The bubble solution interface with minimal temperature (2000 K) is mainly the location of hydroxyl reactions.

$$OH^{\bullet} + \textit{Pesticide} \rightarrow H_2O + CO_2 \quad (8)$$

(3) Mass solution in its entirety at ambient temperature (300 K) is where free radicals migrating from the bubble solution interface are contained. Radicals which may diffuse into the bubble-solution interface or into the mass solution can oxidize the target pesticide. The contained radicals that do not react with the target (pesticide) may also produce hydrogen peroxide in the bulk solution:

$$2OH^{\bullet} \rightarrow H_2O_2 \quad (9)$$

$$2OH_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad (10)$$

Hydroxyl radicals can attack organic pesticides in mass solution, within the imploding cavitation bubble or at the bubble-solution interface, depending on the water affinity of the pesticide or pollutant (Xiao et al., 2014).

Removal and degradation of pesticides by sonication

Ultrasound has been used widely in the removal, degradation of organic pollutants or contaminants (Gopiraman et al., 2015). As an advance oxidation process, ultrasound produces non-selective hydroxyl radicals which attacks and reacts with organic pesticides reducing them to less toxic molecules or mineralizing them mostly into carbon dioxide and water (Babu et al., 2016).

Degradation rate declined as the initial concentration of pesticide increased, in an ultrasonic degradation of diazinon in apple juice. It declined from 51.3 % at a concentration 7.82 µmol/l to 10.8 % at a concentration 65.2 µmol/l after half an hour of ultrasonic treatment at 500 W. This occurrence was attributed to the possible induction of ultrasonic hydroxyl free radicals initiated maximally at the interface of the bubble and minimally in the entirety of the mixture. A degradation pathway involving hydrolysis of the ester moiety, oxidation, hydroxylation, dehydration, and decarboxylation was proposed in the study. Identified degradation products were identified by: (i) means of the NIST mass spectral library, (b) comparing the retention time and the mass spectra of analyte with valid standards, (c) comparing the mass spectra of analyte with previously reported spectra, and (d) interpreting the fragment ions. Products identified included IMP, diazoxon, 2-hydroxydiazinon, isopropenyl derivative of diazinon, hydroxyethyl derivative of diazinon, diazinon methyl ketone and hydroxydiazinon (Zhang et al., 2010b). NIST Mass Spectral Library confirmation revealed malaoxon and chlorpyrifos oxon as degradation products of malathion and chlorpyrifos identified respectively after ultrasonic degradation and GC-MS analysis of these organophosphorus insecticides in apple juice. Malaoxon and chlorpyrifos oxon were subsequently confirmed and proposed as the ultrasonic oxidation pathways of the degraded pesticides (Zhang et al., 2010a). The degradation of phorate (O, O-diethyl S-[(ethylthio) methyl] phosphorodithioate) a commonly used organophosphorus pesticide spiked in apple juice by sonication was investigated and quality implications analysed. Ultrasonic power and sonication time significantly ($p < 0.05$) and effectively aided the degradation and elimination of the pesticide in the juice. Phorate degradation increased with an exponential decrease in concentration, increase in sonication time and ultrasonic power while following first-order kinetics. This was attributed to a corresponding increase in cavitations at increasing ultrasonic power and accelerating hydroxyl radical creation. Gas chromatograph coupled with mass spectrometer (GC–MS) identified phorate-oxon and phorate sulfoxide as the proposed degradation products of phorate. During sonication, phorate made contact with the hydroxyl (OH) radicals and it's P=S and C-S-C bonds oxidised leading to the formation of phorate oxon and phorate sulfoxide (Zhang et al., 2012). The optimal conditions of ultrasonic removal of organochlorine pesticide residues in apples were studied using response surface methodology to evaluate the influence ultrasonic power, degradation time and degradation temperature on the degradation rate and post treatment quality parameters. The best pesticide removal conditions were ultrasonic power, dissipation time and degradation temperature of 609.16 W, 70.46 min, and 15.45 °C respectively. Degradation rate of the organochlorine pesticide (OCP) residues reached 64.32 % under these conditions, indicating the efficiency of ultrasonic irradiation in removing pesticide residue and possibly other organic contaminants (Tianli et al., 2009). Yamashita et al. (2009) examined the use of ultrasonic washing for the purpose of removing chlorothalonil on the exterior of cherry tomato at frequencies of 28, 45 and 100 kHz. The removal rate of the pesticide residue on the surface of the vegetable increased with ultrasonic frequency in the order of 45, 28 and 100 kHz. The elimination rate at 45 kHz with replacement of water reached 86 % in just 2 minutes of washing. Sonolytic washing at 28 kHz and 45 kHz was effective for surface decontamination. At different processing times (1, 2 and 5 min), the effects of decontamination treatments (washing with tap and ozone water, ultrasonic cleaning and boiling) on 16 pesticide (acetamiprid, alpha-cypermethrin, boscalid, bupirimate, chlorpyrifos , cyprodinil, deltamethrin, fenhexamid, fludioxonil , folpet, iprodione, lambda-cyhalothrin, pirimicarb, pyraclostrobin, tetraconazole and trifloxystrobin) residues in fresh strawberry fruits were investigated. Ultrasonic cleaning reduced residual levels of all pesticides up to 91.2 %. The results indicated that ultrasonic cleaning and boiling were the most effective treatments, resulting in possible lower health risk incidences (Lozowicka et al., 2016).

Microbial contamination of fruits and vegetables

With an improvement in processing and packaging techniques, microbiological spoilage has become a key factor for sensory quality failures and criterion for shelf life prediction (wetness and soft rot, surface staining, visual microbial colonies, translucency, moisture loss, off-aroma and flavour, and texture variance) of most fruits and vegetables, especially minimally processed products (O'-Connor-Shaw et al., 1994; Artes and Martinez, 1996; Sapers et al., 2001; Ukuku and Fett, 2002; Allende et al., 2002; Jacxsens et al., 2003). Fruits and vegetables have recurrently been cited in foodborne illnesses and transmissions to humans (CDC, 1979; Martin et al., 1986; Griffin and Tauxe, 1991) as they are easily contaminated with foodborne pathogens on the fields during the preharvest stage, or after growth during harvesting, postharvest handling, processing, and delivery (Beuchat, 1996; Berger et al., 2010). Hence, timely interventional practices during these stages through the use of good agricultural practices (GAP) will provide remarkable decline in losses due to infection and spoilage along the chain of activities (Eckert and Ogawa, 1988). Bacteria, viruses and parasites are the main pathogenic microorganisms implicated in most food born disease outbreaks. These pathogens may be present in irrigation water or in the soil where the produce is grown (Beuchat, 1996; Sivapalasingam et al., 2004; Berger et al., 2010). Despite the varied array of pathogenic microbes capable of contaminating fresh fruits and vegetables at any point in the chain, *Escherichia coli* O157:H7 and *Salmonella* are the main pathogenic microbes of high concern (Warriner et al., 2009). Sprouted seeds, tomatoes and green leafy vegetables remain the most implicated (Doyle and Erickson, 2008) due to their market output volumes (Valentin-Bon et al., 2008). It is however observed that, some pathogenic microbes thrive on specific produce (Bassett and McClure, 2008; CDC, 2008). Outbreak of foodborne illnesses associated with tomatoes (Greene et al., 2008); sprouted seeds, cantaloupes and lettuce (Sivapalasingam et al., 2004; Arthur et al., 2007; Warriner et al., 2009) have been linked to *Salmonella*. *Escherichia coli* O157:H7 has been also linked with sprouted seeds, lettuce, apples and spinach (Sivapalasingam et al., 2004; Valentin-Bon et al., 2008). *Penicillium expansum* (Miedes and Lorences, 2004) and *Botrytis cinerea* (van Kan, 2006) are pathogens of apples, pears, and a number of fruits rich in pectin. Figure 3 depicts the mode of microbial contamination of fruits and vegetables.

Mechanisms of ultrasonic inactivation of microbes

The foremost ultrasonic microbial inactivation capacity was realised in the 1960s when multitudes of fishes died when ultrasonic waves were applied in an anti-submarine experimental warfare (Earnshaw et al., 1995; Piyasena et al., 2003). From then, more studies were channelled towards unravelling the mechanism behind the killing effect of the sound waves (Hughes and Nyborg, 1962) and research on ultrasound inactivation of microorganisms gained grounds afterwards. The bactericidal effect of ultrasound is well-known and widely believed to disrupt cells through cavitation, an important attribute of ultrasound, basically responsible for the removal or dissipation of spoilage and human pathogenic microbes on food surfaces. It encompasses physical, chemical and mechanical effects (Yuting et al., 2013). The mechanism of microbial annihilation is primarily due to the thinning and weakening of cell walls, restricted heating leading to the generation of extreme temperature and pressure and creation of free radicals which eventually acts on the chemical composition of the microorganisms (Fellows, 2000; Chemat et al., 2011). During unsteady cavitation process, the responsible bubbles containing gas and vapour may be excited to extreme temperatures (5000 °C) and pressure (1000 MPa) creating series of hot spot regions in microseconds. This resultant series of implosions generate restricted temperature and pressure conditions within the matrix. The high pressures are deemed liable for cell disruption. The elevated temperatures produced might also have some impact, but as these temperature changes occur shortly during cavitation, only the immediate surrounding medium is heated and consequently only a minimum number of cells would be affected. Imploding bubbles radiate shock waves strong enough to tear and break active cell walls and membrane fortifications. These mechanical shock waves generated through cavitation are mostly responsible for microbial killing in sonication processes (Fellows, 2000). The deoxyribonucleic acid (DNA) of microbes is mostly the prime target of free radicals, which produce breakages and degeneration along the stretch of the DNA strand (Bermudez-Aguirre and Barbosa-Canovas, 2012). The sugar-phosphate support of the DNA chain is mostly attacked by the hydroxyl radical resulting in the division and breakage of the phosphate-ester bonds holding the microbial DNA double strand. This exalts the sonochemical prowess of power ultrasound through its production of major hydroxyl radicals after bubble growth and collapse during cavitation. Free radical creation and electron transfer are major chemical attributes of cavitation and has been involved in the formation of compounds with antimicrobial properties through rearrangements of radicals and atoms. Aside direct removal of microorganism, power ultrasound through its cavitational effects promotes antimicrobial effectiveness by deteriorating the cell wall fortifications and facilitating penetration of chemical cleaning agents (Yuting et al., 2013).

Inactivation of microbes by sonication

Several investigations have been conducted into the bactericidal effects of ultrasound on a variety of human patho-

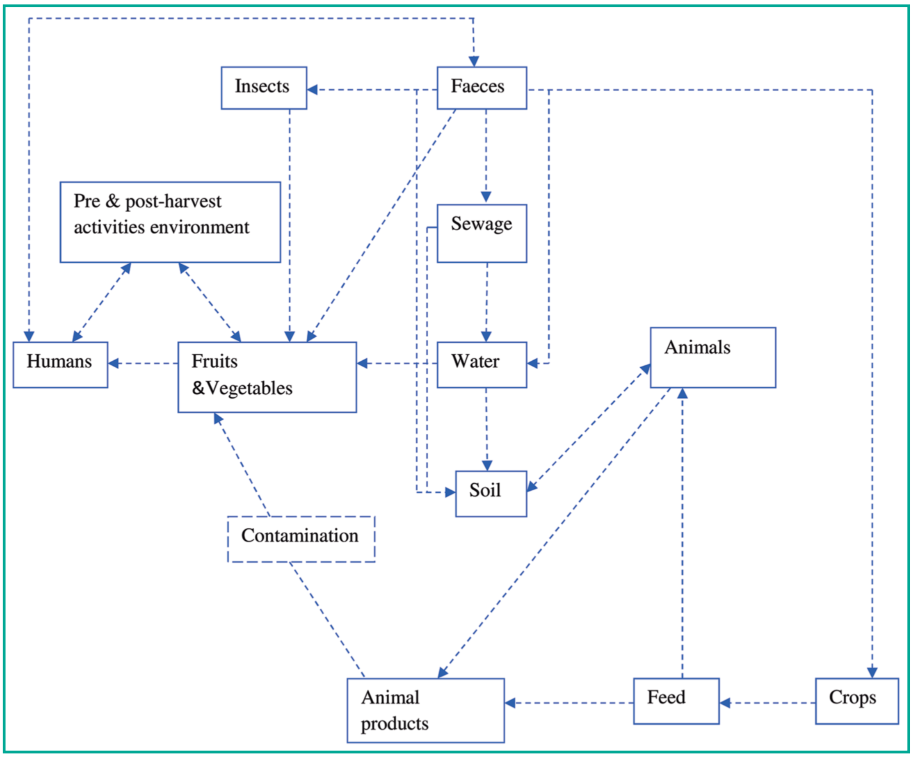

FIGURE 3: *Mode of microbial contamination and transmission in fruits and vegetables (Adapted and modified from Beuchat 1996)*

gens in food produce. Microbes such as *Salmonella* spp., *Listeria monocytogenes, Escherichia coli* O157:H7, *Staphylococcus aureus,* and *Cronobacter sakazakii* have been studied in this regard (Scouten and Beuchat, 2002; Sagong et al., 2011; Birmpa et al., 2013; Chen, 2017). Ultrasound has also been found to be effective against traditional food spoilage microorganisms (Cao et al., 2010a; Cao et al., 2010b; Chen and Zhu, 2011; São José and Vanetti, 2012; Ding et al., 2015; Chen, 2017), including total aerobic and lactic bacteria, yeasts and molds. Green and purple cactus pear juices were treated with ultrasound at 1.5 kW and a constant frequency of 20 kHz, using amplitude levels of between 60–90 % at an interval of 10 % for 1, 3 and 5 min with pulse durations of 2 s on and 4 s off. The investigation was aimed at assessing the efficiency of ultrasound as a preservation technology for the inactivation of *Escherichia coli* in the juices under storage. Ultrasound applied for 1 min reduced bacteria counts by 1–3 log CFU/mL which further improved (3–4 log CFU/mL) under 3 min treatment in both juices. For a period of 48 hours, samples sonicated at 90 % amplitude for 5 min resulted in non-detectable levels of *E. coli* while complete inactivation was achieved in both fruit juices after 5min of sonication at all amplitudes except 60 and 80 % (Cruz-Casino et al., 2016). The efficacy of individual treatments (ultrasound and organic acids) and their combination on reducing foodborne pathogens on raw fresh lettuce leaves inoculated with three strains apiece of *Escherichia coli* O157:H7, *Salmonella Typhimurium,* and *Listeria monocytogenes* was studied. It was reported that leaves treated with ultrasound (40 kHz, 30W/L) alone significantly ($P < 0.05$) reduced the numbers of *E. coli* O157:H7, *S. Typhimurium,* and *L. monocytogenes* on lettuce. There existed significant reduction in pathogens across all treatment times (5–60 min) with the decrease in microorganisms mainly attributed to cavitational effects. The utmost drop in numbers of *E. coli* O157:H7, *S. Typhimurium,* and *L. monocytogenes* on samples were 1.88, 1.64, and 1.81 log CFU/g after 60 min treatments (Sagong et al., 2011). Strawberry fruits were sonicated with 0, 25, 28, 40 or 59 kHz at 20 °C for 10 min. Ultrasonic treatment at 40 kHz significantly ($P < 0.05$) reduced decay occurrence and counts of microorganisms (Cao et al. 2010b). The effect ultrasound on the inactivation of bacteria in freshly harvested lettuce and strawberry was studied by Birmpa et al. (2013). The highest reductions of microbes, recorded in strawberries after treatment with ultrasound (37kHz, 30W/L), were respectively reported as 3.04, 2.41, 5.52 and 6.12 log CFU/g for *E. coli, S. aureus, S. Enteritidis* and *L. innocua. Salmonella enterica* Enteritidis and *Escherichia coli* were removed from green peppers and melons using ultrasound. Ultrasound was confirmed to have enhanced the microbial safety of the products after sonication at 40 kHz. The decimal reductions achieved for both green pepper and melon produce were respectively 1.8 and 1.9 log CFU/cm^2 for *S. enteric* and 2.3 and 1.6 log CFU/cm^2 for *E. coli.* Sonolytic treatments decreased the adherence of the bacteria on the produce surfaces by over 1.4 log CFU/cm^2 (São José et al., 2014b). Ferrario et al. (2015) evaluated the effect of ultrasound (600 W, 20 kHz and 95.2 mm wave amplitude; 10 or 30 min at 20, 30 or 44 ± 1 °C) on the inactivation of *Alicyclobacillus acidoterrestris* ATCC 49025 spores and *Saccharomyces cerevisiae* KE162 inoculated in commercial (pH: 3.5; 12.5 °Brix) and natural squeezed (pH: 3.4; 11.8 °Brix) apple juices was examined and a modest 2.5-log decline observed in *S. cerevisiae* cells after half an hour of sonication at 30 ± 1 °C for both juices and at 44 ± 1 °C for commercial apple juice only while a slightly higher 2.8-log decrease was observed in natural apple juice sonicated at 44 °C. Sonication was however unable to inactivate *A. acidoterrestris* spores, as no reductions were observed after 30 min of treatment.

TABLE 1: *Summary of studies on ultrasound degradation of pesticides and inactivation of microbes in fruits and vegetables.*

Matrix	Contaminant	Operating parameters	Removal rate	Reference
Apple juice	Malathion	500 W, 120 min	47.10 %	Zhang et al. (2010b)
	chlorpyrifos		82.00 %	
	Diazinon	500 W, 30 min	51.30 %	Zhang et al. (2010a)
	Phorate	500 W, 25 kHz, 120 min		Zhang et al. (2012)
Apples	OCP	609.16 W, 70.46 min, 15.45 °C	64.32 %	Tianli et al. (2009)
Cherry tomato	Chlorothalonil	45 kHz, 2 min	86 %	Yamashita et al. (2009)
Strawberry	16 pesticides	240 W, 40 kHz, 1–5 min	91.20 %	Lozowicka et al. (2016)
Lettuce	*E. coli*	40 kHz, 30 W/L, 60 min	1.88 log CFU/g	Sagong et al. (2011)
	S. Typhimurium		1.64 log CFU/g	
	L. monocytogenes		1.81 log CFU/g	
Lettuce and strawberry	*E. coli*	37 kHz, 30 W/L	3.04 log CFU/g	Birmpa et al. (2013)
	S. aureus		2.41 log CFU/g	
	S. enteritidis		5.52 log CFU/g	
	L. innocua		6.12 log CFU/g	
Green peppers and melons	*S. enteritidis*	40 kHz	1.8 log CFU/cm^2	São José et al. (2014b)
	E. coli		1.9 log CFU/cm^2	
Cactus pear juice	*E. coli*	1.5 kW, 20 kHz, 90 % amplitude, 5 min	Non-Detectable	Cruz-Casino et al. (2016)

Inefficiency of Ultrasound as a decontamination technology

Scientific literature reveals that ultrasound alone is less efficient in removing, degrading pesticides and inactivating microorganisms dependably for functions of food preservation and protection. The effectiveness of ultrasound as a decontamination technology can be fully achieved when combined with other treatments (Sango et al., 2014). Ultrasound may be useful in the decontamination of fruits and vegetable surfaces when applied in conjunction with other applications such as: heat, high hydrostatic pressure, ultraviolet radiation, pulsed electric field, pulsed light, gamma and x-ray radiation or chemicals (São José et al., 2014a). It was evidentially important to combine ultrasound with other technologies to improve pollutant removal rate when the roles of individual and hybrid advanced oxidation processes (AOP) removal of sulfamethoxazole were compared (Koda et al., 2003). It is environmentally friendly, operationally simple, efficient, and economically feasible when ultrasound is coupled with other advanced oxidation processes such as photocatalysis, photolysis, Fenton reaction and ozonation (Babu et al., 2016) for purposes of pesticide removal. The efficacy and synergistic effects of combining two or more approaches relies mainly on the intonation of the reaction chemistry (Misra, 2015). In a laboratory-scale experiment, the combined efficacy of ultrasound and ozone on the degradation of two organophosphorous pesticides (Methamidophos and dichorvos), in lettuce were examined and confirmed effective. Parameters including ozone flow rate, water temperature, treatment time and initial concentration of pesticides were selected and studied (Fan et al., 2015). The catalytic removal capacity and mechanism of ultrasound in combination with TiO^2 on pyrethroid pesticides in apple juice were studied and response surface modelling used to optimize the process parameters such as amount of TiO_2, ultrasonic power, treatment time and ultrasonic temperature. High removal rate was recorded with physical and chemical indices remaining unaffected (Yuan et al., 2011).

A variety of microorganisms by their make-up are comparatively resistant to ultrasound effects. The application of ultrasound alone may not by this resistance decrease the microbial loads sufficient enough to satisfy food safety requirements (Alzamora et al., 2011). Vis-à-vis the microbial killing-effect of ultrasound, spore-forming bacteria, fungi, aerobes, cocci and gram positive bacteria are observed to be resistant to sonolysis than vegetative forms, bacteria, anaerobes, bacilli and gram negative bacteria correspondingly. *Listeria monocytogenes* 10403S was cited as the most ultrasound resistant strain among all *L. monocytogenes* strains tested in a study investigating the bactericidal effect of power ultrasound treatments on *Listeria monocytogenes* in apple cider (Baumann et al., 2005). Less than 0.12- log reduction of *A. acidoterrestris* spores was achieved after sonication (10 min, 330 W) of contaminated commercial apple juice concentrate (Djas et al., 2011). This further explains the somewhat low reduction of microbes in most studies that employed ultrasound as a standalone decontamination technology: strawberry; 0.6 log CFU/g (Cao et al., 2010b; Alexandre et al., 2012), cherry tomatoes; 0.8 log CFU/g (São José and Vanetti, 2012), shredded carrot; 1.3 log CFU/g (Alegria et al., 2009), and iceberg lettuce; 1.5 log CFU/g (Seymour et al., 2002). Based on the hurdle technology, ultrasound has been useful in combination with other energy forms and chemical agents (Bermudez-Aguirre and Barbosa-Canovas, 2012; Wordon et al., 2012; Bang et al., 2017). With respect to fruits and vegetables, thermosonication and manosonication are mostly not recommended due to their possible drastic alteration of plant tissue structure (São José et al., 2014a). However, there exist some good amount of scientific evidence in literature confirming the synergistic success in recent times of microbial inactivation by the combination of ultrasound and some chemicals (Sagong et al., 2011; Chen and Zhu, 2011; Coronel et al., 2011; Sagong et al., 2013; Forghani and Oh, 2013; Bang et al., 2017). Ultrasound has been shown to be particularly effective in reducing microbial loads when combined with chemical treatment and offers some advantages including: decrease in chemical utilization, minimal contact with hazardous chemicals, enhanced decontamination and consistency (Mason, 2003). Organic acids, sodium hypochlorite, chlorine dioxide, ethanol and peracetic acid are some of the commonly used chemical agents in combination with ultrasound in microbial load reduction in fresh fruits and vegetables (Kim et al., 2006; Sagong et al., 2011; São José and Vanetti, 2012). When ultrasound is used in combination with chemical sanitizers, the extreme pressure gradients generated cause cell disruption and allow penetration of these agents into the cell membranes of the microorganisms. In addition, cavitation progresses cell degeneration in microbes and thus increase the efficiency of sanitizing chemical agents (Gogate and Kabadi, 2009).

Effects of sonication on product quality characteristics

Major advantages of ultrasound over thermal pasteurization and sterilization include decline in flavour loss, uniformity of treatment and high energy efficiency (Vercet et al., 2001; Chemat et al., 2011). It was hence observed that, treatment with ultrasound (up to 45 min) did not significantly ($p > 0.05$) change the colour of lettuce and strawberry (Birmpa et al., 2013). Parameters of pH, titratable acidity and soluble solids were unaffected after sonication of green and purple cactus pear juices inoculated with *E. coli* (Cruz-Casino et al., 2016). Contents of polyphenolic compounds and sugars, total carotenoids, mineral elements (Na, K and Ca) and viscosity significantly ($P < 0.05$) improved when apple juice samples were sonicated for 0, 30 and 60 min at 20 °C (25 kHz and amplitude 70 %), and there was no observable change in total anthocyanins, zinc and electrical conductivity in the sonicated samples (Abid et al., 2013). Sonication was found to have had no significant ($p > 0.05$) effect on pH, total soluble solids and titratable acidity of apple juice after sonication for 0, 30, 60 and 90 min, at 20 °C and 25 kHz frequency, whilst in the same capacity, application of ultrasound appreciably enhanced ascorbic acid, cloud value, phenolic compounds, antioxidant capacity, DPPH free radical scavenging activity and differences in Hunter colour values (Abid et al., 2013). Sonication (0, 25, 28, 40 or 59 kHz) of strawberry fruits maintained firmness and higher levels of total soluble solids (TSS), total titratable acidity (TA) and vitamin C. Furthermore, treatments with 25 and 28 kHz ultrasound had no considerable effects on fruit decay and quality decline of strawberry fruits, hence ultrasound treatment has the ability to extend shelf-life and maintain quality of fruits (Cao et al., 2010b).

However in one investigation of ultrasonic inactivation of microorganisms, sonication (40 kHz, 30W/L) for ≥30 min significantly affected the overall appearance and texture of lettuce leaves (Sagong et al., 2011). Zhang et al. (2012) reported significant ($p < 0.05$) reduction in toxicity and quality measures such as pH, titratable acidity, electrical conductivity and total soluble solids in the ultrasonic removal of phorate in apple juice. Contents of sucrose, glucose and fructose as well as colour of juice were however not affected. Some mineral elements (P, Mg and Cu) were lost after sonicating apple juice samples (Abid et al., 2013). The rigidity of apple juice spiked with organochlorine pesticide was not significant ($P > 0.05$) whiles total sugar and total acid were significantly impacted, but were within acceptable levels (Tianli et al., 2009). Trends in some experimental results however suggested that sonication may be applied to improve natural phyto-nutrients, quality and safety (Abid et al., 2013).

Future prospects

The unwillingness of professionals in the food industry to fully adapt new technologies such as ultrasound due to inadequate understanding of its working principles is a major limitation to its utilization. However, conventional decontamination methods are gradually becoming less effective despite being increasingly unsafe at a time where food quality concerns are of great importance. From various studies, power ultrasound alone is acknowledged to dislodge biological cells and change chemical compositions of contaminants. However, when combined with some emerging novel technologies (pulse electric field, pulse light, high hydrostatic pressure, non-thermal plasma among others), sanitizers (acidic electrolyzed water, acidified sodium chlorite and chlorine peroxyacetic acid etc), heat (thermosonication), pressure (manosonication), and pressure and heat (manothermosonication), a reduction in treatment duration, intensity and energy expenditure may be achieved. The inclusion of these in new designs of commercial ultrasound equipment by manufacturers could possibly enhance decontamination processes if implemented at various levels of industry. However, for sustainable utilization, process optimization is necessary for possible industrial scale up. The content of this review will possibly increase domestic and industrial utilization of ultrasound in the inactivation of microbes and removal of pesticides in fruits and vegetables.

Conclusion

Due to the growth of global economies, liberalization of food trade, rising consumer demands and advancements in food science and technology, confidence in the safety and integrity of food supply is an important requirement for consumers. In recent years, the food science fraternity has discovered a broad diversity of functions of ultrasound, including decontamination. High intensity ultrasound has been recommended for various applications including pesticide removal and microbial inactivation in food. Methods of handling, processing, packaging, and distribution of fresh produce are gaining awareness in terms of recognizing and controlling possible microbiological and chemical hazards. The results from various investigations reported in this review showed the preservative capacity of ultrasound in decontaminating fruits and vegetables and maintaining product quality. Its efficiency is said to enhance in most decontamination studies especially when combined with other pesticide removing, degrading and microbial inactivating techniques. The optimization of ultrasound processing conditions may have to be investigated with respect to specific fruits and vegetables, in view of the fact that quality could possibly be affected. It is only when a steady impact is struck between safety and quality that, positive results for most products can be achieved.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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