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Ultrasound Degradation Effect on Residual Pesticides and Microorganisms in Commercially Available Fruits and Vegetables

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Abstract: Harvested fruits and vegetables are highly perishable agricultural commodities and their damaged tissues themselves function as an excellent substrate for the growth of spoilage and pathogenic microorganisms. In this research, we focused on the application of power ultrasound combined peracetic acid in the preservation of postharvest fruits and vegetables. Due to the fact that power ultrasound causes the disruption of microorganism cells, eliminates residual pesticides this technology has been applied in food sterilization.

Key words: Ultrasound, peracetic acid, fruits, vegetables, microorganism, pesticide

INTRODUCTION

Ultrasonic devices for pretreatment of fruits and vegetables: Ultrasound is known to cause chemical and physical changes in biological structures (in a liquid medium), owing to the rapid formation and destruction of cavitation bubbles (Alexandre *et al*., 2012a, b; Kentish and Ashokkumar, 2011; Zhou, 2011). So, far, ultrasound technology used for the pretreatment of fruits and vegetables before storage is still in a small scale experimental phase. On the whole, there are two types of devices utilized in current researches. The major device is an ultrasonic cleaner, of which ultrasonic transducer is bonded to the base of a tank. The fruit/vegetable samples are immersed in the solutions in the tank and exposed to uniform distribution of ultrasonic energy (Seymour *et al*., 2002; Yang *et al*., 2011; Chen and Zhu, 2011; Cao *et al*., 2010; Ajlouni *et al*., 2006). Besides, an ultrasonic probe system is also, applied: the ultrasonic probe is placed some distance (usually several centimeters) from the bottom of a beaver which contains the mixtures of samples and treatment solution (Nazari and Weiss, 2010; Zhou *et al*., 2009). For both devices, frequency and power of ultrasound, temperature of treatment solutions and exposure time can be adjusted according to the experimental requirements. This greatly facilitates the optimization of ultrasonic parameters to get better treatment effect.

Use of power ultrasound in preservation of fruits and vegetables

Decontamination of fruits and vegetables: Various microorganisms (spoilage and pathogen), chemical residues and dirt are the main foreign matters exist in postharvest fruits and vegetables. These matters cause the decay of fresh products and threaten people's health. However, power ultrasound has multifunction in reducing spoilage and pathogenic microorganisms and removing other harmful substances.

Sterilization of spoilage and pathogenic microorganisms

Ultrasound used alone: Ultrasonic treatment could significantly reduce the amount of spoilage and pathogenic microorganisms in fruits and vegetables. Actually, microorganisms are directly destroyed or removed by cavitation which is generally a combination of the following effects (Joyce *et al*., 2003); mechanical effects: includes the generation of turbulence, liquid circulation currents and shear stresses; chemical effects; free radicals (H· and OH·) formed during cavitation in aqueous medium attack the chemical structure of microorganism cell wall and weaken the cell wall to the point of disintegration; physical effects: generation of extreme temperature and pressure locally.

Consequently, there are some studies using power ultrasound alone to treat fresh fruits/vegetables before storage. These studies arose since the beginning of the 21st century, a variety of fruits and vegetables such as strawberry, peach, persimmon, bean and green asparagus were pretreated with ultrasound (Ajlouni *et al*., 2006; Cao *et al*., 2010; Wang *et al*., 2006; Alexandre *et al*., 2012a, b; Li *et al*., 2001; Wei, 2010). These results demonstrated that ultrasonic treatment significantly reduced the numbers of spoilage microorganisms such as bacteria, yeasts and molds in fresh products and in turn inhibited decay incidence, extended the storage time of fruits/vegetables.

Ultrasound combined with chemicals: Although, ultrasonic treatment could reduce the microorganism counts to some extent, however, it used alone may not be adequate for industrial application because of its low sterilization effect (Sagong *et al*., 2011). To achieve a satisfying inactivation effect, long treatment time and/or high acoustic energy are required but this could seriously damage the tissues of fresh commodities and make them more susceptible to the infestation of spoilage microorganisms (Seymour *et al*., 2002; Ajlouni *et al*., 2006; Cao *et al*., 2010). In addition, microorganisms detached from fruits and vegetables will be released into the wash water and thus have the potential to cross-contaminate (Seymour *et al*., 2002). In light of these defects, ultrasound is more often combined with other technologies in order to improve sterilization effectiveness.

Among other sterilization methods combined with ultrasound, sanitizers have the most applications. Except killing microorganism cells directly, ultrasound also improves antimicrobial action by weakening the cell wall which facilitates the penetration of sanitizer agents. In addition to the inactivation action, ultrasonic treatment provides an effective removing action on fruits/vegetables, since, cavitation bubble collapse near the solid is non-symmetric and produces a powerful jet which will dislodge microorganism cells. The particular advantage of ultrasonic cleaning is that it can reach crevices that are not easily accessible using conventional cleaning methods (Mason and Lorimer, 2002). Therefore ultrasound waves detach microorganisms from fresh produce into sanitizer solutions which prompts the exposure of micro organisms that locate in creases and crevices to sanitizer solutions. Consequently, the ultrasound/ sanitizers combined treatment shows significant synergistic effect.

Seymour *et al*. (2002) studied the microbial decontamination effectiveness of power ultrasound (25-70 kHz) combined with chlorinated water. Since then, many studies on various fruits and vegetables were reported involving different sanitizers (Ajlouni *et al*., 2006; Huang *et al*., 2006). These sanitizers include synthetic chemicals such as chloric disinfectants (chlorinated water, aqueous ClO_2 and NaClO etc.) H_2O_2, ethanol and natural antimicrobials such as some organic acids (peracetic acid, acetic acid, malic acid, lactic acid and citric acid) were often used. As a potential and applicable post-harvest technology, it has attracted wide attention especially in recent three years, during which many studies frequently arose (Seymour *et al*., 2002; Chen *et al*., 2011; Zhou *et al*., 2009; Sagong *et al*., 2011; Rivera *et al*., 2011; Sao *et al*., 2012). These researches demonstrated that the combinational treatment significantly reduced the amount of different species of microorganisms and ultrasound improved the antimicrobial effectiveness of sanitizers. It was noteworthy that this combinational method was often used to the decontamination of pathogenic bacterias such as *Salmonella typhimurium, Listeria monocytogenes and Escherichia coli* O157:H7 for the sake of its high sterilization effectiveness (Seymour *et al*., 2002; Zhou *et al*., 2009; Sagong *et al*., 2011; Huang *et al*., 2006).

On the other hand, Salicylic Acid (SA) was also, combined with power ultrasound to control postharvest diseases of fruits and vegetables. SA is not only an endogenous hormone that plays a critical part in plant growth and development but a signal molecule that induces the synthesis of plant resistance substance and Pathogenesis-Related proteins (PRs) (Yao and Jiang, 2004). Lots of studies showed that SA could effectively inhibit the decay incidence and prolong the storage life of fresh plant products (Mandal *et al*., 2009). When combined with power ultrasound, the combinational treatment also, presents a synergetic effect. The reason is that ultrasound could facilitate SA penetration into the tissue cells of fruits and vegetables, a quicker and stronger resistance is induced and thus decay incidence is significantly inhibited. Yang *et al*. (2011) and Yao and Jiang (2004) investigated the effects of ultrasound and SA either separately or combined on blue mold caused by *Penicillium expansum* in peach fruit and Yali pear, respectively. Their results showed that SA alone could reduce blue mold, while the use of ultrasound alone had no effect. However, SA combined with ultrasound was more effective than SA treatment alone. In addition, the combined treatment increased the activities of several defense enzymes such as chitinase, β-1, 3-glucanase, Phenylalanine Ammonia-Lyase (PAL), Polyphenol Oxidase (PPO) and Peroxidase (POD).

Besides, combined technologies were also used in the reduction of other types of microorganisms. Iceberg lettuce and carrots inoculated with a cocktail of three strains of *Bacillus cereus* spores were treated with combinations of ultrasound and various concentrations of surfactant solutions for 5 min (Sagong *et al*., 2013).

Some researchers tried to reduce the norovirus which are currently recognized as the most important human foodborne pathogens using ultrasound combined with other technologies, although, these explorations are not very successful. Fraisse *et al*. (2011) reported that a pretreatment step using ultrasound before washing in the presence of disinfectants did not reduce the viral titers on the lettuce more significantly. This demonstrated that the susceptibility of norovirus to ultrasound irradiation was rather low. According to Schultz *et al.* (2012), on fresh raspberries only a 1-log reduction of coliphage, a norovirus surrogate could be achieved after 1 sec of treatment but at this point damage to the texture of the fresh raspberries was evident.

These results presented above showed that ultrasound/chemicals combined treatments significantly increased spoilage and pathogenic microorganisms sterilization in fresh fruits and vegetables with respect to individual treatments. Due to the synergetic effect, an ideal effect can be achieved under shorter treatment time, lower acoustic energy and lower dosage of chemicals. Therefore, the combinational treatments are low energy-consuming, environment-friendly and much safer.

Reducing chemical residues and cleaning dirt: Ultrasound irradiation has been proved degrading organic chemicals through pyrolytic reactions and the formation of free-radical species caused by cavitation (Schramm anf Hua, 2001). Therefore, it has been used in the treatment of wastewater for many years. In recent years, ultrasound was also found effective in reducing chemical residues and dirt in postharvest fruits and vegetables (Chen *et al.*, 2012, Gong *et al.*, 2011; Yue *et al.*, 2009; Zhao *et al.*, 2009). When ultrasonic waves (100 W ultrasound for 10 min) were combined with aqueous chlorine dioxide (40 mg/L ClO_2 for 10 min) in the pretreating of plum fruits before storage, no ClO_2, ClO_2- or ClO_3-residues were detected in the skin or flesh (Chen *et al.*, 2012).

Some studies focused on the degradation effects of organic chemicals of ultrasound and even optimized the ultrasonic treatment conditions or developed this means in order to improve its effectiveness. Gong *et al.* (2011) combined ozone water treatment with ultrasound (power 360 W, temperature 35°C, for 20 min) to degrade the pesticides in commercially available apples and obtained an improved removal rate compared with single ultrasound and single ozone treatment. According to the study of Yue *et al.* (2009), under the optimum technological conditions (power 609.16 W, temperature 15.45°C for 70.46 min), the removal rate for organochlorine pesticide residues in apples reached 64.32%. Besides, ultrasound (40 kHz, 180 W)/detergent treatment was also, used to clean the dirt embeds on mushroom root. Under the optimum condition (detergent 0.67%, sample: water = 1:100, temperature 21.5-25.0°C for 20 min), the cleaning efficiency can be increased 55.5% and more edible parts of the rare mushroom was obtained than that without sonication (Zhao *et al.*, 2009). These results showed that ultrasound significantly reduced chemical residues and dirt in fruits and vegetables, therefore improved their edible safety.

Maintaining quality parameters of fruits and vegetables

Texture, color and nutrients constitute the critical quality attributes of fruits and vegetables. Since fruits and vegetables picked from the whole plants, they went to ripening, senescence and death gradually, followed by the deterioration of texture and color and the decrease of nutrients (Chen *et al.*, 2012; Cao *et al.*, 2010). Current studies showed that ultrasonic pretreatment could inhibit the physiological activities and retard the quality decline of fresh products during storage, presenting as a potential technology in the preservation of fresh fruits and vegetables.

Texture: Texture not only connects with the edible quality of products but also is an indicator of storage property and effect. Firmness is a visual trait that directly represents the texture of fruits and vegetables. Due to the physiological activities such as respiration and transpiration, firmness of fresh products decreases gradually during storage, largely influencing quality and facilitating pathogen infection.

Many studies demonstrated that pretreatment using ultrasound could delay the softening of fruits and vegetables (Chen *et al.*, 2012; Cao *et al.*, 2010; Wang *et al.*, 2006; Alexandre *et al.*, 2012a, b; Wei, 2010; Zhao *et al.*, 2007). One possible reason is that ultrasonic treatment inhibits the activities of enzymes that are largely responsible for fruit softening such as Pectin Methylesterase (PME) and Polygalacturonase (PG) (Cao *et al.*, 2010). However, no direct evidences have been reported to prove this hypothesis. On the other hand, the rate of enzymatic breakdown of cell wall components have been demonstrated depending on the energy produced through respiration (Hertog *et al.*, 2004). Thus, the decrease of respiration might be one of the reasons that the enzymatic activities were reduced. In addition, water loss resulted from the transpiration and ethylene production of fruits and vegetables also influences the firmness of fruits and vegetables (Xin *et al.*, 2009) but studies into the effect of ultrasound on these physiological activities have not been reported yet. Further researches into these questions at molecular and biochemical levels will help us understand the mechanisms about how ultrasound maintains the firmness.

Color: Color is an important sensory quality of fresh fruits and vegetables and depends on the pigments they contain. Chlorophyll, carotenoid and anthocyanin are the major pigments. After harvesting, these pigments began to degrade, so, the color deteriorated gradually which significantly decreased the acceptance of fresh products. Fortunately, recent studies showed that pretreatment with ultrasound could retard the degradation of pigments (Alexandre *et al*., 2012a, b; Wei, 2010). Alexandre *et al*. (2012a, b) discovered the inhibition of anthocyanin declining in strawberries treated with ultrasound. Wei *et al*. (2010) also reported that ultrasound played a positive role in maintaining the color of green asparagus but if the power was too high, ultrasonic treatment would make a reverse effect. Since, the degradation of pigments were processes comprised of a series of enzymatic reactions, it can be inferred that the enzymatic degradation of pigments was inhibited to some extent by ultrasound irradiation.

The effects of ultrasound irradiation on enzyme activities could be confirmed by several studies on enzymatic browning which greatly influenced the color of postharvest fruits. Chen *et al*. (2012) treated the Guiwei litchi with ultrasound at power 120 W for 10 min in water and then stored at room temperature (28°C). They found that application of ultrasound generally reduced the degradation of litchi anthocyanins and inhibited the activities of PPO and POD at the early storage stage and, thus, significantly delayed pericarp browning of litchi fruit. Similarly, the investigation of Jang and Moon (2011) revealed that simultaneous treatment with ultrasound and ascorbic acid had synergistic inhibitory effects on several enzymes related to enzymatic browning including monophenolase, diphenolase and POD.

Nutritional components: It is well known that fruits and vegetables are the main dietary source of Vitamin C (VC) and also are rich in many other nutritional components such as polyphenol, flavonoid, saccharides and organic acids. Thus they are functional in keeping people healthy and have specific flavors. In recent years, many researchers explored the maintenance of these nutritional components through ultrasonic pretreatment before storage (Chen *et al*., 2012; Cao *et al*., 2010; Wang *et al*., 2006; Alexandre *et al*., 2012a, b; Wei, 2010; Zhao *et al*., 2007). In these researches, contents of vitamin C, total flavonoids, total phenols, Total Soluble Solids (TSS), reducing sugars and Titratable Acids (TA) were compared between several types of fruits/vegetables with and without ultrasonic treatment. Results showed that these components were maintained at higher levels in ultrasound treated fresh products. Cao *et al*. (2010) attributed these results to the fact that ultrasound inhibited decay incidence and microbial population of strawberries. However, respiration plays a major role in the loss of these components, since, they are the main substrates of respiration in post-harvest fruits and vegetables. Coincidently, the respiratory intensity can also, be retarded after ultrasonic treatment (Cao *et al*., 2010; Zhao *et al*., 2007), it can be concluded that the inhibition of respiration might be the major mechanism for the maintenance of nutrients.

Mechanism for vegetable responses: As discussed above, the post-harvest fruits/vegetables underwent ripening, senescence and death gradually, followed by the deterioration of texture and color and the decrease of nutrients (Chen *et al*., 2012; Cao *et al*., 2010). Recent discoveries have ascertained that fruit senescence is greatly related to Reactive Oxygen Species (ROS) and incidental oxidative damage of mitochondrial protein (Tian *et al*., 2013). Interestingly, there is a scavenging system of ROS in plants including Superoxide Dismutase (SOD), Catalase (CAT), POD, Ascorbate Peroxidase (APX) and Glutathione Peroxidase (GPX) etc. It was reported that the activities of these enzymes were elevated after ultrasonic treatment (Zhao *et al*., 2007; Li *et al*., 2001). Zhao *et al*. (2007) detected higher POD and SOD activities of fragrant pears with ultrasound and MA packaging than individual treatments and control group during storage. In addition in the study of Ping *et al*. (2007), harvested peaches were treated with ultrasound (50 kHz, 200 W), $CaCl_2$ (3%) immersion or their combination for 3 min. Both ultrasound and $CaCl_2$ treatments increased the activities of SOD and CAT in various extents, decreased the activity of POD, the production rate of $O_2^{\cdot-}$ or the contents of H_2O_2 at different periods of storage. Besides, the combinational treatment presented a synergetic effect as ultrasound facilitated the penetration of $CaCl_2$. However, so, far no study has explored the deep mechanism for the changes of ROS scavenging enzymes after ultrasonic treatment.

Many researchers have studied the effect of ultrasound on biomolecules such as enzymes. In enzymatic reaction systems in vitro, ultrasound plays binary functions (Mason *et al*., 1996) on the one hand, it facilitates the contact between enzymes and substrates, thus improves the efficiency of enzymatic reactions; on the other hand, the mechanic, physical and chemical effects of cavitation could denature the proteins and inactivate the enzymes. However, the responses to ultrasonic waves might be much more complicated when it comes to the multicellular tissues of fruits and vegetables. Till now, few studies have reported the effects of ultrasound on muticellular plant tissues. Further study on the physiological responses to ultrasonic treatment at biochemical and molecular level is urgently needed.

Several outstanding researches regarding to ultrasonication on residual pesticide removal: Gong *et al*. (2011) examined ozone/ultrasound degradation effect on residual pesticides in commercially available apples. Kruve *et al*. (2007) investigated pesticide residues in commercially available oranges and evaluation of potential washing methods. Robina Farooq *et al*. demonstrated ultrasonic induced decomposition of methidathion pesticide. Yamashita *et al*. (2009) studied the removal of pesticide residues in farm products by ultrasonic washing. Yuting *et al*. (2003) optimizied conditions for organochlorine pesticide residues removal in apples using ultrasonic. Khoobdel *et al*. (2010) conducted the effectiveness of ultrasound and ultraviolet irradiation on degradation of carbaryl from aqueous solutions. Dehghani and Fadaei (2013) proved sonochemical kinetic model of diazinon and malathion pesticides degradation in aqueous solution.

Purpose of our research is to demonstrate the effectiveness of ultrasonication combined PAA to remove pesticide residue and microorganism on fruits and vegetables while maintaining their quality parameters.

MATERIALS AND METHODS

Raw material, chemical and equipment

Raw material: Collecting fruits and vegetables in Soc Trang local market:

- Fruit; mandarin, grapefruit, lemon, chinese apple, pear, acelora, grape, tomato, mango
- Vegetable: mustard greens, bitter melon, string bean, long bean, cucumber, spinach, cauliflower, chayote, mushroom, chinese brocoli (kale), celery

Chemical: PAA is supplied from Van Dai Phat company in HCM city

Equipment:

- Ultrasonication cleaner by Zenith/Bluewave
- Penetrator
- Handheld colorimeter (L, a, b)
- Other equipments; centrifugator incubator, colony counter etc

Researching procedure

Ultrasonication combined PAA: According to Plant Protection Department-Vietnam Ministry of Agriculture and Rural Development, most multiple residual pesticides found on fruits and vegetables are cypermethrin, acephate, permethrin indoxacarb, fenobucarb. Notably on:

- Fruits; Fipronil, Chlorpyrifos, Cypermethrin, Carbendazim
- Vegetables; Cypermethrin, Fipronil, Chlorpyrifos, Permethrin

Meanwhile, pathogenic microorganisms available on fruits and vegetables are Coliform, *E. Coli* and *Salmonella* spp.

Effect of different washing methods: (Fruit: Chinese apple/acelora; vegetgable: bitter melon/string bean/mustard greens; target pesticide: cypermethrin; target microorganism: Coliform, target quality: color, texture and Vitamin C).

- Before treatment
- Control: washing in fresh water at temperature (4°C), time (5 min)
- Ultrasonic alone; washing in ultrasonic cleaner at amplitude (30 μm), temperature (4°C), time (5 min)
- PAA alone; washing in bath at PAA concentration (50 ppm), temperature (40°C), time (5 min).
- Combined ultrasonic and PAA treatment: washing in ultrasonic cleaner at amplitude (30 μm), PAA concentration (50 ppm), temperature (4°C), time (5 min).

Residual pesticide removal:

Effect of different parameters of ultrasonic combined PAA in pesticide removal: (Fruit; Chinese apple/acelora; vegetgable; bitter melon/string bean/mustard greens; target pesticide; cypermethrin).

Microbial sanitation

Effect of different parameters of ultrasonic combined PAA in microorganism removal: (Fruit; Chinese apple/acelora; vegetgable; bitter melon/string bean/mustard greens; target microorganism; coliform).

Effect of different parameters of ultrasonic combined PAA on color, texture, vitamin C of fruit and vegetable

Effectiveness of ultrasonication combined PAA: After finding all optimal parameters; amplitude, chemical concentration, temperature and time, I take different fruits and vegetables to washing under ultrasonic combined PAA, analyse other pesticides (cypermethrin, acephate, permethrin indoxacarb, fenobucarb).

- Fruits; Fipronil, Chlorpyrifos, Cypermethrin, Carbendazim
- Vegetables; Cypermethrin, Fipronil, Chlorpyrifos, Permethrin

And other microorganisms (Coliform, *E. Coli, Salmonella*) to prove the effectiveness of ultrasonic combined PAA.

- Fruit: mandarin, grapefruit, lemon, Chinese apple, pear, acelora, grape, tomato, mango

- Vegetable: mustard greens, bitter melon, string bean, long bean, cucumber, spinach, cauliflower, chayote, mushroom, chinese brocoli (kale), celery

Owing to high cost of testing pesticides instead of sampling 9 kinds of fruits and 11 kinds of vegetables, eventually I decide to only choose one kind of fruit (Chinese apple) and one kind of vegetable (bitter melon) to demonstrate again the effectiveness of ultrasonic combined PAA in removing residual pesticides, microbial sanitation while maitaining product quality.

Physico-chemical analysis:

- Determine the firmness by penetrometer
- Determine Lightness (L*) and yellowness (b*) and redness (a*) by CIELAB instrument
- Determine pesticide residue, microorganisms, vitamin C by sending samples to SAC KY HAI Dang Lab (Table 1).

Table 1: Testing methods for pesticide residue, microorganism conducted by SAC KY HAI Dang Science Technology Services Joint Stock company

Discription	Testing method	Limit of detection
Pesticide		
Cypermethrin 1	TK.AOAC 2007.01 (GCMS)	10.0 (μg/kg)
Fipronil	TK.AOAC 2007.01 (GCMS)	20.0 (μg/L)
Chlorpyrifos	TK.AOAC 970.52 (GCMS)	1.0 (μg/kg)
Permethrin	TK.AOAC 2007.01 (GCMS)	10.0 (μg/kg)
Carbendazim	TK.AOAC 2007.01 (LCMSMS)	10.0 (μg/kg)
Indoxacarb	TK.AOAC 2007.01 (LCMSMS)	10.0 (μg/kg)
Fenobucarb	TK.AOAC 2007.01 (LCMSMS)	10.0 (μg/kg)
Acephate	TK.AOAC 2007.01 (LCMSMS)	10.0 (μg/kg)
Vitamin		
Vitamin C	TK.AOAC 985.33:2011 (HPLC-UV)	1.0 (mg/kg)
Microorganism		
Coliforms	AOAC 991.14:2011 (Petrifilm)	10 (CFU/g)
E. coli	AOAC 991.14:2011 (Petrifilm)	10 (CFU/g)
Salmonella spp	TCVN 4829:2008	
	ISO 6579:2007	10 (CFU/g)

Statistical analysis: The experiments were run in triplicate with three different lots of samples. Data were subjected to Analysis of Variance (ANOVA) and mean comparison was carried out using Duncan's Multiple Range Test (DMRT) statistical analysis was performed by the Startgraphics.

RESULTS AND DISCUSSION

Ultrasonication combined PAA: We analyzed the effect of ultrasonication combined with PAA in degradation of residual pesticide and sterilization of microorganism. Our results was illustrated in Table 2. We highly valued the effect of combination between ultrasonic and PAA as a significant synergistic effect.

Residual pesticide removal: Regarding to the effectiveness of residual pesticide removal in Table 3 we clearly noted the positive effect on amplitude 90 μm, PAA 30 ppm in 10 min at 12°C

Microbial sanitation: Regarding to the effectiveness of microbial sanitation in Table 4 we clearly noted the positive effect on amplitude 90 μm, PAA 30 ppm in 10 minutes at 12°C.

Effect of different parameters of ultrasonic combined PAA on color, texture, vitamin C of fruit and vegetable We evaluated different: Parameters of ultrasonic combined PAA on different quality indicators of fruit (Chinese apple) and vegetable (bitter melon). Our results showed in Table 5. Similarly, we got the best quality characteristics on color, texture and vitamin C when we applied amplitude 90 μm, PAA 30 ppm in 10 min at 12°C.

Effectiveness of ultrasonication combined PAA: A combination of ultrasonication with PAA at amplitude

Table 2: Effect of different washing methods on fruit (Chinese apple) and vegetable (bitter melon) regarding to pesticide: cypermethrin; microorganism: coliform, target quality: color, texture and vitamin C

	Experimental parameters				
Washing methods/Fruit and vegetable	Cypermethrin (ppb)	Coliform (CFU/g)	Color (L value)	Texture (kg/cm)	Vitamin C (mg)
Before treatment					
Chinese apple	25.17	3.5×10^3	43.28	5.25	29.17
Bitter melon	32.36	3.1×10^3	58.35	4.79	55.60
Control					
Chinese apple	25.17	3.2×10^3	43.28	5.25	29.17
Bitter melon	32.36	2.8×10^3	58.35	4.79	55.60
Ultrasonic alone					
Chinese apple	0.840	2.7×10^1	48.79	5.22	29.15
Bitter melon	1.050	1.5×10^1	61.47	4.75	55.51
PAA alone					
Chinese apple	18.07	2.6×10^1	47.50	5.25	29.17
Bitter melon	21.34	1.3×10^1	60.05	4.79	55.60
Combined ultrasonic and PAA					
Chinese apple	0.170	Not detected	54.74	5.22	29.15
Bitter melon	0.320	Not detected	68.29	4.75	55.50

[a-c]Each value is the mean of three samples (n = 3). The same characters (denoted above), the difference between them was not significant (α = 5%)

Table 3: Effect of different parameters of ultrasonic combined PAA in pesticide (cypermethrin, ppb) removal on fruit (Chinese apple) and vegetable (bitter melon)

Criteria	Experimental parameters				
Amplitude (30 μm) hemical concentration (vary) temperature (40°C) time (5 min)	10 (ppm)	20 (ppm)	30 (ppm)	40 (ppm)	50(ppm)
Chinese apple	0.73[a]	0.58[b]	0.47[c]	0.47[c]	0.47[c]
Bitter melon	0.95[a]	0.63[b]	0.42[c]	0.42[c]	0.42[c]
Amplitude (vary) chemical concentration (30 ppm) temperature (40°C) time (5 min)	30 (μm)	60 (μm)	90 (μm)	120 (μm)	150(μm)
Chinese apple	0.47[a]	0.25[b]	0.19[c]	0.19[c]	0.19[c]
Bitter melon	0.42[a]	0.38[b]	0.34[c]	0.34[c]	0.34[c]
Amplitude (90 μm) chemical concentration (30 ppm) temperature (vary) time (5 min)	4°C	8°C	12°C	16°C	20°C
Chinese apple	0.19[a]	0.18[b]	0.17[c]	0.17[c]	0.17[c]
Bitter melon	0.34[a]	0.33[b]	0.32[c]	0.32[c]	0.32[c]
Amplitude (90 μm) chemical concentration (30 ppm) temperature (12°C) time (vary)	5 (min)	10 (min)	15 (min)	20 (min)	25 (min)
Chinese apple	0.17[a]	0.09[b]	0.09[b]	0.09[b]	0.09[b]
Bitter melon	0.32[a]	0.11[b]	0.11[b]	0.11[b]	0.11[b]

[a-c]Each value is the mean of three samples (n = 3). The same characters (denoted above), the difference between them was not significant (α = 5%)

Table 4: Effect of different parameters of ultrasonic combined PAA in coliform (cfu/g) removal on fruit (chinese apple)and vegetable (bitter melon)

Criteria	Experimental parameters				
Amplitude (30 μm) chemical concentration (vary) temperature (40°C) time (5 min)	10 (ppm)	20 (ppm)	30 (ppm)	40 (ppm)	50 (ppm)
Chinese apple	2.7×10^1	1.5×10^1	Not detected	Not detected	Not detected
Bitter melon	1.5×10^1	0.4×10^1	Not detected	Not detected	Not detected
Amplitude (vary) chemical concentration (30 ppm) temperature (4°C) time (5 min)	30 (μm)	60 (μm)	90 (μm)	120 (μm)	150 (μm)
Chinese apple	0.6×10^1	0.3×10^1	Not detected	Not detected	Not detected
Bitter melon	0.4×10^1	0.3×10^1	Not detected	Not detected	Not detected
Amplitude (90 μm) chemical concentration (30 ppm) temperature (vary) time (5 min)	4°C	8°C	12°C	16°C	20°C
Chinese apple	0.06×10^1	0.03×10^1	Not detected	Not detected	Not detected
Bitter melon	0.04×10^1	0.01×10^1	Not detected	Not detected	Not detected
Amplitude (90 μm) chemical concentration (30 ppm) temperature (12°C) time (vary)	5 min	10 (min)	15 (min)	20 (min)	25 (min)
Chinese apple	0.02×10^1	Not detected	Not detected	Not detected	Not detected
Bitter melon	0.01×10^1	Not detected	Not detected	Not detected	Not detected

[a-c]Each value is the mean of three samples (n = 3)

Table 5: Effect of different parameters of ultrasonic combined PAA on different quality indicators of fruit (chinese apple) and vegetable (bitter melon)

Criteria	Quality	Experimental parameters			
Amplitude (30 μm) chemical concentration (vary) temperature (4°C) time (5 min) Chinese apple					
Bitter melon					
-	10 (ppm)	20 (ppm)	30 (ppm)	40 (ppm)	50 (ppm)
Color (L value)	54.74[c]	56.93[b]	60.01[a]	60.03[a]	60.04[a]
Texture (kg/cm)	5.22[a]	5.22[a]	5.22[a]	5.22[a]	5.22[a]
Vitamin C (mg)	29.15[a]	29.11[a]	28.85[b]	28.82[b]	28.81[b]
Color (L value)	68.29[b]	70.37[b]	71.19[a]	71.22[a]	71.25[a]
Texture (kg/cm)	4.75[a]	4.75[a]	4.75[a]	4.75[a]	4.75[a]
Vitamin C (mg)	55.50[a]	53.10[b]	49.09[c]	49.00[c]	48.98[c]
Amplitude (vary) chemical concentration (30 ppm) temperature (4°C) time (5 min) Chinese apple					
Bitter melon					
-	30μm	60μm	90μm	120μm	150μm
Color (L value)	60.01[c]	61.34[b]	64.27[a]	64.30[a]	64.31[a]
Texture (kg/cm)	5.22[a]	5.20[ab]	5.19[b]	5.15[c]	5.06[d]
Vitamin C (mg)	28.85[a]	26.47[b]	25.12[c]	25.09[c]	25.08[c]
Color (L value)	71.19[c]	72.08[b]	72.69[a]	72.71[a]	72.74[a]
Texture (kg/cm)	4.75[a]	4.74[a]	4.73[a]	4.55[b]	4.38[c]
Vitamin C (mg)	49.09[a]	47.32[b]	46.18[c]	46.15[c]	46.13[c]
Amplitude (90 μm) chemical concentration (30 ppm) temperature (vary) time (5 min) Chinese apple					
Bitter melon					
-	4°C	8°C	12°C	16°C	20°C
Color (L value)	64.27[b]	66.75[b]	67.83[a]	67.90[a]	67.92[a]
Texture (kg/cm)	5.19[a]	5.07[b]	5.01[c]	5.00[cd]	4.97[d]
Vitamin C (mg)	25.12[a]	24.45[b]	24.39[c]	24.38[c]	24.38[c]
Color (L value)	72.69[c]	73.11[b]	73.74[a]	73.77[a]	73.78[a]
Texture (kg/cm)	4.73[a]	4.73[a]	4.60[b]	4.59[b]	4.59[b]
Vitamin C (mg)	46.18[a]	43.01[b]	40.39[c]	40.35[c]	40.32[c]
Amplitude (90 μm) chemical concentration (30 ppm) temperature (12°C) time (vary) Chinese apple					
Bitter melon					
-	5 (min)	10 (min)	15 (min)	20 (min)	25 (min)
Color (L value)	67.83[c]	69.15[b]	69.17[ab]	69.20[a]	69.20[a]
Texture (kg/cm)	5.01[a]	4.25[b]	4.23[c]	4.23[c]	4.22[c]
Vitamin C (mg)	24.39[a]	22.01[b]	21.96[c]	21.95[c]	21.95[c]
Color (L value)	73.74[c]	74.95[b]	74.98[ab]	75.01[a]	75.01[a]
Texture (kg/cm)	4.60[a]	4.19[b]	4.18[b]	4.18[b]	4.17[b]
Vitamin C (mg)	40.39[a]	37.11[bc]	37.04[c]	37.01[c]	37.01[c]

Each value is the mean of three samples (n = 3). The same characters (denoted above), the difference between them was not significant (α = 5%)

Table 6. Effectiveness of ultrasonic combined PAA on different pesticide residues/microorganisms/quality indicators on various fruit/vegetable

		Chinese apple		Bitter melon	
Criteria	Description	Before treatment	After treatment	Before treatment	After treatment
Pesticides					
	Cypermethrin (ppb)	25.17	0.09	32.36	0.11
	Fipronil (ppb)	17.59	0.02	29.34	0.08
	Chlorpyrifos (ppb)	43.29	0.24	67.13	0.38
	Carbendazim (ppb)	12.09	0.01	8.22	0.01
Microorganisms					
	E. Coli	1.2×10^1	Not detected	1.4×10^1	Not detected
	Coliform	3.5×10^3	Not detected	3.1×10^3	Not detected
	Salmonella	0.7×10^1	Not detected	0.5×10^1	Not detected
Quality indicators					
	Texture	5.25	4.25	4.79	4.19
	Color	43.28	69.15	58.35	74.95
	Vitamin C	29.17	22.01	55.60	37.11

Each value is the mean of three samples (n = 3). The same characters (denoted above), the difference between them was not significant (α = 5%)

90 μm, PAA 30 ppm in 10 min at 12°C we saw a signification redustion of residual pesticide and microoraganism while maintaining fruit quality (Table 6).

CONCLUSION

Microorganisms cause the decay of fresh fruits and vegetables or serve as medium of human disease. For a long time, control of microbiological stability of harvested fruits and vegetables largely relies on synthetic chemical fungicides. However, growing concern over the abuse of synthetic fungicides on horticultural products because of their hazards on human health and the emergence of pathogen resistance to fungicides have been putting great limits on this traditional method. Thus a worldwide trend to explore nonchemical alternatives has been occurring. Ultrasound technology is a rather recent addition to the physical methods used in the decontamination and preservation of postharvest fruits and vegetables.

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