EFFICACY OF ELECTROLYZED WATER IN DEGRADING AND REMOVING PESTICIDE RESIDUES ON FRESH PRODUCE

By

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(Under the Direction of Yen-Con Hung)

ABSTRACT

Pure solution of diazinon, cyprodinil, and phosmet were treated with electrolyzed oxidizing (EO) water and electrolyzed reduced (ER) water. Lower pH, higher available chlorine content (ACC), and longer treatment time of EO water resulted in higher reductions of the three pesticides. ER water was effective in degrading phosmet, slightly effective in reducing diazinon, and ineffective in degrading cyprodinil. Soaking grapes in 12 mg/L mixed pesticide solution for 10 min was found to be the appropriate method for pesticide inoculation. EO water at 500 mL combined with 100 rpm shaking was determined to be the most effective way to wash 200 g contaminated grapes. Then, fresh grapes, spinach, and snap peas contaminated with the three pesticides were washed with EO water at different ACC and treatment time. The results showed both ACC and treatment time were significant ($P \leq 0.05$) factors for EO water in removing the three pesticide residues on produce samples. EO water was more effective than ER water, diluted bleach, Vegwash and DI water. Finally, the effect of EO water treatment on produce quality was evaluated. No significant ($P > 0.05$) color and texture degradation was found for EO water treated produce samples.

INDEX WORDS: Electrolyzed water, fresh produce, diazinon, cyprodinil, phosmet

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A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2015

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December 2015

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my major professor Dr. Yen-Con Hung for his valuable guidance and continuous support on my MS study and the related research work. His help is deeply appreciated.

My sincere thanks also go to my committee members Dr. Qingguo Huang and Dr. Koushik Adhikari for their help and advice. I would also like to thank Dr. Qi Luo for her technique help on LC-MS/MS and Mr. Jerry Davis for his statistic suggestions on data analysis.

Last but not the least, I would like to thank members of my research group, Dr. Veerachandra K. Yemmireddy, Dr. Prashant Singh, Jessica Tatum, Glenn Farrell, Xi Chen, and Chunling Zhang for their help on this project.

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CHAPTER 1

INTRODUCTION

The consumption of fresh fruits and vegetables has been increasing rapidly since the last decade because of the nutritional and health benefits (Rico et al., 2008). It was recommended that fresh fruits and vegetables should occupy half of a meal plate. Along with the increasing consumption of fresh produce, concerns about pesticide that may reside on fresh food product also arise. The use of pesticide in agriculture has increased dramatically as a result of increasing demand of high quality foods. In 2007, about than 400 million kg pesticides were applied in the U.S. (Bohmont, 2003). The use of pesticides helps to decrease the annual U.S. agriculture loss from 50% to 25% (Bohmont, 2003), which ensures enough food supply along with the increasing population (Chen, Lin, & Kuo, 2013). However, pesticides applied on produce may not fully decompose in the environment before harvest. Thus, pesticides would remain on produce and could cause severe adverse effects to human bodies if consumed (Pooja & Latika, 2014). The situation could be even worse as some farmers may apply large amount of pesticides in a very short period of time to control pests and leave substantive pesticide residues on produce (Misra, 2015).

Many symptoms and chronic diseases were reported to be associated with pesticide exposures, including cancer (Bolognesi & Morasso, 2000), Alzheimer’s disease (Zaganas et al., 2013), testicular dysfunctions and female abortion (Culliney, Pimentel, & Pimentel, 1992; Sengupta & Banerjee, 2014), and types II diabetes (Jonggun et al., 2013). Lots of governmental agencies and law acts have been established to regulate pesticide. In the U.S., the Environmental Protection Agency (EPA) is responsible for pesticide registration and setting maximum residue limit (MRL) to control pesticide residues on food crops. However, pesticide residues with detectable and above MRL can still be detected on fresh produce. In a report of FDA, 2.8% domestic and 6.0% imported tested produce were found to contain higher than MRL of pesticide residues (Winter, 2012). According to the U.S. Poison Control Centers (PCCs), there were 130,136 calls received annually associated with pesticide poisonings from 2006 to 2010 with 23 death cases reported each year (Langley & Mort, 2012). And 95.8% of the reported poisonings were unintentional. Food, as one of the most likely source for pesticide residue ingestion, should be effectively cleaned.

Electrolyzed water is a chlorine based solution produced by electrolyzing a diluted salt (usually NaCl) solution in an electrolytic chamber with anode and cathode. Electrolyzed oxidizing (EO) water is produced from the anode side and electrolyzed reduced (ER) water is created from the cathode side. EO water contains available chlorine with pH around 2.5 and oxidation reduction potential (ORP) above +1000 mV, while ER water has a pH above 10.0 and ORP below -800 mV (Huang et al., 2008). Because of the presence of available chlorine, EO water is a very strong antimicrobial agent and has been proved to be effective in inactivating many microorganisms (Hricova, Stephan, & Zweifel, 2008). Because of available chlorine and high ORP, EO water may be effective in degrading pesticides. ER water, on the other hand, may also be able to reduce pesticide through alkaline hydrolysis.

The efficacy of EO and ER water in degrading and removing pesticides from fresh produce were investigated by Hao et al. (2011). In their study, EO water was shown to effectively remove acephate, omethoate, and DDVP on fresh spinach in 30 min with reductions ranging from 59% to 74%. Lin et al. (2006) also found EO water can effectively degrade methamidophos and dimethoate, and ER water was effective in degrading methamidophos. However, both of the studies did not evaluate the potential effect of pH and available chlorine content (ACC) of EO water in degrading pesticides. Also, their EO water ACC was not very high (below 70 mg/L) and the fresh produce items they studied were limited. In addition, the effect of EO water wash with varying ACC and treatment time on produce quality were not investigated.

This study was therefore to systemically evaluate the efficacy of EO and ER water in degrading and reducing pesticides. Three pesticides (diazinon, cyprodinil, and phosmet) and three produce (grape, spinach, and snap peas) were treated with EO water at varying conditions and compare with other commercial produce washing solutions. The three main objectives were:

1. To evaluate the efficacy of EO and ER water in degrading pesticides in aqueous solution and develop appropriate methods for pesticide inoculation and EO water wash condition on grapes.
2. To evaluate the efficacy of EO and ER water in removing pesticide residues on fresh produce and compare the effectiveness with diluted bleach, VegWash, and DI water.
3. To evaluate the effect of EO water treatment on fresh produce quality in comparison with DI water treatment and non-wash control.

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CHAPTER 2

LITERATURE REVIEW

Pesticide

U.S. Environmental Protection Agency (EPA) defines that pesticides are substances intended to prevent, repel, destroy or mitigate any pests (U.S. EPA, 2014c). Living organisms, including insects, fungi, weeds, mice or other animals, and microorganisms that are able to damage crop production or humans or other animals are classified as pests (U.S. EPA, 2014c). The use of pesticides can be traced to 1000 B.C. in ancient Egyptian who used sulfur and salt to control pests and weeds (Bohmont, 2003). Synthetic pesticides were first introduced in the early 1900s and were put into massive production in 1950s (Bohmont, 2003). Based on the target pest species, pesticides can be classified into acaricide, algicide, attractant, avicide, bactericide, defoliant, desiccant, fungicide, growth regulator, herbicide, insecticide, miticide, molluscicide, nematicide, piscicide, predacide, repellents, rodenticide, silvicide, slimicide, and sterilants (Bohmont, 2003). Most of the commonly used pesticides are belong to insecticides, fungicides, herbicides, and rodenticides (Abby et al., 1996).

Commercial pesticides are composed by active ingredients and inert ingredients (Bohmont, 2003). Active ingredients are chemically active in killing or destroying the target organisms; and the inert ingredients are carriers that help in preserving, dissolving and applying the active ingredients (Abby et al., 2004). A mixture of active ingredients and inert ingredients forms a pesticide formulation with different types, including liquid formulation and dry formulation (Bohmont, 2003). The chemical structure of the active ingredient determines the functional target of a pesticide. Based on the chemical structures, there are four major pesticide categories, including organophosphorus pesticides (containing the P=S or P=O bonds), organonitrogen pesticides (containing the R-N-R bond), organochlorine pesticides (containing the C-Cl bonds), and pyrethroid pesticides (U.S. EPA, 2014b). Most of the organophosphorus pesticides and organonitrogen pesticides are insecticides and are able to damage the nervous system by destroying cholinesterases (Chen al., 2002). Pyrethroid pesticides are synthesized based on a natural pesticide pyrethrin, which was found in chrysanthemums (U.S. EPA, 2014b). Compared to the other three pesticides, organochlorine pesticides are more toxic and persistent in the environment. Many organochlorine pesticides have been banned in 1970s because of their potentials to accumulate in food chains and stay in human body for an extended time (Abby et al., 2004; Harner et al., 1999). Some of the banned organochlorine pesticides, such as DDT, heptachlor, and chlordane, can still be found in the atmosphere and some fresh produce (Harner et al., 1999).

Pesticide application

The benefits of applying pesticides in agricultural and related areas are significant. With the application of pesticides, most of insects are destroyed, agricultural production is improved, yields of edible crop are increased, food loss is reduced, the cost of food supply is decreased, and food quality is improved (Fenik, Tankiewicz, & Biziuk, 2011; Topuz, Özhan, & Alpertunga, 2005; Torres et al., 1996). The application of pesticides has decreased the annual U.S. agriculture loss from 50% to 25% (Bohmont, 2003). In the early 1990s, about 1million pounds of 600 hundred different pesticides were applied in the U.S. (Pimentel, Culliney, & Bashore, 2011). However, in 2007, about 400 million kg pesticides were applied in the U.S. (Langley & Mort, 2012). Among the 400 million kg pesticides, about 290 million kg were applied to conventional agriculture crops, 70 million kg were applied to industries and related areas, and around 36 million kg were used in household gardens (Langley & Mort, 2012). Among the major applied pesticides, about 66% are herbicides, 27% are insecticides, and 7% are fungicides (Bohmont, 2003). In Europe and China, the annual pesticide application is about 130 million kg (Fenik et al., 2011), and 1200 million kg (Wu et al., 2007), respectively. Generally, most of the pesticides are applied to fruits and vegetables because they need more pesticides to repel insects and fungi (Culliney, Pimentel, & Pimentel, 1992). The substantive application of pesticides ensures enough food supply along with the rapid increasing population (Chen, Lin, & Kuo, 2013). However, the intense application of pesticides also has many drawbacks, including pesticide contamination in food crops and environment, generation of pesticide resistant pathogens and pests, elimination of beneficial organisms, and potential hazards to human health (Fenik et al., 2011; Wu et al., 2007).

Pesticide regulation

In the U.S., the first federal law passed to help control and regulate pesticides is the Federal Insecticide Act of 1910 (Bohmont, 2003). But this law only covers insecticides and some fungicides. In 1947, in order to better regulate the entire pesticide industry, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was created and used as the major law for pesticide regulation (Abby et al., 2004; Bohmont, 2003). In 1948, The Food and Drug Administration (FDA) started to establish residue tolerance on foods (Bohmont, 2003). In the following decades, several laws and amendments were created to fully revise FIFRA, including the Pesticide Chemicals Amendment of 1954, the Food Additive Amendment of 1958, the Federal Environmental Pesticide Control Act of 1972, and the Food Quality Protection Act (FQPA) of 1996 (Abby et al., 2004; Bohmont, 2003; Katz & Winter, 2009). Under the regulation of FIFRA, all pesticides must be evaluated for their toxicity and health effects before they can be registered and distributed in the U.S. (Abby et al., 2004).

Currently in the U.S., there are three governmental agencies that are responsible for regulating pesticides, which are EPA, FDA and U.S. Department of Agriculture (USDA) (U.S. FDA, 2008). EPA was created in 1970s and is responsible for pesticide registration and establishing Maximum Residue Limit (MRL) for all registered pesticide on particular food commodities as required by FIFRA (Abby et al., 2004; Blasco, Font, & Picó, 2006; Bohmont, 2003). MRL is the maximum tolerance level that a pesticide or its toxic metabolite can remain on foods (Gandhi, 1999). MRL is based on pesticide toxicity and pesticide's Reference Dose (RfD), which is the maximum exposure level a person can receive to a particular pesticide in a 70-year period without having any significant short-term and long-term adverse effect (Bohmont, 2003). Intense legislative acts have been done to ensure MRL is accurate and reasonable for human health. The latest Food Quality Protection Act (FQPA) of 1996 requires EPA to consider more deeper things related to pesticide toxicology and the effects on infants and children (Winter, 2012). By 2006, about 99% reassessments on pesticide residue tolerances have been finished by EPA as required by FQPA (Winter, 2012).

While EPA registers and sets tolerances to different pesticides, FDA and USDA are responsible for monitoring and reporting the real pesticide residues on foods (U.S. FDA, 2008). FDA is responsible for monitoring pesticide residues in raw produce, dairy product, fish and processed food and creating the FDA Regulatory Monitoring Program to report their findings (Gandhi, 1999). On the other hand, the Food Safety and Inspection Service (FSIS) of USDA is in charge of pesticide residues on meat and certain egg and poultry products (Gandhi, 1999). Also, the Agricultural Marketing Service of USDA created the Pesticide Data Program (PDP) to report pesticide residues on fresh produce (Gandhi, 1999). More than nine states joined the PDP and the final results are reported to EPA to help estimate potential risks of pesticides (U.S. Department of Agriculture, 2011).

Pesticide residues

Pesticide residue refers to the pesticides that remain on foods after application (U.S. EPA, 2014a). Any pesticide residue that exceeds the MRL is considered to be violative (Winter, 2012). Even the United States and many other countries have established comprehensive pesticide regulations and monitoring programs to help control pesticides, there are still many food products that are detected to contain detectable or violative pesticide residue levels. For example, 106 orange samples collected from a local farm in Spain were analyzed for ten pesticide residues, and 50% of the samples were found to contain at least one of the ten pesticide residues and 19% of the samples had at least two of the ten pesticide residues (Blasco et al., 2006). According to the results of FDA pesticide monitoring program, 0 to 2.7% of tested domestic fruits were found to contain violative pesticide residues, whereas 3.7 to 5.6 % of tested imported fruits were shown with violation levels (Winter, 2012). Similar results were also found in vegetables: 1.4 to 2.8% of domestic test vegetables were found to contain violative levels of pesticide residues and 4.4 to 6.9% of imported vegetables were found with violation rates (Winter, 2012).

In the report of 2012 FDA pesticide monitoring program, a total of 5523 food samples containing 1158 domestic food samples and 4365 imported food samples from 104 different countries were analyzed for pesticide residues. About 43.0% of domestic and 33.6% of imported collected food samples were found to contain detectable pesticide residues (U.S. FDA, 2012).

Food samples with pesticide violations were found in 2.8% of domestic foods and 11.8% of imported foods (U.S. FDA, 2012). And 96.7% and 97.5% of domestic and imported food samples with pesticide violations were found to contain pesticide residues that have no published EPA tolerance; the rests were found to contain pesticide residues above MRL. Similar findings were also found in the 2013 PDP report, where 10104 samples from 11 states were tested. About 60% of all collected food samples were found to have detectable pesticide residues; 3.23% food samples were found to have pesticide violations with 0.23% of exceeding MRL and 3.0% having no published tolerance levels (U.S. Department of Agriculture, 2013).

Even the reports from FDA and USDA seemed to tell consumers that pesticide residues are under control, the data presented in their reports may not be representative. Only less than 6000 thousand food samples were collected in the 2012 FDA pesticide monitoring program report (U.S. FDA, 2012). The 2012 program did not cover all the foods consumed and pesticides used in the U.S. (U.S. FDA, 2012). The collected sample number was also much lower than that of the 2003 FDA pesticide monitoring program report, in which about 8000 thousand samples were collected (U.S. FDA, 2003). It is highly possible that the real pesticide residue violation rate is underestimated (Langley & Mort, 2012).

Besides the government annual report on pesticide residues, Environmental Working Group (EWG), a U.S. based organization, also influences the pesticide monitoring programs and consumers' choice (Winter, 2012). EWG started to release the annual Shopper Guide to Pesticides since 1995, which introduced fresh produce with most pesticide residues and least pesticide residues (U.S. EWG, 2014). In its annual report, it introduces a dirty dozen list, which covers a small number of fresh produce that contain the most concentrated pesticide residues (U.S. EWG, 2014). In the 2014 Shoppers Guide to Pesticides, the dirty dozen list covered 14 food items, including apple, strawberries, grapes, celery, peaches, spinach, sweet bell peppers, nectarines-imported, cucumbers, cherry tomato, snap peas-imported, potatoes, hot peppers, and kale/collard greens, with the order from highest contamination level descending to lower levels (U.S. EWG, 2014).

Pesticide poisoning

The use of pesticides have become the basis in agriculture around the world because it ensures the crop yield and food quality. However, pesticides are not only toxic to pests, but also dangerous to humans and other wildlife if applied inappropriately (Fenik et al., 2011; Langley & Mort, 2012). Pesticides are able to cause adverse effect to human even present in minute amounts (Pooja & Latika, 2014). Among the most 12 toxic and persistent chemicals, 9 are pesticides (Pooja & Latika, 2014). There are many pesticide-caused poisoning incidences in the United States and other parts of the world. In 1977, a pesticide related poisoning outbreak happened in an Indian village, in which 8 cases of grand mal seizers were diagnosed and found to be caused by ingestion of hexachlorocyclohexane (HCH)-contaminated wheat (Pooja & Latika, 2014). In 1985, a pesticide called aldicarb, caused over 1000 cases of human poisoning incidencse in America, and it was due to illegal and inappropriate application of aldicarb on watermelons (Winter, 1996). Similar cases also happened in Nigeria in 2008, where two pesticide related poisoning incidences happened. More than 100 people were poisoned when consuming food contaminated with an unknown pesticide and resulted in 2 children death cases; 120 students were having pesticide poisoning symptoms after consumption of lindane contaminated beans (Laumann, 2012). It cannot be denied that foods with violative pesticide residues are concerns and potential hazards to the entire human welfare.

According to the U.S. Poison Control Centers (PCCs), there were 130,136 calls reported annually on pesticide poisonings from 2006 to 2010 and about 23 death cases were recorded each year (Langley & Mort, 2012). Among the PCCs calls each year, about 50,761 calls were related with insecticides and 95.8% poisonings were unintentional (Langley & Mort, 2012). People under 5-year-old were involved in 42% of those calls, which indicated younger children and infants were at high risk of pesticide poisonings (Langley & Mort, 2012). Similar reports were also found in the latest 2012 American Association of Poison Control Centers (AAPCC) annual report, which indicated 88,078 cases of pesticide poisonings with 36,056 happened in children under age of 5 and 16 death cases in total (Mowry et al., 2012). Also, about 14,600 of these poisoning cases were treated in health care facilities, which include about 1400 hospitalizations and 7385 emergency room visits (Langley & Mort, 2012; Mowry et al., 2012). U.S. Agency for Healthcare Research and Quality (AHRQ) reported that about 200 million USD were spent annually to treat all the pesticide exposures. However, all these numbers could be much underestimated because of demographic gaps, which make the report only represent 73% of the total pesticide poisoning cases (Culliney et al., 1992). The pesticide poisoning conditions are much worse in developing countries, such as China, where about half million pesticide poisoning cases are happening each year with about 500 death cases (Pooja & Latika, 2014).

Exposure to pesticides can result in both acute and chronic diseases. Acute health problems and symptoms include but not limit to abdominal pain, dizziness, headaches, nausea, vomiting, eye and skin itches (Pooja & Latika, 2014). Long term and chronic diseases can be induction of cancer, immune system damage, neurological disorder, memory disorder, depression and reproductive defects (Culliney et al., 1992; Pooja & Latika, 2014).

Pesticide and Cancer

Numerous studies have been done to investigate the association between human exposure to pesticides and the induction of cancers. Till today, more than fifty pesticides have been proved to be carcinogenic to laboratory used animals and potential carcinogens to humans (Bolognesi & Morasso, 2000). Exposure to pesticides can cause cancers to both adults and children. In an agricultural health study, three organophosphorus insecticides (fonofos, malathion, and terbufos) and one organochlorine insecticide (aldrin) were proved to be strongly associated with aggressive prostate cancer (Koutros et al., 2013). In another study, two organochlorine pesticides, hexachlorocyclohexane (HCH) and para-para - dichlorodiphenyltrichloroethane (p,p'-DDT) were found to be significantly related to urinary bladder cancer because of their potent ability to disrupt endocrine (Sharma et al., 2013). A study done in southeastern Michigan also found positive relationship between pancreas cancer and exposure to ethylan (Fryzek et al., 1998). Women are also susceptible to pesticide exposures. Strong associations have been found on both city and agriculture women breast cancer rate and exposure to increased amount of organochlorine pesticides (El-Zaemey, Heyworth, & Fritschi, 2013; Yang, Hu, & Wang, 2013). On the other hand, children are also at high risks of pesticide-induced cancer. Leukemia cancer on children under age of 2 was found to be highly related to maternal exposure to permethrin, a pyrethroid pesticide (Dantas et al., 2013). Parental exposure to pesticides were found to be related to the induction of brain cancer for children under age of 10 (Shim, Mlynarek, & Wijngaarden, 2009). In addition, exposure to pesticides are also able to cause kidney, liver, lung, and skin cancers (Pooja & Latika, 2014).

Pesticide and Neurological Problems

Evidences were found to prove associations between pesticide exposure and neurological problems. Some pesticides are toxic to nervous system by inducing neurodegenerative diseases and causing cognitive impairment, depression, anxiety, inability to concentrate, and spatial disorientation (Beseler & Stallones, 2003; Juricek & Coumoul, 2014). A report from China have shown that farmers exposed to high level of pesticides tend to develop neurological dysfunctions, such as sensations of numbness or prickling symptoms (Li et al., 2014). Same results were also found in an earlier study, in which the neurological damaging effects of organophosphorus pesticides were found in California from 1982 to 1990 (Steenland et al., 1994). In addition to those sensation losses, more severe diseases, such as Parkinson's disease and Alzheimer's disease (Pooja & Latika, 2014; Zaganas et al., 2013) can be developed by pesticide exposure. One animal test conducted on male mice found that, by exposing to a common fungicide maneb before birth and to herbicide paraquat when mice were grown up, significant damages were found in cells producing dopamine and more than 90% of their motor function were lost (Lew, 2006). Human cells produce dopamine to control muscle movement, and therefore, Parkinson's disease will develop without enough dopamine (Lev et al., 2013). Similar results were also found on human subjects. One study conducted on use of household pesticides found that, frequent use of household pesticide increased the odds of Parkinson's disease by 47%, and frequent use of organophosphorus pesticides increased the odds of Parkinson's disease by about 93% (Narayan et al., 2013). On the other hand, Alzheimer's disease is also a concern because organophosphorus pesticides are able to cause acetylcholinesterase inhibition and lead to microtubule derangements and tau hyperphosphorylation, which are hallmarks of Alzheimer's disease (Zaganas et al., 2013).

Pesticide and Reproductive Problems

Pesticides are also able to impact human reproductive systems by causing birth defects, fetal death and abnormal fetal growth (Pooja & Latika, 2014). An organochlorine pesticide dibromochloropropane (DBCP), which has been banned in 1980s, was proved to cause testicular dysfunction in laboratory animals and linked to human infertilities (Culliney et al., 1992). More details with pesticide effects on reproductive systems have been summarized in the review of Sengupta and Banerjee (2014), which introduced more than 15 different pesticides and their impact on male reproductive systems, including atrazine (conversion of androgen to estrogen and delayed maturation of gonadotrophic system), benomyl (decrease in testicular and epididymal weights and epididymal sperm counts), carbaryl (decrease in quantity and quality of sperm cells), carbofuran (decrease in sperm motility), chlorpyrifos (reduction in epididymal and testicular sperm counts), endosulfan (delay in sexual maturity and hormone synthesis), dimethoate (decrease in testicular weight), HCH (increase in abnormal sperm counts), lindane (disruption of testicular morphology and decrease in testicular steroidogenesis), and malathion & mancozeb (reduction of testes weight, epididymis, seminal vesicle, and ventral prostate). Other pesticides, such as some organophosphorus and pyrethroid pesticides, are also associated with sperm DNA damage and female abortions (Sengupta & Banerjee, 2014).

Pesticide and other Health Effects

In addition to the effects above, pesticides can also impact many other human body systems. A study found that women who consumed ground water contaminated with aldicarb in a chronic period had developed immune dysfunctions (Culliney et al., 1992). Some studies also indicated that pesticides may be able to cause hypersensitivity, allergy, and autoimmunity. In another study, imidacloprid, a neonicotinoid pesticide, has been indicated to be able to cause insulin resistance and lead to type II diabetes (Jonggun et al., 2013). Similar results also showed that, by increasing the intake of imidacloprid, the risk of getting diabetes also dramatically increased (Pooja & Latika, 2014). Problems can also be found in different organs, such as kidney, heart, and lungs (Culliney et al., 1992; Pooja & Latika, 2014). However, the research on pesticides effect on human health is still limited and the long term toxic effect of pesticides can be much underestimated.

Pesticide Risk for Children

Children and infants, on the other hand, could be at higher risk than adults. Children's potential pesticide exposure level could be much higher than adults because the amount of food children consume is relative larger to their body weight when compared to adults (Winter, 1996). Also, as the diet of children and infant usually have less food items, children could be at significant risks if a major food item of the diet contains high level of pesticide residues (Winter, 1996). For example, children are often suggested to consume large amounts of fruits and vegetables, such as apple. However, a study done in Poland investigated the pesticide residues on apple from 2005 to 2013, and they found out that 66.5% of the 696 apple samples contained detectable pesticide residues of 34 different species (Lozowicka, 2015). And 3% of these apples had pesticide residue levels higher than the MRL (Lozowicka, 2015). On the other hand, children and infants could be at higher risks because of their different susceptibilities to pesticides. Studies have shown the toxicities of pesticides on children and adults are different (Winter, 1996). The immune system, nervous system, and metabolic system are less developed in children, and this may make children unable to detoxify the excess pesticides in foods (Winter, 1996). A study done in Iowa found that the risk of childhood cancer is significantly ($P \leq 0.05$)

increased when children got more exposure to pesticides (Flower et al., 2004). The pesticide effects can also be passed from parents to their offspring. Strong association between the rate of children brain tumor and cancer and parent pesticide exposure have been found (Shim et al., 2009; Van, Hoet, & Lison, 2013). Other diseases, such as acute lymphoblastic leukemia and regular leukemia, can also be linked with parental and maternal pesticide exposures (Bailey et al., 2014; Glass et al., 2012).

Diazinon

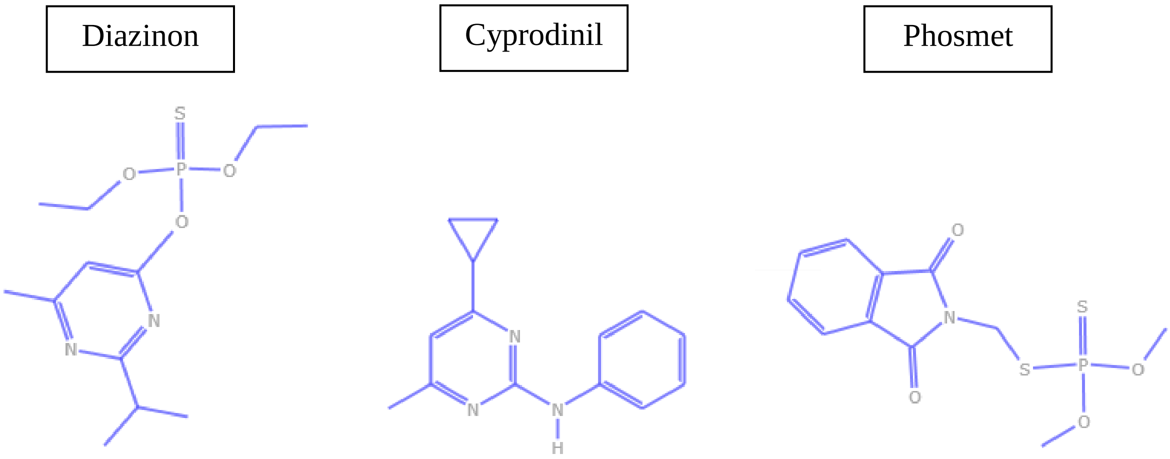
Diazinon (Figure 2.1) is a synthetic organophosphorus insecticide and was first registered in 1956 in U.S. and banned for residential use in 2004 (NPIC, 2009). About 13 million pounds of diazinon were used as the active ingredient in pesticides annually and they are usually used to control foliage and soil insects on many agricultural crops, including many fruits and vegetables, nuts, forage, and field crops (U.S. EPA, 2012). The registered name of diazinon in the International Union of Pure and Applied Chemistry (IUPAC) is O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (Worthing & Walker, 1986). Pure diazinon is colorless oil with molecular weight of 304.3 g/mol and solubility of 40 mg/L in water; it is stable in neutral pH and stable to photolysis. (NPIC, 2009). Diazinon can kill insects through contact by inhibiting acetylcholinesterase (AChE) and alter normal neurotransmission in the nervous system (NPIC, 2009). Diazinon can be oxidized into diazoxon, which is a stronger AChE inhibitor (NPIC, 2009). The acute lethal dose (LD_{50}) is about 1200 mg/kg in rats (NPIC, 2009). Low level of exposure to human can cause some acute symptoms, such as nausea, dizziness, and confusion; these symptoms can progress into vomiting, abdominal cramps, diarrhea and muscle twitching (NPIC, 2009; U.S. EPA, 2012). When the exposure level is high, severe symptoms, such as respiratory paralysis, can happen and death may occur (U.S. EPA, 2012). Although the U.S. EPA has classified diazinon into "Group D - not classifiable as to human carcinogenicity", diazinon is still able to cause many other chronic health problems in humans, including pancreatic atrophy, birth defect, infertility, memory loss and depression (NPIC, 2009).

Cyprodinil

Cyprodinil (Figure 2.1) is a synthetic organonitrogen fungicide and was first registered by EPA in 1998 (U.S. EPA, 1998). Its name in IUPAC is 4-cycloproplyl-6-methyl-N-phenyl-pyrimidinamine, and molecular weight is 225.3 g/mol (Worthing & Walker, 1986). This fungicide is stable under alkaline condition, sunlight and elevated temperature, but unstable in acidic conditions (U.S. EPA, 1998). It is primarily applied to the foliage of almonds, grapes, stone fruit crops, and pome fruit crops to prevent and control plant diseases (U.S. EPA, 1998). The primary target fungi is scab and brown rot blossom (U.S. EPA, 1998). Cyprodinil can kill fungi by inhibiting protein synthesis, particular on methionine biosynthesis (Worthing & Walker, 1986). Based on scientific research, cyprodinil only poses little risk to human health and is negative in mutagenicity and teratogenicity; but chronic effects on reproductive systems can be found (U.S. EPA, 1998). The oral acute LD_{50} is >2000 mg/kg for rats (Worthing & Walker, 1986). It is classified as "Not Likely" (E) carcinogen but can cause dermal problems (U.S. EPA, 1998; Worthing & Walker, 1986).

Phosmet

Phosmet (Figure 2.1) is a synthetic insecticide within the organophosphorus group. It was first registered in U.S. in 1966 with main focus on pest control of fruits (U.S. EPA, 2006). Its IUPAC name is O,O-dimethyl S-phthalimidomethyl phosphorodithioate with a molecular weight of 317.3 g/mol (Worthing & Walker, 1986). Phosmet can be rapidly hydrolyzed under alkaline condition and is relative stable under acidic condition (Worthing & Walker, 1986). Its mechanism of killing insects is through cholinesterase inhibition. Phosmet can cause moderate to low acute oral, dermal and eye/skin irritation by direct contact (Worthing & Walker, 1986). The oral acute LD_{50} is 113 mg/kg for rats and inhalation LC_{50} (1h) is 2.76 mg/L air for rats. Besides, it is not a skin sensitizer (Worthing & Walker, 1986). Phosmet is classified as a tentative category C carcinogen and was found to be related with elevated incidence of liver tumors. Phosmet oxon was the only metabolite of phosmet that draw toxicity concerns and is not stable in the environment (U.S. EPA, 2006).



Diazinon

Cyprodinil

Phosmet

Figure 2.1. Chemical structures of diazinon, cyprodinil and phosmet.

Approaches to Reduce Pesticide Residues on Fresh Fruits and Vegetables

Usually after harvest, farmers or produce industry will use water to wash the collected produce to remove dirt and debris (Lopez-Fernandez et al., 2013). Most consumers will also use water to wash the fresh produce before consumption (Gouri, Tyagi, & Gupta, 2012). However, this traditional washing method is not efficient in removing pesticide residues (Phongchai, Anek, & Sasithorn, 2008). Many chemicals have been used as additives in wash water to improve pesticide removal efficacy. One study investigated the effectiveness of several household chemicals in residue removal of methomyl and carbaryl on Chinese kale. In their results, 0.9% NaCl, 0.1% $NaHCO_3$, and 0.1% acetic acid solutions had residue reductions of 39%, 43%, and 43% for methomyl; 91%, 91%, and 90% for carbaryl, respectively (Phongchai et al., 2008). All the washing solutions were more effective than regular tap water, of which the reductions were 38% and 88% in methomyl and carbaryl, respectively (Phongchai et al., 2008). The highest reduction rate was found on 0.001% $KMnO_4$, which was 48% and 93% in methomyl and carbaryl, respectively. Similar results were also found in another study, which investigated the efficacy of using 0.9% NaCl, 0.1% 0.1% $NaHCO_3$, 0.1% acetic acid, 0.001% $KMnO_4$, 0.1% ascorbic acid, 0.1% malic acid, and 0.1% oxalic acid to wash tomato, bean, okra, eggplant, cauliflower, and capsicum contaminated with organophosphate pesticides, including malathion, fenitrothion, formothion, parathion, methyl parathion, and chlorpyrifos (Gouri et al., 2012). The final pesticide removal percentage were between 20-89% with the highest reduction rate achieved by 0.001% KMnO4 (Gouri et al., 2012). Both of the articles assumed the strong oxidizing power of KMnO4 made it be able to degrade pesticides rapidly (Gouri et al., 2012; Phongchai et al., 2008). This assumption was also made in another research, which studied using hydrogen peroxide, acetic acid, and ammonium hydroxide to remove mancozeb residues from lettuces (Lopez-Fernandez et al., 2013). Their results showed hydrogen peroxide was more efficient than sodium hypochlorite and that may be due to the higher oxidizing power of hydrogen peroxide (Lopez-Fernandez et al., 2013). On the other hand, boiling method was also found to be very effective in removing pesticide residues; however, boiling would destroy the texture, taste, and nutrients of fresh produce (Gouri et al., 2012).

Other chemicals with strong oxidizing power have also been studied in pesticide removal. In one study, chlorine dioxide was added to regular tap water to investigate its efficiency in removing phorate and diazinon on lettuce. The results showed 40-80% and 10-20% reduction on phorate and diazinon using 20 mg/L chlorine dioxide solution and was more effective than regular tap water (Chen et al., 2014). The efficacy was also influenced by several factors, such as pH, temperature, and reaction time (Chen et al., 2014). Chlorinated water and ozonated water were also studied. One study showed 50 mg/L chlorinated water and 2 mg/L ozonated water were able to reduce 50-100% of azinphos-methyl, captan, and formetanate hydrochloride on apples (Ong et al., 1996). Wu et al. (2007) found that 1.4 mg/L ozonated water was able to oxidize more than 60% of methyl parathion, parathion, diazinon, and cypermethrin in aqueous solutions with trace amount of paraoxon and diazoxon as primary byproducts. They also tested the ozonated water (1.4-2.0 mg/L) on reducing these pesticides on Pak Choi (*Brassica rapa*) and found the 2.0 mg/L ozonated water was the most effective one in all cases (Wu et al., 2007). In addition, pure ozone gas was used to remove chlorfluazuron and chlorothalonil on Chinese white cabbage (Chen et al., 2013). They found that 51% chlorfluazuron and 53% chlorothalonil were reduced in 15min when ozone production rate was 250 mg/h. And when the ozone production rate was increased to 500 mg/h, 75% and 77% reduction was found for chlorfluazuron and chlorothalonil, respectively (Chen et al., 2013). All the results have demonstrated water with added chemicals can be significantly more effective in removing pesticide residues on fresh fruits and vegetables than using regular tap water.

Electrolyzed water

Electrolyzed water has gained tremendous reputation in the food industry due to its strong antibacterial effect. It has been deemed as an advanced sanitizing technology in food safety. The idea of electrolyzed water was initiated in Japan (Al-Haq, Sugiyama, & Isobe, 2005; Huang et al., 2008). But other scientists believe that electrolyzed water was first conceptualized in Russia (Hricova, Stephan, & Zweifel, 2008). Electrolyzed water can be divided into electrolyzed oxidizing (EO) water and electrolyzed reduced (ER) water, while some other scientists prefer to call them acidic electrolyzed water (AEW) and basic electrolyzed water (BEW). The antimicrobial activity of EO water has been proved by many food scientists and makes it applicable in agriculture, medicine, dentistry and food industry (Huang et al., 2008).

Electrolyzed water generation

The generation of electrolyzed water only requires a simple equipment (electrolytic cell) and a diluted salt (NaCl/KCl/MgCl) solution (Al-Haq et al., 2005). The process starts by passing the diluted salt solution through the electrolytic cell chamber, in which a diaphragm (septum or membrane) is used to separate the anode and cathode (Huang et al., 2008). A porous metal oxide catalyst layer is coated on the titanium made electrodes to improve their stability, corrosion resistance and operating life (Thorn et al., 2012). Direct current voltages are subjected to the electrodes to initiate electrolysis. As the electrolysis occurs, ions in the salt solution start to move. Chloride, hydroxide and other negatively charged ions move to the anode to give up electrons and turn into chlorine gas, oxygen gas, hypochlorite ion, hypochlorous acid, and hydrochloric acid. On the other side, positively charged ions, including hydrogen and sodium, move to the cathode to take up electrons and turn into hydrogen gas and sodium hydroxide (Huang et al., 2008). Two types of water with contrasting properties are produced. EO water is produced from the anode part while ER water is produced from the cathode part. EO water has a low pH (2.3-2.7), high oxidation reduction potential (ORP > 1000 mV), high dissolved oxygen and available chlorine content (ACC) with varying concentrations (dependent on the generator settings). ER water, on the other hand, has a high pH (>10.0), high dissolved hydrogen, and low ORP (-800 to -900 mV) (Huang et al., 2008). EO water is mainly used for sanitizing purposes due to its high ORP while ER water is often used to wash food processing equipment to remove dirt and grease (Huang et al., 2008). A schematic diagram of electrolyzed water generation is shown in Figure 2.2. In addition, by mixing the EO and ER water or using a single electrolytic cell chamber, neutral electrolyzed water (NEW) can be produced, which has a neutral pH (7-8) and an ORP around 750 mV (Hricova et al., 2008).

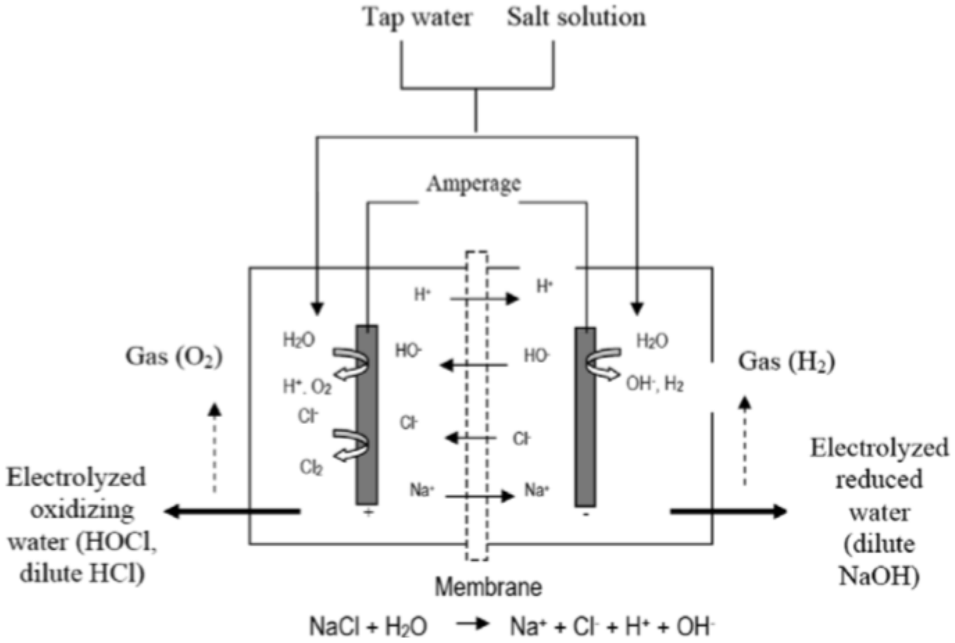


Figure 2.2. Schematics of the electrolytic cell chamber of an electrolyzed water generator and the final compounds in EO and ER water (Adapted from Huang et al., 2008).

Electrolyzed water generation systems

In general, electrolyzed water producing machines can be divided into two types, which are those contain a diaphragm producing EO and ER water and those have no diaphragm producing NEW (Al-Haq et al., 2005; Hricova et al., 2008). In industry, commercial electrolyzed water can be categorized into three types according to their control systems. The first type can adjust voltages and amperages automatically while the users are allowed to change brine flow rate. The second category does the contrary thing, which allows users to change amperages and voltages while generators adjust brine flow rate. The last type allows the users to directly select the final ACC level of EO water while the machine adjusts brine flow rate, amperages, and voltages accordingly (Hsu, 2003; Huang et al., 2008).

The relationship between water flow rate, water temperature and salt concentration and their effect on electrolysis efficiency and separation efficiency of an electrolyzed water generator was investigated by Hsu (2003). He found that water flow rate, water temperature and salt concentration did not affect the electric potential (7.9-15.7 V) and power consumption (16-120 W) of the electrolytic cell. But electric current was affected by water temperature and water flow rate. Also, the increase of water flow rate and salt concentration in the feed solution dramatically decreased the electrolysis efficiency and separation efficiency of the ion exchange diaphragm (Hsu, 2003). In his another study, he indicated that increase of salt concentration and decrease of water flow rate can lead to increases of ACC (Hsu, 2005).

Advantages and disadvantages of EO water

Safety is the major advantage of EO water because the generation requires only tap water and salt (Huang et al., 2008). EO water is also much safer to human bodies compared to hydrochloric acid or sulfuric acid, as EO water is not corrosive to skin, mucous membrane and organic material (Huang et al., 2008). EO water can be generated on site and thus needs no special transportation of concentrated chemicals, such as sodium hypochlorite which would cause skin irritation, membrane irritation and other acute symptoms (Hricova et al., 2008; Huang et al., 2008). Unlike other commonly used sanitizers, such as formaldehyde gas and glutaraldehyde which may pose severe adverse effect to human health and environment, EO water has no such strong adverse effects and will turn into regular water after contacting with organic matter, reverse osmosis water or regular tap water (Huang et al., 2008). In addition, the cost of EO water production is much cheaper. After the initial cost of EO water generator, all needed for EO water generation are salt water and electricity. Al-Haq et al. (2005) reported that the unit cost per kilogram of chlorine production was \$1.6 for liquefied chlorine gas, \$2.7 for sodium hypochlorite solution (15% w/v), \$2.87 for dry calcium hypochlorite, and \$0.34 for electronically generated chlorine. More importantly, EO water kills microorganisms through physical mechanism and has no selectivity, which activates no bacterial resistance (Al-Haq et al., 2005; Hricova et al., 2008). Furthermore, as a non-thermal technique, EO water does not negatively affect the organoleptic properties in many food products, such as fresh produce and fish (Al-Haq et al., 2005; Hricova et al., 2008).

Although the advantages of EO are numerous, its disadvantages are also obvious. The major problem of EO water is its low stability. The ACC level in EO water decreases during storage and hence lower the antimicrobial activity (Huang et al., 2008). The chlorine loss of EO water is much faster at lower pH and higher ACC (Len et al., 2002). Thus, EO water must be produced on site to maintain its maximum efficacy. The efficiency of EO water would also drop when contact with organic matters, such as protein (Al-Haq et al., 2005; Hricova et al., 2008). On the other hand, the use of EO water can cause metal corrosion and synthetic rein degradation because of the strong acidity. But stainless steel is resistant to EO water corrosion, and washing food equipment with sterile water after EO treatment can prevent metal corrosion (Huang et al., 2008). In addition, chlorine gas will be formed during electrolysis and may be emitted to the environment, which would cause discomfort to the operators. A standard extractor fan is sufficient to remove the emitted chlorine gas (Huang et al., 2008).

Principle of EO water antimicrobial activity and possible mechanisms to degrade pesticide

ACC, pH, and ORP are the three main factors that make EO water a strong antimicrobial reagent and are possibly related to pesticide degradation. The chlorine in EO water exists in three forms, including chlorine gas (Cl_2), hypochlorous acid (HOCl) and hypochlorite ion (ClO^-) (Huang et al., 2008). Depending on the pH, the chlorine species will vary dramatically. Figure 2.3 illustrates a detailed chlorine change pattern upon pH change. When pH stays within 3 to 6.5, HOCl is the major chlorine specie. ClO^- starts to dominate as pH increases from 7. Among the three chlorine species, HOCl is considered to be the most effective form because of its ability to penetrate microbial cell membranes and subsequently cause metabolic system dysfunction by supplying hydroxyl radicals (Al-Haq et al., 2005). Other proposed modes of HOCl include: damage on nucleic acid, purines, and pyrimidine, oxidative decarboxylation of amino acids, destruction of key enzymes, and disruption of protein synthesis (Huang et al., 2008).

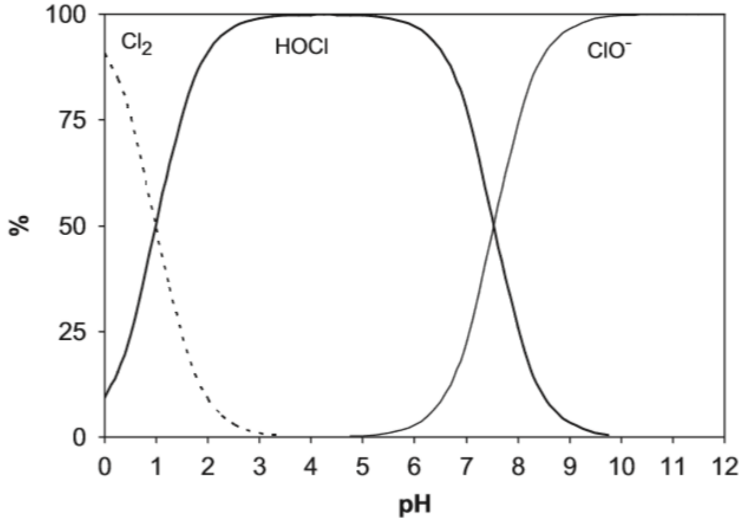


Figure 2.3. Distribution of chlorine species in aqueous solution at 25 ^{o}C with ACC of 177.5 mg/L (Adapted from Deborde & Gunten, 2008).

Another key factor that contributes to the antimicrobial activity of EO water is pH. At low pH, microbes are less likely to grow and more importantly, microbial cell membranes become more sensitive to HOCl and easier for HOCl penetration (Hricova et al., 2008).

Other scientists also suggested that the high ORP in EO water contributed the most in inactivating microbes. ORP is the indicator of the ability to oxidize or reduce. High ORP indicates strong oxidizing power. Generally, microbes can survive between ORP of +200 mV and +800 mV. The high ORP in EO water may be caused by the oxygen generated during electrolysis and from the rupture of unstable bonds between hydroxyl and chloric radicals (Hricova et al., 2008). High ORP is able to cause sulfhydryl compounds oxidation and disrupt cell metabolic pathways (Hricova et al., 2008). However, it was also found that high ORP alone is not effective in killing microorganisms compared with EO water at lower ORP and certain amount of ACC (Al-Haq et al., 2005). Also, high ACC solution with low ORP is also not as effective as EO water with low ACC and high ORP (Al-Haq et al., 2005). Therefore, all these three factors are synergistic to each other in inactivating microorganisms.

On the other hand, the effect of EO water on non-living organisms, such as pure organic chemicals, was not fully investigated. But, numerous research findings have proved that HOCl is also the dominant reactive form of chlorine when reacting with organic compounds (Deborde & Gunten, 2008). Three kinds of reaction can be triggered by HOCl because of its oxidizing power and Cl-O bond polarization structure ($Cl^{\sigma+} \rightarrow OH^{\sigma-}$), which are oxidation, unsaturated bond addition, and electrophilic substitution (Deborde & Gunten, 2008). HOCl addition reaction on unsaturated bonds are usually slow. The reaction is initiated by transferring a Cl^+ to the double bond to form a chloronium ion and then an OH^- was added (Deborde & Gunten, 2008). A detailed reaction mechanism of unsaturated bond addition is illustrated in Figure 2.4. Epoxides may also be formed if excess OH- are provided (Deborde & Gunten, 2008).

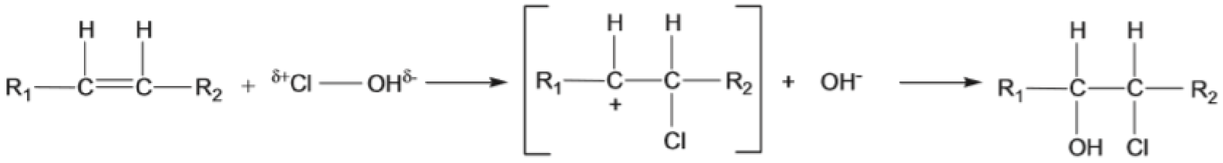


Figure 2.4. Mechanism of HOCl addition on unsaturated bond, where R_2 is a better electron donor than R_1 (Adapted from Deborde & Gunten, 2008).

Compared with unsaturated bonds, HOCl is more likely to react with sulfur or nitrogen containing compounds. Oxidation reactions are often found on reduced sulfur moieties with the presence of chlorine. Sulfoxides, disulfide and sulfonic acid can be produced. Aliphatic amines can react with chlorine rapidly and produce chloramines. However, only the reaction between

HOCl and neutral amine form is significant. HOCl is also very reactive on activated aromatic compounds. Electrophilic attack is considered to be the most significant kinetic during chlorination. Electrophilic substitution are often found in the *ortho* and *para* positions of phenol rings. Only small modification can be observed on substrate parent structure in the primary attack (Deborde & Gunten, 2008). Pesticides, on the other hand, are organic compounds and most of them contain aromatic phenol rings, neutral amine structures, unsaturated bonds, or reduced sulfur or phosphorus moieties. Therefore, EO water with dissolved available chlorine has great potentials to react with and degrade pesticides.

EO water application in the food industry

EO water, as an advanced and environmental friendly technique, has been extensively tested for its disinfection efficacy on different microorganisms and different subjects, such as food processing equipment, fresh produce, and meat and poultry products. Studies also focused on the effect of treatment time, ACC, pH, agitation and temperature. A lot of bacteria, virus, and toxins can be effectively reduced with EO water treatment.

EO water application on food processing equipment

EO water was reported to reduce *E. coli* O157 and *L. monocytogenes* on plastic cutting boards with a magnitude up to 5 log CFU/cm^2 in 20 minutes (Venkitanarayanan et al., 1999). Wooden boards were also tested for removal of *E. coli*, *S. aureus*, *P. aeruginosa*, and *L. monocytogenes* by NEW and it was found that a 4 log CFU/cm^2 was achieved in 5 minute-washing (Deza, Araujo, & Garrido, 2007). Kim et al. (2001) found that EO water (pH of 2.60, ORP of 1060 mV, and ACC of 56 mg/L) treatment on stainless steel contaminated with *L. monocytogene* biofilm was able to get a 10 log CFU/82.5 cm^2 reduction in 300 s. The removal were even more effective when an ER treatment was performed before the EO water wash (Ayebah, Hung, & Frank, 2005). Also, no adverse effect was found on the EO water (pH of 2.42, ORP of 1077 mV, and ACC of 50 mg/L) treated stainless steel (Beatrice, Ayebah & Hung, 2005). Similar test was also conducted on milk producing pipelines where a 10 min wash with ER water followed by a 10 min wash with EO water reduced all detectable bacteria (Huang et al., 2008).

EO water application on fresh produce

Lots of studies have been done on utilizing EO water for disinfection purposes on fresh produce. EO water at ACC 45 mg/L was found to be able to achieve 2.41 and 2.65 log10 CFU reduction for *E. coli* O157:H7 and *L. monocytogenes* per lettuce leaf in 3 min (Park et al., 2001). Other studies also indicated that EO water was able to reduce *E. coli* O157:H7 by up to 1.9 and 2.2 log CFU/g on strawberries and broccoli (Hung, Tilly, & Kim, 2010) and 4.4 log CFU/g on blueberry with only ACC of 30 mg/L (Pangloli & Hung, 2013). In addition, EO water was also found to be effective in reducing *Salmonella* on alfalfa seeds and their sprouts (Kim et al., 2003). On the other hand, EO water seems to have no adverse effect on the quality of fresh produce compared with chlorinated water. One study demonstrated that, lettuce treated with EO water at ACC 45 mg/L had no quality difference compared to those treated with regular tap water (Park et al., 2001). Another study showed no quality difference were found for strawberries and broccoli treated with EO water at ACC 55 and 100 mg/L during a storage time of 13 days (Hung et al., 2010).

EO water application for reducing pesticide residue

Electrolyzed water, including electrolyzed oxidizing (EO) water and electrolyzed reduced (ER) water, has been tested for its potential in reducing pesticide residues on fresh produce. Hao et al. (2011) found that EO water (pH 2.3, available chlorine 70 ppm, ORP 1170 mV) and ER water (pH 11.6, ORP -860 mV) can effectively remove pesticides (acephate, omethoate, and dichloroviny phosphate) on fresh spinach without loss of Vitamin C. Similar results were also achieved on methamidophos and dimethoate on leafy cabbage and green pepper (Lin et al., 2006). However, research on using electrolyzed water to remove pesticide residues on fresh produce is very limited. Systemically evaluation on electrolyzed water to remove and degrade pesticide residues on fresh fruits and vegetables is needed.

Color and texture of fresh produce

One of the most important properties for fresh produce is the appearance, as it is the first sensation consumers can perceive. Appearance includes many terms, such as size, texture, color, and shape. Color and texture might be the most important factors as they can directly tell consumers the quality and ripeness of fresh produce. For fresh produce, they usually undergo very limited process before put into the market. The maintenance of color and texture are very important for fresh produce in order to attract consumers. The chlorine content in EO water has very strong oxidizing power and may react with the pigments on produce surface to cause color and texture deterioration. Therefore, an evaluation of color and texture for produce after EO water treatment is needed.

Food Color

Color, as an important visual property for produce, has strong influence on how consumers evaluate food (Nisha, Singhal, & Pandit, 2011) and make decisions on accepting or rejecting food. Other attributes, such as sensory, visual, and nutritional defects can also be correlated with color (Francis, 1995). Color is a feature of light that can be measured in terms of intensity and wavelength (Pathare, Opara, & Al-Said, 2013). A strike from a luminous source on the surface of food is required to make color visible (Sahin & Sumnu, 2006). The major source of color on fresh produce is from natural pigments in the produce skin, which can change through the process of produce maturation and ripening (Pathare et al., 2013). The primary pigments that contribute to produce color are chlorophyll (green), anthocyanin (red, blue), carotenoid (yellow, orange, and red), flavonoid (yellow), and betalain (red) (Barrett, Beaulieu, & Shewfelt, 2010).

Several color coordinate systems can be used to describe an object's color, including Hunter *L a b*, Commission Internationale de l'Eclairage's (CIE) *L** *a** *b**, CIE XYZ, CIE *Yxy*, and CIE LCH (Pathare et al., 2013). The difference between these systems are the symmetry of the color space. In CIE concepts, there are three receptors on human eyes for red, green, and blue and the combination of the three color can form all the colors. In CIE XYZ system, XYZ are used to denote the amounts of red, green, and blue needed for any specific color, which are also called tristimulus values (Pathare et al., 2013). A chromaticity diagram (Figure 2.5) is used to designate different colors.

The Hunter *L a b* and the modified CIE system called CIELAB color scales are different models to describe color and are commonly used in the food industry (Pathare et al., 2013). The CIELAB color space (Figure 2.6) is used in this system. Color can be directly quantified in this system. In the CIELAB system, *a** and *b** are the two color coordinates and *L** is the index of lightness. Positive *a** represents reddish colors and negative *a** represents greenish colors, and positive *b** represents yellowish colors and negative *b** represents bluish colors. *L** measures the luminosity, which is “the property according to which each color can be considered as equivalent to a member of the greyscale, between black and white” (Pathare et al., 2013).

Based on the value of L^* a^* b^*, several attributes of color can be calculated. Chroma (C^*) is the quantitative attribute of colorfulness. It is calculated as $C^* = \sqrt{}\,(a^{*2} + b^{*2})$. Higher chroma value means higher color intensity a subject can be perceived by humans. Hue angle (h^*) is the qualitative attribute of color. It is used to present the traditional defined color, such as reddish and greenish. It is calculated as $h^* = \tan^{-1}(b^*/ a^*)$. Different angles represent different colors: red (0^{o} or 360^{o}), yellow (90^{o}), green (180^{o}), and blue (270^{o}). The change of color on the same subject can also be calculated based the CIELAB system. Total color difference (ΔE^*) is the concept used to measure the color change between stored and control samples. It is calculated as $\Delta E^* = \sqrt{}\,(\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})$. Analysis of total color difference can be: very distinct ($\Delta E^* > 3$), distinct ($1.5 < \Delta E^* <3$) and small difference ($1.5 < \Delta E^*$) (Pathare et al., 2013).

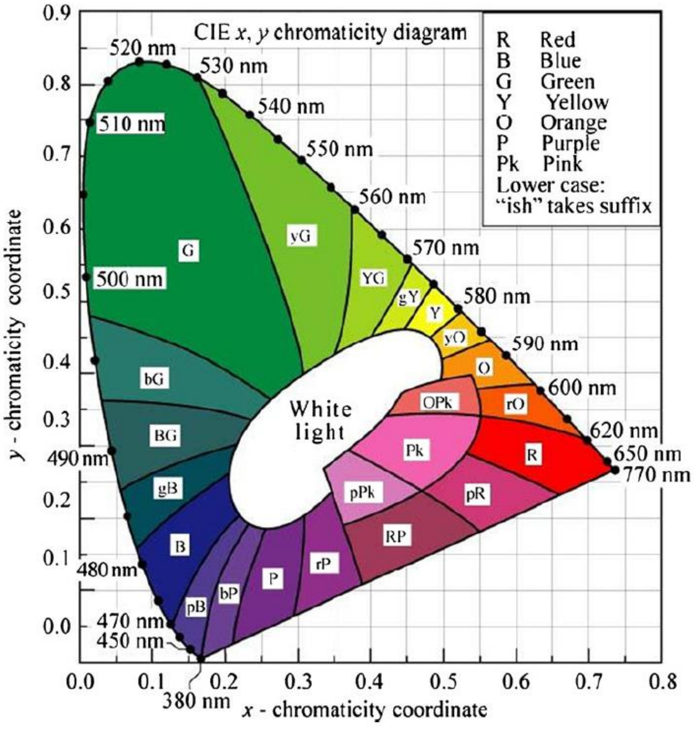


Figure 2.5. CIE chromaticity diagram (Adapted from Pathare et al., 2013).

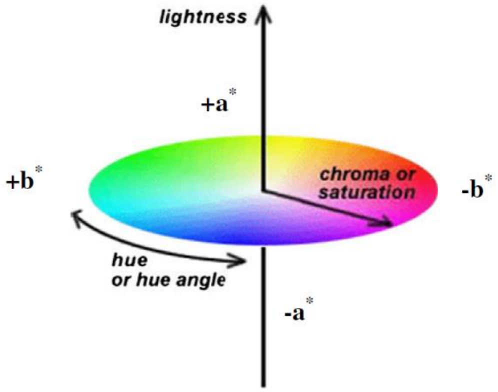


Figure 2.6. CIELAB color space (Adapted from Pathare et al., 2013).

Food Texture

Texture is a key attribute for evaluating the quality and acceptability of fresh and processed food. Texture of fresh produce can also be used to evaluate the readiness of harvest and assess the impact of different postharvest handling process on fresh produce shelf life (Chen & Opara, 2013). According to Lawless and Heymann (2010), food texture is defined as “all the rheological and structural (geometric and surface) attributes of the product perceptible by means of mechanical tactile, and where appropriate, visual and auditory receptors”. The texture of foods can be evaluated by subjective (sensory evaluation) and objective (instrumental) analyses.

Sensory evaluation of foods are often carried out using sensory panels consisting 6 to 20 trained sensory panelists (Lawless & Heymann, 2010). The trained panelists will usually be asked to use common sensory terms to describe the texture of the food samples, such as crispness and hardness. Sensory scales are also often incorporated to give better results. The intensity of the texture of a food sample can be evaluated using the sensory scale (value range from 0 to 10 or 100) (Lawless & Heymann, 2010). However, sensory evaluation has many limitations. As sensory evaluation is subjective, it can be affected by adaption and fatigue, as well as training levels a participants received (Chen & Opara, 2013; Peleg, 2006). Also, the cost of time and money is very high.

Objective evaluation of food texture using instrumental approaches has been developed to overcome the sensory evaluation limitations. Most of the instrumental texture approaches are based on the mechanical and rheological of food attributes as the perceived texture in mouth is largely dependent on the mechanical behavior of food (Chen & Opara, 2013). Both destructive and non-destructive instrumental approach can be used. Compression and puncture tests are the most commonly used methods for food texture measurement. The foods being tested can be solid or semi-solid. Lots of food have been tested using the compression and puncture test, including gels, apple, cornflakes, cheese, carrot, kiwifruit, potato slices, and cereal snacks (Chen & Opara, 2013). On the other hand, non-destructive texture analysis are also used to monitor and control product quality. Non-destructive measurement includes quasi-static force-deformation, impact response, finger compression, and bio-yield detection (Chen & Opara, 2013). Ultrasound and optical techniques are common non-destructive techniques used to evaluate and detect maturation and defects of foods.

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CHAPTER 3

EVALUATION OF ELECTROLYZED WATER EFFICACY IN DEGRADING PESTICIDES AND DEVELOPMENT OF METHODS FOR PESTICIDE REMOVAL CHALLENGE STUDY ON GRAPES

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Abstract

This study aimed to investigate the efficacy of electrolyzed oxidizing (EO) water and electrolyzed reduced (ER) water in degrading diazinon, cyprodinil, and phosmet in aqueous solution. The effect of pH, available chlorine content (ACC), and treatment time of EO water was tested. The study also investigated the appropriate methods for pesticide inoculation and EO water wash condition for grapes. Pesticide extraction was carried out using the Quechers method and pesticide residue was determined by an ultra-performance liquid chromatography with a tandem mass detector. EO water at pH 2.8 was significantly ($P \leq 0.05$) more effective than at pH 5.0 and 6.0 in degrading diazinon, cyprodinil, and phosmet in solution. Higher ACC and longer treatment time also enhanced the reduction of the tested pesticides. ER water, however, was only shown to be effective in hydrolyzing phosmet (99.7% reduction), slightly effective in reducing diazinon (7.4% reduction), and ineffective in degrading cyprodinil (0.0% reduction). The oxidizing power of available chlorine in EO water may be the main reason for its effectiveness in degrading pesticides. The difference in the tested pesticides chemical structure may also play an important role in determining the sensitivity of each pesticide against EO and ER water treatment. For grape inoculation treatment, soaking grapes in 12 mg/L mixed pesticide solution for 10 min was found to be the most consistent method for pesticide inoculation. Washing grapes (200 g) in 500 mL EO water with 100 rpm shaking was found to be the best condition to remove pesticide residues.

Key words: electrolyzed water, diazinon, cyprodinil, phosmet, chlorine, grape

Introduction

Synthetic pesticides were first introduced in the early 1900s and put into massive production in 1950s (Bohmont, 2003). In the last 30 years, there has been a dramatic increase of pesticide use all over the world. The annual amount of pesticides applied in U.S. has increased from about 0.5 million kg to nearly 400 million kg since the 20th century (Bohmont, 2003; Pimentel, Culliney, & Bashore, 2011). The substantive application of pesticides helps decrease agriculture loss and ensure enough food supply for the rapidly increasing population (Bohmont, 2003; Chen, Lin, & Kuo, 2013). However, the intensive application of pesticides would also leave pesticide residues on crops and cause severe adverse effects to human health.

Even though the U.S. and many other countries have established comprehensive pesticide regulations to control pesticides, pesticide residues are still big concerns. Most fresh produce were put into the market soon after harvest, which would leave substantive pesticide residues on produce (Misra, 2015). According to a report of FDA pesticide monitoring program, about 2.8% domestic and 6.0% imported of tested produce were found to contain violative rate of pesticide residues (Winter, 2012). Pesticides could be extremely toxic to humans as they are able to cause adverse effect even when present in minute amount (Fenik, Tankiewicz, & Biziuk, 2011; Pooja & Latika, 2014). According to the U.S. Poison Control Centers (PCCs), there were 130,136 calls received annually associated with pesticide poisonings from 2006 to 2010 with 23 death cases recorded each year (Langley & Mort, 2012). More importantly, 95.8% of the reported poisonings were unintentional. Also, about 200 million USD were spent annually to treat all the pesticide exposures (Langley & Mort, 2012). Food, as one of the most likely source for pesticide ingestion, should be effectively cleaned.

Electrolyzed water, as an advanced sanitizing agent, has gained significant reputation in the food industry. Electrolyzed water is generated from electrolysis of a diluted salt solution (usually NaCl). Electrolyzed oxidizing (EO) water is produced from the anode side and electrolyzed reduced (ER) water is created from the cathode side. EO water contains available chlorine with pH around 2.5 and oxidation reduction potential (ORP) above +1000 mV, while ER water has a pH above 10.0 and ORP below -800 mV (Huang et al., 2008). Some electrolyzed water generators can also mix EO and ER water to make near neutral pH EO water with pH around 6.0 and ORP around +900 mV. The available chlorine in EO water also exists in different forms depending on the pH. When pH is between 3.0 and 6.5, the major chlorine species is hypochlorous acid, which is also the most reactive species in inactivating microorganisms and oxidizing organic compound (Deborde & Gunten, 2008; Huang et al., 2008). Lots of scientific studies have shown EO water has very strong antimicrobial effect against many microbes, such as *E. coli* O157:H7 (Venkitanarayananet al., 1999), *S. typhimurium* (Fabrizio & Cutter, 2003), *C. jejuni* (Park, Hung, & Brackett, 2002), and *L. monocytogenes* (Fabrizio & Cutter, 2003). Because of the presence of available chlorine in EO water and the high pH of ER water, EO and ER water have great potentials to degrade and hydrolyze pesticides (Deborde & Gunten, 2008; Dyguda, Roszak, & Sokalski, 2014).

Diazinon, cyprodinil, and phosmet (Figure 3.1) are the three pesticides involved in this study. The reasons why these three pesticides were selected are: 1) their main application is on fresh produce; 2) the chemical structure of cyprodinil is different from diazinon and phosmet, and thus they may have different sensitivities to electrolyzed water treatment; 3) even though diazinon and phosmet have similar structures, their stabilities in high pH conditions is quite different. Diazinon and phosmet are both organophosphorus insecticides and are primarily applied on fresh produce for pest control purposes through acetylcholinesterase inhibition (Chen et al., 2014; Worthing & Walker, 1986). Phosmet is sensitive to alkaline hydrolysis while diazinon is relative stable in alkaline conditions (Worthing & Walker, 1986). Cyprodinil is an organonitrogen fungicide and widely used on fruits (Esteve-Turrillas et al., 2015). Cyprodinil is extremely stable in alkaline conditions.

In addition, methods of appropriate pesticide inoculation on fresh produce might be challenging for researchers as many labs may not have laboratory fields to grow produce and spray pesticides to contaminate produce evenly. For pesticide removal challenge study, factors such as soaking, shaking, rubbing, and washing solution volume, could also affect the pesticide removal efficacy. The purposes of the present study are aimed to determine the efficacy of both EO and ER water in degrading diazinon, cyprodinil, and phosmet in aqueous solution and develop appropriate way for pesticide inoculation and washing condition for pesticide removal challenge study.

Materials and methods

Chemicals

Standard diazinon (purity 98.5%), cyprodinil (purity 99.9%), phosmet (purity 99.7%), and analytical grade sodium thiosulfate ($Na_2S_2O_3$) (purity 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC/MS grade formic acid (purity 99.5%) was from Fisher Scientific (Fair Lawn, NJ, USA). Primary secondary amine (PSA) was from Agilent Technologies (Santa Clara, CA, USA). ACS grade sodium chloride (NaCl) and USP grade magnesium sulfate anhydrous ($MgSO_4$) were from Amresco® (Solon, OH, USA). ACS grade of sodium phosphate dibasic anhydrous (Na_2HPO_4) and sodium phosphate monobasic monohydrate

($NaH_2PO_4 \cdot H_2O$) (purity 99+%) were from Acros Organics (NJ, USA). HPLC grade acetonitrile was from EMD Millipore Corporation (Billerica, MA, USA). Standard stock solutions (1000 mg/L) of each pesticide were prepared in acetonitrile and stored in amber glass bottles at 4 ^{o}C. Working solutions of each pesticide were prepared by diluting the standard stock solutions with deionized (DI) water.

Preparation of electrolyzed water

EO and ER water were generated by electrolyzing a 0.03% NaCl solution with an electrolyzed water generator (Model #P30HST44T, EAU, GA, USA). All EO and ER water were freshly made and used within 3 h. The pH and ORP of EO and ER water were measured using an Accumet pH meter (AR50, Fisher Scientific, Pittsburgh, PA, USA) with pH and ORP electrodes. The ACC (all existing chlorine species: Cl_2, HOCl, and ClO^-) of EO water was measured using a DOD-FEAS titrimetric method (Hach Co., Loveland, Colo., USA). DI water dilution was applied to EO water with ACC higher than the target values to achieve specified levels. Drops of 1N HCl and 1N NaOH were also applied to adjust the pH of EO and ER water.

EO and ER water treatment

Working solutions of diazinon, cyprodinil, and phosmet were diluted to a final concentration of 25 mg/L, respectively. EO water at pH 2.8, 5.0, and 6.0 with ACC of 20 mg/L; EO water at ACC 10, and 5 mg/L with pH of 2.8; and ER water at pH 11.5 were collected into 250 mL glass bottles covered with aluminum foil. The treatment was initiated by mixing 1 mL pesticide working solution with 4 mL treatment solution in a 50 mL centrifuge tube. After target treatment times, 5 mL neutralizing buffer (0.32% Na_2HPO_4, 1.3% NaH_2PO_4, 9% NaCl, and 0.1% $Na_2S_2O_3$, pH around 5.60) was added to quench the reaction. The final 10 mL solution was subjected to pesticide extraction. DI water was also used to treat pesticide working solutions as a control.

Development of methods for pesticide inoculation and EO water washing procedure to remove pesticides on grapes

Four steps were involved for the method development. Fresh red seedless grapes (imported from Chile) were purchased from a local grocery store and stored at 4 ^{o}C until use (maximum 72 h). Grapes were soaked in a pesticide working solution for specified length of time to inoculate pesticides. The first step was to evaluate the effect of grape size on pesticide deposition during soaking. Grapes were divided into three size groups based on their weight: large (> 7 g), medium (5 – 7 g), and small (< 5 g). All grapes were completely soaked in 12 mg/L mixed pesticide solution for 10 min followed by 14 h air drying under a fume hood. Grapes were then analyzed for pesticide concentration.

The second step was to evaluate if the efficacy of EO water wash can be affected by the number of pesticides deposited on grapes. Fresh grapes of medium size were soaked into two kinds of pesticide solutions: solution with mix of three pesticides (diazinon, cyprodinil, and phosmet) at 12 mg/L of each; and solutions with only one type of pesticide at 12 mg/L. The soaking time was 10 min followed by the same drying process as described above. Then, the contaminated grapes (200g) were then soaked into 500 mL EO water at ACC 120 mg/L and pH 2.8 in a glass beaker for 15 min. After EO water soaking, all grapes were transferred into 1000 mL tap water and soaked for 5 s to remove residual chlorines and then analyzed for pesticide residue. Non-washed inoculated grapes (200g) were analyzed for the initial pesticide concentration.

The third step was to evaluate whether soaking time during pesticide inoculation can affect the amount of pesticide deposited on grapes and whether pesticide will penetrate into grape flesh during soaking. Grapes of medium size were soaked in 12 mg/L of mixed three pesticide solution for 5, 10, 15, and 30 min followed by the same drying procedure as described above. For each soaking time, two batches of 200 g grapes were picked. One was directly analyzed for pesticide concentration and the other one was peeled to remove the outer skin to analyze the pesticide concentration in grape flesh.

The final step was to evaluate the effect of washing method (soaking, rubbing, and shaking) and grape to EO water ratio on pesticide reduction. Medium size grapes were soaked in 12 mg/L of mixed three pesticide solution for 10 min followed by the drying process. The contaminated grapes (200 g for each treatment) were subjected to four different EO water washing conditions. In the first condition, grapes were completely soaked in 500 mL ER water at pH 11.5 in a plastic zip-lock bag for 1 min with continuous hand rubbing on grape surface. Then all grapes were soaked into tap water for 5 s followed by soaking in 500 mL EO water at ACC 120 mg/L and pH 2.8 for 15 min. In the second condition, grapes were only soaked in 500 mL EO water for 15 min. In the third condition, grapes were soaked in 500 mL EO water in a glass beaker for 15 min with continuous shaking at 100 rpm in a reciprocal shaking bath (Model 2870, Thermo Fisher Scientific, Waltham, MA, USA). In the fourth condition, grapes were soaked in 2000 mL EO water for 15 min. After EO water soaking, all grapes were transferred into 1000 mL tap water and soaked for 5 s to remove residual chlorines. Finally, the pesticide reduction for each treatment condition were compared.

Pesticide extraction

Quechers method (Anastassiades et al., 2003) was used for pesticide extraction. Briefly, 10 mL acetonitrile was added to the tube containing the final 10 mL solution or 10 g blended grape puree. The tube was shaken vigorously on a vortexer for 1 min. Then, 4 g $MgSO_4$ and 1 g NaCl were added followed by vigorous shaking for another 1min. The tube was centrifuged at 3200 rcf for 5 min. After centrifugation, 1 mL of the upper clear layer was transferred to a mini-centrifuge tube containing 150 mg $MgSO_4$ and 50 mg PSA, followed by vigorous shaking for 1 min. The mini-centrifuge tube was then centrifuged at 3500 rcf for 5 min. Finally, 0.6 mL of the upper layer was mixed with 1.5 mL HPLC water and filtered through a 0.2 µm nylon syringe filter (Restek, Bellefonte, PA, USA) into a 1.8 mL HPLC vial.

UPLC-MS/MS analysis

An ultra-performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS) was used to determine diazinon, cyprodinil, and phosmet. An Acquity UPLC-I Class System (Waters Corp., Milford, MA, USA) with an Acquity UPLC Bridged Ethylene Hybrid (BEH) C_{18} reverse-phase column (2.1 × 50 mm, 1.7 µm particle size, Waters Corp., Milford, MA, USA) was utilized for separation. The UPLC mobile phase consisted of 0.1% formic acid in HPLC water and acetonitrile. The flow rate of mobile phase was 0.3 mL/min with gradient elution as follows: 0 min to 0.1 min, linear gradient, changing acetonitrile from 10% to 35%; 0.1 min to 4.0 min, linear gradient, increasing acetonitrile from 35% to 90%; 4.0 min to 4.1 min, linear gradient, decreasing acetonitrile from 90% to 10%; 4.1 min to 6.0 min, holding acetonitrile at 10%. The total run time for each cycle was 6.0 min. The column was kept at 40 ^{o}C and the temperature in the sample manager was maintained at 20 °C. The injection volume for each sample was 3 µL.

A triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) with an ESI source operated in positive ion mode was used to detect each pesticide. The capillary voltage was 3.0 kV, source temperature 120 °C, and desolvation temperature 350 °C. The flow rate of nebulizer and desolvation gas was set at 50 and 500 L/h. All pesticides were detected by MRM with a dwell time of 50 ms. The MS parameters for each pesticide were optimized by individual tuning and are shown in Table 3.1. Quantification of pesticides in pure solution was based on five-point external calibration curves prepared in acetonitrile and quantification of pesticides on grapes was based on five-point external calibration curves prepared in grape matrix matched acetonitrile. The limit of detection (LOD, S/N = 3:1), was determined to be 0.01, 0.2, and 0.12 µg/L for diazinon, cyprodinil and phosmet in pure solution, respectively. The limit of quantification (LOQ, S/N = 9:1) was determined to be 0.04, 0.5, and 0.2 µg/L for diazinon, cyprodinil, and phosmet in pure solution, respectively.

Statistical analysis

Duplicate samples were conducted for each treatment in the pesticide degradation study within each replication. The entire experiment for both pesticide degradation and method development for grape challenge studies were repeated three times. Means and standard deviations were calculated using Excel (Micro software, USA). SAS software 9.4 (SAS Institute Inc., Cary, NC, USA) with proc anova procedure was used to conduct one way analysis of variance followed by Duncan’s test for multiple comparisons between each means. A $P \leq 0.05$ was considered to be significantly different.

Results and discussion

EO and ER water properties

The final pH of EO water at ACC 20 mg/L was adjusted to 2.80 ± 0.05, 5.00 ± 0.05, and 6.00 ± 0.05 with ORP of 1130.5 ± 6.7, 929.2 ± 7.0, and 873.0 ± 5.1 mV, respectively. The ORP for EO water at pH 2.80 ± 0.05 with ACC of 10 and 5 mg/L were 1111.8 ± 6.6 and 1099.9 ± 6.0 mV. The pH of ER water was adjusted to 11.50 ± 0.05 with ORP -885.5 ± 8.8 mV.

Effect of EO water pH in pesticide degradation

EO water at ACC 20 mg/L with pH at 2.8, 5.0, and 6.0 were applied to the three tested pesticides for 3 min. DI water was also used as a control. In our results, no pesticide degradation was found by DI water. The three tested pesticides were found to be degradable upon EO water treatment. The effect of pH in degrading diazinon, cyprodinil, and phosmet was significant ($P \leq 0.05$) (Table 3.2). When pH was changed from near neutral to acidic, the reduction was increased from near 98.5% to 99.9, 77.1% to 99.0%, and 99.1% to 99.9% for diazinon, cyprodinil, and phosmet, respectively. The hydroxyl radicals (HO•) produced by the hypochlorous acid (Huang et al., 2008) in EO water may be the major compound to explain pesticide degradation. Hydroxyl radicals are strong reactive intermediates to oxidize organic compounds (Chiron et al., 2000). The presence of hydroxyl radicals in ozone, ozonated water and pulsed electric field has been considered to be the main reason of the pesticide degradation ability for these techniques (Misra, 2015). Hypochlorous acid may also react with pesticides through unsaturated double bonds addition and electrophilic substitution (Deborde & Gunten, 2008).

The pH dependent property of EO water in degrading the three pesticides might be because the reaction between hypochlorous acid and pesticides is catalyzed by acid (Deborde & Gunten, 2008). Also, the reactivity of hydroxyl radicals against organic molecules is highly pH dependent (Chiron et al., 2000). The different ORP of EO water at different pH may also explain the reduction difference. The ORP of EO water is obviously higher at acidic pH (above +1100 mV) than at near neutral pH (around +900 mV) showing there is a greater oxidation reduction potential for EO water at lower pH. This explanation can be supported by the study of Lopez-Fernandez et al. (2013), which concluded the oxidant character was the determinant for washing solutions to remove mancozeb residues from lettuce. Their results showed hydrogen peroxide (higher ORP) was more effective than sodium hypochlorite (lower ORP) in reducing pesticide residues. In addition, compared with diazinon and phosmet, cyprodinil showed more sensitive to EO water pH effect (Table 3.2). Its reductions were also obviously lower than the other two pesticides at neutral pH. Chlorines are easier to react with reduced sulfur moieties than with nitrogen containing moieties (Deborde & Gunten, 2008). Both diazinon and phosmet have the P=S double bond structures (Figure 3.1) and thus are easier to be oxidized by hypochlorous acid even at neutral pH. However, cyprodinil does not contain such structures and its reaction with hypochlorous acid could be extremely slowed down at neutral pH.

Effect of EO water ACC in pesticide degradation

Because EO water was previously shown to be more effective at acidic conditions, we kept EO water at pH 2.8 to study the effect of EO water ACC. Acidic EO water at ACC 20, 10, and 5 mg/L were applied to the three pesticides for 3 min. As shown in Table 3.3, ACC has significant ($P \leq 0.05$) effect in degrading diazinon, cyprodinil, and phosmet. The reduction of diazinon significantly ($P \leq 0.05$) dropped from 99.9% to 99.2% when ACC was reduced from 20 to 5 mg/L (Table 3.3). The ACC effect was more obvious for the other two pesticides as there were about a 6.0% and 3.0 % decrease of reduction when ACC dropped from 20 to 5 mg/L for cyprodinil and phosmet, respectively (Table 3.3). While there were significant ($P \leq 0.05$) differences for the reductions of the three tested pesticides upon EO water treatment at different ACC, there were not too much difference of EO water ORPs (+1099 to 1130 mV). This may indicate that ORP is the only determinant for EO water to degrade the tested pesticides. The concentration of strong oxidizing compound, such as the available chlorine in EO water, is also important in degrading pesticides. Similar results were also found in the study of Chen et al. (2014) using chlorine dioxide (a strong oxidizing reagent) to degrade pesticides. They found the reductions of diazinon and phorate were increased by 20% and 40% when the concentration of chlorine dioxide was increased from 10 to 20 mg/L. Results presented in Tables 3.2 and 3.3 indicated that EO water was more effective at acidic pH and higher ACC to degrade diazinon, cyprodinil, and phosmet.

Effect of EO water treatment time in pesticide degradation

By keeping the pH at 2.8 and ACC at 20 mg/L, the effect of EO water treatment time at 1 min, 3 min, and 5 min was studied. Table 3.4 shows the reductions of all the three pesticides were greater than 98% for all treatments. Longer treatment time did not result in higher reductions except between 1 min and 3 min treatment for diazinon (99.3 vs. 99.9%) and cyprodinil (98.1 vs. 99.0%). However, for phosmet, no reduction difference was found between each treatment time (Table 3.4). Also, different degradation rate was found for the three pesticides, being phosmet the quickest and cyprodinil the slowest. The structural difference might be the main reason, as the P=S, P-O and P-S bonds of phosmet (Figure 3.1) are easier to be attacked by chlorine, while the –NH– bond of cyprodinil is harder for oxidizing reagent to attack (Deborde & Gunten, 2008). The three pesticides tested in current study were shown to be more susceptible to EO water treatment when compared with the results in the study of Lin et al. (2006), where only 93.6% of methamidophos and 96.0% of dimethoate were reduced by acidic EO water at ACC 50 mg/L in 8 min with a mixing ratio of 1:9 (pesticide : EO).

Efficacy of ER water in pesticide degradation

ER water at pH 11.5 was applied to each pesticide for 3 min. No degradation was found for cyprodinil and only 7.4% reduction was found for diazinon while 99.7% reduction was achieved for phosmet (Table 3.2). This is in accordance with the described property of diazinon and phosmet in Worthing and Walker (1986) that diazinon has a half-life of 29 days at pH 9.0 and phosmet has a half-life of only 4 h at pH 8.0. Cyprodinil, on the other hand, is hydrolytically stable with a half-life beyond one year at pH 4-9 (U.S. EPA, 1998). The difference in chemical structures of the tested pesticides may explain their different stabilities at alkaline conditions. The P-O and P-S bonds of diazinon and phosmet can undergo alkaline hydrolysis through nucleophilic substitution (Dyguda-Kazimierowicz et al., 2014), while the chemical structure of cyprodinil does not have reactive site for such reaction. In addition, even diazinon and phosmet are both organophosphorus pesticides and have similar structures, their degradation rates under alkaline conditions were very different (Table 3.2). This demonstrates that pesticides with similar chemical structures could undergo different reduction rate upon ER water treatment. Similar results were also found in the work of Lin et al. (2006), where the reductions for two organophosphorus pesticides, methamidophos and dimethoate, were 99.5% and 44.6% when subjected to ER water treatment at pH 11.24 with a reaction time of 16 min. In addition, no significant ($P > 0.05$) difference was found between EO (ACC 20 mg/L and pH 2.8) and ER water in degrading phosmet, indicating the great potential of ER water in pesticide residue removal on real foods.

Effect of grape size on pesticide deposition

To inoculate pesticide on fresh produce, soaking methods were commonly used (Chen et al., 2014; Hao et al., 2011; Lin et al., 2006; Lopez-Fernandez et al., 2013). However, this method might not be able to get very consistent initial pesticide residue levels on produce. In the case for grapes, the size might be one key factor as smaller grapes have a larger surface area to volume ratio than larger grapes for a same total weight sample. That means, at the same total weight, small grapes have larger total surface area than large grapes and thus have more contact with pesticide solution during the soaking process. Therefore, smaller grapes may be deposited with relatively more total amount of pesticides during the soaking process. In our results, there was an obvious increase on the amount of deposition for the three pesticides due to grape size. The diazinon deposited on small grapes (0.159 mg/kg) was nearly twice as the diazinon deposited on large grapes (0.084 mg/kg) and also higher than the pesticide amount on the medium size grapes (0.123 mg/kg). For cyprodinil and phosmet, not too much difference of deposition amount were found between large (1.195 and 0.139 mg/kg, respectively) and medium grapes (1.198 and 0.139 mg/kg, respectively) while small grapes have an obvious higher pesticide deposition, being 1.428 and 0.148 mg/kg for cyprodinil and phosmet, respectively. These results indicate that grape size are important for pesticide deposition in the soaking process. Smaller size can achieve higher initial pesticide concentration. However, small grapes (< 5 g) were only present in less than 10 % of entire purchased grapes. Thus, for the future studies, we selected medium size grapes.

EO water efficacy in reducing multiple and individual pesticides on grapes

Previously, it was shown that EO water can effectively degrade diazinon, cyprodinil, and phosmet in pure solution. However, all these treatments were conducted on individual pesticide solutions. For real food samples, they may contain multiple pesticide residues. Mixed pesticide residues may have interfering effects and lower the degrading efficacy of EO water. Table 3.5 shows the results of EO water wash in reducing the three pesticides on grapes prepared with either mixed or individual pesticide solutions. The overall reductions were around 77% (diazinon), 65% (cyprodinil), and 81% (phosmet). No significant difference ($P > 0.05$) on pesticide reductions were found between grapes prepared either with mixed or individual pesticide solutions. This indicates the efficacy of EO water in degrading each individual pesticide would not be affected by the presence of other pesticides on fresh produce and also the presence of multiple pesticides would not affect the reduction efficacy of each individual pesticides on produce samples. Therefore, in the future studies, we inoculated all produce samples with mixed pesticides.

Effect of soaking time on pesticide deposition and pesticide penetration into grapes

Different studies in the literature have used different soaking time to inoculate pesticides. Lopez-Fernandez et al. (2013) used 1 h soaking time to inoculate mancozeb on lettuce while Chen et al. (2014) used only 5 min soaking time to inoculate pesticides on lettuce. Also, pesticides may penetrate into produce instead of remaining on produce surface and this penetration effect may be enhanced by longer soaking time. Table 3.6 shows the results of pesticide residues on grapes after different soaking time and the percentage of pesticide penetrated into grape flesh. Both the total pesticide residue level before peeling and the pesticide residue level inside the grape flesh increased with increasing soaking time, indicating more pesticides can be deposited on and also penetrated into grapes with increased soaking time. However, significant ($P \leq 0.05$) increase of pesticide deposition was only fond between 30 min and 15 min soaking for diazinon and between 30 min and 10 min soaking for cyprodinil. On the other hand, no significant ($P > 0.05$) rate of penetration were found among all soaking times. The overall penetrations were ranged from 4.5% to 9.4% for the three pesticides, which are very low and indicates most of the pesticides remained on the skin of grapes after the soaking process. Ten min soaking time was selected to inoculate pesticides for the future studies based on its lowest average penetration rate.

Effect of different EO water wash condition in reducing pesticides on grapes

Many produce wash studies soaked produce in washing solutions combined with shaking to create more physical contact to enhance pesticide removal. The volume of EO water for produce wash could also be important as higher volume of EO water has higher amount of dissolved chlorine. Rubbing on produce surface could be important as it is a very commonly used washing method by consumers to enhance physical contact. A combination of EO and ER water wash might also enhance the pesticide removal as ER water has good cleaning abilities (Lin et al., 2006). Table 3.7 shows the reductions of each pesticide on grapes after different EO water washing treatment. The reductions ranged from 68.8% to 75.7%, 59.2% to 66.9% and 82.6% to 85.7% for diazinon, cyprodinil, and phosmet, respectively. For all the three pesticides, no significantly ($P > 0.05$) different reductions were found among all four washing treatment conditions. This indicates, rubbing in ER water before EO water soaking may not have any advantages in pesticide removal than just EO water soaking. Higher volume of EO water would also not lead to significantly ($P > 0.05$) higher reductions of pesticide residues. The use of shaking during EO water soaking treatment also did not cause significant ($P > 0.05$) increase in pesticide reductions than soaking treatment. Based on the results in the current study, we therefore decided to choose 500 mL volume of EO water combined with 100 rpm shaking to wash grapes for our future studies.

Conclusions

The present study demonstrates EO water can effectively degrade diazinon, cyprodinil, and phosmet in aqueous solution. The efficacy of EO water was affected by pH, ACC, and treatment time. The higher of ACC, the lower of pH, and the longer treatment time, the higher reductions of pesticides can be achieved. ER water was only effective in hydrolyzing phosmet and slightly effective in degrading diazinon, while not effective in reducing cyprodinil. Their different chemical structures may be the main reason for the different reduction rate and different susceptibility under each conditions. In addition, the appropriate way to inoculate the three pesticides on grapes was found by completely soak medium size (5-7 g) grapes in 12 mg/L mixed pesticide solution for 10 min followed by 14 h air drying at room temperature and the appropriate washing condition was to soak 200 g grapes in 500 mL EO water with 100 rpm shaking.

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Table 3.1. UPLC-MS/MS parameters for each pesticide analyzed.

Compound	Precursor ion (m/z)	CV (V)	Quantification ion (m/z)	CE 1 (eV)	Qualifier ion (m/z)	CE 2 (eV)
Diazinon	305.0	35	169.0	20	153.0	20
Cyprodinil	226.0	38	108.0	33	93.0	40
Phosmet	318.0	33	160.0	15	133.0	35

Table 3.2. Effect of EO water pH and the efficacy of ER water in degrading diazinon, cyprodinil, and phosmet.

EO at ACC 20 mg/L	Reduction%		
	Diazinon	Cyprodinil	Phosmet
pH 2.8	99.9±0.0^{A}	99.0±0.2^{A}	99.9±0.0^{A}
pH 5.0	98.5±0.5^{B}	90.1±1.3^{B}	99.2±0.2^{B}
pH 6.0	98.4±0.3^{B}	77.1±4.6^{C}	99.1±0.2^{B}
ER at pH 11.5	7.4±2.4^{C}	0.0±0.0^{D}	99.7±0.1^{A}

Means in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 3.3. Effect of EO water ACC in degrading diazinon, cyprodinil and phosmet.

EO at pH 2.8	Reduction%		
	Diazinon	Cyprodinil	Phosmet
ACC 5 mg/L	99.2±0.2^{C}	92.7±1.8^{B}	96.6±0.8^{B}
ACC 10 mg/L	99.7±0.1^{B}	98.0±0.2^{A}	99.8±0.1^{A}
ACC 20 mg/L	99.9±0.0^{A}	99.0±0.2^{A}	99.9±0.0^{A}

Means in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 3.4. Effect of EO water treatment time in degrading diazinon, cyprodinil and phosmet.

EO at ACC 20 mg/L and pH 2.8	Reduction%		
	Diazinon	Cyprodinil	Phosmet
1 min	99.3±0.1^{B}	98.1±0.6^{B}	99.9±0.0^{A}
3 min	99.9±0.0^{A}	99.0±0.2^{A}	99.9±0.0^{A}
5 min	99.9±0.0^{A}	99.3±0.1^{A}	99.9±0.0^{A}

Means in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 3.5. Reductions of pesticides by EO[a] water treatment on grapes prepared with mixed pesticide solution and individual pesticide solution.

	Reduction (%)	
	Mixture[b]	Individual[c]
Diazinon	75.1 ± 7.8^{A}	79.1 ± 7.9^{A}
Cyprodinil	64.8 ± 10.1^{A}	66.2 ± 8.7^{A}
Phosmet	85.2 ± 5.4^{A}	78.4 ± 3.9^{A}

Means in the same row with different superscript letters are significantly different ($P \leq 0.05$); the initial concentrations were 0.095±0.038, 1.467±0.264, and 0.238±0.039 mg/kg on grapes soaked in mixed pesticide solution and 0.136±0.058, 1.371±0.246 and 0.266±0.029 mg/kg on grapes soaked in individual pesticide solution for diazinon, cyprodinil, and phosmet, respectively.

[a] EO water was at ACC 120 mg/L and pH 2.8, and treatment time was 15 min.

[b] Mixed pesticide solution (12 mg/L of each) was used to contaminate grape samples.

[c] Each individual pesticide solution (12 mg/L) was used to contaminate grape samples.

Table 3.6. Concentration of pesticide residues on grapes after soaking in pesticide solution for different times.

Pesticide	Soaking Time (min)	Pesticide residue		Penetration (%)
		Before Peeling (mg/kg)	After peeling (mg/kg)	
Diazinon	5	0.071 ± 0.029^{B}	0.004 ± 0.001^{B}	5.7 ± 0.8^{A}
	10	0.109 ± 0.012^{B}	0.005 ± 0.000^{B}	4.6 ± 0.7^{A}
	15	0.138 ± 0.043^{B}	0.006 ± 0.003^{B}	4.5 ± 0.7^{A}
	30	0.267 ± 0.049^{A}	0.013 ± 0.001^{A}	5.0 ± 1.3^{A}
Cyprodinil	5	1.189 ± 0.251^{B}	0.110 ± 0.004^{A}	9.4 ± 1.7^{A}
	10	1.477 ± 0.267^{B}	0.097 ± 0.016^{A}	6.8 ± 2.0^{A}
	15	1.775 ± 0.509^{AB}	0.123 ± 0.070^{A}	6.6 ± 2.3^{A}
	30	2.181 ± 0.402^{A}	0.146 ± 0.018^{A}	6.9 ± 1.7^{A}
Phosmet	5	0.186 ± 0.063^{A}	0.011 ± 0.001^{B}	6.5 ± 1.7^{A}
	10	0.255 ± 0.042^{A}	0.013 ± 0.001^{B}	5.3 ± 0.9^{A}
	15	0.266 ± 0.049^{A}	0.019 ± 0.004^{A}	7.4 ± 2.8^{A}
	30	0.280 ± 0.051^{A}	0.023 ± 0.002^{A}	8.3 ± 1.0^{A}

Means in the same column of each pesticide category with different superscript letters are significantly different ($P \leq 0.05$).

Table 3.7. Reductions of each pesticide on grapes after different EO[a] water washing treatment.

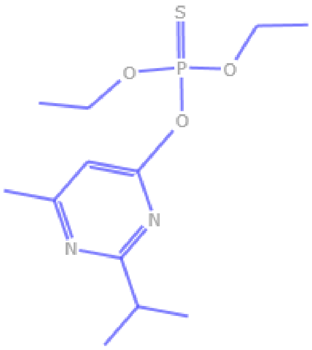
	Reduction %		
Treatment	Diazinon	Cyprodinil	Phosmet
Rubbing[b]	75.7±14.8^A	65.8±7.6^A	85.7±7.6^A
500 mL Soaking	68.8±14.4^A	59.2±4.9^A	82.6±8.0^A
500 mL Shaking	72.4±12.7^A	64.0±7.8^A	83.2±6.9^A
2000 mL Soaking	72.3±11.8^A	66.9±7.2^A	84.2±7.2^A

Means in the same column with different superscript letters are significantly different ($P \leq 0.05$) and the initial pesticide concentrations are 0.108±0.015, 1.677±0.092, and 0.232±0.029 mg/kg for diazinon, cyprodinil, and phosmet, respectively.

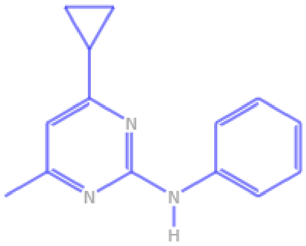
[a] EO water was at ACC 120 mg/L and pH 2.8.

[b] Grapes in this treatment was first soaked in ER water at pH 11.5 for 1 min in a plastic bag with continuous surface rubbing by hands followed by 15 min soaking in EO water for 15 min.

Diazinon



Cyprodinil



Phosmet

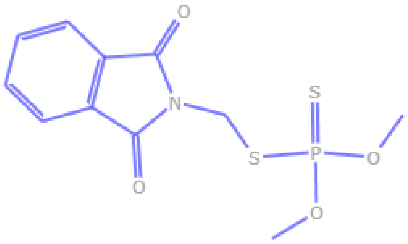


Figure 3.1. Chemical structures of diazinon, cyprodinil, and phosmet.

CHAPTER 4

EFFICACY OF ELECTROLYZED OXIDIZING WATER IN REMOVING PESTICIDE RESIDUES ON FRESH PRODUCE

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Abstract

This study evaluated the efficacy of electrolyzed oxidizing (EO) water in removing pesticide (diazinon, cyprodinil, and phosmet) residues on grapes, spinach, and snap peas. The effect of available chlorine content (ACC) and treatment time of EO water was investigated. Other washing solutions, including electrolyzed reduced (ER) water, diluted bleach, VegWash, and DI water, were also used to compare with EO water in removing pesticide residues. The results showed ACC and treatment time were both significant ($P \leq 0.05$) factors in determining the efficacy of EO water in reducing pesticide residues on fresh produce. Higher ACC and longer treatment time led to higher residue reductions of the three pesticides on the produce. EO water wash at ACC 120 mg/L for 15 min achieved reductions of 37.1%, 31.5%, and 49.4% on grapes; 59.2%, 43.8%, and 85.7% on spinach; 66.5%, 50.0%, and 73.0% on snap peas; for diazinon, cyprodinil, and phosmet, respectively. EO water was also shown to be significantly ($P \leq 0.05$) more effective than ER water, diluted bleach, VegWash, and DI water for removing the three pesticides on the three produce, except cyprodinil on grapes. An example of using surface response model was also presented for the reduction of cyprodinil on spinach to demonstrate the possibility of building mathematical models to predict pesticide residue reductions on fresh produce with EO water treatment. The results from this study indicate EO water has great potentials for application in the food industry and households to remove pesticide residues on produce.

Key words: electrolyzed water, fresh produce, diazinon, cyprodinil, phosmet, chlorine

Introduction

The use of pesticides in agriculture has been increasing dramatically along with the demand of high quality foods by consumers. The application of pesticides can help control insects, crop diseases and enhance crop yield (Bohmont, 2003). It has been reported that more than 400 million kg pesticides were applied in the U.S. in 2007 (Langley & Mort, 2012). However, the use of pesticides also has great potential threats to human health since pesticide residues on foods can have severe adverse health effects even when present in minute amount (Pooja & Latika, 2014). The situation could be even worse since most foods were put into the market soon after harvest to maintain freshness, giving limited time for pesticide residues to decompose in the environment (Misra, 2015). Farmers may also apply high amount of pesticides in order to control pests in short periods, resulting in pesticide residue levels above the maximum residue limit (MRL) required by EPA (Misra, 2015). In the 2011 FDA pesticide monitoring program report, 39.5% of sampled domestic and 35.5% of sampled imported foods were found to contain detectable pesticide residues, with violation rate of 1.6% in domestic and 7.1% in imported foods (U.S. FDA, 2011).

The consumption of fresh produce has increased significantly over the last decade because of the nutritional and health benefits (Afari, Hung, & King, 2015). This trend also renders consumers to greater pesticide exposures since most of the fresh fruits and vegetables only undergo limited process before consumption (Claeys et al., 2011). After harvest, most of the fresh produce are only rinsed with tap water to remove dirt and debris, which has limited effect on pesticide residue removal (Hao et al., 2011). The pesticide residues on fresh produce may reside and accumulate inside human bodies for extended period of time and cause chronic diseases (Carozza et al., 2009). Many symptoms and chronic diseases have been shown to be associated with pesticides, including cancer (Bolognesi & Morasso, 2000), Alzheimer's disease (Zaganas et al., 2013), testicular dysfunction and female abortion (Culliney, Pimentel, & Pimentel, 1992; Sengupta & Banerjee, 2014), and type II diabetes (Jonggun et al., 2013). Numerous sanitizers and washing agents have been developed for fresh produce wash. Chlorine based washing solutions have been widely used because of the inexpensiveness and strong antimicrobial activity (Lopez-Fernandez et al., 2013).

Electrolyzed water, an advanced technology that has been applied in agriculture, medicine, dentistry and food industry (Huang et al., 2008), could be a potent washing agent for pesticide residue removal on fresh produce. The generation of electrolyzed water is by electrolyzing a dilute salt (usually NaCl) solution in an electrolytic chamber, in which a diaphragm separates the anode and cathode. Electrolyzed oxidizing (EO) and electrolyzed reduced (ER) water are produced from the anode and cathode side, respectively. EO water has a low pH (around 2.5), high oxidation reduction potential (ORP > +1000 mV) and available chlorine content (ACC) (total concentration of Cl_2, HOCl, and ClO^-). ER water has a high pH (above 10.0) and low ORP (-800 to -900 mV). EO water is an effective sanitizer in inactivating many foodborne pathogens, such as *E. coli* O157, *L. monocytogenes* (Park et al., 2001), and *Salmonella* (Kimet al., 2003). EO water also has many advantages, such as safe and easy to operate, cheap to produce, and no negative effect on organoleptic properties of many food products (Hricova, Stephan, & Zweifel, 2008). The instability and corrosion to metal are the disadvantages of EO water. But they can be overcome by producing onsite and using stainless steel equipment (Huang et al., 2008).

The efficacy of EO water and other chlorine based agent in removing pesticide residues on fresh produce have been studied. Hao et al. (2011) found that EO water at pH 2.3 with ACC

70 mg/L removed 59-74% of acephate, omethoate, and DDVP on fresh spinach in 30 min. Chlorinated water was found to be able to reduce 50-100% of captan, azinphos-methyl, and formetanate hydrochloride on fresh apple (Ong et al., 1996). However, the effect of ACC and pesticide type on the final reduction efficacy were not fully examined. Also, as different produce have different surface textures, the efficacy of washing treatment could be very different upon produce types. The aim of this study was to investigate the efficacy of EO water in reducing diazinon, cyprodinil, and phosmet on fresh grapes, spinach, and snap peas. The three pesticides were selected because they are primarily applied on produce and have different chemical structures and different sensitivities against chlorine. Other washing solutions, including ER water, diluted bleach solution, VegWash (a commercial produce wash solution), and DI water were also used to compare with EO water in reducing pesticide residues. The effect of surface properties of different produce on pesticide removal were also discussed.

Materials and methods

Chemicals

Standard diazinon (purity 98.5%), cyprodinil (purity 99.9%), and phosmet (purity 99.7%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC/MS grade formic acid (purity 99.5%) was from Fisher Scientific (Fair Lawn, NJ, USA). Primary secondary amine (PSA) was from Agilent Technologies (Santa Clara, CA, USA). ACS grade sodium chloride (NaCl) and USP grade magnesium sulfate anhydrous ($MgSO_4$) were from Amresco® (Solon, OH, USA). HPLC grade acetonitrile was from EMD Millipore Corporation (Billerica, MA, USA). Concentrated bleach solution was from Clorox (Oakland, CA, USA). A commercial fresh produce washing solution was from VegWah (Irvine, CA, USA). Standard stock solutions (1000 mg/L) of each pesticide were prepared in acetonitrile and stored in amber glass bottles at 4 ^{o}C. Pesticide working solutions were prepared by diluting the standard stock solutions with deionized (DI) water to achieve target concentrations.

Preparation of electrolyzed water and other washing solutions

EO and ER water were generated by electrolyzing a 0.03% NaCl solution with an electrolyzed water generator (Model #P30HST44T, EAU, GA, USA). All EO and ER water were freshly made and used within 3 h. The pH and ORP of EO and ER water were measured using an Accumet pH meter (AR50, Fisher Scientific, Pittsburgh, PA, USA) with pH and ORP electrodes. The ACC of EO water was measured using a DOD-FEAS titrimetric method (Hach Co., Loveland, Colo., USA). DI water dilution was applied to EO water with ACC higher than the target values to achieve specified levels. Drops of 1N HCl and 1N NaOH were also applied to adjust the pH of EO and ER water. EO water at pH 2.8 with ACC of 20, 70, and 120 mg/L and ER water at pH 11.5 were collected into glass bottles covered with aluminum foil. Concentrated bleach was diluted with DI water to make a final ACC of 120 mg/L with pH around 7.5. VegWash solution was also diluted according to its label: 8 mL VegWash in 1000 mL DI water.

Pesticide spike on fresh produce

Fresh red seedless grapes (imported from Mexico), organic spinach, and organic snap peas were purchased from a local grocery store and kept at 4 ^{o}C until use (maximum 72 h). All three produce did not contain the pesticide residues to be tested. For grapes, a pesticide working solution containing 12 mg/L diazinon, cyprodinil, and phosmet was made and all grape samples were soaked in the mixed pesticide working solution for 10 min and air-dried under a fume hood at room temperature (approximately 22 ^{o}C) for about 14 h. For spinach, 15 g spinach leaves were selected for each treatment. 3 mL of 20 mg/L diazinon, cyprodinil, and phosmet working solution were applied to the adaxial-side of the spinach surface of each 15 g spinach leaves by depositing droplets with a 200-μL micropipette. All spiked spinach leaves were air dried for 3 h under a fume hood at room temperature. Then all contaminated spinach leaves were covered with a layer of aluminum foil and kept in a refrigerator set at 4 ^{o}C for about 14 h to prevent dehydration. For snap peas, 50 g samples were selected for each treatment and 1.6 mL of 40 mg/L diazinon, cyprodinil, and phosmet were applied to one side of each snap pea surface of the 50 g snap peas samples by depositing droplets as described for spinach. All snap peas then followed the same drying procedure described for spinach.

Washing treatment

The effect of treatment time and ACC of EO water was investigated in this study. Treatment time of 1, 8, and 15 min with EO water at ACC 20, 70, and 120 mg/L were applied. A total of 9 possible treatment combinations were therefore conducted. For grapes, 200 g spiked samples were picked and soaked in 500 mL of EO water with specified treatment time. For spinach, our preliminary study showed that 1000 mL EO water can achieve much higher reductions than 500 mL EO water. Thus 15g of spiked spinach leaves were soaked in 1000 mL of EO water. And for snap peas, as they have similar surface area to weight ratio as grapes, we used 500 mL EO water to treat contaminated snap peas. A plastic wire mesh was used to cover the spinach and snap peas to prevent floating. All washing treatment was conducted in 1000 mL glass beakers placed on a reciprocal shaking bath (Model 2870, Thermo Fisher Scientific, Waltham, MA, USA) set at 100 rpm. After washing, all produce were immediately transferred into 1000 mL tap water and soaked for about 5 s to remove residual chlorine.

ER water, diluted bleach, VegWash, and DI water were also used to wash the three tested produce to compare their pesticide residue reducing efficacy with EO water. 15 min washing was used for all the four solutions with the same washing procedure as described for EO water. Their pesticide reduction efficacy were compared with EO water at ACC 120 mg/L for 15 min. After the washing treatment, all produce were air dried for about 1 h at room temperature before pesticide extraction.

Pesticide extraction

To extract the pesticides from produce samples, Quechers method (Anastassiades et al., 2003) was applied. Briefly, each produce was first homogenized into puree with a blender (Hamilton Beach, Mexico). For spinach and snap peas, as they were too dry to be blended, DI water was added to improve the blending process. 85 mL and 100 mL DI water were added to each 15 g spinach leaves and 50 snap peas, respectively, before blending. Then, 10 g subsamples from the blended puree were transferred into a 50 mL centrifuge tube followed by addition of 10 mL acetonitrile. The tube was shaken vigorously on a vortexer for 1 min. Then, 4 g $MgSO_4$ and 1 g NaCl were added followed by vigorous shaking for another 1min. The tube was centrifuged at 3200 rcf for 5 min. After centrifugation, 1 mL of the upper clear layer was transferred to a mini-centrifuge tube containing 150 mg $MgSO_4$ and 50 mg PSA, followed by vigorous shaking for 1 min. The mini-centrifuge tube was then centrifuged at 3500 rcf for 5 min. Finally, 0.6 mL of the upper layer was mixed with 1.5 mL HPLC water and filtered through a 0.2 μm nylon syringe filter (Restek, Bellefonte, PA, USA) into a 1.8 mL HPLC vial. Unwashed produce were also analyzed to determine the initial pesticides concentration.

UPLC-MS/MS analysis

An ultra-performance liquid chromatography-tandem mass spectrometric (UPLC-MS/MS) was used to determine diazinon, cyprodinil, and phosmet. An Acquity UPLC-I Class System (Waters Corp., Milford, MA, USA) with an Acquity UPLC Bridged Ethylene Hybrid (BEH) C_{18} reverse-phase column (2.1 × 50 mm, 1.7 μm particle size, Waters Corp., Milford, MA, USA) was utilized for chromatographic separation. The UPLC mobile phase consisted of 0.1% formic acid in HPLC water and acetonitrile. The flow rate of mobile phase was 0.3 mL/min with linear gradient elution as follows: 0 min to 0.1 min, changing acetonitrile from 10% to 35%; 0.1 min to 4.0 min, increasing acetonitrile from 35% to 90%; 4.0 min to 4.1 min, decreasing acetonitrile from 90% to 10%; 4.1 min to 6.0 min, holding acetonitrile at 10%. The total run time was 6.0 min. The column was kept at 40 ^{o}C and the temperature in the sample manager was maintained at 20 ^{o}C. The injection volume was 3 μL. A triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA) with an ESI source operated in positive ion mode was used to detect each pesticide. The capillary voltage was 3.0 kV, source temperature 120 ^{o}C, and desolvation temperature 350 ^{o}C. The flow rate of nebulizer and desolvation gas was set at 50 and 500 L/h. All pesticides were detected by MRM with a dwell time of 50 ms. The MS parameters for each pesticide were optimized by individual tuning and are shown in Table 3.1. Quantification of each pesticide was based on five-point external standard curves prepared in matrix matched acetonitrile.

Recovery test

Fresh grapes, spinach and snap peas that contained no diazinon, cyprodinil, and phosmet were used for the recovery test. Each produce was blended into puree. For spinach and snap peas, DI water was added with the same ratio as described in the pesticide extraction section. Ten g of each blended produce puree was transferred into a 50 mL centrifuge tube and spiked with a mixed pesticide standards at three levels: 0.5, 0.1 and 0.05 mg/kg. After spiking, the tube was shaken vigorously on a vortexer for 1 min and then set for 1 h to equilibrate followed by the same extraction process described above. The final recovered concentration of each pesticide was determined by external matrix matched standard curves. Mean recovery (%) and relative standard deviation (RSD%) were calculated.

Statistical analysis

Three subsamples from each treatment group were taken for pesticide analysis and averaged to give representative pesticide concentration within each replication. The entire experiment was repeated three times. Means and standard deviations were calculated using Excel (Micro software, USA). One way analysis of variance was used for data analysis followed by Duncan’s multiple range test for means comparisons using SAS software 9.4 (SAS Institute Inc., Cary, NC, USA) with proc anova procedure. A $P \leq 0.05$ was considered to be significantly different. Also, the data for cyprodinil reduction on spinach was used to build a response surface prediction model with a 3-dimensional plot as an example to show how pesticides reductions by EO water treatment can be predicted using mathematical models. JMP Software (Pro 11.0, SAS Institute Inc., Cary, NC, USA) was used for the response surface analysis.

Results and discussion

Recovery test

The mean recoveries (%) and the relative standard deviations (RSD%) of each pesticides on each produce matrix were present in Table 4.1. The overall recoveries were ranged from 91.0% to 108.2%, indicating good accuracy for the currently used Quechers extraction method with UPLC-MS/MS analysis. The RSDs were ranged from 1.5% to 12.4%, which are acceptable values indicating good precisions. Thus, the extraction and analysis method used in this study is reliable for quantifying diazinon, cyprodinil, and phosmet on fresh grapes, spinach, and snap peas. Similar results can also be found in the study of Lehotay et al. (2010) where a lot more pesticides were shown to have good recoveries on fresh produce using the Quechers extraction and LC-MS/MS analysis.

Properties of EO water and other washing solutions

EO water was made to pH 2.80±0.05 with ACC at 20, 70, and 120 mg/L and ORP of 1126.0±7.0, 1144.0±10.0, and 1151.5±11.7 mV, respectively. ER water was at pH 11.50±0.05 and ORP -878.1±0.2 mV. Bleach was diluted to ACC 120 mg/L with pH 7.44±0.08 and ORP 867.9±8.8 mV.

EO water treatment on grapes

Both treatment time and ACC of EO water were found to be significant factors ($P \leq 0.05$) in reducing diazinon, cyprodinil, and phosmet on grapes. Reductions from 37.1% to 10.2% were achieved for diazinon with EO water treatment (Table 4.2). The highest reduction of diazinon on grapes was achieved after washing with EO water at ACC 120 mg/L for 15 min. No significantly

($P > 0.05$) different diazinon reductions were found between EO water treatment with ACC 120 and 70 mg/L at all three treatment time levels. But EO water at ACC 20 mg/L was always significantly ($P \leq 0.05$) less effective than at ACC 120 mg/L in reducing diazinon. Treatment time of 15 and 8 min showed no significantly ($P > 0.05$) different reductions on diazinon at all three ACC levels, while treatment time of 1 min showed significantly ($P \leq 0.05$) lower reductions at all comparable ACC levels than treatment time of 15 min. Similar results were also found for cyprodinil and phosmet. Reductions between 31.5% and 9.2%, and 49.4% and 21.4% were found for cyprodinil and phosmet, respectively (Table 4.2). The highest reductions for cyprodinil and phosmet were achieved at the highest ACC (120 mg/L) with the longest treatment time (15 min). Also, for cyprodinil and phosmet, reductions achieved by EO water with ACC 120 and 70 mg/L at all three treatment times shower no significant ($P > 0.05$) difference while reductions achieved at ACC 20 mg/L were always significantly ($P \leq 0.05$) lower. About 10% additional reduction can be achieved when ACC was increased from 20 to 120 mg/L for all three pesticides at each treatment time.

As observed in Table 4.2, there were significant ($P \leq 0.05$) increase of reductions between treatment time of 15 and 1 min and between ACC of 120 and 20 mg/L. However, less increase of reductions were achieved between 15 and 8 min and between 120 and 70 mg/L, indicating the increase of ACC and treatment time of EO water may not always achieve much higher pesticide residue reductions on grapes. Table 4.2 also shows the reductions of phosmet at each treatment conditions were always the highest, followed by diazinon and cyprodinil. This is in accordance with the results obtained in CHAPTER 3 that phosmet showed the highest sensitivity upon EO water degradation with diazinon the second and cyprodinil the third.

EO water treatment on spinach

EO water treatment time and ACC were shown to be significant ($P \leq 0.05$) factors in removing diazinon, cyprodinil, and phosmet on spinach. Compared with grapes, the overall reductions of the three tested pesticides on spinach were much higher. Reductions of diazinon were ranged from 59.2% to 8.8% with the highest reduction observed after 15 min EO water treatment with ACC 120 mg/L (Table 4.3). Higher ACC of EO water always resulted in higher diazinon reductions at each treatment time while only the reductions achieved by EO water with ACC 120 and 20 mg/L were significantly ($P \leq 0.05$) different. Increase of ACC from 70 to 120 mg/L did not lead to significantly ($P > 0.05$) higher diazinon reductions. The increase of treatment time for EO water at ACC 120 mg/L significantly ($P \leq 0.05$) improved the reducing efficacy on diazinon. But for EO water at ACC 20 and 70 mg/L, significant ($P \leq 0.05$) increase of reductions were only observed between 1 and 8 min.

For cyprodinil, reductions between 43.8% and 9.7% were obtained on spinach after EO water treatment with the highest reduction achieved after 15 min treatment with ACC 120 mg/L (Table 4.3). Similar to diazinon, longer treatment time and higher ACC resulted in higher cyprodinil reductions. Reductions of cyprodinil between each treatment time at every comparable ACC levels all showed significant ($P \leq 0.05$) difference, indicating longer EO water treatment time on spinach can significantly ($P \leq 0.05$) improve the removal efficacy of cyprodinil on spinach. Also, when treated for 15 min, EO water at ACC 120 mg/L showed significantly ($P \leq 0.05$) higher cyprodinil reduction than at ACC 70 mg/L, which was different from the results shown for diazinon and phosmet (Table 4.3) that no significant ($P > 0.05$) difference of reductions were found between EO water at ACC 120 and 70 mg/L after 15 min treatment. On the other hand, phosmet was shown to be much easier to be removed from spinach when treated with EO water than diazinon and cyprodinil. The reductions of phosmet on spinach ranged from 85.7% to 56.6%. The highest reduction was also achieved at the maximum treatment time and ACC. More than 80% phosmet were reduced when treated with EO water at ACC 120 mg/L for 15 and 8 min; and at ACC 70 mg/L for 15 min. EO water at ACC 120 mg/L achieved about 10% additional phosmet reductions than at ACC 20 mg/L at each treatment time levels. However, no significantly ($P > 0.05$) different reductions were found between each ACC levels at treatment time of 1 min. This indicates the EO water ACC effect may not be significant in very short period of treatment time to reduce phosmet on spinach.

Several studies have demonstrated the efficacy of EO water and other washing solutions with dissolved strong oxidizing compounds in removing pesticide residues on fresh vegetables. In the study of Hao et al. (2011), EO water with ACC 70 mg/L reduced the residue of acephate, omethoate, and dimethyl dichloroviny phosphate (all are organophosphorus pesticides) on spinach by 74%, 62%, and 59%, respectively, in 30 min. These are consistent with the results obtained in this study for spinach, except for cyprodinil, which is an organonitrogen pesticide. As shown in CHAPTER 3, cyprodinil was less sensitive to EO water degradation than diazinon and phosmet, which explains its low reductions on real food samples by EO water treatment. Chen et al. (2014) found washing solution with higher concentration of chlorine dioxide removed more diazinon and phorate residues on lettuce, indicating the concentration of the oxidizing chemicals in washing solution is important to remove pesticide residues from fresh produce.

EO water treatment on snap peas

Similar to grapes and spinach, both ACC and treatment time were significant ($P \leq 0.05$) factors for EO water to reduce the three pesticides on snap peas. Maximum reductions of the three pesticides were achieved by EO water at ACC 120 mg/L with 15 min treatment. Reductions from 66.5% to 12.0% were achieved for diazinon (Table 4.4). More than 60% diazinon were reduced when treated by EO water at ACC 120 and 70 mg/L for 15 min. No significantly ($P > 0.05$) different diazinon reductions were found between the three ACC levels at 1 min treatment. But at 8 and 15 min treatment, EO water with ACC 120 and 70 mg/L were significantly ($P \leq 0.05$) more effective than with ACC 20 mg/L. On the other hand, the increase of EO water treatment time significantly ($P \leq 0.05$) enhanced the diazinon reduction at ACC 70 and 120 mg/L.

For cyprodinil, 50.0% to 12.0 % reductions were achieved on snap peas (Table 4.4). Compared with diazinon, the reductions of cyprodinil were generally lower. At 8 min treatment, EO water with ACC 120 mg/L was significantly ($P \leq 0.05$) more effective than with ACC 70 and 20 mg/L, while no significant ($P > 0.05$) difference of reductions were found between ACC 120 mg/L and ACC 70 mg/L after 15 min treatment. The increase of EO water treatment time from 8 min to 15 min significantly ($P \leq 0.05$) increased the reductions of cyprodinil at all three ACC levels. On the other hand, similar to the results on grapes and spinach, phosmet was easier to be removed from snap peas. A maximum reduction of 73.0% was achieved for phosmet on snap peas with 37.7% the minimum (Table 4.4). The reductions of phosmet on snap peas were higher than diazinon and cyprodinil at all treatment conditions. Same to the other two pesticides, at 1min treatment, there were no significant ($P > 0.05$) difference of phosmet reductions at all three ACC levels. Also, no significant ($P > 0.05$) difference of phosmet reductions were found between EO water at ACC 120 and 70 mg/L at all three treatment times, indicating the increase of EO water ACC may not always lead to significant phosmet reductions on snap peas.

Lin et al. (2006) found that 15 min EO water treatment with ACC 50 mg/L achieved 38.2% and 34.7% reductions for methamidophos and dimethoate on snap beans. Compared with the results in current study, diazinon, cyprodinil, and phosmet showed higher sensitivity than methamidophos and dimethoate upon EO water treatment. In another study, 0.001% KMnO4 solution was used to wash green beans contaminated with several organophosphorus pesticides. They achieved 60% to 90% reductions in 15 min (Gouri et al., 2012), which are very close to our results, indicating solutions with strong oxidizing chemicals may effectively reduce pesticide residues on snap peas.

In addition, compared with the pesticide reduction results on spinach (Table 4.3) and snap peas (Table 4.4), the overall pesticide reductions achieved on grapes (Table 4.2) were obviously lower. Their surface texture difference could be the main reason. Lopez-Fernandez et al. (2013) argued that many pesticides have tendencies to migrate into the wax layer on fruit surfaces which make pesticides less likely to contact with washing solutions and the dissolved oxidizing compounds. Because of the high liposolubility of many pesticides, they tend to strongly bond to the wax layer on fruit skin and become less likely to be washed off during washing treatment. Mourad et al. (2005) found that an intensive washing treatment on tomatoes only showed very limited reductions of pyrifenox, pyridaben and tralomethrin. Lin et al. (2006) also found EO water treatment at ACC 50 mg/L for 15 min only achieved 25.4% and 29.3% reductions of methamidophos on cucumber and green pepper (both contain wax layers on surface), respectively. Therefore, it can be indicated the texture of produce surface is also important in determining EO water efficacy in removing pesticide residues.

Comparison between EO water and other washing solutions

As shown previously, longer treatment time of EO water can reduce more of the three tested pesticide residues. Thus, treatment time of 15 min was selected for different washing solutions to wash the pesticide contaminated produce. Table 4.5 shows that EO water treatment on grapes was significantly ($P \leq 0.05$) more effective than ER water, diluted bleach, VegWash and DI water in reducing diazinon, cyprodinil, and phosmet. The only exception was on cyprodinil that no significantly ($P > 0.05$) different reductions were achieved between EO water and diluted bleach treatment. However, the diluted bleach solution containing the same ACC (120 mg/L) as in EO water in the comparison study was much less effective than EO water treatment in reducing diazinon and phosmet. The pH and ORP difference might be the main reason as EO water has a much higher ORP (1151 mV) at acidic pH while diluted bleach has an obviously lower ORP (868 mV) at neutral pH. This ORP and pH difference can result in significant ($P \leq 0.05$) difference of pesticide degradation rate as demonstrated in CHAPTER 3. DI water was the least effective treatment in removing the three pesticides on grapes, being 14.4%, 11.5% and 23.6% reductions for diazinon, cyprodinil, and phosmet, respectively. ER water was considered to be a good cleaning agent (Hao et al., 2011) and may have potentials in removing pesticides from produce surface. However, as shown in Table 4.5, the efficacy of ER water in reducing the three pesticides on grapes was only slightly higher than DI water and much lower than EO water. Phosmet is very susceptible to alkaline hydrolysis (Worthing and Walker, 1986), and its reduction (38.1%) achieved by ER water treatment on grapes was very close to the results obtained by EO water at ACC 20 mg/L (35.2%) (Table 4.2). However, the efficacy of EO water can be improved by increasing the ACC to 120 mg/L while the efficacy of ER water cannot be improved. VegWash, as a commercial household use washing solution, also showed less efficient in removing the three pesticides on grapes than EO water. VegWash does not contain any strong oxidizing compounds and instead, its main components are saponified organic oils which have good cleaning functionality (e.g. saponified coconut oil and olive oil). But similar to ER water, the lack of strong oxidizing power makes VegWash less effective in removing pesticide residues from grapes than EO water.

The comparison was more obvious on spinach (Table 4.6). EO water showed significantly ($P \leq 0.05$) higher pesticides reducing efficacy on spinach, especially for cyprodinil where the reduction obtained by EO water was at least 23% higher than the other four solutions. Diluted bleach was shown to be the least effective treatment to remove diazinon (25.0%), cyprodinil (12.3%), and phosmet (58.7%) on spinach, which is same to the results on grapes and indicates pH is very important for chlorine based solutions to remove pesticide residues on fresh produce. ER water did not show high reductions of diazinon (26.9%) and cyprodinil (13.5%) on spinach. For phosmet, although a reduction of 70.0% was obtained by ER water treatment, it was still significantly ($P \leq 0.05$) lower than reductions achieved by EO water treatment (85.7%). In addition, the overall reductions of phosmet on spinach were much higher than diazinon and cyprodinil (Table 4.6), indicating phosmet is easier to be removed from spinach in washing treatment.

Similar trends were also found on snap peas that EO water was significantly ($P \leq 0.05$) more effective in reducing the three pesticides than the other four solutions (Table 4.7). Different to grapes and spinach, the mean pesticides reductions obtained from VegWash treatment were the second highest on snap peas, being 53.9%, 37.2, and 56.5% for diazinon, cyprodinil, and phosmet, respectively. Diluted bleach and DI water were the least effective treatment on snap peas, which demonstrates again that pH has big influence on chlorine based sanitizers in reducing pesticide residues. For phosmet, the effectiveness of ER water (50.9%) was still significantly ($P \leq 0.05$) lower than EO water (73.0%). On the other hand, the overall reductions of diazinon and phosmet on snaps (Table 4.7) were generally higher than reductions achieved on spinach (Table 4.6) while the reductions of phosmet on snap peas were generally (Table 4.7) lower than reductions obtained on spinach (Table 4.6). This indicates the surface texture of produce has strong impact on pesticide removal during washing treatment. As different pesticide may have different attachment on different produce surface, the efficacy of EO water and other washing solutions in reducing pesticide residues can be varied.

As shown above, the presence of strong oxidizing compound, ORP and pH of the washing solution, and surface texture of produce, can have strongly impact on pesticide residue reduction. Lopez-Fernandez et al. (2013) found the characters of oxidants present in washing solutions was the most important factor in determining the reduction of mancozeb on lettuce. In their study, hydrogen peroxide (higher ORP) was shown to be more effective than sodium hypochlorite (lower ORP) in reducing mancozeb. Also, they indicated the presence of surface wax on produce and pH of washing solution were determinants for pesticide residue removal. In the study of Phongchai et al. (2008), 0.001% $KMnO_4$ solution was found to be more effective in removing methomyl and carbaryl residues on Chinese-Kale than 0.9% NaCl, 0.1% $NaHCO_3$, and 0.1% acetic acid solution. The oxidative property of $KMnO_4$ was argued to be the main cause for such difference. The efficacy of EO water in reducing pesticide residues was also compared with ER water and other washing solutions in the study of Hao et al. (2011). Both EO and ER waters were found to be more effective than regular tap water and detergent solution. ER water was more effective in reducing acephate and omethoate while EO water was more effective in removing dimethyl dichlorovinyl phosphate. However, the ACC of EO water in their study was only 70 mg/L. The efficacy of EO water could be dramatically enhanced by increasing the ACC.

Response surface model

The results of cyprodinil reduction on spinach by EO water treatment was used to do the response surface analysis and create a 3-dimensional plot to predict the reductions within the experimental range. ACC and treatment time were the independent variables and cyprodinil reduction was the response. An equation [1] was created to fit the experimental data. The analysis of variance results (Table 4.8) for the cyprodinil reduction indicate the prediction model is significant as the P_{model} value is less than 0.0001. The R square present in Table 4.8 shows the coefficient between the experimental and predicted values of the reduction. The value of R square (0.89) indicates 89% of the variability in the data can be explained by the current model. The value of the adjusted R square value (0.87) is also high, indicating the high significance of the model. For the lack of fit test, the $P_{lack\ of\ fit}$ value (0.93) is much higher than 0.05, indicating the chosen model can represent the prediction system appropriately. The response surface (Figure 4.1) plot was then generated using the predicted equation [1]. This 3-dimensional surface sheet clearly shows the trend of reductions based on the change of ACC and treatment time and provides more information about the reduction behavior within the experimental data range. As can be seen in Figure 4.1, the effect of ACC and treatment time are generally linear. The predicted maximum reduction (45.2%) is at ACC 120 mg/L and 15 min treatment, which is very close to the reduction (43.8%) achieved in the real experiment (Table 4.3).

$$\text{Reduction (\%)} = 8.024 + 0.0978 \times \text{ACC} + 1.399 \times \text{Time} + 0.0025 \times \text{ACC} \times \text{Time} \quad [1]$$

Conclusions

In conclusion, higher ACC and longer treatment time of EO water treatment achieved higher reductions of diazinon, cyprodinil, and phosmet on grapes, spinach and snap peas, respectively. At very short treatment time (1 min), the effect of ACC may not be significant ($P > 0.05$). Although EO water at ACC 120 mg/L could always obtain higher reductions than at ACC 70mg/L, their differences were generally not significant ($P > 0.05$). The surface structures of each produce and the sensitivity of each pesticide to EO water are both determinants in deciding the reduction efficacy. Compared with ER water, diluted bleach, VegWash, and DI water, EO water at ACC 120 mg/L showed significantly ($P \leq 0.05$) more reductions for the three pesticides on the three tested produce, except cyprodinil on grapes. Response surface model could also be used to predict pesticide reductions upon EO water treatment. Based on the results obtained in the current study, EO water showed great potentials in both the food industry and household application to remove pesticide residues on fresh produce.

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Table 4.1. Mean recoveries (%) and RSD (%) of diazinon, cyprodinil and phosmet on grapes, spinach and snap peas.

	Spiked level (mg/kg)	Diazinon		Cyprodinil		Phosmet	
		Recovery	RSD	Recovery	RSD	Recovery	RSD
Grapes	0.05	101.7	2.4	101.7	2.9	99.1	12.4
	0.1	100.7	4.1	105.6	8.5	96.0	7.6
	0.5	99.3	2.0	105.9	5.0	100.7	4.6
Spinach	0.05	104.7	3.0	101.4	6.8	91.2	8.5
	0.1	100.6	6.6	102.5	7.8	99.9	11.7
	0.5	103.0	2.7	108.2	2.5	101.0	9.0
Snap peas	0.05	105.1	2.6	97.4	2.2	92.7	8.1
	0.1	101.1	3.7	99.3	3.3	92.2	8.0
	0.5	103.0	2.3	104.2	1.5	91.0	7.4

The recovery test was repeated three times in three different days with two parallel samples conducted within each replication.

Table 4.2. Mean reductions of diazinon, cyprodinil, and phosmet on grapes after EO water treatment.

		Reduction %^{ab}		
Treatment Time (min)	ACC level (mg/L)	Diazinon	Cyprodinil	Phosmet
1	20	10.2±1.8^{E}	9.2±3.6^{E}	21.4±9.7^{F}
	70	18.4±7.4^{DE}	14.9±2.1^{DE}	28.3±4.6^{EF}
	120	22.3±4.2^{CD}	17.8±1.2^{CD}	34.2±5.1^{DE}
8	20	21.7±1.8^{CD}	21.5±3.4^{BC}	28.7±7.1^{EF}
	70	25.5±5.7^{BCD}	22.2±3.8^{BC}	38.5±5.6^{BCD}
	120	29.5±9.9^{ABC}	26.3±6.3^{AB}	44.1±3.2^{ABC}
15	20	27.4±5.3^{BCD}	21.0±4.9^{BCD}	35.2±3.8^{CDE}
	70	31.9±5.3^{AB}	25.2±3.6^{AB}	45.3±1.4^{AB}
	120	37.1±4.1^{A}	31.5±2.6^{A}	49.4±1.3^{A}

[a] Values under this section are the percentage of pesticide residues removed after EO water treatment to the initial pesticide residue concentrations on grapes; the initial concentrations were 0.083±0.014, 0.774±0.091, and 0.133±0.011 mg/kg for diazinon, cyprodinil, and phosmet, respectively.

[b] Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.3. Mean reductions of diazinon, cyprodinil, and phosmet on spinach after EO water treatment.

		Reduction % [ab]		
Treatment Time (min)	ACC level (mg/L)	Diazinon	Cyprodinil	Phosmet
1	20	8.8±5.9^{E}	9.7±3.1^{E}	56.6±2.1^{E}
	70	19.3±5.4^{DE}	17.2±2.7^{D}	57.8±5.5^{E}
	120	29.8±8.4^{CD}	19.4±0.3^{D}	64.3±7.9^{DE}
8	20	33.2±3.5^{C}	21.9±6.4^{D}	70.9±4.5^{CD}
	70	40.3±8.5^{BC}	32.3±2.9^{C}	72.3±6.2^{CD}
	120	47.0±6.1^{B}	34.3±0.3^{BC}	82.0±4.0^{AB}
15	20	33.0±6.7^{C}	30.5±6.3^{BC}	74.7±0.3^{BC}
	70	49.5±6.7^{AB}	38.3±1.8^{B}	83.5±5.7^{A}
	120	59.2±5.5^{A}	43.8±2.9^{A}	85.7±4.8^{A}

[a] Values under this section are the percentage of pesticide residues removed after EO water treatment to the initial pesticide residue concentrations on spinach; the initial concentrations were 1.742±0.461, 4.020±0.393, and 3.727±0.754 mg/kg for diazinon, cyprodinil, and phosmet, respectively.

[b] Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.4. Mean reductions of diazinon, cyprodinil, and phosmet on snap peas after EO water treatment.

		Reduction % [ab]		
Treatment Time (min)	ACC level (mg/L)	Diazinon	Cyprodinil	Phosmet
1	20	12.0±5.2^{D}	12.0±2.9^{F}	37.7±6.7^{G}
	70	11.2±7.0^{D}	12.9±1.0^{F}	46.0±4.4^{EF}
	120	18.5±8.2^{D}	18.0±4.0^{EF}	45.1±8.9^{EFG}
8	20	20.4±2.5^{D}	25.8±4.7^{DE}	43.2±2.5^{FG}
	70	35.7±6.1^{C}	26.9±1.0^{CD}	55.9±3.5^{CD}
	120	55.8±5.5^{B}	40.4±6.9^{B}	63.5±2.5^{BC}
15	20	43.9±7.9^{C}	34.7±2.3^{BC}	52.1±0.1^{DE}
	70	64.0±3.1^{AB}	49.6±6.5^{A}	66.5±2.8^{AB}
	120	66.5±2.5^{A}	50.0±8.2^{A}	73.0±2.7^{A}

[a] Values under this section are the percentage of pesticide residues removed after EO water treatment to the initial pesticide residue concentrations on snap peas; the initial concentrations were 0.306±0.080, 1.207±0.034, and 0.916±0.125 mg/kg for diazinon, cyprodinil, and phosmet, respectively.

[b] Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.5. Comparison of mean reductions of diazinon, cyprodinil, and phosmet on grapes after treatment with different washing solutions.

	Reduction % ^{ab}		
Treatment	Diazinon	Cyprodinil	Phosmet
EO	37.1±4.1^{A}	31.5±2.6^{A}	49.4±1.3^{A}
ER	22.5±5.3^{BC}	14.6±4.9^{C}	38.1±5.8^{B}
Bleach	21.2±7.4^{B}	25.4±5.0^{AB}	25.5±9.6^{C}
VegWash	27.5±1.3^{B}	17.2±6.0^{BC}	29.9±5.5^{BC}
DI	14.4±6.6^{C}	11.5±4.9^{C}	23.6±4.7^{C}

[a] Values under this section are the percentage of pesticide residues removed after treatment to the initial pesticide residue concentrations on grapes; the initial concentrations were 0.083±0.014, 0.774±0.091, and 0.133±0.011 mg/kg for diazinon, cyprodinil, and phosmet, respectively.

[b] Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.6. Comparison of mean reductions of diazinon, cyprodinil, and phosmet on spinach after treatment with different washing solutions.

Treatment	Reduction % [ab]		
	Diazinon	Cyprodinil	Phosmet
EO	59.2±5.5^{A}	43.8±2.9^{A}	85.7±4.8^{A}
ER	26.9±8.3^{C}	13.5±5.7^{BC}	70.0±7.9^{B}
Bleach	25.0±4.3^{C}	12.3±2.7^{C}	58.7±4.8^{C}
VegWash	46.0±3.5^{B}	20.2±1.8^{B}	60.2±1.9^{BC}
DI	20.8±8.8^{C}	15.1±5.1^{BC}	62.9±6.6^{BC}

[a] Values under this section are the percentage of pesticide residues removed after treatment to the initial pesticide residue concentrations on spinach; the initial concentrations were 1.742±0.461, 4.020±0.393, and 3.727±0.754 mg/kg for diazinon, cyprodinil, and phosmet, respectively.

[b] Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.7. Comparison of mean reductions of diazinon, cyprodinil, and phosmet on snap peas after treatment with different washing solutions.

	Reduction % [ab]		
Treatment	Diazinon	Cyprodinil	Phosmet
EO	66.5±2.5^{A}	50.0±8.2^{A}	73.0±2.7^{A}
ER	37.8±4.1^{C}	28.9±2.9^{BC}	50.9±3.0^{B}
Bleach	42.8±7.2^{C}	23.3±3.5^{C}	38.9±6.7^{C}
VegWash	53.9±2.8^{B}	37.2±2.8^{B}	56.5±7.2^{B}
DI	38.1±8.9^{C}	25.2±5.6^{C}	38.6±2.4^{C}

[a] Values under this section are the percentage of pesticide residues removed after treatment to the initial pesticide residue concentrations on snap peas; the initial concentrations were 0.306±0.080, 1.207±0.034, and 0.916±0.125 mg/kg for diazinon, cyprodinil, and phosmet, respectively.

[b] Mean values in the same column with different superscript letters are significantly different ($P \leq 0.05$).

Table 4.8. Analysis of variance of the response surface model for the prediction of cyprodinil removal on grapes.

Source	Sum of squares	DF[a]	Mean squares	*F* value	*P* value
Model	2831.91	3	943.97	62.99	< 0.0001
Error	344.68	23	14.99		
Lack of Fit	109.14	5	21.83	1.67	0.93
Pure Error	235.53	18	13.08		
Cor Total	3176.59	26			
R square	0.89				
R square adj	0.87				

[a] DF: degree of freedom.

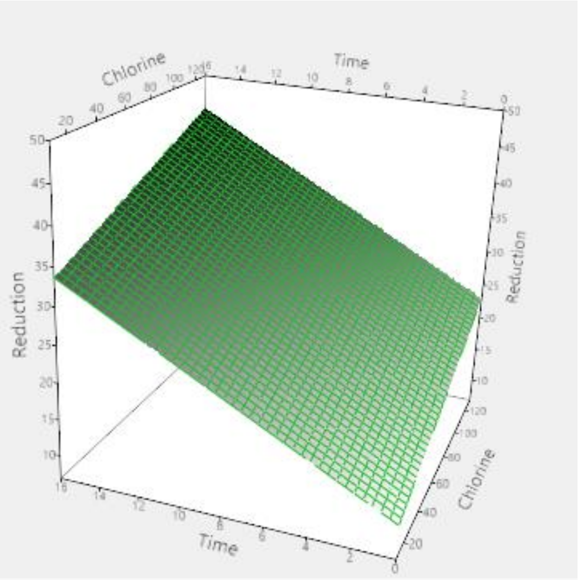

Figure 4.1. Response surface plot showing the effect of EO water ACC (mg/L) and treatment time (min) on removing cyprodinil residues on spinach.

The initial concentration of cyprodinil on spinach was 4.020±0.393 mg/kg.

CHAPTER 5

EFFECT OF ELECTROLYZED OXIDIZING WATER TREATMENT ON THE COLOR AND TEXTURE OF GRAPES, SPINACH, AND SNAP PEAS

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Abstract

EO water has been demonstrated can effectively remove pesticide residues on produce. However, the high ACC level and long treatment time of EO water may have detrimental effect on produce quality. The aim of this study was to evaluate the effect of EO water at ACC 120 mg/L with 15 min treatment, ACC 70 mg/L with 15 min treatment, and ACC 120 mg/L with 8 min treatment on the color of grapes, spinach, and snap peas. The texture (skin hardness and flesh firmness) change of grapes was also measured. Unwashed and DI water washed produce samples were also used for comparison. The results showed that no significant ($P > 0.05$) difference was found among all treatment in terms of color and texture for the three produce. When comparing with the unwashed control, both DI and EO water washed grapes had small total color difference (ΔE^*) (1.4 to 1.7). It was concluded that EO water with ACC below 120 mg/L and treatment time within 15 min will not cause significant quality decay on fresh produce.

Key words: electrolyzed oxidizing water, produce, color, texture

Introduction

Fresh fruits and vegetables are important source of varying nutrients, including vitamins, minerals, and dietary fibers. The presence of fresh produce also adds colors, aromas, flavors, and varieties to consumer's plate (Hung et al., 2010). It is recommended by Dietary Guidelines for Americans that fruits and vegetables should occupy half of a meal plate. More importantly, the intake of fresh fruits and vegetables is associated with the decreased risk of some chronic diseases (Pelucchi et al., 2009). Due to the numerous merits of fresh produce, consumers start to add more fresh produce to their daily diet. The increased awareness of the produce importance as a part for consumer's daily diet has made fresh produce market been rapidly growing in the last decade (Rico et al., 2008).

Grapes, spinach and snap peas are good source of vitamin A, B1, B2, and C, K and potassium (McGill et al., 2013; Zhang et al., 2009) and are popular for consumptions. It has been reported that the intake of grapes is associated with healthier dietary patterns and higher nutrients intake (McGill et al., 2013). However, fresh produce are susceptible to spoilage. Fresh produce do not have long storage time and have to be stored in low temperatures to maintain freshness. Influenced by spoilage, safety issues of foodborne pathogens may arise. CDC has reported that more than 1,500 foodborne diseases occurred in 2010. Pesticide residues are also concerns for fresh fruits and vegetables. FDA reported that, in 2011, 39.5% of sampled domestic and 35.5% of sampled imported foods were found to contain detectable pesticide residues. The intake of pesticide residues can cause severe symptoms and chronic diseases (Carozza et al., 2009). In the food industry, washing treatment with dissolved chlorine based sanitizers are often applied to reduce these potential hazards.

Electrolyzed oxidizing (EO) water has been gaining significant reputations in the food industry as an effective sanitizer. EO water is produced from electrolysis of a diluted NaCl solution. During electrolysis, chlorine ion is oxidized to chlorine gas and dissolved into water to form hypochlorous acid, which is a very effective antimicrobial compound. EO water has shown to be able to inactivate a variety of bacteria (Huang et al., 2008). Also, its effectiveness in degrading pesticides is demonstrated in some studies (Hao et al., 2011; Lin et al., 2006), as well as in CHAPTER 3 and 4. Although EO water is effective for sanitizing purposes, it may have detrimental effect on organoleptic properties on fresh produce as the available chlorine in EO water may react with the nutrients and pigments on produce. Some studies (Park et al., 2001; Yen-Con et al., 2010) have indicated EO water does not have negative effect on produce quality. However, the available chlorine content (ACC) used in these studies were generally low (less than 100 mg/L) and the treatment time was also not long (less than 10 min).

The increase of ACC in EO water would increase its oxidizing power. Longer EO water washing time may also worsen the produce quality. From the results of CHAPTER 4, EO water at ACC 120 mg/L with 15 min treatment achieved the highest reductions of pesticides on grapes, spinach, and snap peas. The aim of this study was to evaluate the effect of EO water treatment (at high ACC and long treatment time) on color of grapes, spinach, and snap peas and on texture for grapes.

Materials and method

Fresh produce

Fresh red seedless grapes, organic spinach, and organic snap peas were purchased from a local grocery store and kept in a refrigerator set at 4 ºC (maximum 72 h). Grapes with weight ranged from 4.5 to 6.5 g without any obvious skin damage and blemish were selected. Spinach with uniform color and no leafy damage were selected. Snap peas with weight ranged from 6 to 9 g were selected.

EO water preparation

EO water was generated from electrolysis of a 0.03 % NaCl solution in an electrolyzed water generator (Model #P30HST44T, EAU, GA, USA). The pH and ORP of EO water were measured using an Accumet pH meter (AR50, Fisher Scientific, Pittsburgh, PA, USA) with pH and ORP electrodes. DOD-FEAS titrimetric method (Hach Co., Loveland, Colo., USA) was used to measure the EO water ACC concentration. DI water was used to dilute EO water with ACC above the desired values to achieve targeted concentrations. Drops of 1N HCl and 1N NaOH were used to adjust EO water pH. EO water at pH 2.8 with ACC of 120 and 70 mg/L were collected into 1000 mL glass bottles. All EO water were freshly made and used within 2 h.

EO water treatment

For grapes, 200 g samples were picked and soaked into 500 mL treatment solution for specified times. For spinach, 15 g samples were soaked into 1000 mL treatment solution. For snap peas, about 50 g samples were soaked into 500 mL treatment solution. All treatment were performed on a reciprocal shaking bath (Model 2870, Thermo Fisher Scientific, Waltham, MA, USA) set at 100 rpm. After the treatment, all samples were immediately soaked into 1000 mL tap water for about 5 s to remove the residual chlorine. DI water was also used to wash all the samples as a comparison. A total of four different washing treatment were conducted for the three produce: EO at ACC 120 mg/L and 15 min treatment, EO at 120 mg/L and 8 min treatment, EO at ACC 70 mg/L and 15 min treatment, and DI water for 15 min treatment. All samples were air dried at room temperature for about 1 h before the color and texture measurement. All treated produce were compared with unwashed samples in terms of color and texture.

EO water at ACC 120 and 70 mg/L with 15 min treatment time were applied in this study because CHAPTER 4 showed these two conditions achieved the highest pesticide reductions. EO water at ACC 120 mg/L with 8 min treatment time was also selected to investigate whether shorter treatment time of EO water has less detrimental effect on produce quality. DI water, without any dissolved oxidizing compound, was served as a comparison.

Color measurement

For spinach and snap peas, color was measured with a Hunter Lab® Miniscan XE™ colorimeter (Model 45/0-L, Hunter Associates Laboratory, Reston, VA, USA) using the CIE color coordinates (L^*, a^*, b^*). Calibration of the colorimeter was performed at the start of each experiment day against a standard white tile and a black tile. For spinach, approximately 10 g of spinach samples were cut into small pieces (roughly 1.5×1.5 cm) and placed in a plastic petridish (9 cm diameter and 10 mm deep) with the adaxial side facing up. For snap peas, about 35 g samples were cut into 1.5 cm long pieces and placed in the petridish. Beneath the petridish a dark background was placed to avoid diffraction. Three readings were taken from above the sample with 120^o rotation after each reading. The three readings were averaged to give representative values of L^*, a^*, b^*. Hue angle was calculated using the equation $\tan^{-1}(b^*/ a^*)$. Chroma was calculated using equation $\sqrt{(a^{*2} + b^{*2})}$.

For grapes, a different color measurement approach was performed as the color on each grapes are not very uniform. Among the 200 g grapes for each treatment group, 10 grapes were randomly selected before the washing treatment and the surface bloom was removed with paper tissues. Each grape was then numbered from 1 to 10 using a black marker. For each grape, color measurement was conducted at three locations around the equatorial belt using a Minolta colorimeter (Model CR200b, Minolta CO., Japan). The colorimeter was calibrated at the start of each experiment day against a standard white tile. The color readings for the three locations on the same grape were averaged to give representative values of L^*, a^*, b^* for each grape. Each site for the color measurement on grapes were circled with a black marker. After the washing treatment, color measurement were conducted again on the same site of each grape to get color readings after the washing treatment for each grape. Then, total color difference (ΔE^*) between the color before and after the washing treatment for each grape was calculated using the equation $\sqrt{(\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2})}$. The ΔE^* obtained from the 10 grapes within the same treatment group were averaged to give representative ΔE^* for each treatment group and compared with other treatment groups. Chroma, and hue angle were also calculated.

Texture measurement on grapes

An Instron Universal Testing Machine (Mode 5544, Instron Corporation, Canton, MA, USA) was used for the texture measurement. For puncture test, individual grape was placed on its equatorial side on a flat plastic washer (105 × 98 mm, 12 mm thick, 6.2 mm hole) in the center of the load cell and punctured with a 1.37 mm diameter probe at 50 mm/min speed and load range of 5 N. The skin hardness was expressed as the peak force required to penetrate the skin in N. Ten grapes from each treatment group were measured and the obtained values for skin hardness were averaged to give representative skin hardness for each treatment group.

For compression test, each grape was placed on its equatorial side on a stainless steel base (125 mm diameter, 25 mm thick). A 36 mm diameter cylindrical probe was used to compress each grape sample at a crosshead and chart speed of 50 mm/min until the cylindrical probe reached 2 mm from the base plate. The energy (mJ) required for the texture analyzer to reach the peak force in compressing the grape samples was used to represent firmness. Ten grapes of each treatment group were measured and the firmness values obtained were averaged to give representative firmness of each treatment group.

Statistical analysis

The entire experiment was repeated three times. Means and standard deviations were calculated using Excel (Micro software, USA). All data was subjected to one way analysis of variance followed by Duncan's multiple range test for means comparisons using SAS software 9.4 (SAS Institute Inc., Cary, NC, USA) with proc anova procedure. A $P \leq 0.05$ was considered to be significantly different.

Results and discussion

EO water properties

EO water was at pH 2.80±0.05 with ACC at 120 and 70 mg/L, and ORP of 1144.0±7.2 and 1132.3±6.5 mV, respectively.

Spinach Color

Mean color values of spinach are shown in Table 5.2. For lightness (L^*), chroma (C^*), and hue angle (h^*), no significant ($P > 0.05$) difference were found between the four different washing treatment. Also, compared with the unwashed spinach group, no significantly ($P > 0.05$) different color values were found for the washed spinach. The lightness of all four treated spinach groups were ranged from 29 to 31. EO water treatment at ACC 120 mg/L for 15 min showed the lowest chroma value (16). But it is not significantly ($P > 0.05$) different compared with DI water washed (17) and unwashed spinach samples (17). It can be indicated that EO water at high ACC (120 mg/L) and long treatment time (15 min) does not have significant ($P > 0.05$) detrimental effect on spinach lightness and brightness. On the other hand, hue angle values of all treated and unwashed spinach were around 114.0^{o} indicating the tested spinach had a yellowish-green tinge. From Table 5.1, no significant ($P > 0.05$) change of hue angle values were found for all spinach groups, indicating the use of EO water did not cause color deterioration on spinach.

Similar results were found in the study of Park et al. (2001), where EO and acidified chlorinated water at ACC 45 mg/L were used to treat fresh lettuce for 3 min and no significant ($P > 0.05$) difference of color were found between the EO water treated, acidified water treated, tap water treated and unwashed lettuce samples through the 14 days storage. Nei et al. (2009) also found that spinach treated with chlorinated water at ACC of 100 mg/L did not show significant ($P > 0.05$) color change compared with the spinach treated by sodium chlorite solution and regular tap water. In addition, Hao et al. (2011) reported that spinach treated with EO water (ACC 70 mg/L) for 60 min did not show vitamin C loss compared with unwashed spinach samples. Based on the results of Table 5.1 and other studies, it can be argued that EO water (ACC $\leq$ 120 mg/L and treatment time $\leq$ 15 min) wash would not have significant ($P > 0.05$) detrimental effect on spinach color.

Snap peas color

Table 5.2 shows the color results for snap peas after different washing treatment. Similar to the results of spinach, no significant ($P > 0.05$) color change were found between each treatment group. The lightness (L*) and chroma (C*) were around 36 and 24 for all the treated and unwashed snap peas without any trend of change, indicating EO water did not negatively affect the snap peas lightness and brightness. Also, compared with the overall lightness and chroma in spinach (Table 5.1), the tested snap peas were lighter and brighter. The hue angle (h*) of snap peas ranged from 107^{o} to 109^{o} among the five treatment groups, indicating the tested snap peas have a yellowish-green tinge. Compared with the results of spinach (Table 5.1), the tested snap peas were more yellowish. The three EO water treatment showed a lower average value of hue angle (around 108^{o}) than DI treated (109.06^{o}) and unwashed (109.17^{o}) samples. However, the difference was not significant ($P > 0.05$).

It has been reported that snap peas treated with chlorinated water at ACC 100 mg/L for 5 min showed no significant ($P > 0.05$) color difference compared with snap peas treated with tap water in a seven days storage (Peres et al., 2011). Chlorine dioxide, a potent oxidizing compound with strong antimicrobial powers, was also investigated in evaluating its antimicrobial effects and quality impact on snap beans. Palharini et al. (2015) found that both *Salmonella* and *E. coli* were significantly ($P < 0.05$) reduced from snap beans with chlorine dioxide (200 and 100 mg/L) treatment for 10 min with no significantly ($P > 0.05$) different browning rate found for chlorine dioxide treated samples compared with conventionally washed samples. The results obtained in current study and other studies may indicate that water wash with dissolved strong oxidizing reagent within certain range would not negatively affect the color properties of snap beans.

Grape color and texture

As the color for the same and between different grapes are not uniformly distributed, the total color difference (ΔE^*) was measured for each grape within different treatment groups to compare if any treatment has significant color changes. Table 5.3 shows the total color difference for each treated grape groups. The total color difference (ΔE^*) ranged from 1.4 to 1.6 for all the four treatment with no significant ($P > 0.05$) difference. This indicates the grapes treated with EO water did not have significantly ($P > 0.05$) different color change compared with the grapes treated with DI water. According to Pathare, Opara, and Al-Said (2013), color difference can be classified as small difference ($\Delta E^* \leq 1.5$), distinct ($1.5 < \Delta E^* < 3$), and very distinct ($\Delta E^* \geq 3$). The ΔE^* obtained from all four treatments were around 1.5, indicating the color change of grapes after each washing treatment were limited. The L^*, chroma, and hue angle were also present in Table 5.4. No significant ($P > 0.05$) difference were found between each treatment in terms of the three color parameters. Similar to the results on spinach and snap peas, the high ACC (120 mg/L) and long treatment time (15 min) of EO water did not cause significant ($P > 0.05$) color change on grapes.

The skin hardness and flesh firmness were also measured for grapes after the washing treatment. In Table 5.4, the results of both compression (firmness) and puncture tests (skin hardness) showed no significant ($P > 0.05$) difference between each treatment group. The energy (compression test) consumed by the texture analyzer to reach the maximum force in compressing the grape samples were ranged from 641 to 655 mJ and the maximum force (skin hardness) required to puncture the grape skins were ranged from 2.38 to 2.59 N. No trend of change were found in both tests, indicating there is no significant ($P > 0.05$) different impact between EO and DI water treatment on grape firmness and skin hardness.

Similar results can be found in other studies in investigating the effect of chlorine based sanitizer wash on fruit quality. Kim et al. (2008) found that fresh grapes treated with aqueous chlorine dioxide (50 mg/L) showed no significant ($P > 0.05$) difference on pH, titratable acidity, and better sensory evaluation scores compared with the control ones. Similar results were also achieved in the study of Yen-Con et al. (2010) where EO water (ACC 100 mg/L) treated strawberries showed same texture and color change with tap water treated strawberries during a 13 days of storage. It was also reported that, other oxidizing reagent treatment, such as ozone, do not have detrimental effect on grape color and texture (Glowacz, Colgan, & Rees, 2015).

Conclusions

In conclusion, EO water treatment did not show significant ($P > 0.05$) detrimental effect on the color and texture properties of fresh grapes, spinach, and snap peas. The high ACC (120 mg/L) and long treatment time (15 min) did not cause significant ($P > 0.05$) change on produce quality. The results obtained in current study can be comparable with many other studies and demonstrate chlorine based sanitizers wash (within certain ACC and treatment time range) would not negatively affect fresh produce quality. Along with the strong antimicrobial and pesticide degrading power, it can be concluded that appropriate EO water application in the food industry can help maintain fresh produce safety and cause no significant ($P > 0.05$) quality deterioration.

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Table 5.1. Results of spinach color before and after the washing treatment.

Treatment[a]	*L** (NS)	Chroma (*C**) (NS)	Hue angle (*h**) (NS)
EO_1	29.54±3.00	16.06±1.94	114.35±1.55
EO_2	30.32±3.44	16.11±2.44	114.06±1.88
EO_3	29.86±3.36	16.96±2.64	114.46±1.53
DI	30.95±3.48	17.62±2.88	114.10±1.63
Unwashed	29.49±2.24	17.28±2.73	113.53±0.80

NS means no significant ($P > 0.05$) difference was found.

[a] EO_1 was at ACC 120 mg/L and 15 min treatment; EO_2 was at ACC 70 mg/L and 15 min treatment, EO_3 was at ACC 120 mg/L and 8 min treatment; DI water was applied for 15 min.

Table 5.2. Results of snap peas color before and after the washing treatment.

Treatment[a]	*L** (NS)	Chroma (*C**) (NS)	Hue angle (*h**) (NS)
EO_1	36.35±2.02	24.62±1.34	107.74±0.64
EO_2	36.01±0.98	23.22±0.34	108.31±0.71
EO_3	35.95±0.99	22.44±0.78	107.83±1.16
DI	36.92±2.30	24.21±2.14	109.06±1.26
Unwashed	35.98±0.74	23.43±1.08	109.17±0.87

NS means no significant ($P > 0.05$) difference was found.

[a] EO_1 was at ACC 120 mg/L and 15 min treatment; EO_2 was at ACC 70 mg/L and 15 min treatment, EO_3 was at ACC 120 mg/L and 8 min treatment; DI water was applied for 15 min.

Table 5.3. Results of grapes color before and after the washing treatment.

Treatment[a]	ΔE^* (NS)	L^* (NS)	Chroma (C^*) (NS)	Hue angle (h^*) (NS)
EO_1	1.4±0.4	32.6±1.2	8.9±0.9	16.9±6.1
EO_2	1.7±0.3	32.9±1.0	9.0±0.7	18.2±6.3
EO_3	1.5±0.4	32.3±0.8	8.5±0.1	16.6±5.1
DI	1.6±0.1	32.3±0.8	9.0±0.8	15.4±4.2
Unwashed		32.7±0.9	10.1±0.4	13.9±6.6

NS means no significant ($P > 0.05$) difference was found.
[a] EO_1 was at ACC 120 mg/L and 15 min treatment; EO_2 was at ACC 70 mg/L and 15 min treatment, EO_3 was at ACC 120 mg/L and 8 min treatment; DI water was applied for 15 min.

Table 5.4. Results of compression and puncture tests for grapes before and after the washing treatment.

Treatment[a]	Compression (mJ) (NS)	Puncture (N) (NS)
EO_1	652.20±61.66	2.56±0.33
EO_2	632.30±75.95	2.38±0.19
EO_3	641.67±40.11	2.45±0.27
DI	655.29±23.32	2.59±0.12
Unwashed	651.51±22.61	2.53±0.13

NS means no significant ($P > 0.05$) difference was found.
[a] EO_1 was at ACC 120 mg/L and 15 min treatment; EO_2 was at ACC 70 mg/L and 15 min treatment, EO_3 was at ACC 120 mg/L and 8 min treatment; DI water was applied for 15 min.

CHAPTER 6

SUMMARY AND CONCLUSIONS

Three research steps were involved in this study. In the first step, the effect of pH, available chlorine content (ACC), and treatment time of electrolyzed oxidizing (EO) water in degrading diazinon, cyprodinil and phosmet in aqueous solution was examined. The efficacy of electrolyzed reduced (ER) water in hydrolyzing the three pesticides was also tested. In addition, appropriate methods of pesticide inoculation and EO water washing condition on grapes were investigated. In the second step, the effects of ACC and treatment time of EO water in removing residues of diazinon, cyprodinil, and phosmet on fresh grapes, spinach and snap peas were evaluated. ER water, diluted bleach, VegWash, and DI water were also used as comparison with EO water treatment. In the third step, effect of EO water wash on quality of grapes, spinach, and snap peas was evaluated.

In the aqueous solution study, EO water was shown to be significantly ($P \leq 0.05$) more effective in degrading the three pesticides in acidic conditions (pH 2.8) than in near neutral conditions (pH 5.0 and 6.0). Higher ACC and treatment time resulted in higher reductions. Reductions above 99.0% were achieved for all three pesticides with 5 min EO water treatment at ACC 20 mg/L and pH 2.8. ER water was effective in reducing phosmet (99.7% reduction), slightly effective against diazinon (7.3% reduction), and ineffective on cyprodinil (0.0% reduction). The chemical structure difference of the three pesticides could be the main reason for the different reduction rate and sensitivity against EO and ER water treatment. On the other hand, soaking medium size grapes in 12 mg/L mixed pesticide solution for 10 min was found to be the appropriate way for pesticide deposition and the appropriate washing condition was to soak 200 g grapes in 500 mL EO water with 100 rpm shaking.

For the produce wash study, EO water at ACC 120, 70, and 20 mg/L with treatment time of 15, 8, and 1 min were applied on grapes, spinach, and snap peas contaminated with the three pesticides. The results showed EO water ACC and treatment time were both significant factors ($P \leq 0.05$) in determining the removal efficacy of the three tested pesticides on the three produce. Higher ACC and longer treatment time could always achieve higher reductions. The reductions obtained for diazinon, cyprodinil, and phosmet with EO water treatment were: 37.1% to 10.2%, 31.5% to 9.2%, and 49.1% to 21.4% on grapes; 59.2% to 8.8%, 43.8% to 9.7%, and 85.7% to 56.6% on spinach; 66.5% to 12.0%, 50.0% to 12.0%, and 73.0% to 37.7% on snap peas, respectively. EO water at ACC 120 and 70 mg/L with 15 min treatment were shown to be generally equally effective in removing the three pesticide residues. The reductions of cyprodinil were always lower than diazinon and phosmet on each produce, which might be due to its low sensitivity upon EO water treatment as shown in the aqueous solution study. The reductions for the three pesticides on grapes were obviously lower than on spinach and snap peas. This might be because the pesticides may migrate into the wax layer on grape surface, which would limit the contact between pesticides and EO water. When compared with other washing solutions, EO water was always significantly ($P \leq 0.05$) more effective than ER water, diluted bleach, VegWash, and DI water on each produce, except for cyprodinil on grapes that the reducing efficacy of EO water was equal with diluted bleach.

For the produce quality study, EO water at ACC 120 mg/L with 15 min treatment, ACC 70 mg/L with 15 min treatment, and ACC 120 mg/L with 8 min treatment were applied on the three produce as well as DI water. The color of grapes, spinach, and snap peas, and the texture of grapes were measured and compared. The results showed that the three EO water treatment did not cause any significant ($P > 0.05$) quality change in terms of color and texture of the three produce when compared with the DI water treated and unwashed produce samples. This indicates EO water at ACC ≤ 120 mg/L with treatment time ≤ 15 min would not cause significant ($P > 0.05$) color and texture deterioration on grapes, spinach, and snap peas.