

CHAPTER 2

LITERATURE REVIEW

2.1 Water Spinach (*Ipomoea aquatica* Forssk.)

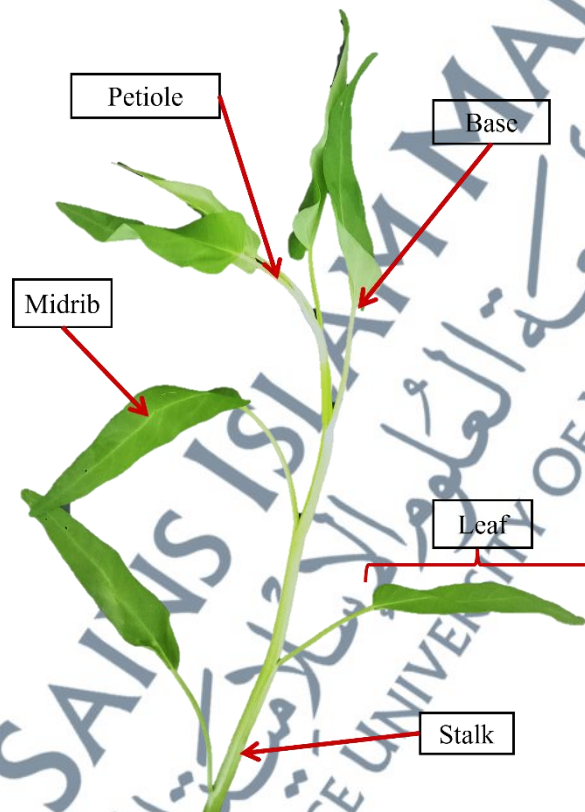


Figure 2.1: Labelled Parts of Water Spinach

Water spinach (*Ipomoea aquatica* Forssk.) is a leafy vegetable from the Convolvulaceae family (USDA, 2019). Water spinach is a staple vegetable in Southeast Asia and parts of China (Ebert, 2011). It is the second most produced vegetables in Malaysia behind spinach in 2023, with the recorded planted area of 4,631 ha and harvest amounting to 48,611 metric tonne and the value of production amounted to RM104,757.00 (Jabatan Pertanian Malaysia, 2023).

In terms of nutritional content, water spinach contains high fibre and is a good source of vitamin B6 and C (Table 2.1). It is also rich in minerals. Ndamitso et al. (2015) found high content of potassium, magnesium, sodium and phosphorus in the leaves and stems. Based on the dietary requirements, water spinach is good sources of potassium, manganese and iron for all categories of people, while the level of magnesium contents can fulfil both adult female and children's requirements (Umar et al., 2007). Bioactive compounds such as flavonoids and phenols are also discovered in water spinach, providing anti-inflammatory and antioxidant properties with medicinal values (Mariani et al., 2019).

Table 2.1: Nutritional Content Per 100g Water Spinach

Nutritional Contents	Amount	% Daily Value*
Total Fat	0.2 g	0
Cholesterol	0 mg	0
Sodium	113 mg	4
Potassium	312 mg	8
Total carbohydrate	3.1 g	1
Dietary fibre	2.1 g	8
Protein	2.6 g	5
Vitamin C	55 mg	91
Iron	1.67 mg	9
Vitamin B6	0.096 mg	5
Magnesium	71 mg	17
Calcium	77 mg	7

*Based on 2000 calorie diet

(Source: USDA, 2019)

According to Federal Agricultural Marketing Authority (FAMA)'s Standard FAMA: FS018-2001, good quality water spinach should be clean of residues and dirt as well as free of blemishes, injuries (from diseases or mechanical) and insects (FAMA, 2008). When kept post-harvest in cold room with temperature between 12-14°C, the shelf life is between 10-12 days with RH values between 90-95% (Chen & Paull, 2015).

2.2 Postharvest Issues in Vegetables

The benefits of consuming fresh fruits and vegetables and minimally processed fruits and vegetables have long been known and largely advocated. Among the benefits are high moisture and nutrients contents such as vitamins, minerals, fibres, and antioxidants (Mendoza et al., 2022). However, the open nature of fresh produce production makes it susceptible to contamination from multiple sources (Murray et al., 2017). The more steps in the handling and processing of a product, the more obvious physiological changes will be in that product, leading to shortened product shelf-life and enhance the growth and survival of microbes associated with the product (Matthews, 2014).

2.2.1 Chemical Contamination

Vegetables are potentially exposed to chemical contaminants from the environment itself such as toxic metal or metalloids, mycotoxins, and pesticides. These chemical contaminants pose food safety risks and has been linked to numerous long-term health complications such as cancer (Thompson & Darwish, 2019). While some factors causing contamination are beyond control such as differences in plant uptake mechanisms from the environment, exposure to pesticides can be regulated.

2.2.1.1 Pesticide residues

Pesticides residues are the most common chemical contamination found on vegetables. The presence of pesticide residues in in the fields, water, and foods can produce harmful effects on human health and the environment (Musarurwa et al., 2019) Ingestion of chemical pesticide residue by human may lead to diseases such as

respiratory disease, neurodevelopmental disorders, cardiovascular diseases, and cancer (Chiu et al., 2019; Hertz-Picciotto et al., 2018). Death from pesticide poisoning is estimated at 200,000 deaths worldwide as reported by Hertz-Picciotto et al. (2018). Residue from crops may also spread to the water, soil, and the atmosphere, causing contamination. Organisms ingesting the pesticide residues may be endangered as a result and leading to the disturbance of the ecological balance (Ogbeide et al., 2019).

Even so, farmers and growers continue to use pesticides recklessly so that the produce is free from damage caused by pests. A study reported by the Consumers' Association of Penang, Malaysia in March 2023 revealed that vegetables sold in supermarkets, hypermarkets, wet markets, mini markets and retail shops has a high presence of up to 35 pesticides compounds in a single vegetable (Dermawan, 2023). Besides using excessive pesticides, farmers are found using banned pesticides on vegetables. Carbofuran (banned from May 2023) which was previously allowed only in rice was detected in some leafy vegetables (Abdul Kader, 2023).

Up to 97.6% of Malaysian farmers preferred the use of pesticides to keep off pests from their crops (Halimatunsadiah et al., 2016). (Ishak et al., 2023) found that five out of six (83.3%) randomly sampled water spinach from local markets contains pesticides, with one sample exceeding the maximum permitted level (MRL) of carbendazim.

2.2.1.1.1 Diazinon

Diazinon is a broad-spectrum contact organophosphorus pesticide used as an insecticide, acaricide, and nematicide to control soil and foliage insects and pests (Aggarwal et al., 2013). The mechanism of action in pests is by inactivating the acetylcholinesterase enzymes (Wang & Shih, 2016). The main properties are

summarised in Table 2.2. Diazinon is the second most used organophosphorus pesticide after malathion (Nematollahi et al., 2022). The persistence of diazinon on plants are known to increase in low temperature condition and can be attributed to the high oil content as well (NPIC, 2009).

Table 2.2: Main Properties of Diazinon

Properties	Values
Empirical formula	C ₁₂ H ₂₁ N ₂ O ₃ PS
CAS - Number	333-41-5
Density	1.116 g/cm ³
Solubility in water	60 mg/L
Soil half-life	40 days
Colour	Colourless amber to brown
Odour	Odourless
Toxicity	Class II (moderately hazardous)

(Source: (Alam et al., 2015; Nematollahi et al., 2022))

Diazinon is detected in many vegetables sold in fresh markets across the world, with studies recorded diazinon as the most pesticide residue detected with values between 33% (Dinede et al., 2023) and 35% (Alam et al., 2015) of total vegetable samples. Even in organic farms, diazinon has been detected on the vegetables claimed to be grown organically. Farina et al. (2018) studied cabbage, cauliflower, broccoli, lettuce, celery, spinach and mustard grown in organic farms in Cameron Highlands, Malaysia and found highest level of diazinon in spinach (200 µg/kg) and lowest in cabbage (13.3 µg/kg), which still exceeded the exceeded the maximum residual limit (MRL) of 0.01 mg/kg.

In Bangladesh, a study by Nahar et al. (2020) found out of 40 samples of cauliflower, 6 samples (15% of the total number of samples) contained pesticide

residues, of which 3 samples contained diazinon and quinalphos residues and 3 samples contained only diazinon residue. 5 out of 6 cauliflower samples exceeded EU MRL (0.146-0.346 mg/kg). 1 out of 40 tomato samples in this study found diazinon exceeding EU MRL (0.119 mg/kg). An older study found the concentration approximately 360 times higher than the MRL in tomato (3.612 mg/kg) and up to 4.514 mg/kg (450 times the allowed concentration) in eggplant (Alam et al., 2015), which is alarming.

Samples from six countries recorded an average concentration of 26.86 µg/kg, with Iran having the most samples contaminated with diazinon and despite mostly found in fruit samples, the highest concentration of diazinon was found in leafy vegetables at 124.91 µg/kg (W. Li et al., 2023). This meta-analysis on organophosphorus pesticides contamination in food samples provided the latest insight on diazinon usage around the world.

2.2.2 Microbial Contamination

According to Matthews (2014), the microbial safety of leafy vegetables is a persistent cause for concern around the world. Foodborne illnesses due to consumption of contaminated leafy vegetables accounted for 22% cases in the United States. Norovirus such as *Escherichia coli* (*E. coli*) is the most common pathogen associated with foodborne illnesses caused by fresh produce (Luna-Guevara et al., 2019).

Postharvest microbial contamination is a great concern because the handling and processing of produce will provide microbes with nutrition and optimum conditions to thrive. *E. coli* O157:H7 has been shown to be able to thrive in water, soil and manure for extended periods and resists changes in moisture, nutrients, temperature, and exposure to sunlight (ultraviolet). Although other foodborne pathogens have similar

survival characteristics, they are not commonly identified as cause of foodborne illnesses from leafy vegetables (Matthews, 2014).

Physical postharvest processing of fruits and vegetables such as cutting and peeling induce the release of cellular contents at the site of wounding that can promote the growth of pathogenic and spoilage microorganisms (Ali et al., 2017). Agricultural factors (pre-harvest) and postharvest factors can introduce microorganisms to leafy vegetables and cause contamination. Sources of microbial contamination are summarised in Table 2.3.

Table 2.3: Sources of Microbial Contamination in Pre-and Postharvest Stage on Raw Vegetables

Stage	Sources of Microbial Contamination
Preharvest	Soil, irrigation water, inadequately composted manure, human handling, reconstituted fungicides and insecticides, seasons (fall, winter, and spring)
Postharvest	Harvesting equipment, transport container, contaminated water used for washing, transport vehicles and processing equipment, unclean implements, poor hygiene in hands, cross contamination (during preparation or storage)

(Source: Luna-Guevara et al., 2019)

Microbial contamination of vegetables has yet to cause foodborne illness outbreak in Malaysia, however a study by Kuan et al. (2017) found that from both organic and conventional vegetables sampled in Malaysia, mesophilic aerobic bacteria count ranged from 5 to 7 log₁₀ CFU/g while total coliforms counts were recorded between 1 to 7 log₁₀ CFU/g. *Salmonella spp.* prevalence in raw vegetables was 21.5%, with 8 samples (31.0%) detected the presence of this microorganism out of 26 water spinach samples (Abatcha et al., 2018) .

2.2.3 Postharvest Quality Loss

The challenges that begin from the cut made to harvest leafy vegetables are to contain loss of quality and stop microbial spoilage. The contamination and quality loss are due to contamination by mycotoxins, respiration and ethylene production rate, water activity of the produce, water loss and the extent of physical damage exerted onto the harvest (Gaudham et al., 2021).

During physical processing of fruits and vegetables that involves wounding of produce, rates of tissue senescence can be enhanced resulting in reduced storage life of fresh-cut fruits and vegetables (Ali et al., 2017).

2.3 Current Postharvest Treatment

Microbial and chemical contamination of fresh produce (fruits and vegetables) may be caused by improper water irrigation, fertilizers, harvesting process, packaging, transport and handling in retail (Freitas et al., 2014; Luna-Guevara et al., 2019). The presence of contamination reduces the quality, shorten the shelf-life and may become unsafe for consumption. Fresh produce, especially leafy vegetables are prone to cause foodborne illness (Murray et al., 2017). Therefore, there is a need for postharvest treatment of these products before further processing or directly entering retail to allow the consumers having them raw or minimally processed.

Postharvest treatment is divided into chemical, physical and combination of both. These treatments are expected to effectively reduce microbial load, extends shelf-life by preserving the texture, visual appearance, flavour, and nutritional value (L.-Z. Deng et al., 2017).

2.3.1 Chemical Postharvest Treatment

Chlorine compounds such as sodium hypochlorite (NaOCl), chlorine dioxide (ClO₂), and chlorine (Cl₂) are used as sanitizing agents added to water used to wash produce. When added to water, they produce hypochlorous acid (HOCl) and other Reactive Chlorine Species (RCS) that simultaneously damage multiple cellular components of bacteria (Ali et al., 2017; Mendoza et al., 2022). Ozone, which falls under chemical postharvest treatment oxidizes the bacterial and fungal cell components, such as proteins, unsaturated lipids, and respiratory enzymes, leading to elimination of odour, mycotoxins, and pesticide residue from surface of fresh produce (Brodowska et al., 2018; Özen et al., 2021; Yan et al., 2021). Electrolyzed water is produced through electrolysis of a salt solution with chlorine to produce reduced substances with strong biocidal activity such as hypochlorous acid (HOCl), hypochlorite ion (OCl⁻), hydroxyl (OH⁻), and superoxide radicals (O₂•⁻) to disinfect fresh produces (Ali et al., 2017; Marçal et al., 2021).

2.3.2 Physical Postharvest Treatment

In decontamination of fresh produce, ultraviolet (UV) light generates pyrimidine dimers, which distorts DNA helix and interferes with cell replication of microorganisms to produce fatality of bacteria, fungi, and viruses (Mendoza et al., 2022; Murray et al., 2017). Irradiation treatment is another physical treatment using ionising radiation, commonly used in decontamination of spices, herbs and condiments. Reactive oxygen species and free radicals generated by ionising radiation produces damage (direct or

indirect) to microbial nucleic acids (DNA), break chemical bonds and alter various molecules, leading to the destruction or deactivation of bacterial components including enzymatic activity (Asghar et al., 2022; L. Deng et al., 2019). Ultrasonic treatment is utilised in fresh produce decontamination and enzymatic degradation. The high-pressure waves induce acoustic cavitation, releasing a high amount of energy that destroys the microbial cell walls and damages the DNA via free radical production (Gallo et al., 2018; Mendoza et al., 2022).

2.4 Postharvest Treatment on Leafy Vegetables

Currently available literatures on leafy vegetables decontamination are on leafy vegetables eaten raw as salad (Chong et al., 2021; Gogo et al., 2017; Jasper et al., 2021; Nousiainen et al., 2016; Santarelli et al., 2018; Xylia et al., 2021). However, due to the broad definition of leafy vegetables, often succulent leafy vegetables and tender green leafy vegetables are treated with the same parameters although there are variations in succulence, cuticle thickness and leaf cell types (Radovich, 2018).

Leaves with low cuticle thickness and non-succulent are such as spinach, mustard leaves, and water spinach are especially fragile (M. Zhang et al., 2013). Excessive handling of these vegetables may damage the physical structure. Postharvest chemical treatment can be the solution to this problem due to the absence of physical handling. However, the disadvantages include absorption of accumulated harmful byproducts such as chlorates and trihalomethanes (Rodrigues Gadelha et al., 2019), mould growth from residual moisture post-treatment (L.-Z. Deng et al., 2017), reduced efficacy in presence of multiple organic compounds in the washing liquid (Nguyen-the & Carlin,

1994) and consumers' hostility towards synthetic chemicals (Mesías et al., 2021; Rico et al., 2007).

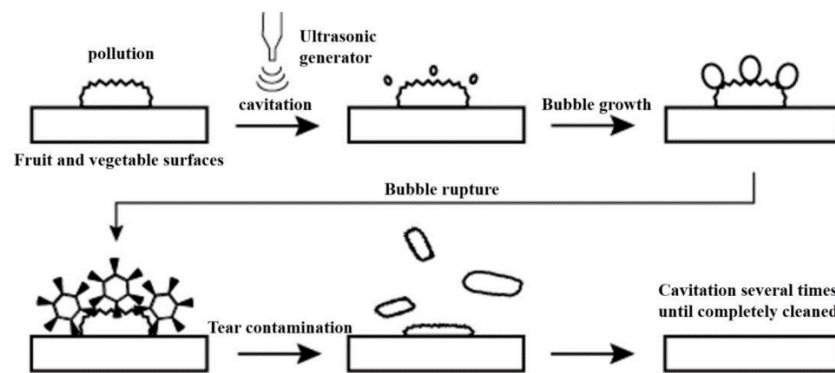
Physical treatments to remove or reduce contamination level involving heat such as sterilisation and blanching usually causes physiological effects such as tissue softening or alteration in biochemical compositions such as chlorophyll degradation and loss of nutrients. (L. Deng et al., 2019) .Therefore, non-thermal physical decontamination treatment is gaining interest. A summary on physical treatments on non-succulent leafy vegetables are shown in Table 2.4.

Table 2.4: Summary of Non-Thermal Physical Postharvest Treatment on Non-Succulent Leafy Vegetables

Vegetables	Treatment	Effects of treatment	Source
Spinach	Ionising electron beam, 0.7 kGy dose in combination with modified atmosphere packaging	Reduction of <i>Salmonella spp.</i> and <i>Listeria spp.</i> microorganisms by 5.0 log CFU/g.	(Gomes et al., 2011)
	UV-B irradiation	Reduced yellowing of leaf and better visual quality compared to control samples.	(Ufuk Kasım & Kasım, 2017)
	Pulsed light	Reduction in the availability of <i>E. coli</i> O157:H7 by 1.7–3.4 log CFU/g.	(Mukhopadhyay et al., 2019)
	Ultrasonic treatment combined with aqueous chlorine dioxide (ClO ₂)	Chlorophyll contents in ultrasonic treatment alone was higher in comparison to combination treatment.	(Mu et al., 2020)
Pak choi	Ultraviolet-C (UV-C) in combination with aqueous chlorine dioxide (ClO ₂)	Reduction of initial total aerobic bacteria by 2.55 log CFU/g	(Kim et al., 2011)
	White LED irradiation	Significant suppression of sensory changes and maintained higher chlorophyll and vitamin C and vitamin C content at 20 °C storage.	(F. Zhou et al., 2020)

2.5 Ultrasonic Treatment

In the food industry, ultrasonication is a physical postharvest treatment that utilises high-power ultrasonic waves (20-100 kHz) to inactivate microorganisms and remove contaminants. The waves produce acoustic bubbles (cavitation bubbles) that propagates through the cellular structure of microorganisms to give a lethal effect (Gao et al., 2014). The cavitation effects also remove dirt particles (Figure 2.2) and accelerates cleaning efficiency due to temperature and pressure change (W. Zhou et al., 2022) Ultrasonic treatment utilized less energy, reduces damage that may affect food quality and extends shelf-life (Yan et al., 2021).



(Source: (W. Zhou et al., 2022))

Figure 2.2: Ultrasonic Treatment Process

2.5.1 Postharvest Ultrasonic Treatment on Fruits and Vegetables

Ultrasonic cleaning has been used on both whole fruits and vegetables as well as fresh-cut ones. By pre-treating fresh produce, physiological activity can be retarded and quality degradation during storage can be delayed (Yuting et al., 2013). Inhibition of peroxidase and polyphenol oxidase (PPO) by ultrasonic treatment is the key to prolong

shelf-life (W. Zhou et al., 2022). The propagation of acoustic bubbles acts to inactivate microorganisms and remove other types of contaminants from fresh produce (São José et al., 2014).

R. Azam et al. (2020) considered ultrasound-assisted cleaning as an effective method to remove pesticide residues, saving time and energy and does not pollute the environment. Liang et al. (2012) applied ultrasonic treatment on cucumbers spiked with five organophosphorus pesticides and recorded removal percentages between 49.8% (dichlorvos) and 82.9% (trichlorfon) after 20 minutes' treatment. Lozowicka et al. (2016) study on strawberries reported that ultrasonic cleaning lowered residues for 16 analysed pesticides with removal of up to 91.2 %.

Ultrasonic treatment not only removes pesticide residue physically, but also assists degradation of the residues. High degradations percentage were recorded for malathion (41.7%) and chlorpyrifos (82.0%) after ultrasonic treatment at 500 W for 120 minutes on spiked apple juice (Y. Zhang, Xiao, et al., 2010). In terms of diazinon degradation percentage, up to 51.3% was reported by Y. Zhang, Zhang, et al. (2010) whereas Wang & Shih (2016) recorded 22% degradation.

Matouq et al. (2008) studied degradation of diazinon-water mixture by 10-minute exposure to 1.7Mhz ultrasonic waves and found that degradation of the compound is a quiet and complex mechanism involving production of hydroxyl radicals by ultrasonic waves followed by reaction with diazinon by hydrogen abstraction or electrophilic addition to double bonds. Peroxy radicals were then formed by reaction of the resulting free radicals with dissolved molecular oxygen and rapidly initiating a series of oxidative degradation reactions. Based on the degradation products identified by GC-MS, the

degradation processes proposed included hydrolysis of the ester moiety, oxidation, hydroxylation, dehydration, and decarboxylation (Y. Zhang, Zhang, et al., 2010)

Table 2.5 summarized reviews of ultrasonic treatment on leafy vegetables in reducing pesticide residues and microbial availability, while preserving the physical quality of vegetables.

Table 2.5: Ultrasonic Treatment on Leafy Vegetables

Sample	Treatment	Findings	References
Pak choi in combination with sodium hypochlorite, NaClO)	Bath,45 kHz, 300 W, 30 min at 25 °C,	Ultrasound treatment could significantly reduce the usage of NaClO solution from 800 ppm to 500 ppm.	(L. Zhang et al., 2022)
Pak choi in modified atmosphere packaging (MAP)	Bath,30 kHz, 2.4 W/g 5 min, 10 min, or 15 min; at 20 °C ± 1 °C	Decreased initial total number of colonies in from 7.11 to 6.01 log CFU/g. Delayed increase in mass loss, colour indices, and accumulation of malondialdehyde (MDA) and reduced the increasing rate of cell membrane permeability, as well as peroxidase and PPO activities.	(X.-T. Zhang et al., 2019)
Spinach leaves	Jet washer, 40kHz, 100W, 15 minutes,	The freshness of spinach leaves was revived and prolonged by stomatal closure after washing with ultrasonic waves.	(Oda et al., 2021)
	Flow ultrasonic washer, 25 kHz, 79.41 W/L, 60 seconds	Reduction of <i>E. coli</i> count to 4.45 log CFU/g sample	(B. Zhou et al., 2012)