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Optimisation of Ultrasound Treatment to Enhance the Postharvest Life of Fresh-cut Pineapple

by

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ABSTRACT

Minimal processing of fresh-cut pineapple (*Ananas comosus* L.) is time and labour consuming as it has thick inedible skin and large crown. Liberation of cellular contents at the site of wounding can promote the growth of microorganisms and limit the storage life of fresh-cut pineapple. Therefore, ultrasound treatment at different power input and exposure time was used to investigate its effectiveness on the storage life of fresh-cut pineapple. To determine the antimicrobial effect of ultrasound on the proliferation of spoilage microorganisms three different ultrasound power inputs (17, 25 and 29 W) and treatment times (5, 10 and 15 min) during 7 days of storage at 7 ^{o}C were applied on co. Josapine. The application of ultrasound at the power input of 25 and 29 W for 10 and 15 min, respectively, inhibited the growth of mesophilic, lactic acid bacteria and yeast and moulds. On day 5, it was found that 29 W for 15 min treatment resulted in the lowest growth of mesophilic, lactic acid bacteria and yeast and mould counts. However, changes in the proliferation of spoilage microorganism were observed among samples treated at 25 W: 10 min, 25 W: 15 min and 29 W: 10 min. The storage life of ultrasound treated fresh-cut pineapple was extended by 2 days in comparison to those washed in distilled water (dH_2O).

The ideal ultrasound power input (25 and 29 W) and treatment time (10 and 15 min) were then used to study the effect of treatment on the production of intracellular ROS and extent of oxidative damage in fresh-cut pineapple. Increments in ROS concentration when exposed to ultrasound treatment negatively correlated with populations of mesophiles, lactic acid bacteria and yeast and mould. Higher ROS contents may have induced disease resistance in fresh-cut pineapple. However, increments in power input and treatment time to 29 W and 15 min resulted in the oxidation of polyunsaturated fatty acid (PUFA) as indicated by higher malondialdehyde (MDA) content during cold storage.

Ultrasound treatment also invoked oxidative stress and induced defence mechanisms in fresh-cut pineapple by regulating phenolic metabolism and recycling of ascorbate concentration. Induction of phenylalanine ammonia lyase and inhibition of polyphenol oxidase and polyphenol peroxidase activity in fresh-cut pineapple upon exposure to ultrasound treatment resulted in higher total phenolic concentration. Similarly, the redox state of ascorbate as indicated by lower concentrations of dehydroascorbic acid was better maintained following ultrasound treatment due to the induction of monodehydroascorbate reductase and dehydroascorbate reductase activity. Likewise, the total antioxidant capacity of fresh-cut pineapple treated at 25 W for 10 was found higher in comparison to other treatments during 5 days of storage at 7 ^{o}C.

The effect of ultrasound treatment on the physico-chemical and physiological changes in fresh-cut pineapple was also assessed in this study. Ultrasound treatment at 25 W for 10 min retained 98% of initial firmness and did not result in changes in the juice leakage, colour parameters, soluble solid concentration and titratable acidity in regards to control after 5 days of storage. Meanwhile, the increment of ultrasound power input and treatment time to 29 W and 15 min had resulted in the lowest SSC concentration with the highest volume of juice leakage and respiration rate in fresh-cut pineapple during 5 days of cold storage.

In conclusion, an ultrasound treatment at 25 W for 10 min optimally inhibited the growth of spoilage microorganisms and extended the storage life for 2 days. Higher reduction of spoilage microorganisms was achieved when the ultrasound power input and treatment time was increased to 29 W and 15 min but it caused higher accumulation of ROS which reduced the nutraceutical concentration of fresh-cut pineapple following 5 days of cold storage.

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LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
ABTS	2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA	Analysis of Variance
APX	Ascorbate peroxidase
ASC	Ascorbate
atm	Atmospheric pressure
ATP	Adenosine triphosphate
BTH	benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester
C*	Chroma value
CDC	Centers of Disease Control and Prevention
CFU	Colony forming unit
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CRD	Completely randomised design
CWDE	Cell wall degrading enzyme
DHA	Dehydroascorbic acid
DHAR	Dehydroascorbate reductase
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAD	Flavin adenine dinucleotide
FAO	Food and Agriculture Organization
FAMA	Federal Agricultural and Marketing Authority
FCC	Free chlorine concentration
FDA	Food and Drug Administration
FRAP	Ferric reducing antioxidant power
FW	Fresh weight
GAE	Gallic acid equivalent
GAP	Good Agricultural Practice
GMP	Good Manufacturing Practice
GR	Gluthathione reductase
GRAS	Generally Recognized as Safe
GSH	Gluthathione
GSSG	Oxidised gluthathione

h^o	Hue angle
ha	Hectare
HAT	Hydrogen atom transfer
HPLC	High performance liquid chromatography
H_2O	Water
H_2O_2	Hydrogen peroxide
HOCl	Hypochlorous acid
IFST	Institute of Food Science and Technology
IU	International unit
K	Kelvin
L*	Lightness
LOX	Lipoxygenase
MAP	Modified atmosphere packaging
MARDI	Malaysian Agricultural Research and Development Institute
MDA	Malondialdehyde
MDHA	Monodehydroascorbic acid
MDHAR	Monodehydroascorbate reductase
MPBI	Malaysian Pineapple Board Industry
MRS	de Man, Rogosa and Sharpe
O_2	Oxygen
$O_2^{\bullet-}$	Superoxide anion
O_2^{2-}	Peroxide ion
OCl^-	Hypochlorite ion
OH^-	Hydroxyl radical
ORP	Oxidation reduction potential
PAL	Phenylalanine ammonia lyase
PCA	Plate count agar
PG	Polygalacturonase
PME	Pectin methyl esterase
POD	Polyphenol peroxidase
PPO	Polyphenol oxidase
PUFA	Polyunsaturated fatty acid
RAE	Retinol activity equivalent
RH	Relative humidity
RID	Refractive index detector

ROS	Reactive oxygen species
RSM	Response surface methodology
SD	Standard deviation
SED	Single electron transfer
SOD	Superoxide dismutase
SSC	Soluble solid content
TA	Titratable acidity
TBARS	Thiobarbituric acid reactive substance
TCD	Thermal conductivity detector
TE	Trolox equivalent
TSS	Total soluble solid
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
WHO	World Health Organization
YGC	Yeast Extract Glucose Chloramphenicol

1 INTRODUCTION

Pineapple (*Ananas comosus* L.), which is a non-climacteric tropical fruit, has gained much attention and popularity among other tropical fruits due to its distinctive flavour, aroma and juiciness. Considering the significant proportion of nutritional and functional compounds such as vitamin A, B and C and minerals and fibres that can be contributed in daily diet, the consumption of pineapple has led to global economic growth (Hossain & Rahman, 2011). In 2012, pineapple was grown in approximately 995,888 ha and the world production quantity was about 23,333,886 tonnes (FAOSTAT, 2015). Malaysia is ranked 18th in the production of pineapple with the production value of approximately 95 million USD (FAOSTAT, 2015). To meet the markets' demand, world production of pineapple has increased by an average of 4.7% per annum between 2002 and 2012 (FAOSTAT, 2015). However, morphological structures of pineapple, which are characterised by thick inedible skin and large crown, often result in laborious processing and hence reduce consumers' acceptability (Azarakhsh et al., 2014a; Benitez et al., 2014). Therefore, processing pineapple into ready-to-eat products has commercial advantages due to the convenience of consumptions among consumers.

The storage life of fresh-cut pineapple is often limited as mechanical operations can enhance the transfer of skin microflora to the fruit flesh (Zhang et al., 2013a). Removal of the natural protective epidermal barrier and increase in moisture and dissolved sugar contents at the site of wounding can provide an ideal environment for the growth and colonisation of microorganisms (González-Aguilar et al., 2004; Mantilla et al., 2013). Although low pH of fruits can hinder the growth of many human pathogenic microorganisms such as *Escherichia coli* (Oms-Oliu et al., 2010), increase in respiratory activity of cut fruits in confined packages can enhance the proliferation of anaerobes such as lactic acid bacteria and yeast and mould (Siroli

et al., 2015). Loss of cellular compartments in injured plant tissues can result in the mixing of intra- and intercellular enzymes and substrates, which cause softening, enzymatic browning, juice leakage and rapid degradation of functional compounds (González-Aguilar et al., 2004; Mantilla et al., 2013). Marrero and Kader (2006) suggested that the marketability of fresh-cut pineapple following storage for 5 days at 10 ^{o}C was unacceptable as signaled by a sharp increase in respiratory activity beyond the threshold limit.

The food industry relies heavily on the application of chlorine to decontaminate the surface of fruits and vegetables due to its antimicrobial activity against microorganisms, availability and low application cost (Gil et al., 2009). However, constant replenishment of chlorine into water with high organic concentration can result in the formation of chlorine off-gas and carcinogenic halogenated compounds such as trihalomethanes and haloacetic acid which pose serious threat to human's health and environment (Luo et al., 2012; Yang et al., 2012). Therefore, alternatives to chlorine treatment are required to prolong the storage life of fresh-cut produce.

Studies have focused on the use of chemical preservatives and additives such as ascorbate and edible coating materials to maintain the quality of fresh-cut pineapple. For instance, Azarakhsh et al. (2014a) reported that 0.56% gellan based edible coating was effective to reduce weight loss and respiration rate of fresh-cut pineapple, but the coating did not inhibit the proliferation of spoilage microorganisms. Likewise, although the physiological changes in cassava starch coated was inhibited, the microbial counts of both the uncoated and cassava starch coated fresh-cut pineapple were above the legal limit for consumption after storage for 9 days (Bierhals et al., 2011). In that study, significant loss of ascorbate concentration throughout cold storage was observed in coated fresh-cut pineapple. Benitez et al. (2014) also demonstrated that the reduction of ascorbate in alginate coated fresh-

cut pineapple was higher than uncoated fruit at the end of cold storage. Therefore, research also should be conducted on the use of sustainable physical treatments to inhibit the growth of microorganisms without compromising the physico-chemical properties of fresh-cut pineapple.

Ultrasound as a clean technology due to its application in medical imaging has received considerable interest in the food industry (Chandrapala et al., 2012; Kentish & Feng, 2014). Based on the operational frequency and amplitude of ultrasound, the wide spectrum of ultrasound application in the food industry can be categorised into low and high intensity ultrasound. Low amplitude of ultrasonic waves at high frequency (>1 MHz) can be used as a non-destructive tool to assess the physico-chemical properties of food such as composition, firmness and other quality control assessments (Sango et al., 2014; Soria & Villamiel, 2010). The latter which operates at low frequency (16 kHz – 100 kHz) with high amplitude of ultrasonic waves has numerous application in food processing such as the inactivation of spoilage microorganisms and food enzymes and modification of food functional compounds (Sango et al., 2014; São José et al., 2014).

The major effects of power ultrasound in food processing application is mainly related to the cavitation phenomena. Propagation of sound waves through the medium can create alternating regions of rarefraction and compression (Soria & Villamiel, 2010). The pressure changes in these regions can result in the formation, alteration and implosion of cavitation bubbles (Pingret et al., 2013). The collapse of cavitation bubbles leads to energy accumulation in hot spots and generate high temperature (5000 K) and pressure (1000 atm) which in turn produce very high shear energy and turbulence in the cavitation zone (Bilek & Turantaş, 2013; Chandrapala et al., 2012). Also, the formation of transitory hot spots can breakdown water molecules and generate highly reactive free radicals that may react and modify other

molecules such as amino acid residues and functional compounds present on various foods (São José et al., 2014; Soria & Villamiel, 2010).

The combination of high temperature, pressure, shear forces and the formation of free radicals during sonication may have a variety effects on growth of microorganisms, physico-chemical, enzyme activity and antioxidant status of fruits and vegetables. An optimisation study was carried out by Cao et al. (2010b) on the decontamination efficiency of ultrasound (40 kHz) at different powers (250, 350 and 400 W) and treatment times (5, 10 and 15 min) on strawberry. Total microbial yeast and mould counts were reduced and the firmness was better maintained in strawberry treated at 250 W of ultrasound for 10 min following 8 days of storage at 5 ^{o}C. Therefore, amplitude of power ultrasound is an important parameter affecting the efficacy of ultrasound. Similarly, application of power ultrasound in fresh-cut broccoli (Ansorena et al., 2014) and lettuce (Ajlouni et al., 2006) showed that antimicrobial effect of ultrasound increased with prolong exposure time. However, limited studies are available on the effect of ultrasound parameters on the growth of spoilage microorganisms in fresh-cut fruits and vegetables.

However, the formation of free radicals during sonication may impose oxidative stress in fresh fruits and vegetables. Toivonen (2004) suggest that the production of intracellular ROS such as $O_2^{\bullet -}$ and H_2O_2 can be enhanced in plant tissues in response to various biotic and abiotic stress. Over accumulation of ROS can accelerate the senescence and deterioration of whole or fresh-cut produce. Also, the presence of hydroxyl radicals (OH^-) due to the sonolysis of water may alter the degree of hydroxylation in various fruits and vegetables and hence influence their antioxidant activity throughout storage (São José et al., 2014). Most of the studies have only demonstrated that the application of ultrasound can induce the antioxidant capacity in fresh produce such as strawberry (Cao et al., 2010b), plum (Chen & Zhu, 2011)

and peach (Yang et al., 2011) fruit without research further into the possible biological stimuli and signals that may activate and control the biotic and abiotic stress response.

1.1 Hypothesis

The application of ultrasound treatment with optimal level of power input and exposure time will inhibit the growth of spoilage microorganisms and enhance the phytochemicals concentration and storage life of fresh-cut pineapple.

1.2 Research objectives

To study the effects of different ultrasound parameters (power inputs and treatment times) on the storage life of fresh-cut pineapple by

1. Investigating the antimicrobial activity of ultrasound treatment on the inhibition of spoilage microorganisms.
2. Studying the effect of ultrasound treatment on the production of intracellular ROS and extent of lipid peroxidation.
3. Studying the effect of ultrasound treatment on the activity of phenolic and ascorbate metabolism enzymes.
4. Studying the effect of ultrasound treatment on the antioxidant activity.
5. Investigating the effect of ultrasound treatment on the physico-chemical and physiological behaviour.

2 LITERATURE REVIEW

2.1 Pineapple (*Ananas comosus* L.)

2.1.1 Taxanomy, morphology and anatomy

Pineapple (*Ananas comosus* L.) belongs the family of *Bromeliaceae* which is the only species in the bromeliad family being grown commercially for its fruit. Widely distributed throughout most tropical and sub-tropical regions, pineapple is also known as piña (Spanish), abacaxi (Portuguese), annachi pazham (Tamil) or nanas (Malaysian) (Paull & Duarte, 2011). It is a xerophytic, succulent, herbaceous, perennial plant which can grow up to 1 – 2 m high in adult stage (Bartholomew et al., 2003). The main morphological structures of pineapples that can be distinguished are stem, roots, leaves, peduncle, multiple fruit, crown and shoot (Figure 2.1). The leaves of pineapple plant is arranged spirally in a dense rosette pattern and the low density stomata is densely covered with furfuraceous trichomes to reduce water loss (Mitra, 1997). The club shaped stem is approximately 25 – 50 cm long with a width of 2 – 5 cm at the base and 5 – 8 cm at the top (Bartholomew et al., 2003). The plant is anchored by adventitious shoot which can spread up to 1 – 2 m laterally and 0.85 m in depth. Individual flowers are hermaphroditic and composed of three sepals, three petals, six stamens and a tricarpellate pistil (Paull & Duarte, 2011). Pineapple is a syncarp derived from the ovaries of several flowers and can be developed parthenocarpically in some self-incompatible commercial cultivars such as 'Smooth Cayenne' (Mitra, 1997).

2.1.2 Origin and production

Pineapple was domesticated in South America with south-eastern Brazil, Paraguay and northern Argentina being taught as the place of origin. It is one of the most important tropical fruit markets around the world with at least 79 countries in the

tropics and sub-tropics produce measurable quantities of pineapple (Paull & Duarte, 2011).

In 2012, pineapple was grown in approximately 995,888 ha and the world production quantity was about 23,333,886 tonnes. Statistical data showed that the world production of pineapple was increased by an average of 5% per annum between 2002 and 2012 (FAOSTAT, 2015). In 2012, the leading pineapple producing countries were Thailand (2,650,000 tonnes), Costa Rica (2,478,178 tonnes), Philippines (2,397,628 tonnes), Indonesia (1,780,889 tonnes) and followed by India (1,456,000 tonnes). Malaysia was ranked 18th in the production of pineapple with the production value of approximately 95 million USD (FAOSTAT, 2015). Cultivar difference is one of the major factor which contribute to the variation in the production and yield of pineapple among countries (Paull & Duarte, 2011).

Pineapple industries of the world are dominated by the cultivar 'Smooth Cayenne' due to its cylindrical shape, shallow eyes, attractive yellow flesh, mild acid taste and high yield which is the standard used for fresh fruit and processing (Bartholomew et al., 2003). However, over dependence on a single cultivar with narrow genetic base may not be favourable in the pineapple industry as the cultivar susceptible to pest and disease infections which reduce yield. Therefore, breeding programmes in many pineapple growing countries have developed new cultivars for the fresh market. In Malaysia, early pineapple improvement programmes focused on the selection of promising variants in the clonal fields which resulted in the development of several cultivars representing the three major group of pineapples such as: Sarawak (Cayenne), Yankee (Queen), Gandul (Spanish), Moris Gajah (Queen), Moris (Queen) and Masmerah (Spanish) (MPBI, 2015).

In 1996, the Josapine hybrid, suitable as table fruit, was successfully developed from the cross between 'Sarawak' and 'Johor' parents (Chan & Lee, 1996).

This hybrid is an improvement over the Sarawak and Moris cultivars due to its resistance to black heart disorder and short fruit maturation period of 120 days from flower induction. Josapine bears medium sized crown with dark purple skin in immature fruit or attractive bright orange red in ripen fruit. It is a cylindrical small fruit and weighing about 1.2 – 1.5 kg with an attractive deep golden yellow flesh. It has high sugar (16 – 18% SSC) with good balance of acid (0.5 – 1.2% citric acid) to give a strong aroma. The plants have dark green-purplish leaves without spines and yield about 35 – 50 tonnes per hectare (MARDI, 1996). Before the release of Josapine, the export revenue of pineapple in 1995 was a mere of 2,618,000 USD. In 2006, the export revenue was increased to 3,664,000 USD, an increment of approximately 3.6% per annum between 1995 and 2006. In 2011, Malaysia ranked 20th in the exportation of pineapple with an export value of 1,727,204,000 USD.

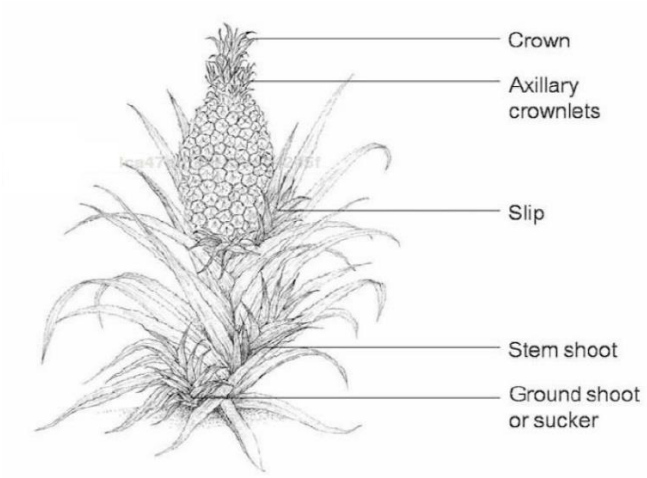


Figure 2.1 Main morphological structures of pineapple plant (Bartholomew et al., 2003).

2.1.3 Nutritional composition

Pineapple is a good source of vitamin C to prevent oxidative damage in body cells by scavenging ROS, vitamin B to aid in digestion, vitamin A, fibre and minerals (Hossain & Rahman, 2011) (Table 2.1). Several studies have revealed that bromelain, a proteolytic enzyme which can be found in pineapple exhibits anti-inflammatory, anti-

tumorous and anti-diarrhea activity (Ketnawa et al., 2012; Maurer, 2001). Also, pineapple is well appreciated around the world due to its distinct flavour and aroma. More than 280 volatile compounds have been found in pineapple, but only a few of these volatiles contribute to the distinct flavour of pineapple. Two minor hydrocarbon compounds, 1-(E,Z)-3,5-undecatriene and 1-(E,Z,Z)-3,5,8-undecatetraen and esters such as methyl and ethyl 2-methylbutanaote, methyl hexanoate, methyl and ethyl 3-(methylthio) propanoate are important contributors to the aroma of fresh-cut pineapple due to their low odour threshold values (Kaewtathip & Charoenrein, 2012).

Table 2.1 Proximate analysis of pineapple (all cultivars) (USDA, 2015)

	Unit	**Amount per 100 g of edible portion**
Proximates		
Water	g	86.00
Energy	Kcal	50.00
Protein	g	0.54
Total lipid	g	0.12
Carbohydrates	g	13.12
Fibre	g	1.40
Sugars	g	9.85
Minerals		
Calcium	mg	13.00
Iron	mg	0.29
Magnesium	mg	12.00
Phosphorus	mg	8.00
Potassium	mg	109.00
Sodium	mg	1.00
Zinc	mg	0.12
Vitamin		
Vitamin C	mg	47.80
Thiamin	mg	0.079
Riboflavin	mg	0.032
Niacin	mg	0.500
Vitamin B6	mg	0.112
Folate	µg	18.00
Vitamin A, RAE	µg	3.00
Vitamin A, IU	IU	58.00
Vitamin E (alpha-tocopherol)	mg	0.02
Vitamin K	µg	0.70

2.2 Problems related to fresh-cut fruits and vegetables

Pineapple is well appreciated globally due to its nutraceutical properties, distinct flavour and juiciness. It can be eaten fresh, canned slices or processed to cut pieces in dessert, dishes, salads and fruit cocktail mixes (Paull & Duarte, 2011). Considering the lengthy preparation of pineapple due to its morphological structures, which make immediate consumption difficult, there is an increasing interest in fresh-cut pineapple (Bierhals et al., 2011). Thick inedible skins and large crowns of pineapple also take up much storage space and result in higher transportation cost (Azarakhsh et al., 2014b). Therefore, processing pineapple into ready-to-eat products may be an alternative to meet consumers' demands. However, the storage life of fresh-cut pineapple is often limited (2 – 3 days) due to increase metabolic activity and delocalisation of substrates and enzymes leading to rapid microbial growth, softening, enzymatic browning and off-flavour (Antoniolli et al., 2007; Bierhals et al., 2011).

2.2.1 Microbial contamination of fresh-cut produce

Native microflora such as mesophilic, psychrotrophic, lactic acid bacteria, coliforms and yeast and moulds are commonly found on the surface of whole or fresh-cut fruits and vegetables. The number of natural microbiota inhabits on the surface of whole or fresh-cut produce may vary from 10^4 – 10^6 CFU g^{-1} and increase rapidly throughout storage period which depend on various pre and postharvest factors (Ramos et al., 2013). As reported in most studies, the initial native microflora (mesophilic, psychrotrophic and yeast and moulds) loads enumerated from different cultivars of fresh-cut pineapple were ranged from 3 – 4 log CFU g^{-1} (Azarakhsh et al., 2014b; Zhang et al., 2013a). Low pH of fresh-cut pineapple is suitable for the growth of yeast and lactic acid bacteria. Particularly, *Candida argentea*, *Candida sake* and *Meyerozyma caribbica* are the spoilage yeast strains that have been isolated from spoiled commercial fresh-cut pineapple (Zhang et al., 2014).

Improper sanisation of whole fruits may increase the likelihood of cross-contamination during peeling and cutting operations which enhance the transfer of foodborne pathogens to the internal tissues of fresh-cut produce. Loss of protective epidermal layers and subsequent damage to the plant tissue increase the susceptibility to invade by spoilage and pathogenic microorganisms (Barry-Ryan & O'Beirne, 1998; Ramos-villarroel et al. , 2012). Storage of product in a package with high humidity and leakage of cellular contents at the site of wounding can provide a nourishing environment for the growth of microorganisms (Oms-Oliu et al., 2010; Rico et al., 2007). Also, the ability of some pathogenic microorganisms such as *Salmonella* strains to form biofilm on plant tissues may protect them against harsh environments and reduce the efficacy of various sanitisation treatment (Abadias et al., 2008; Ölmez & Kretzschmar, 2009). The rate of contamination of fresh-cut products can be further enhance due to the use of unhygienic equipment during processing and improper storage conditions (Fernando et al., 2014; Holvoet et al., 2014).

Consumption of raw and fresh-cut fruits has been associated with the outbreak of foodborne illnesses due to some pathogenic microorganisms such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*. In March 2013, Centers of Disease Control and Prevention (CDC) revealed that approximately 46% of foodborne illness in the USA was attributed by the consumption of raw fruits and vegetables (Painter et al., 2013). In Malaysia, a recent research conducted by the Department of Food Science, Universiti of Putra Malaysia highlighted the concern of foodborne illness associated with the consumption of fresh-cut produce as the prevalence of *Salmonella* spp. in ready to eat fruits found in different fruit stalls and supermarket in Malaysia was 23% and 10%, respectively (Pui et al., 2011).

Often, pathogens such as *L. monocytogenes*, *Bacillus cereus* and *Clostridium botulinum* inhabit naturally in many soil and hence their existence on fresh fruits and vegetables are common (Beuchat & Ryu, 1997). High percentage occurrence of these soil-inhabiting bacteria in various whole produce such as lettuce, cucumber and melons suggested that natural contaminant in the soil is one of the pre-harvest factors which affects the microbial populations intact on the surface of fruits and vegetables (Dobhal et al., 2015; Soon et al., 2012). Besides soil conditions, improper usage of composted manure or untreated sewage water can contribute to the prevalence of *Salmonella* spp. and *E. coli* on fresh produce (Olaimat & Holley, 2012; Taban & Halkman, 2011).

Considering the outbreak of foodborne illnesses is associated with the microbial populations on whole or fresh-cut fruits and vegetables, there is an urge to improve the current pre and postharvest techniques to ensure the safety of foods provided to consumers. Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP) should be implemented throughout the food supply chain to minimise the risk of cross contamination of foodborne pathogens to fresh produce.

2.2.2 Physico-chemical changes in fresh-cut produce

2.2.2.1 Softening

Consumer purchasing intention is often influenced by the firmness and juiciness which affect the mouth feel of fresh-cut fruits and vegetables. Tissue softening is one of the major factors which affect the storage life of fresh-cut produce. For instance, the storage life of fresh-cut papaya was limited to 2 days due to rapid loss of texture even though it was stored at 13 ^{o}C (O'Connor-Shaw et al., 1994). The physical anatomy (cell wall thickness and cell adhesion) and turgor pressure will affect the firmness of intact or fresh-cut fruits and vegetables (Toivonen & Brummell, 2008). Mechanical operations which involve various degree of peeling, cutting or slicing often

result in faster deterioration of physical attributes of fresh-cut produce when compared to that of intact fruits and vegetables (Toivonen & Brummell, 2008).

Tissue damage in fresh-cut fruits and vegetables are often associated with the liberation of intracellular contents at the site of wounding upon exposure to mechanical operations (González-Aguilar et al., 2009; Toivonen & Brummell, 2008). Metabolic activities such as the respiration rate of fresh-cut produce can be triggered at the site of wounding due to the released of cellular substrates (Soliva-Fortuny & Martín-Belloso, 2003). For example, Sangsuwan et al. (2008) reported that the firmness of fresh-cut pineapple was reduced by 20% after only 2 days of storage at 10 ^{o}C and associated with an increase in respiration rate and moisture loss. The results obtained were consistent with previous studies which were also conducted on fresh-cut pineapple (González-Aguilar et al., 2004; Mantilla et al., 2013). Similarly, the liberation of cell contents of fresh-cut fruits and vegetables which resulted in the loss of firmness and increase in respiration rate was also reported in fresh-cut papaya (Karakurt & Huber, 2003; Rivera-Lopez et al., 2005), kiwi fruit (Agar et al., 1999), tomato (Aguayo et al., 2004b), apple (Saftner et al., 2002), jicama (Aquino-Bolaños et al., 2000) and iceberg lettuce (Deza-Durand & Petersen, 2011).

Loss of texture in fresh-cut fruits and vegetables are related to certain enzymatic and non-enzymatic processes. Several ethylene-inducible genes encoding cell wall degrading enzymes (CWDEs) which are responsible for the deterioration and overall texture of fresh-cut produce can be activated due to stimulated ethylene production (Karakurt & Huber, 2003). Pectin methyl esterase (PME) and polygalacturonase (PG) are the major enzymes involved in the degradation of pectins and resulted in softening (Rico et al., 2007). Karakurt and Huber (2007) reported that both α- and β-galactosidase are involved in the modification of cell wall components through hydrolytic and glycosyl transferase reactions.

These studies suggested that besides the activation of enzymatic activities, cellular leakage can also contributed to loss of texture in fresh-cut produce. Water loss is rapid in fresh-cut products as the internal tissues are exposed to abiotic stresses due to the absence of cuticle and sub-epidermal layers (Toivonen & Brummell, 2008; Watada & Qi, 1999). Loss of membrane integrity allows the leakage of cellular osmotic solutes into the apoplastic space and results in turgor loss (Karakurt & Huber, 2003).

2.2.2.2 Enzymatic browning

Undesirable colour changes in fresh-cut fruits and vegetables is one of the major concern among retailers as the aesthetic value of products is affected. Upon exposure to mechanical damage, wound signaling responses (tissue deterioration and senescence) will be mediated through the apoplast of plant tissues (Hodges & Toivonen, 2008; Toivonen, 2004). Subsequent loss of cellular compartmentalisation due to tissue senescence can induce the enzymatic activity of polyphenol oxidase (PPO) and polyphenol peroxidase (POD) which cause browning of plant tissues (Toivonen, 2004). PPO and POD catalyse the hydroxylation of monophenols to diphenols and oxidation of diphenols to quinones (Soliva-Fortuny & Martín-Belloso, 2003; Toivonen & Brummell, 2008). Subsequent oxidation of o-quinones leads to the formation of melanin which results in brown, reddish or black coloration on the surface of fresh-cut produce (Artés et al., 2007). Similarly, exposure of plant tissues to biotic and abiotic stresses such as wounding can increase the activity of phenylalanine ammonia lyase (PAL) which is the key enzyme in phenolic biosynthesis (Hodges & Toivonen, 2008; Rico et al., 2007). Increased production of phenolic compounds will be used as substrate for the oxidative enzymes.

For instance, González-Aguilar et al. (2004) reported that browning beyond market acceptability was observed in fresh-cut pineapple after stored for 14 days at

10 °C. Wu et al. (2013) reported that the activity of PAL and POD was increased after six hours of processing, but colour evaluation was not carried out. However, the activities of PAL, PPO and POD in wounded lettuce, escarole and rocket salad increased and were associated with the incidence of browning during storage at 4 °C (Degl'Innocenti et al., 2007). Similarly, Mishra et al. (2012) reported that the PPO activity of fresh-cut eggplant sliced by knife and blade was increased by 57 and 25% respectively, and was associated with high browning index.

2.3 Biotic and abiotic stresses on the production of reactive oxygen species (ROS) and defence mechanisms

2.3.1 Production of intracellular reactive oxygen species (ROS)

Harvesting and processing of fresh fruits and vegetables can lead to rapid initiation of senescence due to the reduction of energy resources, nutrients and hormones (Lemoine et al., 2008). Senescent fruits and vegetables are susceptible to pathogen invasion and environmental stress which can further deteriorate their quality. Postharvest senescence of fresh produce is a developmental process involving tight regulation of biochemical, physiological and oxidative metabolisms (Zhu et al., 2008). ROS, the primary mediators of oxidative damage in plants, are involved in the progression of senescence (Tian et al., 2013). As shown in Figure 2.2, ROS can be generated at the apoplastic region (cell wall and apoplastic space), cytoplasm and cellular organelles (chloroplast, mitochondria and peroxisomes) when exposed to biotic and abiotic stresses such as pathogen attacks, extreme temperature, ozone, ultraviolet radiation, mechanical stress (Toivonen, 2004). The production of ROS can be enhanced via the activation of plasma-membrane bound NADPH oxidase, cell wall bound peroxidase and amine oxidase mediated by stress-initiated ion fluxes and pH changes in the apoplast (Apel & Hirt, 2004; Toivonen, 2004).

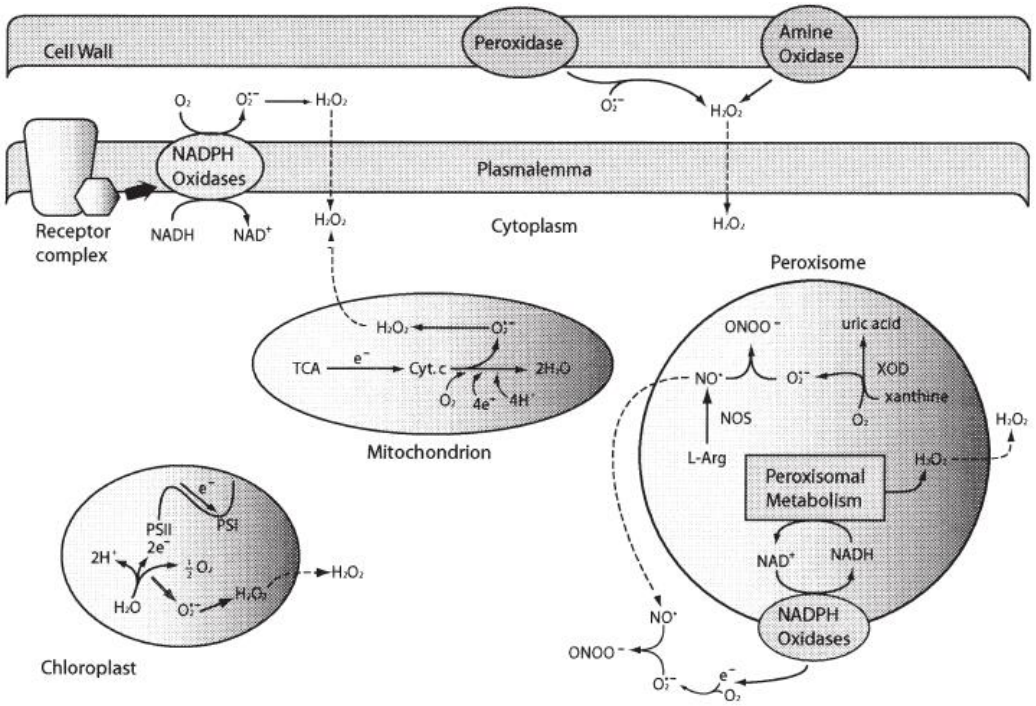


Figure 2.2 Generation of reactive oxygen species (ROS) in various sites of plant cell (Toivonen, 2004).

ROS such as $O_2^{\bullet-}$ and H_2O_2 are continuously produced as byproducts of aerobic metabolism in different cellular compartments such as mitochondria, peroxisomes and chloroplast (Apel & Hirt, 2004; Gill & Tuteja, 2010). It has been suggested that mitochondria is the major site of ROS production since the consumption of 1 – 5% of O_2 leads to the production of H_2O_2 (Gill & Tuteja, 2010; Nyathi & Baker, 2006). Although atmospheric O_2 involved in the respiratory metabolism in plant cells is relatively non- reactive, and it can be converted into active ROS forms either by energy or electron transfer (Blokhina et al., 2003; Gill & Tuteja, 2010) (Figure 2.3). Single electron reduction of O_2 can lead to the formation of $O_2^{\bullet-}$ which has a half-life of approximately 2 -4 μs (Gill & Tuteja, 2010). Dismutation of $O_2^{\bullet-}$ at low pH which generates peroxide ion (O_2^{2-}) can be protonated to form H_2O_2 with a relatively long half-life of 1 ms (Blokhina et al., 2003; Gill & Tuteja, 2010). In the presence of transition metals such as iron and copper, the accumulation of H_2O_2 can act as the

precursor for the production of a highly toxic OH^- through Fenton reaction (Mittler et al., 2004; Toivonen, 2004).

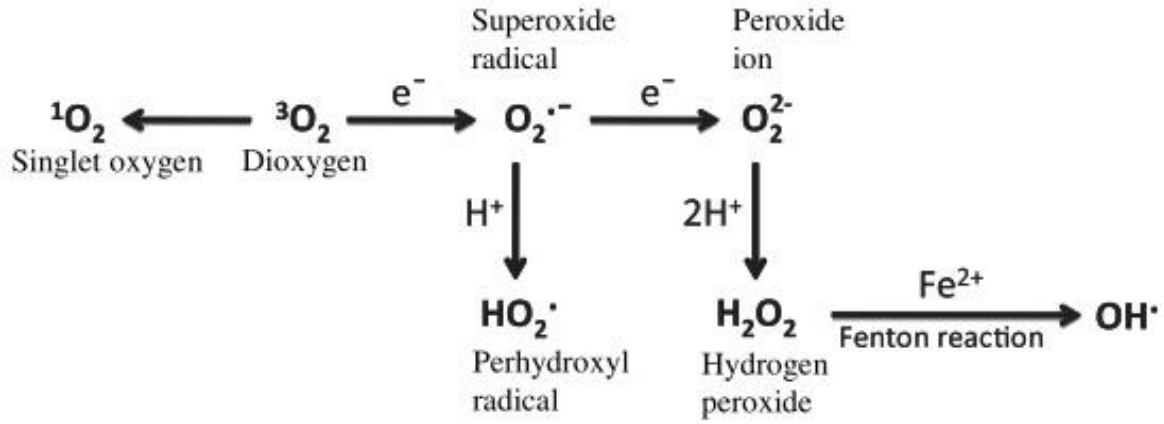


Figure 2.3 Generation of reactive oxygen species (ROS) by energy transfer (Gill & Tuteja, 2010).

Studies have suggested a dual role for ROS in plant biology. In the first, accumulation of ROS can cause oxidative damage to biomolecules such as lipids, proteins, carbohydrates and DNA which often results in programme cell death (Apel & Hirt, 2004; Blokhina et al., 2003). Peroxidation of lipid initiated in the presence of $OH^{\bullet}$ can lead to the formation of cytotoxic aldehydes, alkenals and hydroalkenals such as MDA (Blokhina et al., 2003; Gill & Tuteja, 2010). Oxidation of PUFA often leads to increase leakiness, decrease fluidity of membrane and cause secondary damage to membrane protein (Tian et al., 2013). MDA which is a secondary end product formed through the oxidation and degradation of PUFA by lipoxygenase (LOX) can be used as an indicator to indicate the extent of lipid peroxidation and oxidative stress in cells (Hodges et al., 1999; Singh et al., 2012). Li et al. (2010) observed that the MDA concentration in bruised pear was 37% higher than that of non-bruised fruit which was associated with higher ROS production after stored for 30 days at 16 °C. The accumulation of MDA concentration in pepper ionised with high dosage of electron beam (7 kGy) resulted in the deterioration of chloroplast membrane structure

(Martínez-Solano et al., 2005). Similarly, storage of tomato fruit under cold stress resulted in an increase in chilling index which was associated with the accumulation of MDA concentration throughout storage (Aghdam et al., 2012). Jimenez et al. (2002) also reported that the accumulation of H_2O_2 had resulted in lipid peroxidation in senescent tomato.

At low concentration, however, ROS can act as key regulators of growth, development, hormone signaling and biotic and abiotic stress responses (Gill & Tuteja, 2010; Mittler et al., 2004). It has been proposed that H_2O_2 can act as signals and secondary messengers for the activation of stress defence pathways due to its relatively long half-life and high permeability across membrane (Foyer & Noctor, 2005; Mittler et al., 2011). For instance, elevated concentration of H_2O_2 in benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) treated muskmelon was associated with increased resistance against *Trichothecium roseum* with lower concentration of MDA than control throughout storage (Ren et al., 2012). Similarly, accumulation of H_2O_2 in BTH treated peach fruit was reported to involve in the development of resistance against *Penicillum expansum* during 7 days of storage at 22 ^{o}C (Liu et al., 2005). Torres et al. (2003) also reported that H_2O_2 production and enhancement of defence mechanisms induced in wounded apple resulted in better resistance against *P.expansum*. The possible role of H_2O_2 as a signaling molecule to induce defence mechanisms against postharvest disease and abiotic stress was also observed in pear (Cao & Jiang, 2006) and persimmon (Novillo et al., 2014) fruit.

2.3.2 Enzymatic and non-enzymatic antioxidant system

Although accumulation of ROS can stimulate defence mechanisms of fresh produce against biotic and abiotic stresses, ROS have to be scavenged by various enzymatic antioxidants and low molecular weight metabolites to prevent lipid peroxidation. Enzymatic antioxidant defence systems include a variety of scavengers such as

superoxide dismutase (SOD), ascorbate peroxidase (APX), MDHAR, DHAR, PAL, PPO and POD. Non-enzymatic low molecular weight metabolites such as ASC, glutathione (GSH), carotenoids, and flavonoids are important to maintain the equilibrium between the production and scavenging of ROS (Apel & Hirt, 2004; Blokhina et al., 2003).

ASC is a highly abundant, powerful and water soluble metabolite that plays an important role in plant stress physiology and growth and development. It can help to minimise the cellular damage caused by ROS in plants by directly scavenging singlet oxygen, $O_2^{\bullet -}$ and OH^- and indirectly eliminating H_2O_2 via APX (Blokhina et al., 2003; Conklin, 2001). The ability of apoplastic ASC to react enzymatically and non-enzymatically with a wide range of ROS makes ASC the main ROS detoxifying compound in the aqueous state (Blokhina et al., 2003; Davey et al., 2000). Conklin and Barth (2004) also estimated that approximately 10% of ASC pool is localised within the apoplastic space which makes apoplastic ASC as the first line of defence in plants to govern resistance to abiotic stress.

Detoxification of H_2O_2 to H_2O by APX occurs by the oxidation of ASC to monodehydroascorbic acid (MDHA) which disproportionates spontaneously to DHA (Conklin, 2001; Shigeoka et al., 2002) (Figure 2.4). To maintain the redox state of ASC, MDHA and DHA must be reduced and recycled rapidly by MDHAR and DHAR, respectively (Conklin & Barth, 2004). MDHAR, a flavin adenine dinucleotide (FAD) enzyme catalyses the reduction of MDHA into ASC by using NADPH as an electron donor (Gill & Tuteja, 2010). Reduction of DHA into ASC is mediated by DHAR in the presence of GSH as a reductant (Smirnoff, 1996). Therefore, the combination of *de novo* synthesis of ascorbate by L-galactono-1,4-lactone dehydrogenase in the mitochondria and recycling of ASC via reductases are critical to maintain high ascorbate redox state (Conklin, 2001; Davey et al., 2000).

Several studies also have reported the possible role of APX, MDHAR and DHAR in the detoxification of ROS and the recycling of ascorbate-glutathione system of fresh produce in response to various postharvest treatments. For instance, the activity of APX of strawberry treated with ultraviolet C (UV-C) radiation for 10 min was approximately 1.4-fold higher than that of control on day 10. Similarly, higher APX activity was found in fresh-cut broccoli treated with neutral electrolysed water and UV-C (Martínez-Hernández et al., 2013). You et al. (2012) also reported that the APX activity of fresh-cut water chestnut was higher than that of untreated samples during cold storage. Besides abiotic stress type treatment, the inoculation of *P. membranaefaciens* in citrus fruit had resulted in significant enhancement of APX activity which coincided with increased in disease resistance against green and mould infection (Luo et al., 2013b).

The application of postharvest treatments on the ASC concentration of fresh produce also has been reported in several studies. Cao et al. (2010a) reported that the ASC concentration of ultrasound treated strawberry was 11% higher than control at the end of storage. It was also found that the concentration of ASC in ultrasound treated peach fruit was 2% higher than control after stored for 6 days at 20 ^{o}C (Yang et al., 2011). Wu et al. (2012b) reported that the concentration of ASC in high-pressure argon treated fresh-cut pineapple was higher than that of control throughout the storage. The application of gamma irradiation above 0.50 kGy was also found to induce higher concentration of ASC on day 3 (Hussain et al., 2014).

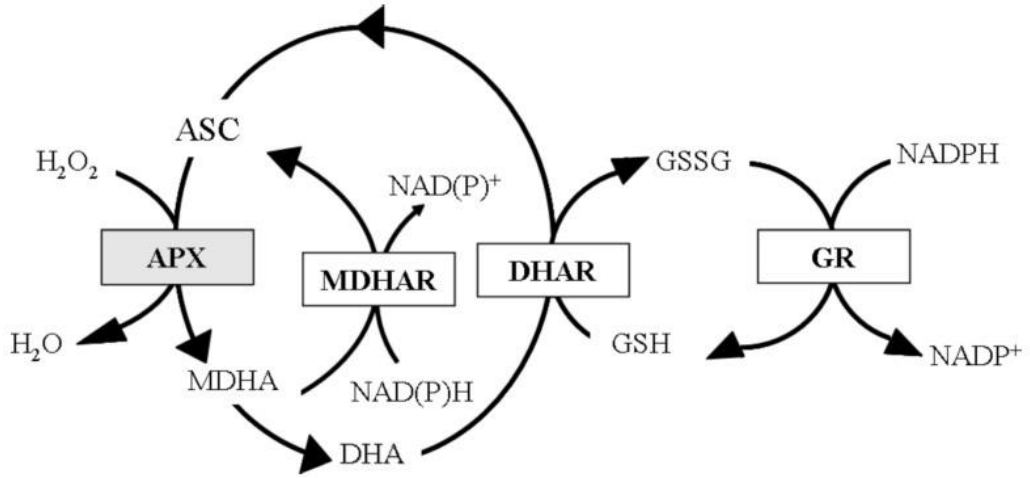


Figure 2.4 Ascorbate-gluthathione cycle. H_2O_2, hydrogen peroxide; H_2O, water; ASC, ascorbate; APX, ascorbate peroxidase; MDHA, monodehydroascorbic acid; DHA, dehydroascorbic acid; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; GR, gluthathione reductase; GSH, gluthathione; GSSG, oxidised gluthathione (Locato et al., 2013).

2.4 Postharvest treatments used to enhance the storage life of fresh-cut produce

Disinfecting and washing are important processes to remove dirt, pesticide residues and spoilage microorganisms, which are detrimental to the quality of fresh-cut fruits and vegetables (Gil et al., 2009). Currently, more than three quarters of the food industry depends on the application of chlorine to kill pathogens and spoilage organisms due primarily to its reliable availability and low application cost (Gil et al., 2009). However, the use of chlorine to wash and disinfect fresh-cut produce requires continuous replenishment of chlorine in high organic concentration wash water and can promote the formation of carcinogenic compounds such as trihalomethanes, which pose a threat to human health and the environment. Over the years, many alternative chemical and physical disinfection treatments have been evaluated for their efficacy to reduce the population of pathogens and extend the shelf-life of fresh-cut produce including their impact on texture, visual appearance, flavour and nutritional value (Rico et al., 2007). In addition, the development of edible coatings also provides new technologies that can contribute to the preservation of the quality

and safety of fresh-cut fruits and vegetables. Therefore, this review will discuss the effectiveness of chlorine and compare it to alternative technologies for washing, sanitising and preserving fresh-cut produce.

2.4.1 Chlorine

Chlorine is typically added as a sanitizing agent to water used to wash fresh-cut produce. The recommended total chlorine concentrations in washing process range from 50 – 200 mg L^{-1} with the maintenance of 2 – 7 mg L^{-1} free residual chlorine after contact (Delaquis et al., 2004). The pH of chlorine based sanitisers must be adjusted in the range of 6 – 7.5 to maintain high concentration of hypochlorous acid and minimise corrosion of equipment (Rico et al., 2007; Van Haute et al., 2013). Existing data about the efficiency of chlorine solution at the recommended concentration are very limited since it can generally reduce the population of spoilage microorganisms by only 1 – 2 log CFU g^{-1} (Alegria et al., 2009; Van Haute et al., 2013).

A study conducted by Akbas and Olmez (2007) reported that the initial population of mesophilic, psychrotrophic and Enterobacteriaceae bacteria in fresh-cut iceberg lettuce was reduced by 1.7, 2.0 and 1.6 log CFU g^{-1}, respectively, following a 2 min dipped in 100 mg L^{-1} chlorine. Changes in physico-chemical properties, including ascorbate and ß- carotene, in chlorine-treated lettuce were comparable to that of untreated lettuce throughout storage. Several studies also reported similar reductions of microbial populations in chlorine treated fresh-cut iceberg lettuce although the contact time and concentration of the chlorine solution used were different (Delaquis et al., 2004; Wulfkuehler et al., 2013). Additionally, the sensory attributes of iceberg lettuce was not adversely affected by chlorine (Delaquis et al., 2004; Vandekinderen et al., 2009). Similarly, a reduction of 1 – 2 log CFU g^{-1} of various pathogenic and spoilage microorganisms was also reported in chlorinated

fresh-cut lettuce (Allende et al., 2008; Posada-Izquierdo et al., 2013), escarole (Allende et al., 2008) and carrot (Klaiber et al., 2005).

In contrast, López-Gálvez et al. (2010) reported that although the initial counts of native microflora were reduced in fresh-cut melon following dipping in 150 mg L^{-1} of chlorine for 1 min, the microbial populations increased gradually and showed no significant difference in those dipped in tap water throughout 10 days of storage at 5 ^{o}C. The inability of chlorine to inhibit the growth of microorganisms was accompanied by a decline in total ASC concentration of chlorine-treated fresh-cut melon during storage (López-Gálvez et al., 2010). Similarly, no changes in the growth of native microbial population was observed between chlorine-treated fresh-cut kiwifruit and untreated fruit following 10 days of storage at 4 ^{o}C (Beirao-da-Costa et al., 2014). Waghmare and Annapure (2015) reported that the storage life of fresh-cut cilantro treated with 100 mg L^{-1} of sodium hypochlorite solution for 2 min was only extended up to 20 days. Meanwhile, the storage life of samples treated with combined treatment (MAP + sodium hypochlorite) was prolonged to 25 days at 5 ^{o}C.

A relatively constant free chlorine concentration must be maintained during commercial fresh-cut wash operations to ensure disinfection efficiency of the sanitiser and prevent cross contamination. However, the increase in chemical oxygen demand (COD) due to the accumulation of plant debris and exudates in the washing solution often leads to increased chlorine consumption, thus increasing the potential of pathogen survival and cross contamination (Luo et al., 2012). For instance, Yang et al. (2012) reported the survival of *E. coli* O157:H7 was enhanced when the concentration of free chlorine in wash solutions was depleted from 35 mg L^{-1} to near zero after washed with 3.6 kg of shredded lettuce in 40 L of sanitising solution. The depletion of free chlorine concentration due to the presence of high organic loads was

also reported in the washing solution of fresh-cut spinach (Gómez-López et al., 2014) and lettuce (Van Haute et al., 2013).

Continuous replenishment of chlorine into high organic concentration wash water can result in the formation of chlorine off-gas and carcinogenic halogenated compounds such as trihalomethanes and haloacetic acid in the processing environment, which pose dangers to workers' health and the environment (Luo et al., 2012; Yang et al., 2012). Although López-Gálvez et al. (2010) revealed that the trihalomethanes concentration in fresh-cut lettuce washed with 100 mg L^{-1} sodium hypochlorite and high concentration of organic matter (COD 700 mg L^{-1}) was negligible, the concentration of trihalomethanes in the process water was beyond the authorised limit set by European legislation (100 µg L^{-1}). Similarly, unacceptable concentrations of trihalomethanes were also detected in process water of fresh-cut spinach (Gómez-López et al., 2014) and lettuce (Van Haute et al., 2013). Although previous studies demonstrated that the concentration of trihalomethanes in fresh-cut produce washed with chlorinated water was below the legislated limit, maintenance of stable free chlorine requires periodic monitoring and intervention during fresh-cut produce processing. Process control failure due to the rapid depletion of free chlorine in the wash system in the presence of high organic loads has urged the food industry to develop economically viable, safe and environmental friendly alternative technologies to prolong the storage life of fresh-cut produce.

2.4.2 Other chemical treatments

2.4.2.1 Ozone

Ozone is a chemically active triatomic allotrope of the element oxygen which can be regenerated by ultraviolet radiation and corona discharge. High energy irradiation can split diatomic oxygen into free radicals that rapidly combine with oxygen to form ozone (Guzel-Seydim et al., 2004; Khadre et al., 2001). In 2001, U. S. Food and Drug Administration (FDA) declared ozone to be a Generally Recognised as Safe (GRAS) substance for the commercial use as a disinfectant and sanitiser in food handling (Aguayo et al., 2014; Khadre et al., 2001). However, ozone can rapidly decompose leaving no residue on food and hence provides an alternative to chlorine based washing solutions. Ölmez and Akbas (2009) revealed that waste water collected from an ozonated wash system of fresh-cut green leaf lettuce had lower COD with lower recovery of mesophilic bacteria when compared with waste water treated with chlorine. Garcia et al. (2003) also suggested that ozonated waste water can be reused and recycled to reduce excessive water consumption by the industry. The application of ozone has received commercial interest in the food industry due to its effectiveness to extend the shelf life of fresh or fresh-cut produce by inhibiting the growth of microorganisms (Beltrán et al., 2005; Silveira et al., 2010), preventing decay (Nadas et al., 2003; Tzortzakis et al., 2008) and removing pesticides and fungicides that reside on the surface of fruits and vegetables (Ikeura et al., 2011; Karaca et al., 2012).

Ozone in the aqueous or gaseous state has been applied to extend the shelf life of fresh-cut produce due to its broad antimicrobial effect. For instance, ozonated water at the concentration of 4 mg L^{-1} reduced the population of mesophilic, psychrotrophic and Enterobacteriaceae bacteria in fresh-cut iceberg lettuce by 1.7, 1.5 and 1.6 log CFU g^{-1}, respectively, after a 2 min treatment (Akbas & Olmez, 2007).

Akbas and Olmez (2007) reported that changes in the quality and nutritional attributes of ozonated fresh-cut iceberg lettuce were comparable to that of untreated lettuce throughout storage. Similarly, Zhang et al. (2005) demonstrated that the microbial load of fresh-cut celery treated with 0.18 mg L^{-1} ozonated water for 10 min was reduced by 1.2 log CFU g^{-1} after 9 days of storage at 4 ^{o}C. Quality of ozone treated fresh-cut celery was maintained throughout storage and the respiration rate and activity of PPO were reduced when compared to the control. The population of native microflora was also reduced in ozonated fresh-cut carrot (Chauhan et al., 2011), cilantro (Wang et al., 2004) and green leaf lettuce (Ölmez & Akbas, 2009).

The reduction of initial microbial loads of fresh-cut produce is mainly due to the antimicrobial effect of ozone that oxidizes the thiol group of cysteine residues in bacterial enzymes involve in respiration and maintenance of the homeostatis environment (Guzel-Seydim et al., 2004). Also, the strong oxidising potential of ozone can degrade the bacterial cell envelope, which consist of various components such as polyunsaturated fatty acids, glycoproteins and glycolipids (Khadre et al., 2001). Hence, a chain reaction triggered by ozone often leads to cell lysis due to subsequent leakage of cell contents and rapid oxidation of cellular protein.

Although Aguayo et al. (2006) reported that cyclic exposure to 4 µL L^{-1} of gaseous ozone for 30 min every 3 h reduced respiration rate and ethylene emission of sliced tomato throughout storage, there was little reduction of yeast population. Fresh-cut melon treated with 0.4 mg L^{-1} ozonated water for 5 min also had little reduction in the population of yeast and mould, while respiration rate increased during storage. Adverse effects of ozone on ASC and phenolic concentration were also reported in fresh-cut carrot (Chauhan et al., 2011) and iceberg lettuce (Beltrán et al., 2005), which accompanied a reduction of microbial populations.

Inconsistent results obtained in various ozone experiments may be due to the leaching of plant exudates from the cut surface of fresh-cut produce. Ozone can readily react with organic compounds and be inactivated before it can react with microorganisms on the cut surface (Alexandre et al., 2011a; Ketteringham et al., 2006). In addition, the low pH of fresh-cut produce can affect the efficiency of ozone since it is more stable and effective in oxidising bacteria cell membranes at low pH (Khadre et al., 2001).

2.4.2.2 Electrolysed Water

Electrolysed water was originally developed in Japan and Russia to decontaminate and regenerate processing water and disinfect medical instruments (Hricova et al., 2008; Huang et al., 2008). It has strong bactericidal effects against a broad spectrum of spoilage microorganisms due to its low pH, high oxidation-reduction potential (ORP) and presence of free residual chlorine (Martínez-Hernández, Navarro-Rico, Gomez et al., 2015). Electrolysed water is generated by electrolysing a dilute sodium chloride (NaCl) solution with a current across an anode and cathode that are separated by a bipolar membrane. Electrolysis of the salt solution can produce reduced substances with strong biocidal activity such as hypochlorous acid (HOCl), hypochlorite ion (OCl^-), hydroxyl (OH^-) and superoxide radicals ($O_2^{\bullet-}$) (Abadias et al., 2008; Pinto et al., 2015).

Ionization resulting from different current voltage at the anode will result in the formation of acidic electrolysed water that has a pH range of 2 – 4, ORP value > 1000 mV and free chlorine concentration (FCC) of up to 50 mg L^{-1} (Abadias et al., 2008; Mukhopadhyay & Ramaswamy, 2012). Whereas, alkaline electrolysed water with a pH range of 10 – 11.5 and an ORP value of -800 mV is formed at the cathode (Huang et al., 2008). In the absence of a bipolar membrane, the mixture of acidic and alkaline solutions produces neutral electrolysed water, which is characterised by

a pH range of 5 – 8.5, ORP value of 500 – 700 mV and FCC of 10 – 30 mg L^{-1} (Graça et al., 2011; Hao et al., 2015).

The application of electrolysed water as an eco-innovative sanitiser has gained interest in the fresh-cut industry considering its potential advantages, including strong bactericidal effect, negligible residual contamination, minimal corrosion of processing equipment or skin irritation and low operational cost (Hao et al., 2015; Hricova et al., 2008). Martínez-Hernández et al. (2015b) observed a 1.6 log CFU g^{-1} reduction of *E. coli* and *S.* enteritidis on inoculated fresh-cut kalian hybrid broccoli following a 2 min exposure to neutral electrolysed water (FCC: 100 mg L^{-1}, pH 7, ORP: +900 mV). Similarly, after 5 min application of neutral electrolyzed water (FCC: 200 mg L^{-1}, pH 8.2, ORP: +846 mV), the native population of mesophilic aerobic and Enterobacteriaceae bacteria in fresh-cut catalogna chicory was inhibited (Pinto et al., 2015). In the cited study, the antimicrobial effect of neutral electrolysed water was prolonged during cold storage, as the population of total mesophilic aerobic and Enterobacteriaceae bacteria was reduced by 0.9 and 2.0 log CFU g^{-1}, respectively, after 3 days of storage at 4 ^{o}C

Hao et al. (2015) also showed that the application of slightly acidic electrolysed water (FCC: 20 mg L^{-1}, pH 6, ORP: +800 mV) reduced the native microflora (total aerobic bacteria, coliforms, and yeast and moulds) of fresh-cut cilantro by approximately 1.5 log CFU g^{-1}. Interestingly, a reduction of 2 log CFU g^{-1} on the initial total aerobic plate count of fresh-cut iceberg lettuce was observed after treatment with neutral electrolysed water (FCC: 30 mg L^{-1}, pH 8, ORP: N/A) and the results obtained were comparable to samples treated with sodium hypochlorite with a high FCC (200 mg L^{-1}) (Vandekinderen et al., 2009). The efficacy of neutral and acidic electrolysed water against artificially inoculated bacteria or native microflora

also has been reported in fresh-cut lettuce (Abadias et al., 2008; Rico et al., 2008), broccoli (Navarro-Rico et al., 2014) and apple (Tomás-Callejas et al., 2011).

Recently, Hao et al. (2015) reported that an additional 1 log CFU g^{-1} reduction in the native microflora (total aerobic bacteria, coliforms and yeast and moulds) of fresh-cut cilantro was observed when subjected to sequential washes of alkaline electrolysed water (FCC: N/A, pH 11.7, ORP: -820 mV) followed by acidic electrolysed water (FCC: 68 mg L^{-1}, pH 2.5, ORP: 1127 mV) for 5 min each. Similarly, the combination of alkaline electrolysed water (FCC: N/A, pH 11, ORP: -800 mV) and 1% citric acid at 50 ^{o}C for 3 min, inhibited the total microbial and yeast and mould counts by approximately 3.7 log CFU g^{-1} in shredded carrot (Rahman et al., 2011) when compared to each treatment alone. In the cited study, authors suggest that mild heat treatment for short duration enhances the effect of sanitising agent to kill or remove spoilage microorganisms on shredded carrot.

High ORP of electrolysed water can inflict damage on cell membrane and allow better penetrability of HOCl through the membrane resulting in bacteria necrosis (Huang et al., 2008; Navarro-Rico et al., 2014). Besides high ORP, low pH of acidic electrolysed water also can induce cell permeabilitisation, which allows the entry of HOCl into the bacterial cell. In the presence of HOCl, OH^{-1} radicals with strong antimicrobial activity are produced and induce the oxidative decarboxylation of amino acids to nitrites and aldehydes, which disrupts of protein synthesis (Huang et al., 2008; Pinto et al., 2015).

2.4.2.3 Hydrogen Peroxide

H_2O_2 has been proposed as an alternative to chlorine in the food industry and can be generated by electrolytic oxidation of sulphuric acid or electrical discharge through a mixture of hydrogen, oxygen and water vapour (FDA, 2000a). It has been classified

as a GRAS substance for use in various food products (milk, dried egg, wine, starch and instant tea) as a bleaching, oxidising and antimicrobial agent in the concentration range of 0.04 – 1.25% (FDA, 2000a). Although the application of H_2O_2 in fresh or fresh-cut fruits and vegetables still awaits approval from the Food and Drug Administration (FDA), considerable research has been conducted on the use of H_2O_2 as an antimicrobial agent against various food spoilage and pathogenic microorganisms on blueberry (Li & Wu, 2013), lettuce (Back et al., 2014), baby spinach (Huang et al., 2012), red bell pepper, strawberry, watercress (Alexandre et al., 2012) and button mushroom (Guan et al., 2013).

Being a potent oxidant, the generation of cytotoxic oxidising species such hydroxyl free radicals can cause lethal damage to bacterial cell by inducing cell permeabilitisation and attacking essential cell components such as lipids, proteins and DNA (Schenk et al., 2012). It also has been proposed that H_2O_2 can facilitate the destruction of microorganisms by destroying biofilms and exposing the microbial cells (Martin & Maris, 2012). Besides antimicrobial activity against a wide range of microorganisms, H_2O_2 can be decomposed into oxygen and water by the enzyme catalase, which is naturally found in plants and hence it does not form carcinogenic residues (Alexandre et al., 2012; Ölmez & Kretzschmar, 2009).

Ukuku (2004) reported that treatment of whole cantaloupe and honey dew melons with 2.5% H_2O_2 for 5 min reduced the transfer of aerobic bacteria to fresh-cut pieces by approximately 2 log CFU g^{-1}. In the cited study, the recovery of *Salmonella* from fresh-cut pieces after washing with 2.5% H_2O_2 was lower than from controls. The application of 0.9% H_2O_2 for 1 min also suppressed decay and browning incidence in fresh-cut Chinese water chestnut during 18 days of storage at 4 ^{o}C (Peng et al., 2008). The reduction of psychrotrophic bacteria and yeast and mould counts in fresh-cut 'Galia' melon treated with H_2O_2 showed no difference with chlorine treated

samples after storage for 10 days at 4 ^{o}C, however, an additional reduction of approximately 0.5 log CFU g^{-1} in the growth of mesophilic and Enterobacteriaceae bacteria was achieved in hydrogen peroxide treated samples when compared with that of chlorine (Silveira et al., 2008).

Although the reduction in the native microflora (mesophilic, psychrotrophic and Enteriobacteriaceae) counts in H_2O_2 (167 mg L^{-1} for 3 min) treated fresh-cut watercress was more effective than chlorine, total phenolic and antioxidant concentration of hydrogen peroxide treated samples were reduced drastically throughout storage (Hinojosa et al., 2013). Kim et al. (2007) also reported that the total phenolic concentration, antioxidant capacity and ASC concentration of fresh-cut tomato treated with 0.4 M of hydrogen peroxide for 1 min were reduced throughout storage compared to unwashed samples. Similarly, a combination treatment of 3% hydrogen peroxide for 5 min and 3.7 kJ m^{-2} UV-C for 7.5 min retained optimal microbiological stability throughout storage at 5 ^{o}C, however, the treatment resulted in higher browning incidence when compared to that of the control (Schenk et al., 2012).

Considering the phytotoxic effects of H_2O_2 which induce browning and degradation of nutraceutical compounds in fresh-cut produce, appropriate physical and chemical methods have to be adopted during processing to remove residual H_2O_2.

2.4.3 Physical treatments

2.4.3.1 Ultraviolet (UV) Radiation

Many studies have revealed that ultraviolet radiation is a promising non-thermal technology used to disinfect and decontaminate food surfaces and packaging. The wavelength range of ultraviolet (UV) radiation (100 – 400 nm) can be further subdivided into short wave UV (UV-C), medium wave UV (UV-B) and long wave UV

(UV-A) (Kim et al., 2013). UV-C light in the range of 200 – 280 nm can be as effective as sodium hypochlorite or ozone to surface decontaminate and prolong the storage life of various fresh-cut produce (Alothman et al., 2009; Maghoumi et al., 2013). The strongest microbiocidal effect of UV-C falls between 250 – 260 nm since the peak effectiveness of UV absorption by DNA is close to that range (Graça et al., 2013; Kim et al., 2013). This alternative technology has attracted attention of the fresh-cut industry since it is inexpensive to setup and operate and it does not generate chemical residues that reside on the surface of fresh-cut products (Allende et al., 2006; Rico et al., 2007).

Research has suggested that UV-C doses ranging from 0.5 – 20 kJ m^{-2} can inhibit the growth of microorganisms by cross-linking aromatic amino acids at the carbon to carbon double bonds that can cause membrane depolarisation, abnormal ionic flow, photochemical oxidation and pyrimidine dimer formation (Allende et al., 2006; Martínez-Hernández et al., 2015a). Alteration of the DNA helix by distortion of the sugar phosphate backbone may inhibit microbial DNA replication and transcription causing cell death and mutation (Artés et al., 2009; Kim et al., 2013). For example, the population of *E. coli*, *L. innocua* and *S. enterica* in artificially inoculated fresh-cut apple was reduced by approximately 1.5 – 1.9 log CFU g^{-1} following UV irradiation at 1.0 kJ m^{-2} (Graça et al., 2013). The counts of the three bacteria strains were lower in irradiated fruit than in control fruit after 7 and 15 days of storage at 4 ^{o}C and the reduction was comparable to that in sodium hypochlorite treated fresh-cut apple. Similarly, when compared with unirradiated samples, 7.5 kJ m^{-2} of UV-C radiation reduced the population of *E. coli* and *S. enterica* on inoculated fresh-cut kalian-hybrid broccoli by 1.3 and 2.1 log CFU g^{-1}, respectively (Martínez-Hernández et al., 2015a). Approximately 1 – 2 log CFU g^{-1} reduction in the population of native microflora also

has been reported in UV-C radiated fresh-cut pomegranate arils (Maghoumi et al., 2013) and watermelon (Artés-Hernández et al., 2010).

In contrast, the application of UV-C doses from 4.54 – 11.35 kJ m^{-2} inhibited the growth of mesophilic and Enterobacteria in fresh-cut spinach, but no residual inhibitory effect was observed on the population of psychrophilic bacteria throughout storage (Artés-Hernández et al., 2009). Allende et al. (2006) also showed that UV-C radiation did not inhibit the growth of yeast in fresh-cut lettuce as the population for all samples was beyond the microbial recommended limit after stored for 5 days at 5 ^{o}C. Similarly, the population of spoilage microorganisms in UV-C radiated showed no difference with unirradiated samples and reached the maximum microbial limit as suggested by the Spanish Legislation following 13 days of storage at 5 ^{o}C (López-Rubira et al., 2005) as well. Inconsistent results obtained in various UV-C treated fresh-cut produce may be due to different composition of produce and site of microorganism attachment on the product (Allende et al., 2006; Tomás-Callejas et al., 2012). It has been suggested that UV-C does not penetrate the produce tissues, so bacteria that are attached in cracks and crevices of fresh-cut produce may not be exposed directly to UV-C radiation (Graça et al., 2013).

In addition to lethal germicidal effects, it has been proposed that UV-C radiation can induce biological stress and the development of defence mechanisms against microorganisms and senescence process in plant tissues (Alothman et al., 2009; González-Aguilar et al., 2007a). These inducible effects are often accompanied by the accumulation of secondary metabolites with increased antioxidant activity and alteration in the activity of degradative enzymes. For instance, the total phenol concentration and antioxidant activity of UV-C irradiated fresh-cut Tatsoi baby leaves were increased by 24% and 9%, respectively, after storage for 4 days at 5 ^{o}C (Tomás-Callejas et al., 2012). Changes in the antioxidant capacity also have been reported

in fresh-cut mango (González-Aguilar et al., 2007a), carrot (Alegria et al., 2012), pineapple, guava, and banana (Alothman et al., 2009). Interestingly, the activity of enzymes (PPO, POD, PME and PG) in 'Galia' melon treated with 4.8 kJ m^{-2} of UV-C radiation was lower than unirradiated samples throughout storage (Chisari et al., 2011). The reduction of these enzymes in UV-C treated melon coincided with higher firmness and lower colour changes when compared with unirradiated samples following 10 days of storage at 5 ^{o}C.

2.4.3.2 High Pressure Processing

High hydrostatic pressure is a non-thermal technology that is increasingly used in the food industry to inactivate a broad spectrum of foodborne pathogens and food deteriorative enzymes with minimal effect on the overall quality, nutraceutical properties or flavour of liquid and solid foods (Jung et al., 2013; Ortega et al., 2013). Pressurisation of food products can be carried out using pressure between 100 – 800 MPa at process temperatures ranging from 0 – 100 ^{o}C with a recommended practical exposure time up to 20 min (FDA, 2000b; Maitland et al., 2011). Studies have suggested that the application of pressure ranges from 300 – 600 MPa can effectively inhibit the growth of most microorganisms and maintain the overall quality of food products (Maitland et al., 2011; Ortega et al., 2013).

Interestingly, chemical changes in high hydrostatic pressurised food products are minimal as the covalent bonds are not broken during treatment (Liu et al., 2013a). Instead, weaker bonds such as van der Waals forces, electrostatic interactions and hydrogen bridges are easily affected by the applied pressure (Mújica-Paz et al., 2011). Another advantage of high hydrostatic pressure processing is pressure at a given position and time during treatment can be distributed instantaneously and uniformly on food products irrespective of different food composition, size and shape (FDA, 2000b; Mújica-Paz et al., 2011). Many studies

have shown that high hydrostatic pressure processing can be efficiently used to preserve and enhance the microbial stability of various food products such as fruit puree (Guerrero-Beltran & Barbosa-Cánovas, 2004; Krebbers et al., 2003), fruit juices (Alphas & Bozoglu, 2000; Lavinas et al., 2008), fresh commodities (Jung et al., 2013; Maitland et al., 2011) and meat (Moerman, 2005).

Germicidal effects of high hydrostatic pressure against various pathogen bacteria have been reported in several fresh-cut fruits and vegetables. For instance, Liu et al. (2013a) reported that the population of total aerobic bacteria and yeast and mould in mango pulp was reduced by approximately 4.5 and 3.9 log CFU g^{-1} after being pressurised at 600 MPa for 1 min. Similarly, the growth of *S. enterica* serovar Braenderup inoculated in diced tomato was reduced by 3.7 log CFU g^{-1} following exposure to high hydrostatic pressure treatment at 550 MPa for 2 min (Maitland et al., 2011). Upon pressurisation at 500 MPa with a holding time for 10 min, the number of *L. monocytogenes*, *S. aureus* and *S*. Thyphimurium inoculated in various fresh-cut vegetables were inhibited to below the detection limit (Jung et al., 2014).

However, irreversible changes in the structure of important macromolecules such as deoxyribonucleic acid (DNA) and protein during high hydrostatic pressure processing can induce permeabilisation of cytoplasmic membrane leading to vegetative cell damage (Mújica-Paz et al., 2011; Ross et al., 2003). High pressure causes tighter packing of the acyl chains within the membrane's phospholipid bilayers and reduces cross sectional area per phospholipid molecules which can promote the permeabilisation of cell membrane (Mañas & Pagán, 2005; Ross et al., 2003). Disruption of the cell membrane often results in extensive leakage of solutes and loss of osmotic responsiveness and hence cell death.

Recently, a novel technique involving the combination of high pressure and inert gas treatment has been reported to enhance the storage life and inhibit the

growth of microorganisms in various fresh-cut produce. For instance, the shelf life of fresh-cut pineapple treated with pressurised argon at 1.8 MPa for 60 min was extended by 6 days as the proliferation of native microflora was slowed down throughout storage when compared with that of control (Wu et al., 2012a). Wu et al. (2013) also reported that the combination of pressurised argon and xenon inhibited the growth of *E. coli* and *S. cerevisiae* inoculated in fresh-cut apple and pineapple throughout 7 days of storage at 10 °C. The formation of inert gas hydrate under high pressure and low temperature in fresh-cut fruits and vegetables can inhibit the intracellular water activity and restrain metabolism and bioactivity in microorganisms (Wu et al., 2012a). Research on this technique may be limited due to its high setup cost.

2.4.4 Edible Coating

Biodegradable and environmental friendly materials that are used to wrap and extend the shelf life of food products with or without further removal, form the basic idea of edible coatings (Azarakhsh et al., 2014a; Mantilla et al., 2013). Edible coatings, which utilise protein, lipid and polysaccharides as their raw materials are a promising alternative technology in the fresh-cut industry to meet challenges related to safety, quality and economic production cost (Ghidelli et al., 2014; Leceta et al., 2015).

The formation of semipermeable barrier by edible coatings can reduce the deleterious effects of minimal processing by slowing down moisture and solute migration, gas exchange, respiration rates, flavour loss and physiological disorders of fresh-cut fruits and vegetables (Benitez et al., 2014; Maya-Meraz et al., 2014). For example, Azarakhsh et al. (2014a) reported that the application of 0.56% gellan based edible coating for 2 min was able to reduce respiration rate, colour change, and maintain firmness and sensory characteristics of fresh-cut pineapple throughout storage at 5 °C. In that study, the formulation of gellan coating showed no

antimicrobial effects against the native microflora of fresh-cut pineapple. Similarly, the growth of aerobic psychrophilic bacteria and yeast and mould in polysaccharides-based (gellan, alginate and pectin) edible coated fresh-cut melon was not inhibited during storage in polysaccharides-based (gellan, alginate and pectin) edible coated fresh-cut melon, but the coating prevented desiccation and maintained fruit firmness (Oms-Oliu et al., 2008). Interestingly, gellan based edible coating was able to maintain high concentration of vitamin C in fresh-cut melon throughout storage in comparison to control. Application of 5% aloe vera dipped for 15 min reduced the respiration rate and maintained the firmness and green pulp of fresh-cut kiwifruit throughout during 11 days of storage at 4 ^{o}C although the growth of mesophiles and yeast and mould was only reduced by 0.21 – 0.99 log CFU g^{-1} (Benítez et al., 2013).

Studies also have suggested that some coating materials can improve food appearance and inhibit the growth of pathogenic and spoilage microorganisms (Alvarez et al., 2013; Martiñon et al., 2014). González-Aguilar et al. (2009) reported that the population of mesophilic bacteria and yeast and mould was reduced by 2.4 and 2.3 log CFU g^{-1}, respectively, in chitosan coated (2% medium molecular weight for 2 min) fresh-cut papaya after storage for 7 days at 5 ^{o}C. Changes in firmness and colour of chitosan treated papaya were reduced compared to control fruit and polygalacturonase activity was 30% less. Similarly, the growth of native microflora (mesophilic, psychrotrophic, coliforms and yeast and mould) in chitosan dipped (2% medium molecular weight chitosan for 3 min) fresh-cut broccoli was effectively inhibited by 0.8 – 2.5 log CFU g^{-1} throughout 20 days of storage at 5 ^{o}C (Moreira et al., 2011).

Considering the potential of polysaccharides based edible coatings to form an effective semi protective barrier, studies have suggested the incorporation of different active agents such as natural antioxidants and antimicrobial agents to further

enhance the polysaccharides formulation (Mantilla et al., 2013; Oms-Oliu et al., 2008). Maya-Meraz et al. (2014) reported that the addition of isoleucine to an alginate coating enhanced the production of 2 methyl-1-butanol and 2-methyl butyl acetate in fresh-cut apples, which were the main volatile compounds that contribute to apple flavour. The incorporation of 0.3% of lemongrass oil in 1.29% of alginate coating solution reduced total plate and yeast and mould count while maintaining the firmness, colour and sensory attributes of fresh-cut pineapple (Azarakhsh et al., 2014b).

2.5 Ultrasound

2.5.1 Principle of ultrasound

Ultrasound technology is a form of vibrational energy in the frequency range of 20 – 100 kHz which is beyond the threshold of human hearing (Awad et al., 2012). Based on the amount of energy used which is often measured by sound power (W), intensity (W m^{-2}) and energy intensity (W s^{-1} m^{-3}), the application of ultrasound in various industries can be categorised into low and high intensity ultrasound (O'Donnell et al., 2010; São José et al., 2014). Low energy ultrasound is characterised by power intensity less than 1 W cm^{-2} at high frequency (>1 MHz) and commonly used in medical diagnostic (São José et al., 2014). In the food industry, small amplitude of sound waves is used to conduct non-destructive analysis and access physico-chemical properties of food materials such as their composition, viscosity and structure (Soria & Villamiel, 2010). Contrarily, high power ultrasound with the power intensity range from 10 – 1000 W cm^{-2} at low frequency (16 – 100 kHz) has wide application in food processing, preservation and safety (Pingret et al., 2013; Sango et al., 2014). High amplitude of sound waves can induce and alter the properties of food physically or chemically (Soria & Villamiel, 2010). The disruptive properties of power ultrasound have been applied in the extraction of bioactive compounds, inactivation of microbial growth and enzymes activity, emulsification, and surface cleaning (Awad et al., 2012).

2.5.2 Microbial inactivation of ultrasound

During sonication process, longitudinal waves are created when sound energy passed to liquid medium thereby creating regions of alternating compression and rarefraction. These regions of pressure change cause cavitation to occur and bubbles are formed from gas nuclei existing within the fluid. These bubbles have a large surface area during the expansion cycle as the pressure is at minimum, which

increases the diffusion of gas and causes the bubbles to expand (Patist & Bates, 2008; Piyasena et al., 2003). Stable changes in the size of bubbles during the oscillation of ultrasonic waves can result in stable cavitation and generate acoustic micro-agitation on the medium (Cárcel et al., 2012; Kentish & Feng, 2014). However, these bubbles become unstable and collapse violently when the ultrasonic energy provided resonates with the fluctuation of the bubble wall (Sango et al., 2014). The condition within these implosion of bubbles leads to energy accumulation in hot spots and generate high temperature (5000 K) and pressure (1000 atm) which in turn produce very high shear energy waves and turbulence in the cavitation zone (Bilek & Turantaş, 2013; Chandrapala et al., 2012). An illustration of the cavitation phenomenon is shown in Figure 2.5.

Disruption of cellular structure and subsequent leakage of cellular components due to high pressure and temperature can lead to cell lysis (Chemat et al., 2011; São José et al., 2014). Chandrapala et al. (2012) suggested that the hydrophobic surface of microorganism also can promote the collapse of cavitation bubbles and lead to severe damage on the cell wall. The presence of multi-layered hydrophobic cuticles and uneven surfaces of some fresh fruits and vegetables may provide some protection for the bacterial cells and make an effective sanitising of the fresh produce to be difficult (São José et al., 2014). Acoustic microstreaming associated with strong shear force which is produced during the oscillation of ultrasound waves and subsequent collapse of cavitation bubbles can be used to remove microorganisms on the surface and cracks and crevices of fresh produce (Kentish & Feng, 2014; Soria & Villamiel, 2010). The shear force can break the cell wall and membrane of microorganism up to the point of cell lysis (Bilek & Turantaş, 2013; São José et al., 2014).

Another important effect is that water molecules can be broken generating highly reactive free radicals that may react and modify with other molecules (Awad

et al., 2012; Soria & Villamiel, 2010). Free radicals such as OH^- can react with the sugar phosphate backbone of the DNA chain by removing hydrogen atoms from the sugars (Bilek & Turantaş, 2013). The double stranded microbial DNA will be broken through the scission of the phosphate ester bond (Chandrapala et al., 2012; Mañas & Pagán, 2005).

The physical forces and highly reactive free radicals generated by the oscillation of ultrasound waves have received considerable interest in the food industry. *In vivo* studies demonstrated that sonication at low frequency (20 kHz) for 10 min reduced the individual viable *Bacillus subtilis* counts (Joyce et al., 2003). A number of studies also have reported that the antimicrobial mechanisms of ultrasound is effective against a wide range of pathogenic microorganisms which cause deteriorative changes in various fruit juices. For instance, the growth of *E. coli* O157:H7 and *S.* enteritidis inoculated in mango juice was reduced approximately 5 and 9 log CFU g^{-1} following exposure to sonication at 20 kHz, 200 W for 7 min (Kiang et al., 2013). A 5 log cycle reduction of *E. coli* was also reported in ultrasound treated (24 kHz, 85 W cm^{-2}, 10 min) apple juice (Salleh-Mack & Roberts, 2007). The efficacy of ultrasound against various spoilage microorganisms also has been shown in ultrasound treated orange (Valero et al., 2007), tomato (Adekunte et al., 2010) and blackberry (Wong et al., 2010) juices.

Besides fruit juices, spoilage microorganisms that often limit the storage life of intact or fresh-cut fruits and vegetables can be inactivated using ultrasound waves as well. As shown in Table 2.2, ultrasound frequency ranges from 20 kHz – 45 kHz and treatment time from 1 – 10 min are commonly applied in the washing procedures of intact or fresh-cut fruits and vegetables. The combination of different ultrasound parameters such as power, temperature and time can result in 0.5 – 2.0 log of microbial reduction (Bilek & Turantaş, 2013). In most of studies, lettuce which is

commonly associated with the outbreak of foodborne illness is used to study the decontamination efficiency of ultrasound (Elizaquível et al., 2012; Forghani et al., 2013).

Cao et al. (2010a) reported that the decay incidence of ultrasonicated strawberry (40 kHz, 350 W, 10 min) was reduced by 44% when compared with that of control. At the end of storage, the microbial population of treated strawberry was decreased by 0.80 log CFU g^{-1}. Based on the results obtained, authors showed that frequency is one of the parameters affecting the efficacy of ultrasound. São José et al. (2014) suggested that the size of bubbles formed during ultrasonication is inversely proportional to acoustic frequency. Formation of larger bubbles at lower frequency of ultrasonic waves can result in higher localised pressure and temperature due to more collapsed event of cavitation bubbles (Kentish & Feng, 2014).

Another optimisation study was carried out by Cao et al. (2010b) on the decontamination efficiency of ultrasound (40 kHz) at different powers (250, 350 and 400 W) and treatment times (5, 10 and 15 min) on strawberry. After storage for 8 days at 5 ^{o}C, strawberry treated with 250 W of ultrasound for 10 min showed the highest reduction in total microbial and yeast and mould counts which were 2.42 and 2.45 log CFU g^{-1}, respectively. Based on the response surface methodology (RSM) analysis, authors found that ultrasound at 250 W for 10 min was the optimised conditions to extend the storage life of strawberry by inhibiting the growth of spoilage microorganisms. Kentish and Feng (2014) suggested that stagnant cloud bubbles can be formed upon too much of energy is being applied to the ultrasonic probe which can prevent the uniform transmission of acoustic energy.

Birmpa et al. (2013) showed that ultrasonication (37 kHz, 30 W) for 45 min resulted in a reduction of more than 2 log cycles in the population of *E. coli*, *L. innocua*, *S.* enteritidis and *Staphylococcus aureus* inoculated on lettuce and

strawberry. Similarly, the microbial count of *L. innocua* inoculated on red bell pepper was reduced by 1.9 log CFU g^{-1} upon exposure to ultrasonication operated at 35 kHz and 350 W for 2 min. However, a reduction of only 1.4 log cycle was achieved when the samples were washed with water (Alexandre et al., 2013). Seymour et al. (2002) also reported that upon exposure to ultrasonication (32 – 40 kHz, 10 W L^{-1}) for 10 min, the counts of *S.* typhimirium inoculated on fresh-cut iceberg lettuce was reduced by 1.6 log CFU g^{-1} and the result obtained was comparable with that of chlorine which decreased by 1.7 log CFU g^{-1}.

However, some studies have revealed that the combination of ultrasound with other chemical sanitisers such as organic acid, chlorine dioxide, calcium propionate and salicylic acid may result in higher reduction of microbial population. For instance, the combination of ultrasound (40 kHz, 30 W L^{-1}, 5 min) with different organic acids (2% malic, lactic and citric acid) resulted in an additional 0.8 – 1.0 log CFU g^{-1} reduction of *E. coli* O157:H7, *S.* typhimurium and *L. monocytogenes* inoculated in fresh lettuce (Sagong et al., 2011). Similarly, Huang et al. (2006) demonstrated that an additional of 1.0 – 1.2 log cycle reduction on the growth of *Salmonella spp* and *E. coli* O157:H7 inoculated on apple and lettuce was achieved upon exposure to combined treatment of ultrasound (170 kHz, 10 min) with 40 mg L^{-1} chlorine dioxide.

Recently, Ding et al. (2015) showed that the antimicrobial efficacy of ultrasound (40 kHz, 250 W, 10 min) against mesophiles and yeast and mould counts in cherry tomato and strawberry was improved by an additional of 0.88 – 1.06 log cycle of reduction when combined with slightly acidic electrolysed water (SAEW: 34 mg L^{-1} FCC; ORP: 854 mV; pH 6.5). The microbial counts of mesophilic, psychrotrophic and yeast and mould of plum fruit were reduced by 3.7, 3.9 and 2.9 log CFU g^{-1}, respectively, after exposed to ultrasound (40 kHz, 100 W, 10 min) combined with 40 mg L^{-1} chlorine dioxide treatment (Chen & Zhu, 2011). In the cited

study, the reduction of native microflora was higher in combined treatment in regards to ultrasound or chlorine dioxide alone.

Considering the non-uniformity distribution of acoustic energy in the ultrasonic treatment chamber due to the blockage of ultrasonic waves by the product to be processed, Zhou et al. (2012) designed a pilot scale continuous-flow ultrasonic washing system with three pairs of transducer operating at different frequency (25, 40 and 75 kHz) to ensure uniform distribution of acoustic energy. In the cited study, the reduction of aerobic bacteria, yeast and mould and *E.coli* population was increased by 38, 13 and 19%, respectively, when compared to that of chlorine treatment alone.

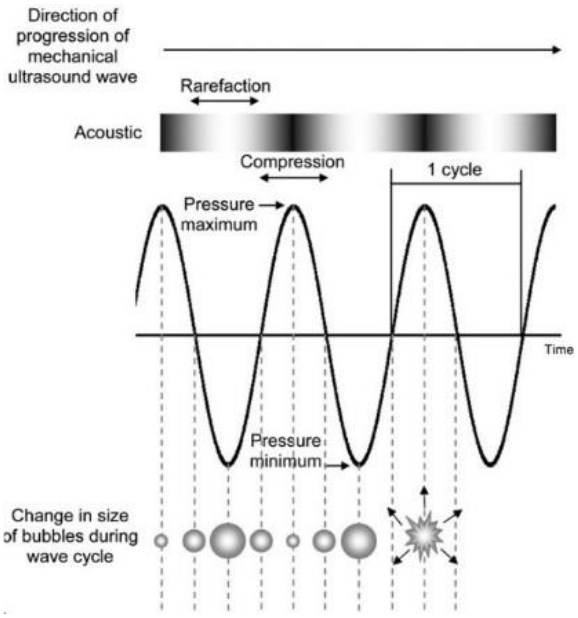


Figure 2.5 Formation of cavitation bubbles during thousand cycles of oscillating ultrasound waves (Soria & Villamiel, 2010).

2.5.3 Effect of ultrasound on fresh/fresh-cut fruits and vegetables

Formation of free radicals during sonication may react easily with the food components such as phenols and hence affect quality of foods. However, several studies have suggested that the presence of free radicals can affect the degree of hydroxylation in fresh fruits and vegetables and enhance the antioxidant activity of other components such as flavonoids (Awad et al., 2012; Soria & Villamiel, 2010).

For instance, Cao et al. (2010a) reported that low decay incidence in ultrasound treated (40 kHz, 350 W, 10 min) strawberry was associated with 26% of higher ascorbate concentration in regards to control after storage for 8 days at 5 ^{o}C. The total phenolic concentration of peach fruit treated with ultrasound (40 kHz, 350 W, 10 min) combined with 0.05 mM salicylic acid was 42.9% higher than that of untreated samples (Yang et al., 2011). Similarly, ultrasound was also found to induce the total flavonoids and ascorbate concentration of plum fruit during storage (Chen & Zhu, 2011).

Besides the induction of antioxidant activity in various fresh fruits and vegetables, mechanical and chemical effects of acoustic cavitation can lead to the inactivation and activation of enzymes. Acoustic streaming which is associated with high shear forces can disrupt the hydrogen bonding and Van der Waals forces of polypeptide chain and result in the conformational change of the secondary and tertiary structure of protein (Chandrapala et al., 2012). Binding of hydroxyl and hydrogen free radicals generated during sonication with some of the amino acid residues that are responsible for substrate binding, stability and catalytic of enzymes can result in the alteration of biological activity (Kentish & Feng, 2014; São José et al., 2014). Studies have suggested ultrasound mechanisms of ultrasound inactivation is dependent on the amino acid composition and conformational structure of protein

(O'Donnell et al., 2010). Hence, different enzymes have different resistance against ultrasound.

For instance, the application of ultrasound (20 kHz) at high temperature (85 ^{o}C) resulted in higher inhibition of POD activity in watercress when compared to that of blanching alone (Cruz et al., 2006). Similarly, Jang and Moon (2011) reported that the synergistic effect of ultrasound (40 kHz) and 1% ascorbate had inhibited the activity monophenolase, diphenolase and PPO better than that of individual treatment which showed limited inhibitory effects on the enzymes. The inhibitory effect of ultrasound was also observed in melon juice in which the activities of PPO, POD and APX was reduced following ultrasound treatment (19 kHz, 376 W cm^{-2}) for 10 min (Fonteles et al., 2012).

Therefore, chemical effects of acoustic cavitation may be either detrimental or beneficial which depend on the dynamic of ROS accumulation and induction of enzymatic and antioxidant capacity in ultrasound treated fresh or fresh-cut fruits and vegetables.

Table 2.2 Parameters of ultrasound or combination of ultrasound treatment on various fresh or fresh-cut fruit and vegetables.

Treatment	Parameters	Products	References
US alone	Bath: 40 kHz; 200 W; 5 min	Fresh-cut potato	Amaral et al., 2015
US + slightly acidic electrolysed water	Bath: 40 kHz; 250 W; 10 min SAEW: 34 mg L^{-1} FCC; ORP: 854 mV; pH 6.5	Cherry tomato Strawberry	Ding et al., 2015
US + oregano essential oil	Probe: 26 kHz; 200 W; 5 min EO: 0.025% (v/v)	Lettuce	Millan-Sango et al., 2015
US + ozone US + ClO_2	Probe: 20 kHz; 30 W; 5 min Ozone: 0.075 mg L^{-1} ClO_2: 6 mg L^{-1}	Strawberry	Aday & Caner, 2014
US + heat shock + citric acid	Bath: 40 kHz; 180 W; 7.5 min Heat shock: 50 ^{o}C; 3 min Citric acid: 1.5% (w/v); 3 min	Fresh-cut broccoli	Ansorena et al., 2014
US + Tsunami	Probe: 25 kHz; 2000 W; 1 min Tsunami: active ingredient peroxyacetic acid	Iceberg Romaine	Salgado et al., 2014a
US + chlorine US + Tsunami	Probe: (25, 40 and 75 kHz); 2000 W; 2 min Chlorine: 60 mg L^{-1} FCC Tsunami: 80 mg L^{-1} peroxyacetic acid	Fresh-cut iceberg	Salgado et al., 2014b
US + citric acid	Bath: 40 kHz Citric acid: 1% (w/v)	Green peppers Melon	São José et al., 2014
US alone	Probe: 40 kHz; 30 W; 5 min	Strawberry	Aday et al., 2013

Treatment	Parameters	Products	References
US alone	Bath: 37 kHz; 30 W; 45 min	Romaine lettuce Strawberry	Birmpa et al., 2013
US + electrolysed water	Bath: 40 kHz; 400 W L^{-1}; 3 min Electrolysed water: 5 – 10 mg L^{-1} FCC; 660 -770 mV; pH 7.4; 3 min	Lettuce	Forghani et al., 2013
US + Tween 20	Bath: 40 kHz; 30 W L^{-1}; 5 min Tween 20: 0.1% (v/v)	Lettuce Carrot	Sagong et al., 2013
US + CaO	Bath: 20 kHz; 130 W; 10 min CaO: 2% (w/v)	Lettuce Radish sprout Apple slices	Yoon et al., 2013
US + peracetic acid	Bath: 45 kHz; 20 W; 10 min Peracetic acid: 40 mg L^{-1}	Cherry tomato	São José & Dantas Vanetti, 2012
Continuous flow US + chlorine	Probe: 25, 40 and 75 kHz; 2000 W; 1 min Chlorine: 50 mg L^{-1}	Baby spinach	Zhou et al., 2012
US + heat	Bath: 35 kHz; 130 W; 2 min Temperature: 65 °C	Red bell pepper Strawberry Watercress	Alexandre et al., 2011b
US + ClO_2	Bath: 40 kHz; 100 W; 10 min ClO_2: 40 mg L^{-1}	Plum fruit	Chen & Zhu, 2011
US	Probe: 20 kHz; 600 W; 5 min	Grape berry	Fava et al., 2011

Treatment	Parameters	Products	References
US + ascorbate	Bath: 40 kHz ascorbate: 1% (w/v)	Fresh-cut apple	Jang & Moon, 2011
US + heat + calcium propionate	Bath: 40 kHz; 400 W; 65 ^{o}C; 15 -17 min Calcium propionate: 2% (w/v)	Fresh-cut celery	Kwak et al., 2011
US + organic acid (malic, lactic and citric acid)	Bath: 40 kHz; 30 W L^{-1}; 5 min Organic acids: 2% (w/v)	Lettuce	Sagong et al., 2011
US + salicylic acid	Bath: 40 kHz; 350 W; 10 min Salicylic acid: 0.05 mM	Peach	Yang et al., 2011
US alone	Bath: 40 kHz; 350 W; 10 min	Strawberry	Cao et al., 2010a
US alone	Bath: 40 kHz; 250 W; 10 min	Strawberry	Cao et al., 2010b
US alone	Bath: 45 kHz; 1 min	Shredded carrot	Alegria et al., 2009
US + acidified sodium hypochlorite	Probe: 21.2 kHz; 200 W L^{-1}, 2 min Sodium hypochlorite: 200 mg L^{-1}	Spinach leaves	Zhou et al., 2009
US	Bath: 20 kHz; 2 min	Lettuce	Ajlouni et al., 2006
US + ClO_2	Bath: 170 kHz; 10 min ClO_2: 40 mg L^{-1}	Apple Lettuce	Huang et al., 2006
US + chlorine	Bath: 40 kHz; 10 – 15 W L^{-1}; 10 min Chlorine: 100 mg L^{-1}	Fresh-cut vegetables	Seymour et al., 2002

3 GENERAL MATERIALS AND METHODS

This chapter presents the common materials and methods that were used throughout the study. Specific protocols and procedures which were conducted in some experiments were detailed in their respective chapters.

3.1 List of standard solutions

Henderson–Hasselbalch equation was used throughout the experiment in the preparation of buffer solutions as below:

$$\mathbf{pH = pK_a + \log \frac{[A^-]}{[HA]}}$$

pK_a = $-\log_{10}(K_a)$ where K_a is the acid dissociation constant

[HA] = molar concentration of undissociate weak acid

$[A^-]$ = molar concentration of acid's conjugate base

A list of solution used in the extraction of plant material and enzymatic assays are listed as below:

50 mM sodium phosphate buffer (pH 7.8): 12 g sodium dihydrogen phosphate (NaH_2PO_4) and 56.8 g disodium hydrogen phosphate (Na_2HPO_4) were dissolved in 450 ml of distilled water (dH_2O). pH of the solution was adjusted to 7.8 with 1 M of sodium hydroxide (NaOH) and final volume was made up to 500 ml in a volumetric flask.

100 mM sodium borate buffer (pH 8.8): 2.2 g boric acid and 3.02 g sodium tetraborate were dissolved in 450 ml of dH_2O. pH of the solution was adjusted to 8.8 with 1 M of NaOH and final volume was made up to 500 ml in a volumetric flask.

100 mM sodium phosphate buffer (pH 6.5): 15.6 g NaH_2PO_4 and 71.2 g Na_2HPO_4 were dissolved in 450 ml of dH_2O. pH of the solution was adjusted to 6.5 with 1 M of NaOH and final volume was made up to 500 ml in a volumetric flask.

100 mM potassium phosphate buffer (pH 7.0): 4.1 g potassium dihydrogen phosphate (KH_2PO_4) and 3.5 g dipotassium hydrogen phosphate (K_2HPO_4) were dissolved in 450 ml of dH_2O. pH of the solution was adjusted to 7.0 with 1 M of NaOH and final volume was made up to 500 ml in a volumetric flask.

90 mM potassium phosphate buffer (pH 7.5): 2.0 g KH_2PO_4 and 5.2 g K_2HPO_4 were dissolved in 450 ml of dH_2O. pH of the solution was adjusted to 7.5 with 1 M of NaOH and final volume was made up to 500 ml in a volumetric flask.

90 mM potassium phosphate buffer (pH 7.0): 3.4 g KH_2PO_4 and 3.5 g K_2HPO_4 were dissolved in 450 ml of dH_2O. pH of the solution was adjusted to 7.0 with 1 M of NaOH and final volume was made up to 500 ml in a volumetric flask.

4 mM hydrogen peroxide (H_2O_2): 160 µl H_2O_2 (30%) was aliquot and final volume was made up to 500 ml in a volumetric flask.

20 mM guaiacol: 225 µl guaiacol (99%) was aliquot and final volume was made up to 100 ml in a volumetric flask.

1 M sulphuric acid (H_2SO_4): 28.1 ml of H_2SO_4 (purity 95%) was aliquot and final volume was made up to 500 in a volumetric flask.

40 mM hydrochloric acid (HCl): 1.8 ml of HCl was aliquot and final volume was made up to 500 in a volumetric flask.

3.2 Preparation of plant materials

Twenty-five pineapples (*A. comosus* L. cv. Josapine; Figure 3.1) were purchased from Exotic Star Sdn. Bhd in Selangor Wholesale Market, Malaysia. Fruit with uniform size (weighed about 1.3 – 1.5 kg) and shape and of maturity index 4 (Table 3.1) as recommended by FAMA (2015) were selected and used in this experiment. Pineapples were washed under running tap water for 1 min and allowed to air dry at room temperature (Figure 3.2). To minimise cross contamination during processing, the blossom and stem end of each pineapple was discarded. Then, fruit was transversely cut into three rings of 2 cm thick (Figure 3.3). Each ring was further diced into eight triangular cubes of 2.5 cm with a handheld stainless steel dicer (Figure 3.4 – 3.5). The weight of the cubes was 15 ± 1 g. The cutting method adopted in the study was in accordance to Finnegan and colleagues (2013). Peeler, knife, dicer and cutting board were sterilised with 0.1% (v/v) sodium hypochlorite solution prior to use.

A total of 600 cubes were obtained after 3 hours of processing. All cubes were combined, mixed and randomly selected for subsequent analyses which will be detailed in their respective chapters. Due to long hours of processing and treatment time, samples were temporary kept in a sanitised cold room at 10 °C and 80 ± 5% relative humidity (RH) during experiment.

Figure 3.1 Pineapple used in the experiment.

Figure 3.2 Drying of washed pineapples at room temperature

Figure 3.3 Pineapple ring of 2 cm thickness.

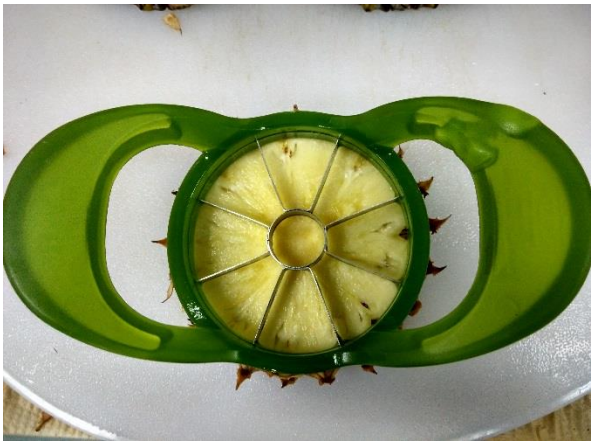


Figure 3.4 Stainless steel pineapple dicer

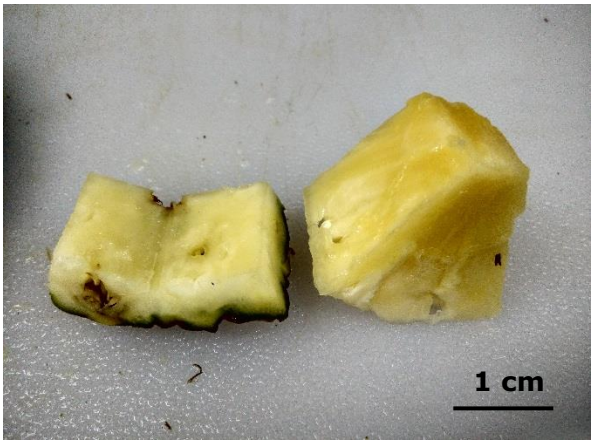


Figure 3.5 2.5 cm pineapple triangular cubes

Table 3.1 Maturity indices of pineapple (FAMA, 2015)

	**Index 1:** Immature fruit. The skin and pineapples eyes are fully green. Flesh is hard and unlikely to ripen well. Not ready for harvest.
	**Index 2**: Premature fruit. Pineapple eyes are green with traces of yellow between the eyes at the blossom end. Flesh is hard and suitable for pickles and export.
	**Index 3**: Mature fruit. 1 – 2 pineapple eyes appear yellowish at the blossom end. Suitable for export.
	**Index 4**: Fruit begins to ripen. Approximately 25% of the eyes from the blossom end is yellowish orange. Flesh is firm.
	**Index 5**: Half ripe fruit. Approximately 50% of the eyes from the blossom end turns to yellowish orange. Fruit is firm and juicy.
	**Index 6**: Ripe fruit. More than 75% of the eyes turns to yellowish orange. Fruit is soft and juicy. Not suitable for export.
	**Index 7**: Over ripe fruit. All of the eyes turn into yellowish orange. Fruit is too soft and juicy. Not suitable for marketing.

3.3 Ultrasound treatment and storage of fresh-cut pineapple

Ultrasonication was carried out in 240 mm x 137 mm x 100 mm (width x length x height) ultrasound water bath (Figure 3.6; Elmasonic P30, Elma Hans Schmidbauer GmbH & Co. KG, Germany) with sample to water ratio of 200 g to 1 L. In the optimisation of ultrasound parameters (Chapter 4), cubes were randomly selected and treated at different power amplitude (60, 80 and 100%) and exposure time (5, 10 and 15 min) at a constant frequency of 37 kHz. The amount of power dissipated at 60, 80 and 100% was 17, 25 and 29 W, respectively as determined using calorimetry. Washing water was changed every 5 min and replaced with pre-cooled distilled water (dH_2O) in order to maintain the temperature of the ultrasound water bath at 25 ± 1 ^oC. As controls, cubes were dipped in dH_2O for 5, 10 and 15 min, respectively. Ultrasound water bath was surface sterilised with 70% (v/v) ethanol before usage. After treatment, fruit was air dried at room temperature for approximately 10 min. Then, the samples were packed in a 9.0 cm x 9.0 cm x 4.0 cm (width x length x height) polystyrene containers (three pineapple cubes in each container) and stored at 7 ^oC and 80 ± 5% relative humidity (RH) for 7 days. To determine the storage life of treated and untreated pineapple cubes, microbial analysis was carried out on day 0, 1, 3, 5 and 7, respectively.

Based on the results obtained for microbial analysis (Chapter 4), the ideal ultrasound power input (25 and 29 W) and treatment time (10 and 15 min) were used in the experimental design and analyses (Figure 3.7) for Chapter 5, 6, 7 and 8, respectively. After treatment, fruit was stored at 7 ^oC and 80 ± 5% RH for 5 days. Sampling was carried out on day 0, 1, 3 and 5, respectively.

Figure 3.6 Ultrasound water bath (Elmasonic P 30 H, Germany)

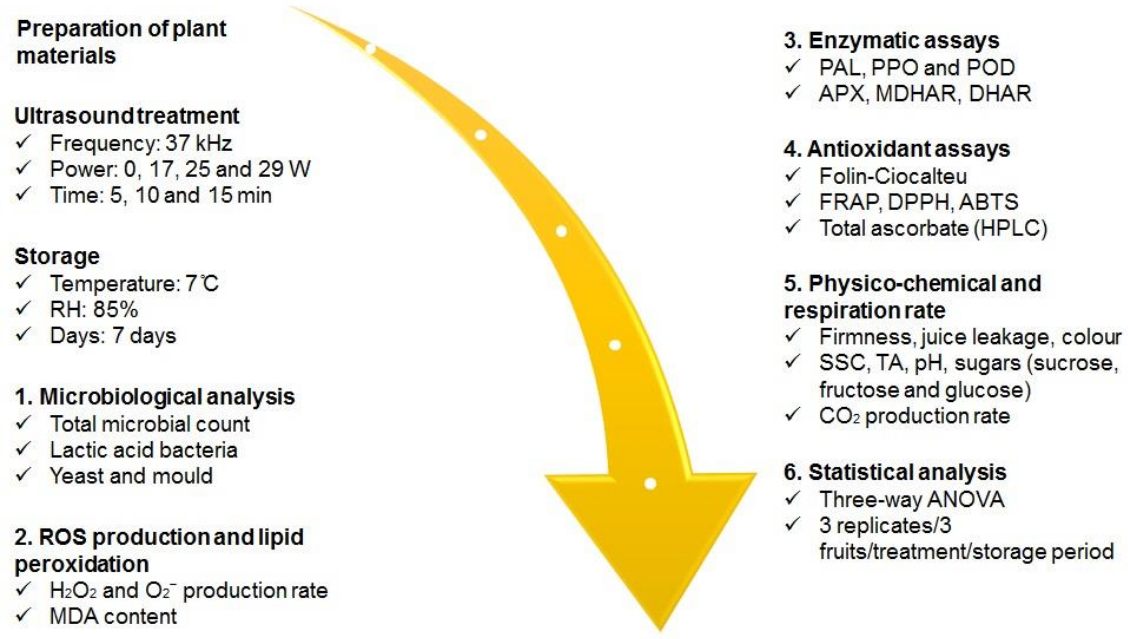


Figure 3.7 Flow chart of analyses that were being carried out throughout experiment.

3.4 Experimental design and statistical analysis

The data obtained in all experiments were subjected to Shapiro-Wilk and Bartlett's test for normality and homogeneity of variances. The effect of power, treatment time and storage period (P x T x S) were subjected to three way analysis of variance (ANOVA) using GenStat Version 16.1.0 (VSN International Ltd., Hemel Hemstead, UK). Significant level of P=0.05 was used to determine the main and interaction effects among the three factors. Significant differences of means among treatment were carried out using Duncan Multiple Range Test (DMRT). All experiments were arranged in a completely randomised design (CRD) with three replications. To ensure balance data based on the number of variables involved and replications collected in all experiments, ANOVA tables for the optimisation study (Chapter 4) and subsequent analyses (Chapter 5, 6, 7 and 8) were outlined as shown in Table 3.2 and Table 3.3.

Table 3.2 ANOVA table outlined for Chapter 4 (4 power input x 3 treatment time x 5 storage period x 3 replicates)

Sources of variation	Degree of freedom (d. f.)	Sum of square (S.S)	Mean square (M.S)	F value	Probability (P)
Power (P)	3				
Treatment time (T)	2				
Storage day (S)	4				
P x T	6				
P x S	12				
T x S	8				
P x T x S	24				
Error	120				
Total	179				

Table 3.3 ANOVA table outlined for Chapter 5, 6, 7 and 8 (3 power input x 2 treatment time x 4 storage period x 3 replicates)

Sources of variation	Degree of freedom (d. f.)	Sum of square (S.S)	Mean square (M.S)	F value	Probability (P)
Power (P)	2				
Treatment time (T)	1				
Storage day (S)	3				
P x T	2				
P x S	6				
T x S	3				
P x T x S	6				
Error	48				
Total	71				

4 ANTIMICROBIAL EFFECT OF ULTRASOUND ON SPOILAGE MICROORGANISMS OF FRESH-CUT PINEAPPLE

4.1 Introduction

The storage life pineapple is often limited as various degree of cutting and peeling operations enhance the transfer of skin microflora to the fruit flesh (Zhang, et al., 2013a). Loss of protective epidermal layer and leakage of nutrient laden juice at the site of wounding can provide a nourishing environment for the growth of microorganisms (González-Aguilar et al., 2004; Mantilla et al., 2013). The proliferation of anaerobes microorganism can enhance the production of volatile organic compounds which affects the sensory quality of fruit (Zhang et al., 2014).

Recent research has focused on the application of edible coatings to extend the storage life of fresh-cut pineapple. Edible materials such as polysaccharides, protein and lipids have been used to form a semi permeable barrier to slow down respiration rate, ethylene production and water loss from fresh-cut commodities (Bierhals et al., 2011; Rojas-Graü et al., 2009a). Azarakhsh et al. (2014a) reported that gellan based edible coating reduced weight loss and respiration rate of fresh-cut pineapples, but the coating did not inhibit the proliferation of spoilage microorganisms. Likewise, the microbial counts of both the uncoated and cassava starch coated fresh-cut pineapple were above the legal limit for consumption after storage for 9 days (Bierhals et al., 2011). Treatment of fruit with ascorbate also has been tested to extend the storage life of fresh-cut pineapple. González-Aguilar et al. (2004) reported that 0.1 M of L-ascorbic acid prevented browning and reduced decay incidence of fresh-cut pineapple, but the proliferation of spoilage microorganisms throughout storage was not measured.

The application of ultrasound has received commercial interest in food industry due to its effectiveness to maintain the quality and safety of food products by

inhibiting the growth of microorganisms and inactivating several enzymes. The generation, growth and collapse of cavitation bubbles may result in the formation of localised hot spots that are characterised by extreme temperature (5000 K) and high pressure (1000 atm) can inhibit the growth of microorganisms (Kentish & Feng, 2014; Bilek & Turantaş, 2013). Interestingly, the generation of free radicals due to sonolysis of water also results in strong antimicrobial effect against microorganisms that reside on fresh fruits and vegetables (Awad et al., 2012). For example, Cao et al. (2010b) reported that the decay incidence and microbial populations were reduced in strawberry treated with 250 W of ultrasound for 10 min. The application of ultrasound alone or in combination with other treatments to inhibit the growth of microorganisms also has been demonstrated in lettuce (Millan-Sango et al., 2015), tomato (São José & Dantas Vanetti, 2012) and apple (Huang et al., 2006).

In accordance to Malaysia Food Act 1983 and Food Regulations 1985, ready to eat foods should not contain bacteria (total plate, coliforms and *E. coli*) in numbers greater than the one specified in Regulation 38 (Fifteenth Schedule) (MOH, 2016). Also, based on previous studies, the presence of lactic acid bacteria and yeast and mould often limits the storage life of fresh-cut pineapple (Montero-Calderon et al., 2008; Zhang et al., 2014). However, low pH of pineapple is unsuitable for the growth of *E.coli*. Therefore, the objective of this study was to investigate the effects of ultrasound at different powers (17, 25 and 29 W) and treatment times (5, 10 and 15 min) on the number of total plate, lactic acid bacteria and yeast and mould counts in fresh-cut pineapple.

4.2 Materials and methods

This study was to determine the storage life of ultrasound treated fresh-cut pineapple based on the microbial populations enumerated from samples. In accordance with the Institute of Food Science and Technology (IFST), 6 $\log_{10}$ CFU g^{-1} FW was the

maximum limit of acceptance used to determine the storage life of fresh-cut pineapple (Azarakhsh et al., 2014b; Bierhals et al., 2011). Pineapple cubes were prepared and processed as described previously in Section 3.2. The fruits were treated and stored as described previously in Section 3.3. The experiment was a completely randomised design which was replicated three times each with three fruits for each observations. The fruits were sampled for total microbial, lactic acid bacteria and yeast and mould count on 0, 1, 3, 5 and 7 days of storage. The data were subjected to three way analysis of variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test at P=0.05.

4.2.1 Microbiological analysis

The microbial counts of ultrasound treated and untreated samples were carried out as described by Zhang et al. (2013a) with some modifications. Briefly, 10 g of sample was transferred into a 400 ml stomacher bag (BagLight® PolySilk®, Interscience, France) and added with 90 ml of 0.1% (w/v) buffered peptone water. The mixture was stomached manually for 1 min and transferred into a 50 ml falcon tube. The mixture was serially diluted (10-fold dilution) with 0.1% (w/v) peptone water and vortex mixed prior to spreading. The number of colonies formed was counted using a colony counter.

4.2.1.1 Total microbial count

The enumeration of mesophilic bacteria was carried out by transferring and spread plating of 0.1 ml of diluent onto plate count agar and incubated at 35 ^{o}C for 48 h. Microbial counts were expressed as $\log_{10}$ CFU g^{-1} FW.

4.2.1.2 Lactic acid bacteria count

Lactic acid bacteria was enumerated by spread plating 0.1 ml of diluent onto de Man, Rogosa and Sharpe agar and incubated at 25 ^{o}C for 48 h. Microbial counts were expressed as $\log_{10}$ CFU g^{-1} FW.

4.2.1.3 Yeast and mould counts

Yeast Extract Glucose Chloramphenicol agar was used to enumerate yeast and mould. 0.1 ml of diluent was spread plated and incubated at 25 ^{o}C for 5 days. Results were expressed as $\log_{10}$ CFU g^{-1} FW.

4.3 Results and discussion

The effect of ultrasound at different power and treatment time on the number of mesophilic, lactic acid bacteria and yeast and mould counts of fresh-cut pineapple is shown in Table 4.1 – 4.3. Initial counts of mesophilic, lactic acid bacteria and yeast and mould of fresh-cut pineapple were in the range of 3.9 – 4.3, 4.1 – 4.22 and 3.6 – 3.8 log CFU g^{-1}, respectively.

Regardless of treatment applied, the microflora of fresh-cut pineapple increased gradually throughout storage. Exposure to ultrasound treatment delayed microbial growth of samples during cold storage. After ultrasound treatment at 29 W for 15 min, the total viable counts of mesophilic, lactic acid bacteria and yeast and mould on fresh-cut pineapple were reduced by 1.3, 1.3 and 0.8 log CFU g^{-1}, respectively.

In accordance with the Institute of Food Science and Technology (IFST), 6 log CFU g^{-1} is the maximum limit of acceptance used to determine the shelf life of fruit based products (Azarakhsh et al., 2014b; Bierhals et al., 2011). In this study, the total mesophilic and lactic acid bacteria counts of control and ultrasound treated samples was beyond the acceptance limit after stored for 5 and 7 days, respectively. Whereas, the maximum limit of acceptance for yeast and mould counts in control and ultrasound treated fresh-cut pineapple was reached on day 5 and 7, respectively.

Table 4.1 Total microbial count of ultrasound treated fresh-cut pineapple during storage (7 °C and 80 ± 5% RH) for 7 days. Values (means ± SD of three replicates) followed by different significant letters are significantly different (P=0.05)

		Total Microbial Count (log_{10} CFU g^{-1} FW)				
Power (W)	**Time (min)**	**0**	**1**	**3**	**5**	**7**
0	5	3.92 ± 0.37 q-z	4.31 ± 0.10 n-u	5.11 ± 0.24 f-m	6.65 ± 0.27 b-d	7.19 ± 0.07 ab
	10	4.32 ± 0.13 n-t	4.59 ± 0.09 l-q	5.35 ± 0.05 f-j	6.71 ±0.15 b-d	6.88 ±0.19 b-d
	15	4.12 ± 0.05 p-x	4.62 ± 0.21 k-q	5.31 ± 0.06 f-k	6.25 ± 0.34 de	6.88 ± 0.21 a-d
17	5	3.78 ± 0.13 r-z	4.65 ± 0.65 k-p	5.00 ± 0.05 g-n	5.27 ± 0.43 f-k	7.52 ± 0.17 a
	10	3.59 ± 0.20 v-z	5.14 ± 0.02 f-m	4.39 ± 0.40 n-s	5.10 ±0.82 f-m	6.42 ± 0.41 cd
	15	3.49 ± 0.15 w-z	4.14 ± 0.55 p-w	4.51 ± 0.15 m-q	4.88 ± 0.74 i-n	6.99 ± 0.50 a-c
25	5	3.63 ± 0.33 t-z	4.18 ± 0.19 o-w	4.37 ± 0.05 n-s	4.97 ± 0.08 h-n	6.40 ± 0.26 cd
	10	3.39 ± 0.34 y-A	3.60 ± 0.21 u-z	4.04 ± 0.08 p-y	4.95 ± 0.56 h-n	5.62 ± 0.50 e-h
	15	3.68 ± 0.10 s-z	3.78 ± 0.65 r-z	4.68 ± 0.48 j-p	4.53 ± 0.41 m-p	5.69 ± 0.37 e-g
29	5	3.43 ± 0.86 x-A	3.91 ± 0.42 q-z	4.37 ± 0.36 n-s	5.64 ± 0.67 e-h	6.49 ± 0.20 cd
	10	3.27 ± 0.03 zA	3.72 ± 0.07 s-z	4.19 ± 0.42 o-w	4.64 ± 0.24 k-p	5.75 ± 0.33 ef
	15	2.84 ± 0.32 A	3.67 ± 0.13 s-z	4.21 ± 0.58 o-v	4.46 ± 0.18 m-r	5.56 ± 0.12 f-i

Least significant differences of means at P = 0.05	
Power (P)	0.15*
Time (T)	0.13*
Storage day (S)	0.17*
P x T	0.26*
P x S	0.33*
T x S	0.29
P x T x S	0.58*

*indicate that the difference is significant at P = 0.05.

Table 4.2 The number of lactic acid bacteria count of ultrasound treated fresh-cut pineapple during storage (7 °C and 80 ± 5% RH) for 7 days. Values (means ± SD of three replicates) followed by different significant letters are significantly different (P=0.05)

		Lactic Acid Bacteria Count (log_{10} CFU g^{-1} FW)				
Power (W)	**Time (min)**	**0**	**1**	**3**	**5**	**7**
0	5	4.08 ±0.04 m-u	4.16 ± 0.06 m-u	4.91 ± 0.07 j-m	6.56 ± 0.30 c-e	7.22 ± 0.18 ab
	10	4.22 ± 0.02 m-s	4.36 ± 0.04 l-p	5.26 ± 0.07 h-j	6.68 ± 0.23 b-d	7.61 ± 0.15 a
	15	4.19 ± 0.02 m-t	4.24 ± 0.06 m-r	5.02 ± 0.15 i-k	6.19 ± 0.37 d-f	7.02 ± 0.23 a-c
17	5	3.77 ± 0.38 p-x	3.71 ± 0.26 p-x	4.00 ± 0.15 n-v	5.04 ± 0.34 i-k	7.24 ± 0.58 ab
	10	3.54 ± 0.15 s-y	4.05 ± 0.37 m-v	4.30 ± 0.24 l-q	5.14 ± 0.95 ij	6.93 ± 0.50 bc
	15	3.03 ± 0.07 yz	3.52 ± 0.34 t-y	4.00 ± 0.27 n-v	4.71 ± 0.55 j-m	6.05 ± 0.70 e-g
25	5	3.48 ± 0.14 u-y	3.63 ± 0.09 q-y	4.04 ± 0.18 m-v	5.24 ± 0.62 h-j	6.02 ± 0.35 e-g
	10	3.14 ± 0.51 x-z	3.52 ± 0.21 t-y	4.09 ± 0.14 m-u	4.93 ± 0.38 j-m	5.55 ± 0.37 g-i
	15	3.11 ± 0.74 x-z	3.58 ± 0.78 r-y	3.99 ± 0.20 n-v	4.47 ± 0.10 k-o	5.54 ± 0.46 g-i
29	5	3.21 ± 0.55 w-z	3.75 ± 0.08 p-x	4.62 ± 0.07 j-n	5.20 ± 0.h-j	6.28 ± 0.06 d-f
	10	3.01 ± 0.05 yz	3.39 ± 0.03 v-z	4.24 ± 0.45 m-r	4.63 ± 0.21 j-n	5.82 ± 0.15 f-h
	15	2.83 ± 0.24 z	3.63 ± 0.27 q-y	3.86 ± 0.13 o-w	4.19 ± 0.46 m-t	5.52 ± 0.15 g-i

Least significant differences of means at P = 0.05	
Power (P)	0.14*
Time (T)	0.12*
Storage day (S)	0.16*
P x T	0.25*
P x S	0.32*
T x S	0.28
P x T x S	0.56

*indicate that the difference is significant at P = 0.05.

Table 4.3 Yeast and mould counts of ultrasound treated fresh-cut pineapple during storage (7 °C and 80 ± 5% RH) for 7 days. Values (means ± SD of three replicates) followed by different significant letters are significantly different (P=0.05)

		Yeast and Mould (log_{10} CFU g^{-1} FW)				
Power (W)	**Time (min)**	**0**	**1**	**3**	**5**	**7**
0	5	3.57 ± 0.21 l-q	3.81 ± 0.07 k-p	4.99 ± 0.18 f-h	6.29 ± 0.28 e	7.71 ± 0.19 ab
	10	3.71 ± 0.19 k-p	3.90 ± 0.04 k-n	5.15 ± 0.19 f	6.74 ± 0.10 c-e	7.58 ± 0.07 ab
	15	3.77 ± 0.19 k-p	3.96 ± 0.19 k-m	4.88 ± 0.31 f-h	6.76 ± 0.14 c-e	7.48 ± 0.31 ab
17	5	3.83 ± 0.06 k-p	3.85 ± 0.18 k-o	3.96 ± 0.15 k-m	4.93 ± 0.15 f-h	7.97 ± 0.49 a
	10	3.50 ± 0.17 l-r	3.94 ± 0.25 k-m	4.26 ± 0.23 i-k	5.10 ± 0.82 fg	7.49 ± 0.40 ab
	15	3.27 ± 0.11 p-r	3.67 ± 0.33 l-q	4.02 ± 0.34 j-l	4.65 ± 0.09 f-i	7.75 ± 0.69 a
25	5	3.11 ± 0.33 qr	3.46 ± 0.31 l-r	3.97 ± 0.13 k-m	5.03 ± 0.28 f-h	7.19 ± 0.40 bc
	10	3.44 ± 0.12 l-r	3.54 ± 0.06 l-q	3.88 ± 0.32 k-o	4.90 ± 0.13 f-h	6.82 ± 0.23 cd
	15	3.40 ± 0.16 m-r	3.87 ± 0.83 k-o	3.88 ± 0.18 k-o	4.67 ± 0.01 f-i	6.80 ± 0.35 c-e
29	5	3.35 ± 0.56 n-r	3.57 ± 0.13 l-q	4.50 ± 0.40 h-j	5.20 ± 0.20 f	6.95 ± 0.17 cd
	10	3.30 ± 0.22 o-r	3.52 ± 0.16 l-r	3.70 ± 0.11 k-p	4.70 ± 0.30 f-i	6.83 ± 0.30 cd
	15	2.98 ± 0.06 r	3.67 ± 0.17 l-q	3.66 ± 0.17 l-q	4.58 ± 0.10 g-i	6.59 ± 0.13 de

Least significant differences of means at P = 0.05	
Power (P)	0.12*
Time (T)	0.10
Storage day (S)	0.13*
P x T	0.21*
P x S	0.27*
T x S	0.23
P x T x S	0.47

*indicate that the difference is significant at P = 0.05.

Icnreased power input and exposure time increased the antibacterial activity of ultrasound. Optimal inhibition on the growth of microflora was observed when power input and exposure time were increased to 25 W for 10 min. Further increased in ultrasound power input and treatment time to 25 W and 15 min, respectively did not result in changes in the population of mesophiles, lactic acid bacteria and yeast and mould of fresh-cut pineapple. Lower inhibition on the growth of mesophiles, lactic acid bacteria and yeast and mould was observed in samples treated at 17 W for 5 min in comparison to those treated at 25 and 29 W.

The results from this study support those from Cao et al. (2010b) who found that the ultrasound power output affected the growth of spoilage microorganisms on strawberry. In that study, strawberry treated at 250 W of ultrasound for 10 min showed the highest reduction in total microbial and yeast and mould counts which were 2.4 and 2.5 log CFU g^{-1}, respectively, after 8 days of storage at 5 ^{o}C. Based on the response surface methodology analysis, the authors found that ultrasound at 250 W for 10 min was the optimum conditions to extend the storage life of strawberry by inhibiting the growth of spoilage microorganisms. Kentish and Feng (2014) suggested that stagnant cloud bubbles can be formed upon too much of energy is being applied to the ultrasonic probe, which can prevent the uniform transmission of acoustic energy.

Similarly, the native microbial counts of cherry tomato was reduced by approximately 1 log CFU g^{-1} after 10 min of ultrasound treatment (45 kHz) (São José & Dantas Vanetti, 2012). The application of ultrasound to inhibit the growth of microorganisms also has been reported in fresh-cut broccoli (Ansorena et al., 2014) and lettuce (Ajlouni et al., 2006). Both studies demonstrated that the antibacterial activity of ultrasound increased with treatment time which were consistent with the findings in this study.

Uneven surfaces of fresh-cut pineapple, especially the underside, may provide some protection for the bacterial cells and make an effective sanitising of the fruit to be difficult. The attached and entrapped microorganisms which tightly adhere on the surface of fresh commodities can be removed using mechanical force generated by shock wave and microstreaming (Kentish & Feng, 2014). Shock wave can shear and break the cell wall and membrane structures of microorganisms up to the point of cell lysis (Chemat et al., 2011; Soria & Villamiel, 2010). The results from this study support those from São José and Dantas Vanetti (2012) who demonstrated that the presence of adherent *S.* typhimurium cells with biofilm formation on cherry tomato was removed after treated at 40 kHz of ultrasound for 30 min.

Another important effect is that water molecules can be broken generating highly reactive free radicals that can react and modify with other molecules (São José et al., 2014; Soria & Villamiel, 2010). OH^- radicals can react with the sugar phosphate backbone of the DNA chain by removing the hydrogen atoms from the sugars (Bilek & Turantaş, 2013). The double stranded microbial DNA will be broken through the scission of the phosphate ester bond (Chandrapala et al., 2012; Mañas & Pagán, 2005).

In conclusion, ultrasound power input at 25 W and treatment time for 10 min was the optimal treatment to delay the growth of spoilage microorganisms in fresh-cut pineapple. Although ultrasound meets the requirement for an antimicrobial agent to reduce a minimum of 2 log microbial population in fresh-cut pineapple, the effect is not lethal due to the rapid growth of yeast and mould during cold storage. Hence, the storage life of ultrasound treated fresh-cut pineapple was only extended by 2 days at 7 ^oC.

Findings from this study have proven that ultrasound treatment is a potential alternative to achieve food safety in fresh-cut pineapple. However, considering the

mechanical forces and formation of free radicals during ultrasound treatment, a deeper understanding towards these impacts on the production of intracellular ROS to induce resistance and result in lower microbial counts in fresh-cut pineapple during storage should be investigated. Also, rapid induction of intracellular ROS in fresh-cut pineapple can result in browning and off-flavour development. As so, the extent of oxidative damage due to the accumulation of ROS during sonication should be studied as well. It is decided not to increase the power output above 29 W as much juice leakage was observed in samples treated at 29 W during storage. Therefore, ultrasound power outputs of 25 and 29 W and exposure times of 10 and 15 min were chosen to carry out further study on the production of intracellular ROS and extent of lipid peroxidation in fresh-cut pineapple.

5 EFFECT OF ULTRASOUND ON THE PRODUCTION OF INTRACELLULAR REACTIVE OXYGEN SPECIES (ROS) AND LIPID PEROXIDATION

5.1 Introduction

Postharvest senescence of fresh produce is a developmental process involving the regulation of biochemical, physiological and oxidative metabolisms (Zhu et al., 2008). ROS such as $O_2^{\bullet-}$ and H_2O_2 which are the primary mediators of oxidative damage in plants are involved in the progression of senescence (Tian et al., 2013). ROS are continuously produced as byproducts of aerobic metabolisms in different cellular compartments such as mitochondria, peroxisomes and chloroplast (Apel & Hirt, 2004;Toivonen, 2004). It has been suggested that approximately 1 – 5% of oxygen consumption can lead to the production of H_2O_2 (Gill & Tuteja, 2010; Nyathi & Baker, 2006). When exposed to various biotic and abiotic stresses such as pathogen attacks, extreme temperature, ozone, ultraviolet radiation and mechanical stress, the production of ROS can be enhanced via the activation of plasma-membrane bound NADPH oxidase, cell wall bound peroxidase and amine oxidase in the apoplast (Apel & Hirt, 2004; Toivonen, 2004).

Studies have suggested a dual role for ROS in plant biology. At low concentration, ROS can act as key regulators of growth, development, hormone signaling and biotic and abiotic stress responses (Gill & Tuteja, 2010; Mittler et al., 2004). It has been proposed that H_2O_2 can act as signals and secondary messengers for the activation of stress defence pathways due to its relatively long half-life and high permeability across membrane (Foyer & Noctor, 2005; Mittler et al., 2011). For instance, higher accumulation of $O_2^{\bullet-}$ and H_2O_2 in *Pichia membranaefaciens* (yeast type biocontrol agent) inoculated citrus fruit resulted in better resistance against postharvest green and blue mould infection (Luo et al., 2013b). Similarly, Gómez et al. (2008) showed that early induced H_2O_2 concentration following heat shock treatment at 40 ^{o}C for 3.5 min delayed senescence of spinach leaves when compared

with the control. Elevated concentrations of H_2O_2 in BTH treated peach fruit was associated with the development of resistance against *Penicillum expansum* during 7 days of storage at 22 ^oC (Liu et al., 2005).

Over accumulation of ROS can cause oxidative damage to biomolecules such as lipids, carbohydrates and DNA which often results in cell death (Apel & Hirt, 2004; Blokhina et al., 2003). Oxidation of polyunsaturated fatty acid (PUFA) often leads to increase leakiness, decrease fluidity of membrane and cause secondary damage to membrane protein (Tian et al., 2013). MDA which is a secondary end product formed through the oxidation and degradation of PUFA by LOX can be used as an indicator to indicate the extent of lipid peroxidation and oxidative stress in cells (Hodges et al., 1999; Singh et al., 2012). Li et al. (2010) observed that the MDA concentration in bruised pear was 37% higher than that of non-bruised fruit, which was associated with higher ROS production after storage for 30 days at 16 ^oC. The accumulation of MDA concentration in pepper ionised with high dosage of electron beam (7 kGy) resulted in the deterioration of chloroplast membrane structure (Martínez-Solano et al., 2005). Similarly, storage of tomato fruit in cold stress resulted in an increase in chilling index which was associated with the accumulation of MDA concentration throughout storage (Aghdam et al., 2012).

Although the efficacy of ultrasound to inhibit the growth of microorganisms is well elucidated in strawberry (Cao et al., 2010b), cherry tomato (Ding et al., 2015), lettuce (Sagong et al., 2011), apple (Huang et al., 2006) and red bell pepper (Alexandre et al., 2013), the dynamic of intracellular ROS accumulation in ultrasound treated fruits and vegetables has not been conducted. Also, the formation of malondialdehyde (MDA) has been used in fruits such as mango (Djioua et al., 2009) and kiwi fruits (Zhu et al., 2008) to indicate the extent of oxidative damage.

Therefore, the objective of this study was aimed to study the production of intracellular ROS and MDA concentration in ultrasound treated fresh-cut pineapple.

5.2 Materials and methods

The fruits used in this study were prepared as described in Section 3.2. Ultrasound treatments used power outputs of 25 and 29 W and exposure times of 10 and 15 min. Treatments were applied as described in Section 3.3. Three replications of three fruits for each treatment were analysed on 0, 1, 3 and 5 days of storage at 7 ^{o}C and 80 ± 5% relative humidity (RH). Although the microbial counts of dH_2O washed controls were beyond the maximum acceptance limit on day 5, these fruits were still sampled for analyses to investigate the underlying reasons that resulted in lower microbial counts during cold storage. The data were subjected to three way analysis of variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test at P=0.05.

5.2.1 Production rate of superoxide anion ($O_2^{\bullet-}$)

$O_2^{\bullet-}$ production rate was determined using the method described by Liu et al. (2013b) with some modifications. 1 g of flesh tissue was ground with 10 ml of 50 mM sodium phosphate buffer (pH 7.8) using a mortar and pestle. The homogenate was centrifuged at 10,000 x g for 20 min at 4 ^{o}C. 1 ml of supernatant was mixed with 1 ml of 1 mM hydroxylamine hydrochloride in 50 mM phosphate buffer (pH 7.8). After incubation at 25 ^{o}C for 1 h, 1 ml of 17 mM sulfanilic acid and 1 ml of 7 mM p-naphthylamine (dissolved in 75% (v/v) acetic acid) were added and incubated at 25 ^{o}C for 20 min. The absorbance of the mixture was read at 530 nm with a microplate spectrophotometer. A standard curve (R^2 = 0.9983; Appendix A.1) was established by using 1 ml of different concentrations of sodium nitrite ($NaNO_2$). Briefly, 7 mg of $NaNO_2$ was dissolved in 10 ml of 50 mM phosphate buffer (pH 7.8) to obtain a final concentration of 10 mM. The stock solution of $NaNO_2$ was diluted to 2.5, 5.0. 7.5, 10,

20, 40, 60, 80 and 100 µM, respectively. The production rate of $O_2^{\bullet-}$ was determined against the standard $NaNO_2$ calibration curve and the absorbance value was converted to nmol min^{-1} g^{-1} FW.

5.2.2 Determination of hydrogen peroxide (H_2O_2)

The H_2O_2 concentration was determined according to Ren et al. (2012). 3 g of flesh tissue was ground with 3 ml of cold acetone using a mortar and pestle. The homogenate was centrifuged at 10,000 x g for 20 min at 4 °C. 1 ml of supernatant was added with 0.2 ml of 20% (w/v) titanium tetrachloride and 0.2 ml of concentrated ammonia solution to precipitate the titanium-hyperoxide complex in the presence of H_2O_2. Precipitate was washed with pre-chilled acetone for three times and pellet was redissolved in 3 ml of 1 M H_2SO_4 followed by centrifugation at 10,000 x g for 10 min at 4 °C. The absorbance reading of the supernatant was measured at 410 nm. The experiment was performed similarly in the preparation of standard curve using H_2O_2 as a standard (R^2 = 0.9932; Appendix A.2). Briefly, 0.78 ml of 30% (v/v) H_2O_2 was added and made up to 100 ml with dH_2O to obtain a final concentration of 1 M. The stock solution of H_2O_2 was diluted to 0.25, 1.00, 2.00, 4.00, 6.00, 8.00, 10.00 and 20.00 mM, respectively. H_2O_2 concentration was determined against the standard curve and expressed as µmol of H_2O_2 g^{-1} FW.

5.2.3 Determination of lipid peroxidation by malondialdehyde (MDA) concentration

Lipid peroxidation was determined by using thiobarbituric acid reactive substance (TBARS) concentration according to Hodges et al. (1999). 1 g of flesh tissue was ground with 10 ml of 80% (v/v) ethanol using a mortar and pestle followed by centrifugation at 10,000 x g for 10 min at 4 °C. 1 ml of aliquot was either added to a test tube with 1 ml of **i)** + thiobarbituric acid (+TBA) solution comprised of 0.65% (w/v) thiobarbituric acid (TBA), 20% (w/v) trichloroacetic acid (TCA) and 0.01%

(w/v) butylated hydroxytoluene (BHT) or **ii)** –TBA solution comprised of 20% (w/v) TCA and 0.01% (w/v) BHT. The mixture was vortexed and then heated at 95 ^{o}C for 25 min in a water bath. Homogenates were cooled immediately in cracked ice and centrifuged at 3000 x g for 10 min at 4 ^{o}C. The absorbance was read at 440 nm, 532 nm and 600 nm, respectively. The concentration of MDA was expressed as nmol of MDA equivalents g^{-1} FW and calculated in the following manner:

1. A = [(Abs 532 nm $_{+TBA}$ – Abs 600 nm $_{+TBA}$) – (Abs 532 nm $_{-TBA}$ – Abs 600 nm $_{-TBA}$)]
2. B = [(Abs 400 nm $_{+TBA}$ – Abs 600 nm $_{+TBA}$) (0.0571)]
3. MDA equivalents (nmol g^{-1} FW) = [(A – B)/ 157 000] 10^{6}

5.3 Results and discussion

5.3.1 Production of intracellular ROS

As shown in Figure 5.1, $O_2^{\bullet-}$ production rate of control decreased steadily throughout storage. Following ultrasound treatment at 25 and 29 W of power input, production rate of $O_2^{\bullet-}$ increased on day 1 and decreased during subsequent storage days. Although the highest peak in the $O_2^{\bullet-}$ production rate was observed in fresh-cut pineapple treated at 25 W for 15 min (8 nmol min^{-1} g^{-1} FW), no changes was observed among ultrasound treated samples. Following 5 days of storage at 7 ^{o}C, the production rate of $O_2^{\bullet-}$ of fresh-cut pineapple treated at 25 and 29 W of power input was higher than that of control. Increased power and treatment time did not result in differences among the $O_2^{\bullet-}$ production in fresh-cut pineapple.

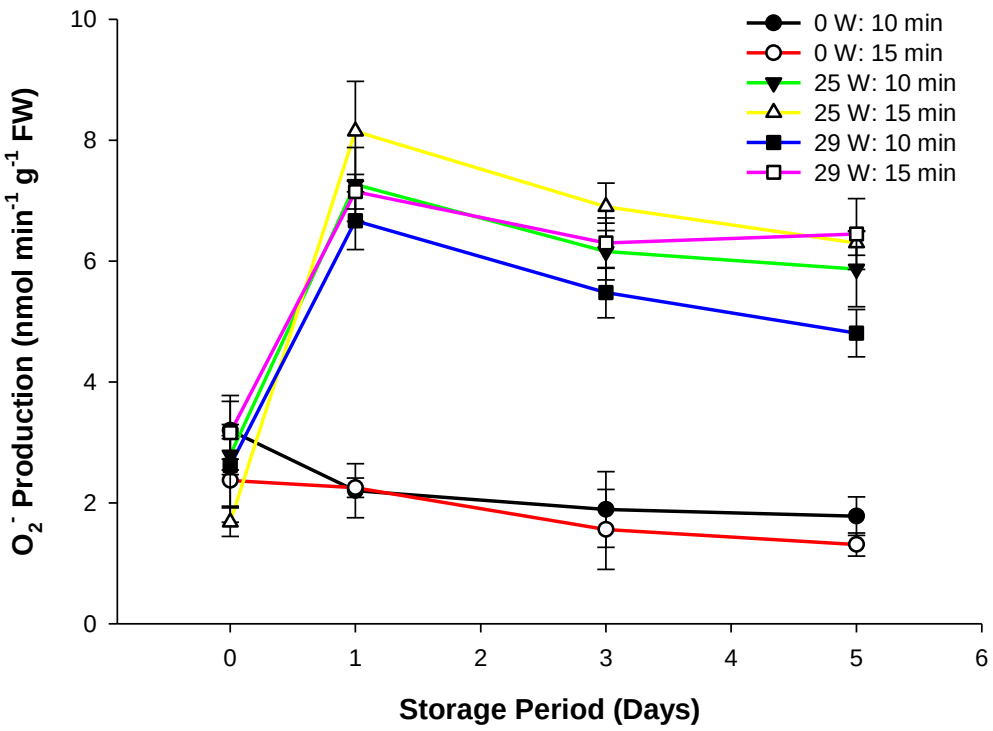


Figure 5.1 Production rate of $O_2^{\bullet-}$ in fresh-cut pineapple treated at different powers and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Immediately after ultrasound treatment operated at 29 W for 15 min, the concentration of intracellular of H_2O_2 was 65% higher than the control (Figure 5.2). Similar increment in the concentration of H_2O_2 was also observed in other ultrasound treatments. As shown in Figure 5.2, the production of H_2O_2 in ultrasound treated samples reached a peak on day 1 and decreased during subsequent storage days. Meanwhile, the intracellular concentration of H_2O_2 in control decreased steadily throughout storage. Following 5 days of storage at 7 °C, the highest concentration of H_2O_2 (20 μmol g^{-1} FW) was observed in samples treated at 29 W for 15 min, however, no changes were observed among ultrasound treatment.

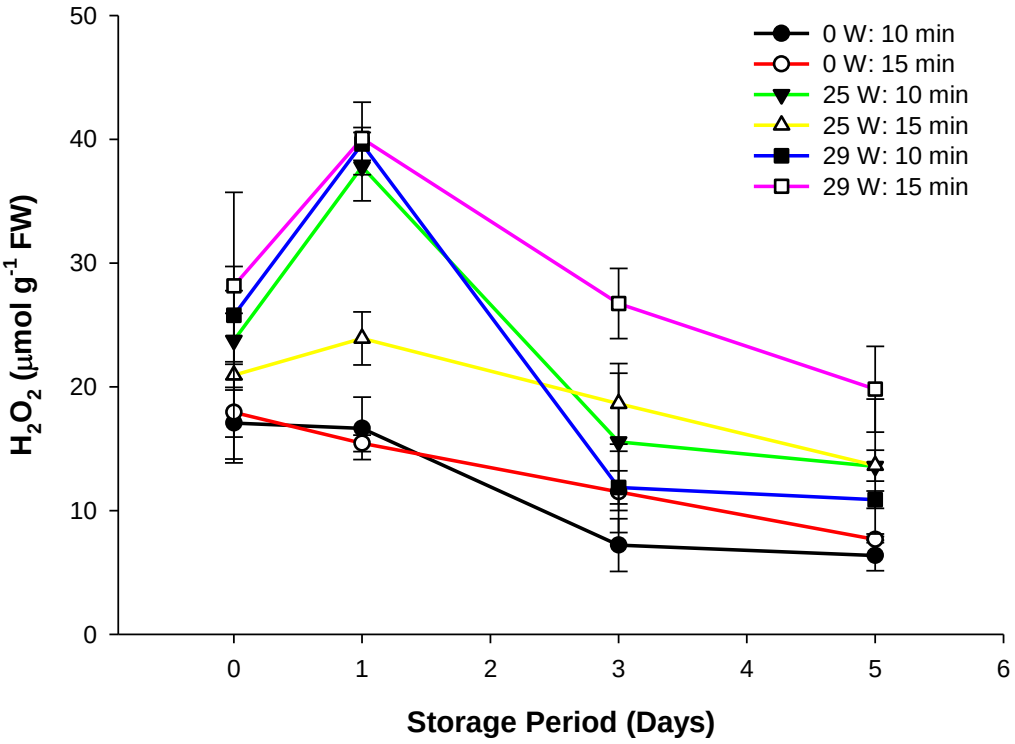


Figure 5.2 H_2O_2 concentration in fresh-cut pineapple treated at different powers and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Increased $O_2^{\bullet-}$ and H_2O_2 production in ultrasound treated fresh-cut pineapple may be due to the production of free radicals due to sonolysis of water. The collapse of cavitation bubbles can result in high temperature and pressure which can be used to dissociate water molecules and generate hydrogen radicals, hydrogen atom and hydrogen peroxide (Awad et al., 2012; Kentish & Feng, 2014). The formation of free radicals and collapse of cavitation bubbles are highly dependent on the sound wave amplitude (Kentish & Feng, 2014). Ultrasonic waves with large amplitudes can facilitate the displacement of molecules and collapse pressure and hence promote the formation of free radicals in aqueous solution (Sauter et al., 2008). No cavitation bubbles or free radicals can be formed when the ultrasound intensity is below the cavitation threshold (Xia et al., 2002).

The accumulation of intracellular ROS in ultrasound treated fresh-cut pineapple suggests that ultrasound treatment at different amplitude and treatment time can induce different level of oxidative stress in fresh-cut commodities. Consistent with the findings in this study, research has revealed that the exposure to abiotic and biotic stresses can enhance the production of intracellular ROS in fresh commodities. Novillo et al. (2014) reported that the production rate of $O_2^{\bullet -}$ and H_2O_2 concentration of persimmon was doubled, upon exposure to deastringency treatment with CO_2 which induced oxidative stress. Mechanical injuries which induced the production of ROS in fresh produce was reported in cut lettuce tissues (Toivonen et al., 2012) and bruised pears (Li et al., 2010) as well. Li et al. (2010) reported that the concentration of H_2O_2 in bruised pear was 96% higher than control following 15 days of storage at 16 ^oC.

Gill and Tuteja (2010) suggested that over accumulation of intracellular ROS can disrupt normal metabolism by oxidising lipid, proteins, nucleic acid, or carbohydrates, affecting the integrity of cell membranes and inactivating key cellular function. However, enhanced $O_2^{\bullet -}$ and H_2O_2 production can increase host disease resistance against various postharvest pathogen, act as signals and secondary messengers for the activation of stress defence pathways, activate gene expression of various antioxidant enzymes (APX, PAL, and SOD) and non-enzymatic metabolites (AA, carotenoids and flavonoids).

In this study, the increased in the H_2O_2 concentration was negatively correlated with the decreased in total microbial count ($r = -0.6413$, $P<0.001$, Appendix B.6), lactic acid bacteria ($r = -0.6252$, $P<0.001$, Appendix B.7) and yeast and mould counts ($r = -0.5955$, $P<0.001$, Appendix B.8) of fresh-cut pineapple during cold storage. The results from this study support those of Toivonen et al. (2012) who showed that the transient increased in H_2O_2 concentration in lettuce was associated

with the low survival rate of *E. coli* O157:H7 when subjected to mechanical stress. The authors suggested that wound-generated H_2O_2 can be associated with bacterial survival on fresh-cut produce. Similarly, the application BTH as an elicitor of systemic acquired resistance (SAR) had greatly enhanced the disease resistance of muskmelon which coincided with the accumulation of H_2O_2 concentration (Ren et al., 2012). Consistently, the accumulation of $O_2^{\bullet-}$ and H_2O_2 in BTH treated peach fruit was also conferred better resistance against *Penicillum expansum* (Liu et al., 2005). The inoculation of citrus fruit with biocontrol agents such as *Pichia membranaefaciens* (yeast) (Luo et al., 2013b) and *Pantoea agglomerans* (gram-negative bacteria) (Torres et al., 2011) had resulted in greater $O_2^{\bullet-}$ and H_2O_2 accumulation, which induced resistance against blue and green mould infection.

Although a negative correlation was found between the concentration of ROS and survival of microbial growth in fresh-cut pineapple, oxidative stress-induced ROS metabolism can also be associated with lipid peroxidation which results in quality deterioration. Nonetheless, study conducted by Nukuntornprakit et al. (2015) also showed that the product of lipid peroxidation, MDA concentration is served as an indicator of membrane peroxidation in pineapple. Therefore, the extent of lipid peroxidation in ultrasound treated samples was further discussed in section 5.3.2.

5.3.2 Lipid Peroxidation

Regardless of treatment applied, the MDA concentration of control and ultrasound treated fruit steadily decreased throughout storage (Figure 5.3). Similar reduction in MDA concentration was also observed in fresh-cut pineapple treated at 29 W for 10 and 15 min, respectively, however, a sharp increase in the concentration of MDA was observed on day 5. Particularly, MDA concentration of sample treated at 29 W of ultrasound for 15 min was higher than control at the end of storage.

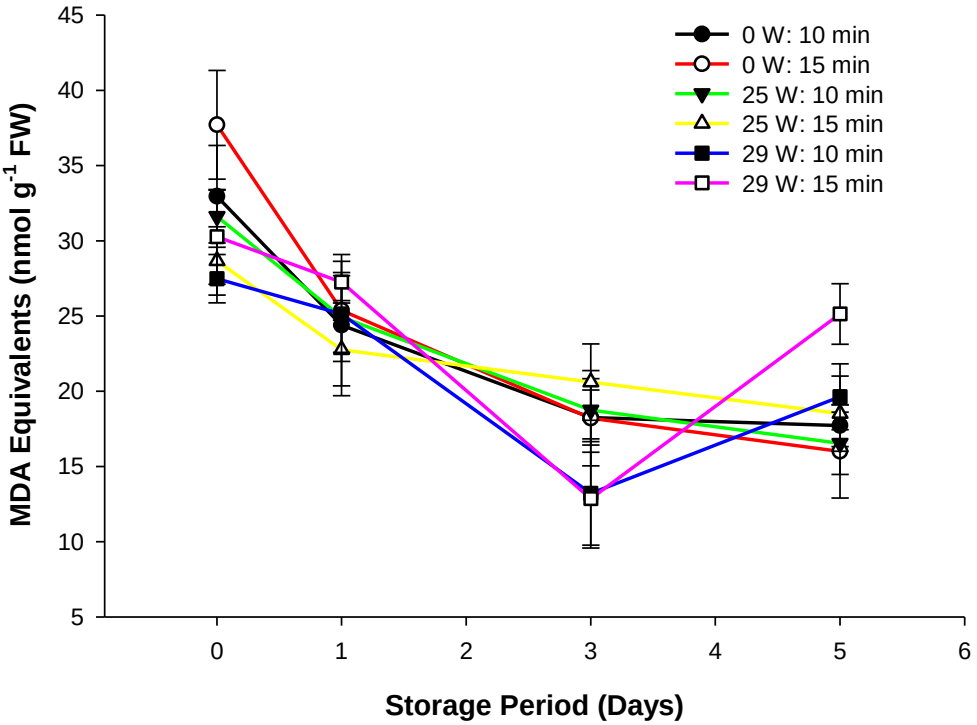


Figure 5.3 Ultrasound treatment at different power inputs and treatment times on the MDA concentration in fresh-cut pineapple 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

ROS accumulation above the threshold level is directly involved in lipid peroxidation which often results in the loss of cell membrane integrity and affects normal cellular function and hence leads to senescence (Apel & Hirt, 2004; Gill & Tuteja, 2010). MDA which is a secondary end product formed through the oxidation and PUFA by LOX can be used to indicate the extent of lipid peroxidation and oxidative stress in cells (Hodges et al., 1999; Singh et al., 2012). MDA concentration of fresh-cut pineapple treated at 29 W for 10 and 15 min, respectively, was higher than that of control at the end of storage. The extent of lipid peroxidation is dose-dependent since the concentration of MDA concentration increases as amplitude of ultrasound is increased with prolonged treatment time.

Similar trends were also observed in electron beam ionised bell pepper where MDA concentration was higher than control when higher dosage (7 kGy) was applied (Martínez-Solano et al., 2005). Djioua et al. (2009) also reported that long duration of heat treatment enhanced the formation of MDA and hence induced senescence process in fresh-cut mango. Research conducted Bi et al. (2011) showed that prolonged exposure of fresh-cut carrot to high pressure of carbon dioxide at 1.5 and 3.0 MPa, respectively, induced the accumulation of MDA concentration.

In conclusion, the formation of free radicals and high shear forces during sonication can induce the production of ROS such as H_2O_2 and $O_2^{\bullet-}$ in fresh-cut pineapple during 5 days of storage at 7 ^{o}C. Lower microbial growth in ultrasound treated fresh-cut pineapple was associated with higher $O_2^{\bullet-}$ and H_2O_2 concentration. Thus, higher $O_2^{\bullet-}$ and H_2O_2 may have induced disease resistance in fresh-cut pineapple. Results indicated that ultrasound treatment at 25 W for 10 min are the optimised condition to ensure food safety and induce better resistance in fresh-cut pineapple without causing oxidative damage. Considering the role of intracellular ROS as signalling molecules to induce plant defence responses against various biotic and abiotic stresses in fresh produce, it would be interesting to further explore into the role of ROS scavenging enzymes which prevent lipid peroxidation due to excess ROS production. Therefore, the effect of ultrasound treatment on the activity of phenylpropanoid (PAL, PPO and POD) and ascorbate recycling (APX, MDHAR, DHAR) enzymes was carried out (Chapter 6).

6 EFFECT OF ULTRASOUND ON THE PHENOLIC METABOLISM AND ASCORBATE RECYCLING ENZYMES

6.1 Introduction

ROS such as $O_2^{\bullet-}$ and H_2O_2 are produced as a result of biochemical, physiological and oxidative metabolisms in fresh fruits and vegetables (Hu et al., 2014). Several postharvest treatments have demonstrated that the early induction of ROS can enhance the defence mechanisms in fresh or fresh-cut produce against senescence and postharvest diseases. For instance, the accumulation of ROS in BTH treated muskmelon (Ren et al., 2012) and chitosan coated navel oranges (Zeng et al., 2010) was found to stimulate disease resistance and prolong storage life. However, excess ROS must be scavenged to prevent the activation of LOX and MDA concentration in fruit tissue which can increase cell permeability and off-flavours (Blokhina et al., 2003; Hu et al., 2014).

Defence against stress-induced ROS in plant is counteracted with enzymatic ROS scavenging system and non-enzymatic antioxidant compounds. Enzymatic antioxidant defense system includes a variety of scavengers such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), polyphenol peroxidase (POD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR). Non-enzymatic low molecular metabolites such as phenolic compounds and ascorbate are important to maintain the equilibrium between the production and scavenging of ROS (Apel & Hirt, 2004; Blokhina et al., 2003).

Studies have suggested that in response to various abiotic and biotic stresses, a vast array of phenolic compounds which scavenge free radicals are stimulated and accumulated in plant tissues via the phenylpropanoid pathways (Heredia & Cisneros-Zevallos, 2009; Luna et al., 2012). PAL is the initial rate controlling enzyme in the

phenylpropanoid pathway to enhance the accumulation of bioactive phenolic compounds in response to environmental stress. An increase in PAL activity has been reported in wounded carrot (Surjadinata & Cisneros-Zevallos, 2012), highly pressurised fresh-cut pineapple under xenon and argon (Wu et al., 2013) and UV radiated fresh-cut carrot (Du et al., 2012) and mango (González-Aguilar et al., 2007b). Although the increase in POD activity has been associated with the decrease in phenolic compounds, it has been suggested that the enzyme may involve in the activation of phenolic precursors which are important for lignin synthesis and repair damaged tissue (Aquino-Bolaños & Mercado-Silva, 2004; Eichholz et al., 2012).

Ascorbate is a highly abundant, powerful and water soluble metabolite which plays an important in plant stress physiology and growth and development (Gill & Tuteja, 2010). It can help to minimise the cellular damage caused by ROS in plants by directly scavenging singlet oxygen, $O_2^{\bullet-}$ and $OH^{\bullet}$ and indirectly eliminating H_2O_2 via APX (Blokhina et al., 2003; Conklin, 2001). For instance, expression of APX and was found to be up-regulated in persimmon fruit after treated with CO_2 which induced the production of $O_2^{\bullet}$ and H_2O_2 (Novillo et al., 2014). Therefore, the oxidation and recycling of ascorbate involving the enzymatic activity of APX, MDHAR and DHAR represent major key factor in the inducible defence mechanisms of plants in response to various stresses.

However, there are limited literature reviews on the effect of ultrasound on the enzymatic activities of fresh-cut fruits and vegetables. Also, studies conducted on the effect of sonication on the enzyme activity of fruit juices and fresh-cut produce have resulted in different conclusions regarding the appropriate ultrasound experimental conditions such as power intensity, exposure time, temperature and pressure. For instance, Cheng et al. (2007) demonstrated that the application of 35 kHz of ultrasound for 30 min resulted in higher PPO activity in guava juice.

Contradictorily, the residual activity of PPO, POD and APX in cantaloupe melon juice was reduced following ultrasound treatment at 373 W cm^{-2} for 10 min (Fonteles et al., 2012). The inhibition of PPO and POD activity was also reported in fresh-cut apple (Jang & Moon, 2011) and potato (Amaral et al., 2015) following ultrasound treatment. Therefore, the effect of ultrasound treatment on the activity of PAL, PPO, POD, APX, MDHAR and DHAR was investigated in this study. As the mechanisms of ultrasound may have resulted in the activation or inhibition of different enzymes based on their chemical structures, the findings of the result in this chapter were discussed in a different subsection.

6.2 Materials and methods

This study was to determine the activity of PAL, PPO, POD, APX, MDHAR and DHAR in ultrasound treated fresh-cut pineapple. Pineapple cubes were prepared and processed as described previously in Section 3.2. The fruits were treated and stored as described previously in Section 3.3. Three replications of three fruits for each treatment were sampled for enzymatic activity on 0, 1, 3 and 5 days of storage. The data were subjected to three way analysis of variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test at P=0.05.

6.2.1 Enzymatic assays for phenolic metabolism enzymes

6.2.1.1 Extraction and assay of phenylalanine ammonia lyase (PAL) activity

Extraction and determination of PAL activity was carried out as described by Wu et al. (2013) with slight modifications. Briefly, 3 g of pineapple cubes was homogenised with 5 ml of pre-chilled 0.1 M sodium borate buffer (pH 8.8) containing 5 mM ß-mercaptoethanol, 2 mM ethylene diaminetetraacetic acid (EDTA) 1% (w/v) polyvinylpolypyrrolidone (PVPP). After incubation for 1 h at 4 ^{o}C, the homogenised sample was centrifuged at 10,000 x g for 25 min at 4 ^{o}C. 12.5 µl of supernatant was transferred and mixed with 137.5 µl of 60 mM L-phenylalanine in 0.1 M sodium borate

buffer (pH 8.8) in a 96-well microplate. Homogenates were incubated for 1 h at 40 ^{o}C and the reaction was stopped by adding 5 µl of 6 M HCl. The increase in absorbance at 290 nm due to the formation of trans-cinnamate was measured using a microplate spectrophotometer. One unit of enzyme activity was defined as the amount that resulted an increase of 0.001 absorbance unit per hour and the result was expressed as unit h^{-1} mg^{-1} FW.

6.2.1.2 Extraction and assay of polyphenol oxidase (PPO) activity

PPO was extracted and assayed based on the method of Lu et al. (2011) with some modifications. Briefly, 3 g of pineapple cubes was homogenised in 8 ml of pre-cooled 50 mM sodium phosphate buffer (pH 7.8) and 0.05% (w/v) PVPP. Then, the homogenate was centrifuged at 10,000 x g for 25 min at 4 ^{o}C. Determination of PPO activity was carried out by adding 5 µl of supernatant with 145 µl of 0.1 M catechol in 0.1 M sodium phosphate buffer (pH 6.8). The increase in absorbance at 420 nm was monitored for 4 min 25 ^{o}C using a microplate spectrophotometer. One unit of PPO activity was defined as the amount of enzyme that resulted an increase of 0.01 absorbance unit per min and the result was expressed as unit min^{-1} mg^{-1} of FW.

6.2.1.3 Extraction and assay of polyphenol peroxidase (POD) activity

The activity of POD was measured according to Wu et al. (2013) with some modifications. Extraction of POD was carried out by homogenising 5 g of pineapple cubes with 10 ml of 0.2 mM sodium phosphate buffer (pH 6.5). The mixture was centrifuged at 10,000 x g for 15 min at 4 ^{o}C. POD activity was measured by adding 7.5 µl of enzyme extract and 142.5 µl of 0.1 M sodium phosphate buffer (pH 6.0) blended with 20 mM guaiacol and 4 mM H_2O_2. The increase in absorbance at 470 nm was monitored for 4 min at 25 ^{o}C using microplate spectrophotometer. One unit of POD activity was defined as the amount of enzyme that resulted an increase of 0.001 absorbance unit per min and the result was expressed as unit min^{-1} mg^{-1} of FW.

6.2.2 Enzymatic assays for ascorbate recycling enzymes

6.2.2.1 Crude enzyme preparation

3 g of pineapple cubes was homogenised with 5 ml of precooled 0.1 M potassium phosphate buffer (pH 7.0) consisting of 1 mM sodium ascorbate (freshly added) and 1 mM EDTA along with 0.5 g of PVPP and 0.5 g of inert sand in a mortar and pestle. The homogenate was centrifuged at 10,000 x g for 25 min at 4 ^{o}C. The supernatant was kept on ice throughout the experiment and used for the assays of APX, MDHAR and DHAR as described by Singh et al. (2012) with slight modifications.

6.2.2.2 Ascorbate peroxidase activity (APX)

APX activity was assayed in a 200 µl mixture contained of 90 mM potassium phosphate buffer (pH 7.0), 0.65mM sodium ascorbate, 0.1 mM EDTA and 1.0 mM H_2O_2. The reaction was initiated by adding 20 µl of enzyme extract. The decrease in absorbance at 290 nm due to the oxidation of ascorbate was monitored for 5 min using microplate spectrophotometer. APX activity was calculated using a molar extinction coefficient of 2.8 mM^{-1} cm^{-1} and expressed as mmol ascorbic acid oxidised min^{-1} g^{-1} FW.

6.2.2.3 Monodehydroascorbate reductase activity (MDHAR)

The reaction mixture of MDHAR assay consisted of 90 mM potassium phosphate buffer (pH 7.5), 0.25 unit ascorbate oxidase from Cucurbita sp. (freshly prepared) 0.01 mM EDTA, 0.2 mM nicotinamide adenine dinucleotide (NADH) (freshly prepared), 2.5 mM sodium ascorbate (freshly prepared) and 0.0125% (v/v) Triton X-100. The reaction was initiated by adding 20 µl of enzyme extract with 200 µl of reaction mixture. The reaction rate was monitored by measuring the decrease in absorbance at 340 nm for 5 min due to the conversion of NADH to NAD^{+}. MDHAR activity was calculated using a molar extinction coefficient of 6.2 mM^{-1} cm^{-1} and expressed as mmol NADH oxidised min^{-1} g^{-1} FW.

6.2.2.4 Dehydroascorbate reductase activity (DHAR)

The activity of DHAR was measured by monitoring the increase in absorbance at 265 nm for 5 min due to the glutathione-dependent reduction of DHA. Reaction was initiated by adding 20 µl of enzyme extract with 200 µl of buffer solution (pH 7.0) which contained 90 mM potassium phosphate, 0.1 mM EDTA, 5.0 mM reduced gluthathione (GSH) (freshly made) and 0.2 mM DHA (freshly made). DHAR activity was calculated using a molar extinction coefficient of 14.7 mM^{-1} cm^{-1} and expressed as mmol DHA reduced min^{-1} g^{-1} FW.

6.3 Results

6.3.1 Phenolic metabolism enzymes

6.3.1.1 PAL

Changes in the activity of PAL in fresh-cut pineapple treated at different ultrasound power input and treatment time are shown in Figure 6.1. Immediately after ultrasound treatment for 10 and 15 min, respectively, increased PAL activity was observed. A peak in the activity of dH_2O controls was only observed on day 1 which showed a delay in the increment of enzyme activity. The highest activity of PAL (5.99 unit h^{-1} mg^{-1} FW) was observed in samples treated at 25 W for 15 min and followed by those treated at 29 W: 15 min, 25 W: 10 min and 25 W: 15 min. On day 3 and 5 of storage, no difference between the PAL activities of ultrasound treated samples and dH_2O controls was observed.

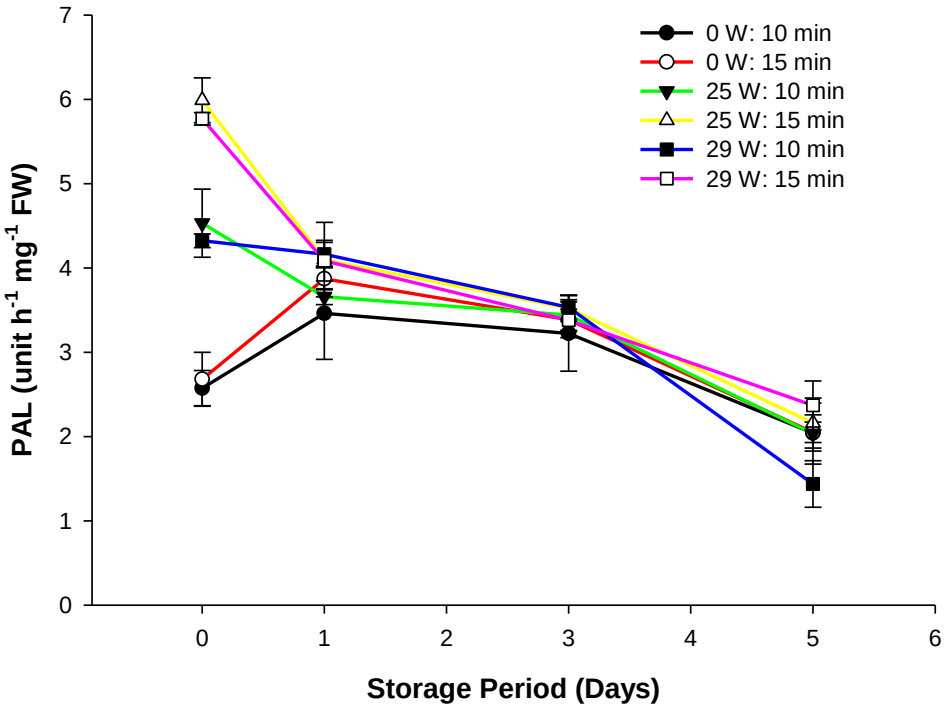


Figure 6.1 Different ultrasound power inputs and treatment times on the PAL activity of fresh-cut pineapple during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

6.3.1.2 PPO

The effect of ultrasound at different power input and treatment time on the PPO activity in fresh-cut pineapple is shown in Figure 6.2. Irrespective of treatment time, PPO activity in samples treated at 25 and 29 W of ultrasound was approximately 88 and 72% lower than dH_2O controls. As shown in Figure 6.2, the activity of PPO in dH_2O controls was relatively higher than ultrasound treated samples throughout cold storage. However, a similar pattern was observed whereby the PPO activity in dH_2O controls and ultrasound treated samples reached a peak on day 3 and decreased during subsequent storage. On day 3, an increased in power input and treatment time to 29 W and 15 min resulted in higher PPO activity when compared to other ultrasound treatments. At the end of storage, the PPO activity of ultrasound treated fresh-cut pineapple at 25 and 29 W was lower than dH_2O controls.

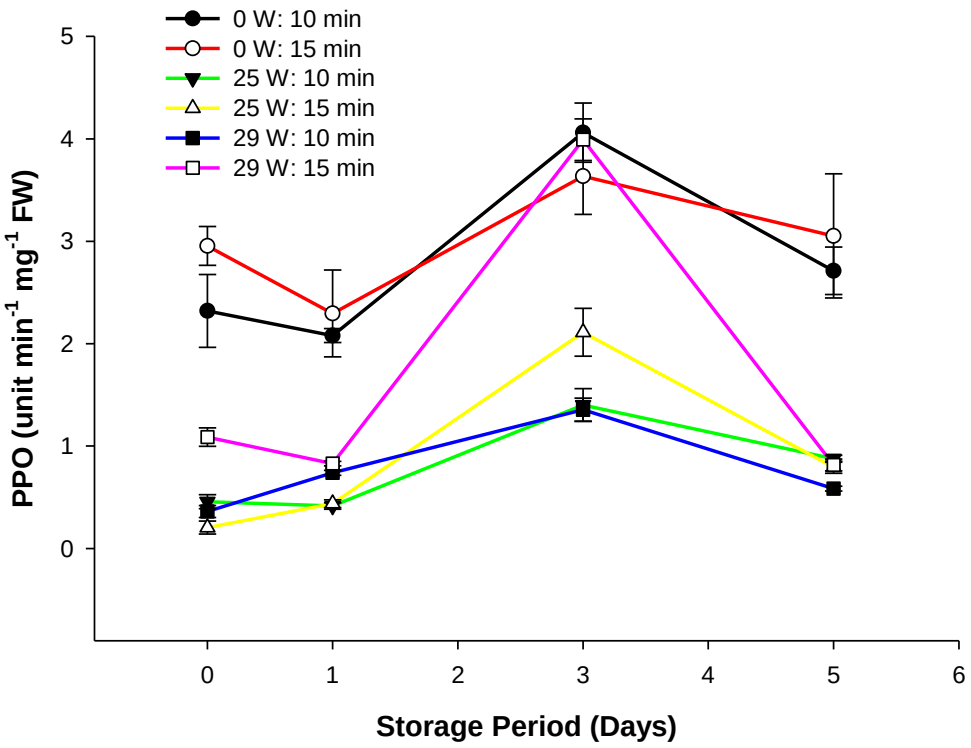


Figure 6.2 Different ultrasound power inputs and treatment times on the PPO activity of fresh-cut pineapple during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

6.3.1.3 POD

The effect of ultrasound at different power inputs and treatment times on the POD activity in fresh-cut pineapple is shown in Figure 6.3. Samples washed with dH_2O for 10 and 15 min maintained a relatively stable POD activity after 3 days and increased during subsequent storage. However, immediately after ultrasound treatment at 25 and 29 W, the POD activity of fresh-cut pineapple was 47 and 59%, respectively, lower than dH_2O control. Increment in ultrasound power input and treatment time caused a delay on the peak of POD activity in fresh-cut pineapple to day 3 when compared to sample treated at 25 W for 10 min. The activity POD in dH_2O controls was higher than those treated at 25 and 29 W of ultrasound.

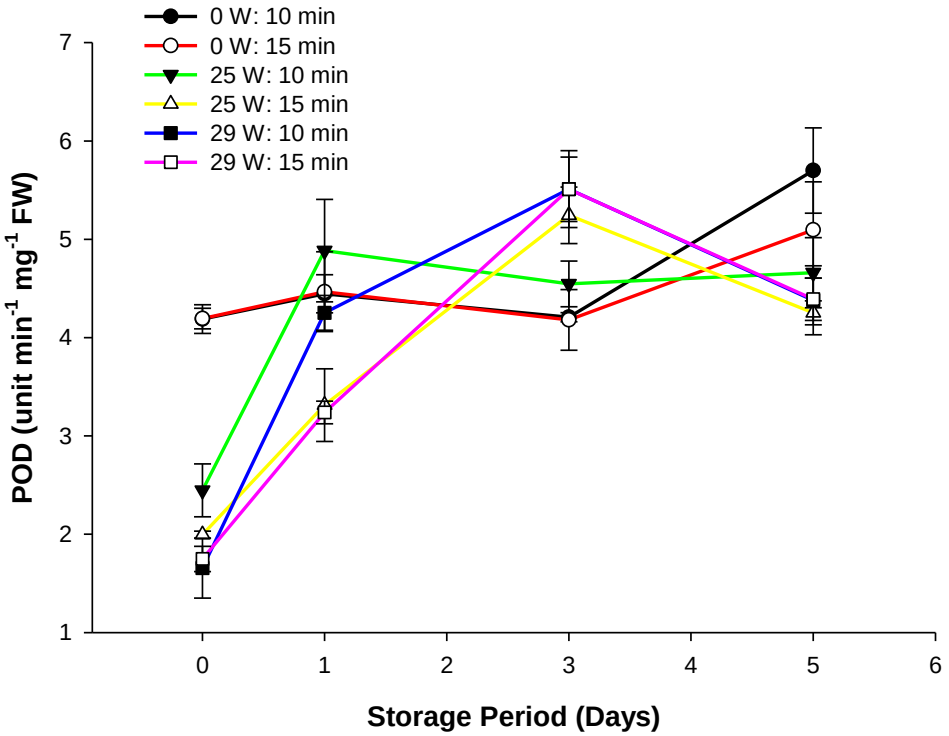


Figure 6.3 Different ultrasound power inputs and treatment times on the POD activity of fresh-cut pineapple during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

6.3.2 Ascorbate recycling enzymes

6.3.2.1 APX

The effect of ultrasound at different powers and treatment times on the activity of APX is shown in Figure 6.4. Regardless of treatment applied, the activity of APX in fresh-cut pineapple reached a peak on day 1 and then decreased during subsequent storage. However, ultrasound treated samples retained higher APX activity than controls. Following ultrasound treatment at 29 W for 10 and 15 min, the APX activity was higher in comparison to dH_2O controls.

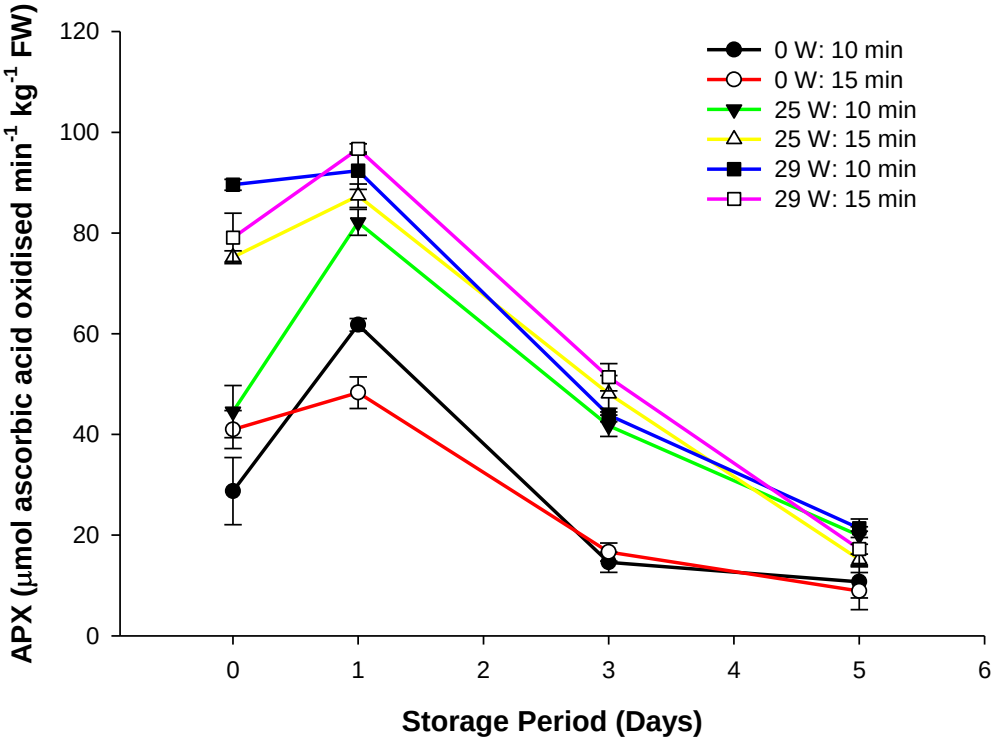


Figure 6.4 APX activity in ultrasound treated fresh-cut pineapple at different power inputs and times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replications.

An increased in power input and treatment time enhanced the activity of APX of fresh-cut pineapple. The highest peak in the activity of APX (97 mmol ascorbic acid oxidised min^{-1} g^{-1} FW) was observed in samples treated at 29 W for 15 min and followed by those treated at 29 W: 10 min, 25 W: 15 min and 25 W: 10 min. On day 5 of storage, the activity of APX in ultrasound samples treated at 25 and 29 W was 79 and 97% higher than the controls. In this study, regression analysis showed that the activity of APX was positively correlated with the accumulation of H_2O_2 ($r = 0.7661$, $P<0.001$, Appendix B.14) in fresh-cut pineapple.

6.3.2.2 MDHAR

Changes in the MDHAR activity in fresh-cut pineapple treated at different ultrasound power inputs and treatment times are shown in Figure 6.5. Following ultrasound treatment at 25 and 29 W for 10 and 15 min, respectively, MDHAR activity increased and then decreased during subsequent storage period. Meanwhile, a peak in MDHAR activity of dH_2O controls was only observed on day 1 which showed a delay of increased enzyme activity. Fruit treated at 29 W of ultrasound for 15 min retained the highest activity of MDHAR throughout 5 days of storage at 7 ^oC. Although the MDHAR activity of fresh-cut pineapple treated at 25 W for 10 and 15 min was also enhanced, the production rate was not higher than those treated at 29 W for 15 min. Regression analysis showed that MDHAR activity was only positively correlated with the accumulation of H_2O_2 ($r = 0.6767$, $P<0.001$, Appendix B.16) in fresh-cut pineapple.

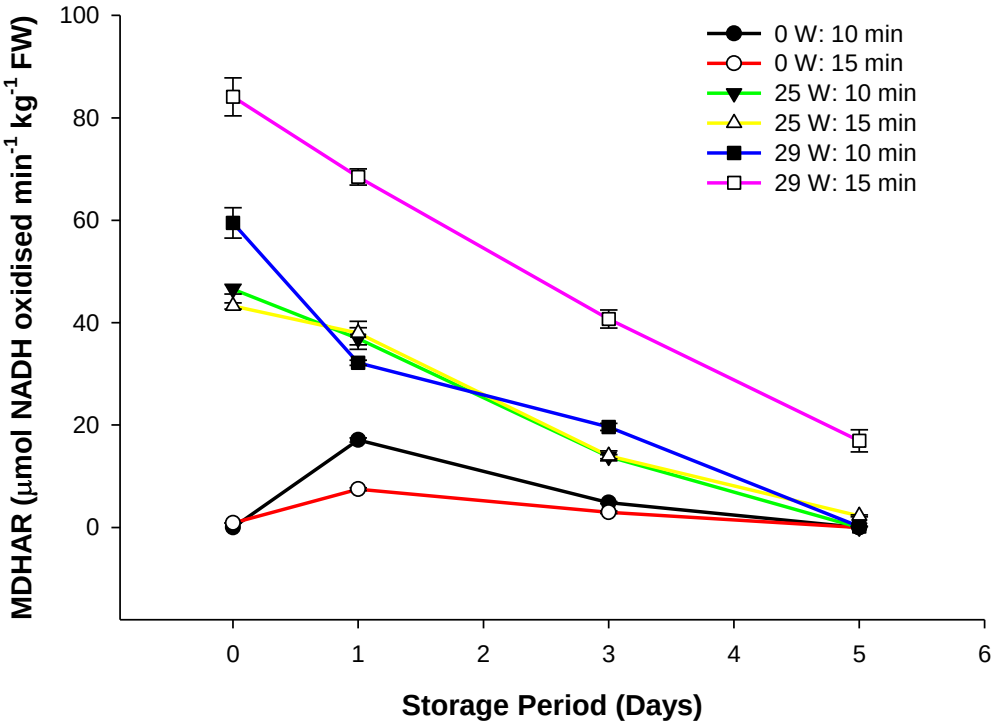


Figure 6.5 MDHAR activity in ultrasound treated fresh-cut pineapple at different power inputs and times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replications.

6.3.2.3 DHAR

The effect of ultrasound at different power inputs and treatment times on the activity of APX is shown in Figure 6.6. Upon exposure to 25 W of ultrasound for 10 and 15 min, the DHAR activity of fresh-cut pineapple increased by 42 and 39%, respectively. Further increment in ultrasound power input and treatment time to 29 W and 15 min enhanced the activity of DHAR in comparison to other ultrasound treatments. At the end of storage, the DHAR activity of ultrasound treated fresh-cut pineapple was higher than dH_2O controls. Samples treated at 29 W of ultrasound for 15 min recorded the highest (7mmol of DHA reduced min^{-1} g^{-1} FW) DHAR activity. Regression analysis showed that the activity of MDHAR activity was only positively correlated with the accumulation of H_2O_2 ($r = 0.6227$, $P<0.001$, Appendix B.18).

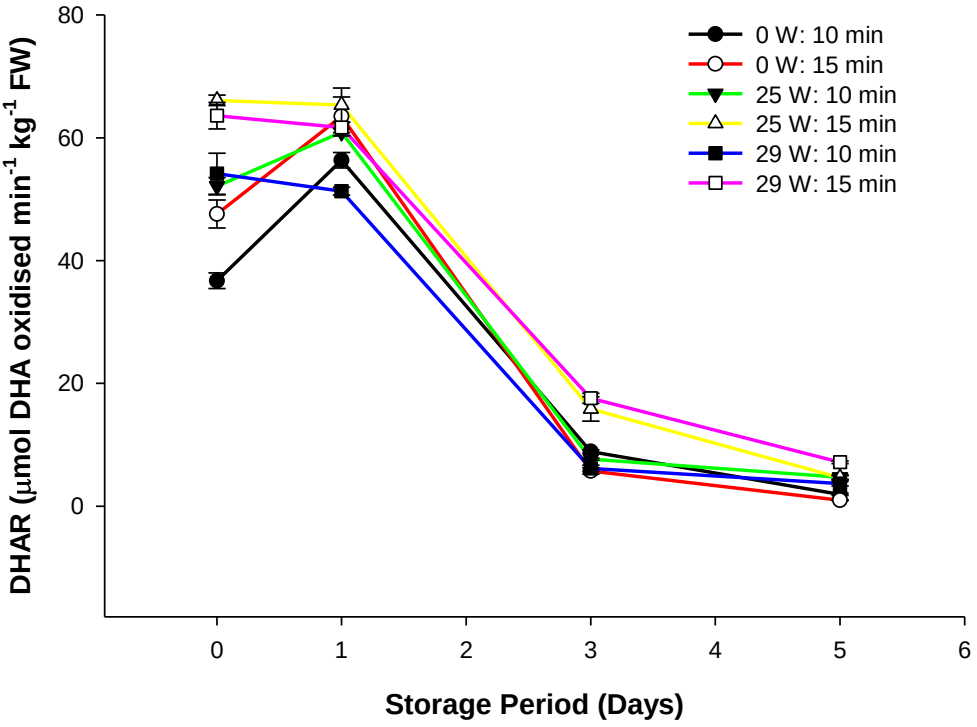


Figure 6.6 DHAR activity in ultrasound treated fresh-cut pineapple at different power inputs and times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replications.

6.4 Discussion

The generation of ROS beyond the capacity of plant to maintain cellular redox homeostasis can cause lipid peroxidation, DNA damage and accelerate plant senescence (Foyer & Noctor, 2005; Hodges et al., 2004). The induction of one or more cellular antioxidant machinery plays important roles in plant tolerance against stress conditions. In particular, the metabolism of phenolic compounds and ascorbate is essential for ROS detoxification when subjected to various biotic and abiotic stress conditions (Gill & Tuteja, 2010).

The accumulation of secondary metabolites such as flavonoids, lignins, coumarins and other phenolic compounds plays a major role in the inducible defence mechanisms of plants (Heredia & Cisneros-Zevallos, 2009; Siboza et al., 2014). PAL

is the first committed step in the phenylpropanoid metabolism to catalyse the deamination of L-phenylalanine to trans-cinnamic acid which is necessary for the biosynthesis of various phenylpropanoid-derived secondary products (Tomás-Barberán & Espín, 2001; Vogt, 2010).

PAL activity of ultrasound treated fresh-cut pineapple was higher in comparison to dH_2O control on day 3 of cold storage. The results from this study support those of Yang et al. (2011) who demonstrated that the application of ultrasound treatment at 350 W for 10 min resulted in higher PAL activity although the changes were insignificant in comparison to control. Authors found that the combination of salicyclic acid and ultrasound treatment induced the activity of PAL following 6 days of storage at 20 ^{o}C. Thus, results suggest that the formation of free radicals due to the sonolysis of water may impose oxidative stress to the plant cells system and induce higher PAL activity to protect the cells from oxidative damage.

Regression analysis also showed that the changes in PAL activity was positively correlated ($r = 0.4878$, $P<0.05$, Appendix B.19) with H_2O_2 concentration. An increased in PAL activity may have enhanced the production of phenolic compounds which quench and reduce intracellular H_2O_2 concentration. Therefore, in the equilibrium state between production and scavenging of ROS, gene expression of PAL may not be upregulated and decreased during subsequent storage.

Several studies have also reported a similar increment in the activity of PAL in various fruits and vegetables after exposed to oxidative stress. Stevens et al. (1999) reported that the induction of PAL activity in sweet potato following exposure to low dosage of UV-C irradiation resulted in better resistance against *Fusarium solani*. A higher peak in the expression and activity of PAL was observed in UV-C irradiated (4 kJ m^{-2}) tomato fruit after 1 day of storage at 20 ^{o}C (Zhang et al., 2013b). Similarly, the application of UV-B and UV-C radiation was also found to increase the

PAL activity of fresh-cut carrot (Du et al., 2012) and white asparagus (Eichholz et al., 2012), respectively. Results suggest the important role of PAL as a key enzyme in the biosynthesis of phenylpropanoid derived secondary metabolites to defend plants against oxidative stress.

An increase in the activity of PPO and POD in dH_2O controls during cold storage may be due to various degree of mechanical operations which impose mechanical stress to the fruits. Various mechanical operations involve in the minimal processing of asparagus (Chen et al., 2010), celery (Zhan et al., 2013), pineapple (Wu et al., 2012b) and sweet potatoes (Ojeda et al., 2014) were also reported to induce the activity of PPO and POD during cold storage. On day 3, samples treated at 29 W for 15 min had the highest PPO and POD activities. This may be due to the rapid accumulation of ROS which serve as signalling molecules to upregulate the gene expression of PPO and POD.

Upon subjected to ultrasound treatment, PPO and POD activities were lower than controls. The findings from this study support those in Amaral et al. (2015) who demonstrated that the PPO activity of fresh-cut potato was inhibited following ultrasound treatment at 200 W for 5 min. Similarly, it was found that the application of 23 kHz ultrasound probe system operated at 75% power input for 90 s completely inhibited PPO activity in tomato extract (Ercan & Soysal, 2011). Nonetheless, the inhibition of PPO and POD activities was also found in other ultrasound experiments which were applied in cantaloupe melon juice Fonteles et al. (2012), mushroom (Lagnika et al., 2013) and dried longan (Rithmanee & Intipunya, 2012).

The inactivation of enzymes by sonication is mainly attributed by the physical and chemical effects of cavitation. High shear forces generated from the collapsed of cavitation bubbles can disrupt the hydrogen bonding and van der Waals interaction in the polypeptide chains and hence lead to the modification of secondary and tertiary

structure of the protein (Chandrapala et al., 2012; Kentish & Feng, 2014). In addition, sonolysis of water can generate high energy intermdiates such as $O_2^{\bullet-}$ and H_2O_2 which can react with some of the amino acid residues that are involved in enzyme stability, substrate binding or enzyme catalytic activity and consequently result in the change in the biological activity (Kentish & Feng, 2014).

Besides ultrasonication, the application of UV-C treatment at a dosage of 4.8 kJ m^{-2} for 120 s resulted in lower PPO and POD activity in fresh-cut melon throughout 10 days of storage at 5 ^{o}C (Chisari et al., 2011). Chauhan et al. (2011) also demonstrated that the activity of PPO and POD in fresh-cut carrot was 1.4 and 3.7-fold lower than control upon exposure to 10 µl L^{-1} ozone treatment. Similarly, PPO and POD activity of fresh-cut celery and pineapple was inhibited following exposure to high light intensity (Zhan et al., 2013) and high pressure argon treatment (Wu et al., 2012a), respectively.

Contradictorily, it was found that ultrasound alone was insufficient to inhibit the PPO and POD activity in fresh-cut apple throughout 12 days of storage at 10 ^{o}C (Jang & Moon, 2011). The authors reported that simultaneous treatment of ultrasound and 1% ascorbate inhibited the activity of PPO and POD in fresh-cut apple during cold storage. The study suggests that the disruption of cell wall of tissue by ultrasound allows better penetration of ascorbate and hence inhibits the activity of both enzymes due to enhance antioxidant machinery. Cruz et al. (2006) also showed that the application of ultrasound treatment at low temperature induced the activity of POD in water cress. Inhibition of POD activity was only observed when the temperature of ultrasound treatment was increased above 85 ^{o}C.

Therefore, studies conducted on the effect of sonication at different power intensity, exposure time, temperature and pressure on the enzyme activity of fruit juices and fresh produce can result in different conclusions regarding the appropriate

ultrasound experimental conditions. O'Donnell et al. (2010) suggested that low power ultrasound can stimulate enzyme production whereas, high power ultrasound can inactivate enzymes due to intense cavitation that breaks the Van der Waals interaction in the polypeptide chains. Also, the effectiveness of ultrasound inactivation depends on the chemical structure of protein and different enzymes marked differences in their resistance to ultrasound treatment (Kentish & Feng, 2014; O'Donnell et al., 2010).

APX is an important enzyme in protecting cells against highly toxic free radicals. It is involved in the dismutation of H_2O_2 to water by reducing ascorbate to MDA which disproportionates spontaneously to DHA (Conklin, 2001; Foyer & Noctor, 2011). Increased of APX activity in dH_2O controls and ultrasound treated fresh-cut pineapple on day 1 of cold storage may be due to the mechanical operations which triggered the production of ROS. Similar trends was also observed in pear where the APX activity was found to increase rapidly and reached a peak on day 1 of storage after cutting into slices (Hu et al., 2014). The rapid increase in the activity of APX due to various degree of minimal processing was also found in fresh-cut baby spinach (Cocetta et al., 2014) and *Zizania latifolia* (Luo et al., 2013a). These studies have showed that the role of APX to decompose H_2O_2 accumulated when exposed to stress conditions.

The activity of APX in ultrasound treated fresh-cut pineapple was higher than control during cold storage. Also, regression analysis showed that the changes in the APX activity and H_2O_2 concentration in fresh-cut pineapple were positively ($r = 0.7661$, $P<0.001$) correlated. Several studies also have showed a similar enhancement of APX activity in fresh or fresh-cut fruits and vegetables following postharvest treatments. Martínez-Hernández et al. (2013) reported that the APX activity of fresh-cut broccoli treated with the combination of UV-C and neutral

electrolysed water and packaged under superatmospheric oxygen was 1.9-fold higher than control following 19 days of storage at 5 ^{o}C. Yang et al. (2014) also demonstrated that the activity of APX in peach fruit was enhanced after 1 day of storage in response to UV-C radiation at a dosage of 3 kJ m^{-2}. Similarly, higher APX activity (21.9%) in UV-C radiated red bell pepper that control at the end of refrigerated storage also resulted in better tolerance against chilling stress (Andrade Cuvi et al., 2011). Thus, it can be suggested that the role APX may be responsible to modulate ROS concentration in plant cells for signaling purposes upon exposure to stress conditions.

To maintain the redox state of ascorbate, MDHA and DHA must be reduced and recycled rapidly by MDHAR and DHAR, respectively (Conklin & Barth, 2004). MDHAR, a FAD enzyme catalyses the reduction of MDHA into ascorbate by using NADPH as an electron donor (Gill & Tuteja, 2010). Reduction of DHA into ascorbate is mediated by DHAR in the presence of gluthatione as a reductant (Smirnoff, 1996). Regardless of treatment applied, the transient increase in the activity of MDHAR and DHAR following 1 day of storage may suggest the role of these enzymes in the regeneration of ascorbate to restore the antioxidant potential of pineapple upon exposure to various degree of processing. Similarly, Cocetta et al. (2014) reported that the expression of MDHAR and DHAR baby spinach increased 3 hours after cutting. Authors observed that the activity of both enzymes in fresh-cut baby spinach was higher than uncut sample on day 1 of storage at 4 ^{o}C. The increase transcripts and enzymatic activity of MDHAR and DHAR was also reported in acerola fruit when exposed to cold and salt stress (Eltelib et al., 2011). Tsaniklidis et al. (2014) reported a similar increase in the transcript level of MDHAR and DHAR in cherry tomato when exposed to low temperature also demonstrated that the vital role of these enzymes in the defence response against abiotic stress .

In this study, the activity of MDHAR and DHAR in ultrasound treated fresh-cut pineapple was higher than control during day 5 of cold storage. Also, regression analysis showed that the changes in the concentration of H_2O_2 was positively correlated with the activity of MDHAR ($r = 0.6767$, $P<0.001$) and DHAR ($r = 0.6227$, $P<0.001$) in fresh-cut pineapple. Results demonstrated that the vital role of these enzymes in the maintenance of redox potential of ascorbate in fresh-cut pineapple against oxidative stress generated by ultrasound. Similar increment in the activity of MDHAR and DHAR was also observed in UV-C treated strawberry fruit (Erkan et al., 2008).

In conclusion, it was demonstrated that ultrasound treatment at 25 W for 10 min enhanced the enzymatic activity of PAL, APX, MDHAR and DHAR in fresh-cut pineapple in comparison with other ultrasound treatments. Meanwhile, it was observed that PPO and POD activity was lower than that of control throughout cold storage. Results suggest that the effectiveness of ultrasound inactivation depends on the chemical structure of protein and different enzymes marked differences in their resistance to ultrasound treatment. In this study, the inhibition of PPO and POD may be due to the physical and chemical effects of sonication which disrupt the Van der Waals forces and hydrogen bonding of polypeptide chains. On the contrary, formation of free radicals during ultrasound treatment may impose oxidative stress in fresh-cut pineapple and hence increase the activity of PAL, APX, MDHAR and DHAR that may be involved in defence mechanisms against intracellular ROS.

The induction or inhibition of different antioxidant enzymes due to the mechanisms of ultrasound treatment may have resulted in the changes of antioxidant capacity of fresh-cut pineapple throughout cold storage. Considering the important role of low molecular metabolites such as phenolic compounds and ascorbate to prevent oxidative damage and lipid peroxidation by scavenging intracellular ROS, it

would be interesting to further explore into the changes in total phenolic and ascorbate concentration as well as antioxidant capacity in ultrasound treated fresh-cut pineapple. Also, the interplay between phenolic metabolism enzymes (PAL, PPO and POD) and total phenolic concentration or ascorbate recycling enzymes and ascorbate concentration in response to oxidative stress could be better understood by conducting a correlation analysis in the following chapter.

7 EFFECT OF ULTRASOUND ON THE ANTIOXIDANT ACTIVITY OF FRESH-CUT PINEAPPLE

7.1 Introduction

Pineapple is a good source of vitamin C to prevent oxidative damage in body cells by scavenging reactive oxygen species, vitamin B to aid in digestion, vitamin A, fibre and minerals (Hossain & Rahman, 2011). Considering the significant proportion of total antioxidants that can be contributed in daily diet, the consumption of pineapple has risen perpetually. However, antioxidant constituents of pineapple are susceptible to degradation when subjected to various degree of mechanical operations prior to packaging and storage (Gil et al., 2006). Therefore, considerable efforts have been taken in the field of food science to maintain or improve the antioxidants of fresh-cut fruits and vegetables through postharvest handling and processing.

Wu et al. (2012b) demonstrated that high pressure argon and nitrogen treatment induced higher and reduced the degradation of ascorbate of fresh-cut pineapple following 20 days of storage at 4 ^{o}C. Although the effect of UV radiation and ozone treatment on fresh-cut pineapple with prolonged storage was not carried out, both treatments effectively induced the total phenolic concentration with reduced ascorbic acid concentration in sample. These studies have demonstrated that the application of postharvest treatment can activate plant stress defence system and enhance the nutraceutical concentration of fresh fruits and vegetables (Cisneros-Zevallos, 2003; Gonzalez-Aguilar et al., 2010).

Interestingly, the generation of free radicals such as OH^- and H_2O_2 during sonication may impose oxidative stress to fresh produce and hence induce the antioxidant potential of fresh fruits and vegetables (Awad et al., 2012; São José et al., 2014). Cao et al. (2010b), reported that low decay incidence was associated with

an increase of vitamin C concentration in ultrasound treated strawberry. Similarly, ultrasound was also found to induce the total flavonoids and ascorbate concentration of plum fruit during storage (Chen & Zhu, 2011). Therefore, present study was conducted to study the effect of ultrasound treatment on the total phenolic concentration, ascorbate concentration and total antioxidant capacity of fresh-cut pineapple during cold storage.

7.2 Materials and methods

Fruits were prepared as previously described in Section 3.2. Ultrasound treatment operated at the power input of 25 and 29 W and exposure time of 10 and 15 min were used and applied as described in Section 3.3 Three replications of three fruits for each treatment were sampled for extraction and analyses on 0, 1, 3 and 5 days of storage at 7 ^{o}C and 80 ± 5% RH. The data were subjected to three way analysis of variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test at P=0.05.

7.2.1 Extraction of polyphenols

The recovery of phenolic compounds from fresh-cut pineapple was carried out using extraction method as described by (Wu et al., 2013). Briefly, 1 g of pineapple cubes was ground with 10 ml of 100% (v/v) methanol in a mortar and pestle. Homogenates were vortexed and centrifuged at 10,000 x g for 15 min at 4 ^{o}C. Supernatant was filtered with Whatman paper no. 1 and used for Folin-Ciocalteu and antioxidant capacity assays.

7.2.2 Total phenolic concentration

Total phenolic concentration of extract was performed using Folin-Ciocalteu assay as described by Du et al. (2009) with some modifications. Briefly, 0.1 ml of extract was added with 6.0 ml of dH_2O and 0.5 ml of 2 N Folin-Ciocalteu reagent. After incubated

at room temperature for 4 min, 1.5 ml of 7% (w/v) sodium carbonate and 1.9 ml of distilled water were added into the mixture. The solution was vortexed and incubated at 37 °C for 2 h. Blank was prepared by replacing 0.1 ml of pineapple extract with 0.1 ml of dH_2O. The absorbance value of the blue-coloured complex formed was measured against a blank at 765 nm using a microplate spectrophotometer. The experiment was performed similarly in the preparation of standard curve using gallic acid as a standard (R^2 = 0.9986; Appendix A.3). Briefly, 10 mg of gallic acid was mixed with 10 ml of dH_2O to obtain a final concentration of 1000 µg ml^{-1}. The stock solution of gallic acid was diluted to 25, 50, 100, 200, 400, 600, 800 and 1000 µg ml^{-1}, respectively. Total phenolic concentration was determined against the standard curve and expressed as mg of gallic acid equivalents (GAE) 100 g^{-1} FW.

7.2.3 Determination of total ascorbate (AA + DHA), ascorbic acid (AA) and dehydroascorbic acid (DHA)

Extraction of AA and DHA was carried out as described by Franck et al. (2003) with some modifications. Approximately 0.2 g of pineapple cubes was homogenised with 1 ml of pre-cooled extraction buffer consisting of 3% (w/v) metaphosphoric acid (MPA) and 1 mM EDTA in a mortar and pestle. Homogenates was thoroughly vortexed and centrifuged at 10,000 x g for 10 min for 4 °C. The supernatant was filtered through polyvinylidene fluoride (PVDF) filter with 0.2 µm pore size, loaded into 2 ml glass injection vial and analysed immediately with HPLC.

The concentration of DHA was determined by subtracting AA from the total ascorbate (AA + DHA) after reduction of extracts with dithiothreitol (DTT) as described by Davey et al. (2003). Briefly, 500 µl of extract in MPA/EDTA extraction solvent was added and homogenised with 250 µl of DTT in 400 mM of Tris-base. After incubated at room temperature for 15 min, the reaction was stopped by adding 250

µl of 8.5% (v/v) o-phosphoric acid which resulted in two-fold dilution of the original extract. The reduced samples were directly analysed for total ascorbate by HPLC.

The determination of AA and total ascorbate was carried out on a Series 200 HPLC system using the ZORBAX SB-C8 column as described by Davey et al. (2003). 10 µl of the extract was injected using an autosampler. The mobile phases [A: 0.5% (v/v) methanol (MeOH), 1 mM EDTA, and 400 µl L^{-1} o-phosphoric acid; B: 30% (v/v) acetonitrile, 70% (v/v) A)] were membrane filtered using regenerated cellulose filter membrane and pumped through the column at a rate of 1.0 ml/min. The applied gradient elution time for mobile phase B was: 0 min, 0%; 3.5 min, 0%; 6.5 min, 100%; 8.5 min, 100%; 10.5 min, 0%; 15 min, 0%. AA and total ascorbate was detected using UV/VIS detector at 242 nm.

Chromatographic peak of samples (Appendix A.6) was identified by comparing its retention time with that of L-AA standard (Appendix A.5). A standard curve (R^2 = 0.9975; Appendix A.4) was established by using 1 ml of different concentrations of L-AA. Briefly, 0.5 mg of L-AA was dissolved in 5 ml of MPA/EDTA extraction buffer to obtain a final concentration of 100 µg ml^{-1}. The stock solution of L-AA was diluted to 20, 40, 60, 80 and 100 µg ml^{-1}, respectively. The concentration of AA and total ascorbate was compared against standard and expressed as mg of L-AA 100 g^{-1} FW.

7.2.4 Determination of total antioxidant activity

7.2.4.1 Ferric Reducing Antioxidant Power (FRAP)

Antioxidant capacity of polyphenol extracts was carried out using FRAP assay as described by Alothman et al. (2009) with some modifications. The FRAP reagent comprised of 2.5 ml of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 2.5 ml of 20 mM iron (III) chloride ($FeCl_3$) and 25 ml of 0.3 M acetate buffer pH 3.6. Briefly, 20 µl of extract was added with 200 µl of FRAP reagent into the 96-well microplate

and the homogenate was incubated at 37 °C for 4 min. Blank was prepared by replacing 0.1 ml of polyphenol extract with 0.1 ml of dH_2O. The absorbance value of the blue-coloured complex formed was measured against a blank at 593 nm using a microplate spectrophotometer. The experiment was performed similarly in the preparation of standard curve by using 20 µl of different concentration of iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7H_20$). Briefly, 27.8 mg of $FeSO_4 \cdot 7H_20$ was dissolved in 10 ml of dH_2O to obtain a final concentration of 10 mM. The stock solution of $FeSO_4 \cdot 7H_20$ was diluted to 25, 50, 75, 100, 150, 200, 400, 600, 800 and 1000 µM, respectively. FRAP value was determined from the standard curve (R^2 = 0.9993; Appendix A.7) and expressed as mmol Fe (II) g^{-1} FW.

7.2.4.2 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant capacity of polyphenols extract was carried out using DPPH assay as described by with Wu et al. (2012b) some modifications. 0.025 g/L of methanolic DPPH was freshly prepared before analysis was carried out. A 20 µl of aliquot of the extract was mixed with 200 µl of DPPH in a 96- well microplate and incubated in the dark at room temperature for 30 min. Blank was prepared by replacing 20 µl of extract with 20 µl of methanol. The absorbance of value was measured against blank at 517 nm using a microplate spectrophotometer. A standard curve (R^2 = 0.9924; Appendix A.8) was established by using 20 µl of different concentrations of trolox. Briefly, 25 mg of trolox was dissolved in 100 ml of methanol to obtain a final concentration of 1 mM. The stock solution of trolox was diluted to 75, 100, 150, 200, 300, 400 and 500 µM, respectively. The decrease in absorbance of samples in DPPH solution due to the scavenging of DPPH free radicals was determined from the standard curve and results were expressed as mM of trolox equivalent (TE) g^{-1} FW.

7.2.4.3 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)

Radical scavenging activity of pineapple extract against ABTS radical cation was measured by using the method of Thaipong et al. (2006) with some modifications. Briefly, an ABTS radical solution was prepared by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate solution at a ratio 1:1 (v/v). The mixture was vortex mixed and then allowed to stand in dark condition for 12 – 16 h to give a dark green solution which was used for analysis within 2 d. The absorbance of ABTS radical solution was calibrated to an absorbance of 0.7 ± 0.02 at 734 nm by diluting with absolute ethanol before used. An aliquot of 20 µl of extract was added to 200 µl of $ABTS^{+}$ radical cation solution in a 96-well microplate. The absorbance of the mixture was read immediately at 734 nm after incubated at room temperature for 6 min. Blank was prepared by replacing 20 µl of extract with 20 µl of methanol. A standard curve (R^2 = 0.9957; Appendix A.9) was established by using 20 µl of different concentrations of trolox. Briefly, 25 mg of trolox was dissolved in 100 ml of methanol to obtain a final concentration of 1 mM. The stock solution of trolox was diluted to 50, 75, 100, 150, 200, 300, and 400 µM, respectively. The decrease in absorbance of samples in ABTS solution due to the scavenging of ABTS free radicals was determined from the standard curve and results were expressed as mM of trolox equivalent (TE) g^{-1} FW.

7.3 Results and discussion

7.3.1 Total phenolic concentration

The effect of ultrasound at different power inputs and treatment times on the total phenolic concentration is shown in Figure 7.1. After ultrasound treatment at 25 and 29 W for 10 and 15 min, respectively, total phenolic concentration of fresh-cut pineapple was increased on day 0 and then decreased during subsequent storage period. The highest total phenolic concentration (133 mg GAE 100 g^{-1} FW) was recorded in fruit treated at 29 W of ultrasound power input for 15 min. On day 3, the total phenolic concentration of those treated at 29 W for 10 and 15 min, respectively, was 8 and 16% higher than dH_2O controls.

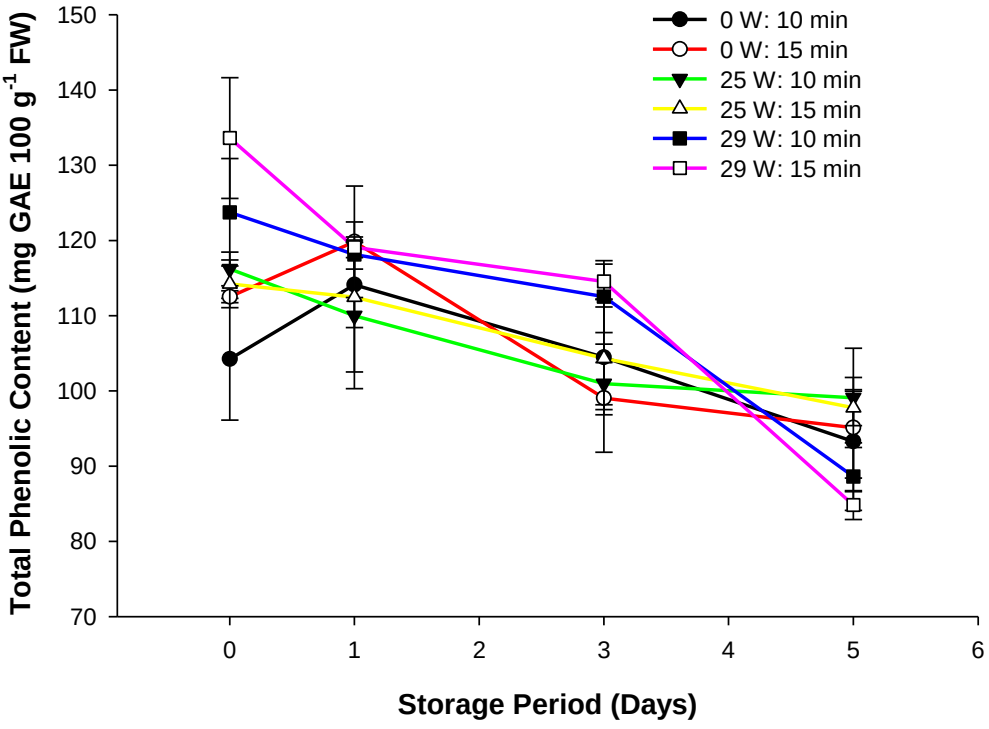


Figure 7.1 Total phenolic concentration of ultrasound treated fresh-cut pineapple during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Irrespective of treatment time, the total phenolic concentration of fresh-cut pineapple treated at 29 W was 8 and 12% lower than dH_2O control and fruit treated at 25 W, respectively, after 5 days of storage at 7 ^oC. Also, samples treated at 25 W of ultrasound power input was 5% higher than dH_2O control.

Biosynthesis and accumulation of secondary metabolites such as phenolics, terpenes and nitrogen containing compounds in response to various environmental stimuli play essential roles in plant inducible defence system (Barros & Saltveit, 2013; Du et al., 2014). In particular, polyphenol compounds which can inhibit lipid peroxidation, chelate redox-active metals and regulate redox status of plant tissues are important class of antioxidant compounds ubiquitous in fruits and vegetables (Amodio et al., 2014; Wu et al., 2012b).

Application of postharvest treatments, which induce oxidative stress can also result in increased total phenolic concentration in various fresh-cut commodities. Du et al. (2014) demonstrated that the application of UV-B irradiated (3.1 kJ m^{-2}) increased the soluble phenolic concentration in fresh-cut lettuce by 2.5-fold following 10 days of storage at 4 ^oC. Higher total phenolic concentrations were also reported in highly pressurised fresh-cut carrot (Bi et al., 2011), heat shock treated fresh-cut carrot (Alegria et al., 2012), hot air and UV-C treated fresh-cut broccoli (Lemoine et al., 2010) and electrolysed water disinfected fresh-cut mizuna baby leaves (Tomás-Callejas et al., 2011).

Higher total phenolic concentration of ultrasound treated fresh-cut pineapple may be due to the induction of PAL activity which is the key metabolic enzyme in the phenylpropanoid pathway to synthesise various monomeric or polymeric phenolics to repair damaged tissues and prevent further invasion by pathogens (Surjadinata & Cisneros-Zevallos, 2012; Wang et al., 2015).

Irrespective of treatment applied, decreased total phenolic concentrations in fresh-cut pineapple may be due to increased PPO and POD activities after 1 day of storage. Both enzymes catalyse the oxidation of o-diphenols and monolignols (Toivonen & Brummell, 2008). In this study, increment of ultrasound power input decreased the total phenol concentrations of fresh-cut pineapple after 5 days of cold storage. Ultrasound waves with large amplitudes can facilitate the displacement of molecules and collapse pressure and hence promote the formation of free radicals in aqueous solution (Sauter et al., 2008). Exposure of fresh-cut pineapple to oxidative stress may have perturbed the redox balance and led to the rapid oxidation of phenolic compounds to neutralise the free radicals in plant tissues. The findings of this study support Martínez-Hernández et al. (2011) who also demonstrated that the application of UV-C dose above 4.5 kJ m^{-2} resulted in reduction of total phenolic concentration in fresh-cut broccoli following 19 days of storage at 10 ^{o}C. Similarly, it was found that the total phenolic concentration of fresh-cut pineapple was found to decrease markedly after prolonged exposure to UV-C and ozone treatment for 30 min (Alothman et al., 2009, 2010). Thus, evidence suggest that hormetic dosage of postharvest treatments can improve the phytochemical concentration of fresh-cut produce.

7.3.2 Total ascorbate (AA + DHA), ascorbic acid (AA) and dehydroascorbic acid (DHA) concentration

The effect of ultrasound at different power inputs and treatment times on the total ascorbate of fresh-cut pineapple is shown in Figure 7.2. After ultrasound treatment at 25 for 10 min, total ascorbate of samples was 11% higher than dH_2O control. However, increased treatment time resulted in lower ascorbate concentration in comparison to ultrasound treated samples for 10 min.

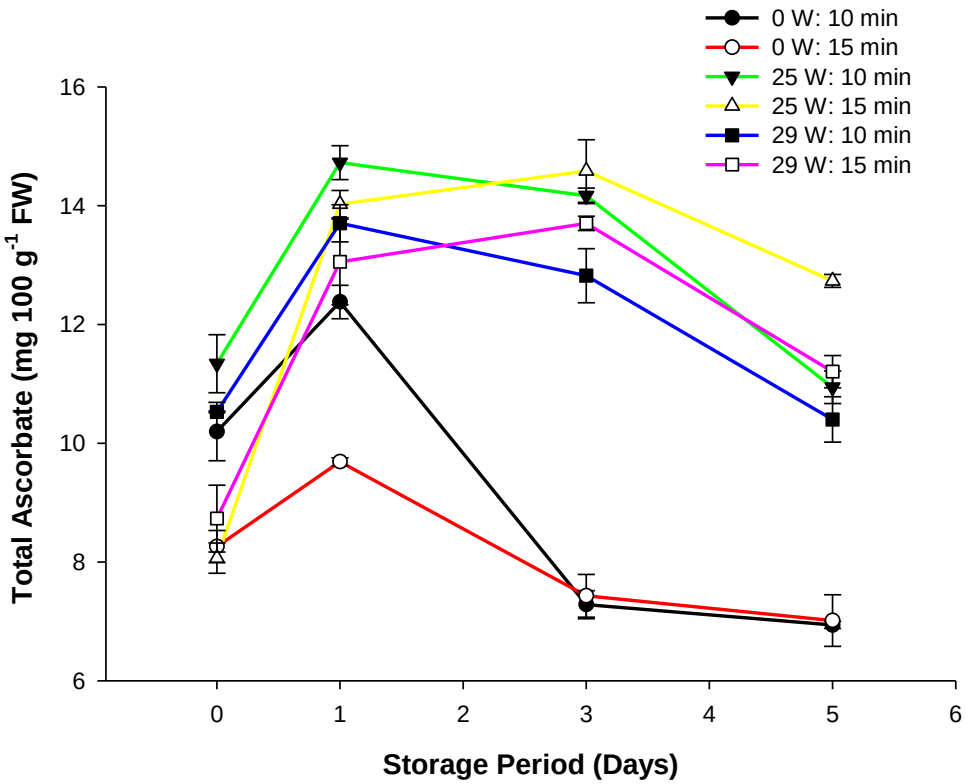


Figure 7.2 Total ascorbate concentration in ultrasound treated fresh-cut pineapple at different power inputs and times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replications.

Regardless of treatment applied, total ascorbate of fresh-cut pineapple was increased continuously, reached a peak on day 1 and decreased during the subsequent storage period. As shown in Figure 8.2, the highest peak of total ascorbate (15 mg 100 g^{-1} FW) was recorded in fruit treated at 25 W of ultrasound for 10 min after stored for 1 day and the value was 19% higher than dH_2O control. Total ascorbate of dH_2O controls decreased on day 3 and 5 but the magnitude of decline was lower in ultrasound treated fruits. After storage for 5 days, total ascorbate of fresh-cut pineapple treated at 25 and 29 W was higher than control.

Changes in the AA concentration of fresh-cut pineapple treated at different ultrasound power input and treatment time are shown in Figure 7.3. Variation in the concentration of AA in dH_2O controls and ultrasound treated fresh-cut pineapple followed a similar trend as reported in total ascorbate. It was observed that the highest increment of AA concentration was recorded for fruit treated at 25 and 29 W of ultrasound. AA concentration was decreased in both control and ultrasound treated fruit on day 3 and 5. However, those treated at 25 and 29 W of ultrasound was at least 2.9 and 3.4-fold higher than dH_2O control on day 3. Meanwhile, after 5 days of storage, the concentration of AA in ultrasound treated samples at 25 and 29 W was 2.1 and 2.3-fold higher than dH_2O controls.

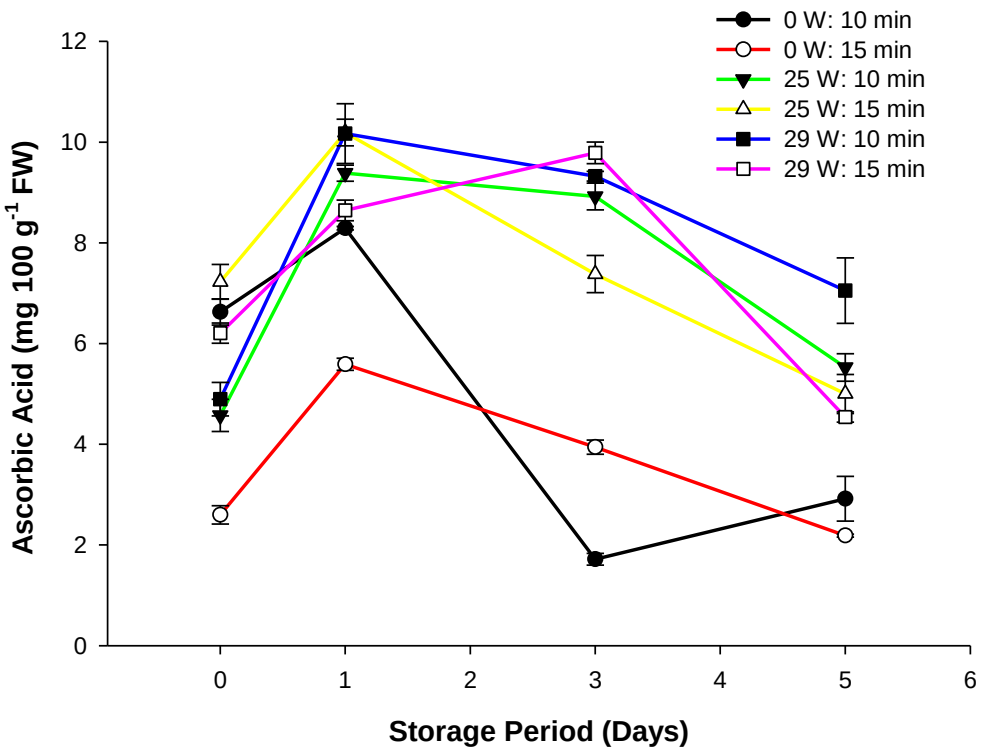


Figure 7.3 Ascorbic acid concentration in ultrasound treated fresh-cut pineapple at different power inputs and times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replications.

The effect of ultrasound at different power inputs and treatment times on the concentration of DHA is shown in Figure 7.4. After ultrasound treatment at 25 and 29 W for 10 min, the DHA concentration of samples was 90 and 58% higher than control washed with dH_2O for 10 min. An equilibrium state was achieved whereby the DHA concentration was remained constant throughout subsequent storage period. However, lower DHA concentration was observed in fresh-cut pineapple after exposed to ultrasound treatment at 25 and 29 W for 15 min. The DHA concentration of dH_2O controls and ultrasound treated samples at 25 and 29 W for 15 min increased on day 5.

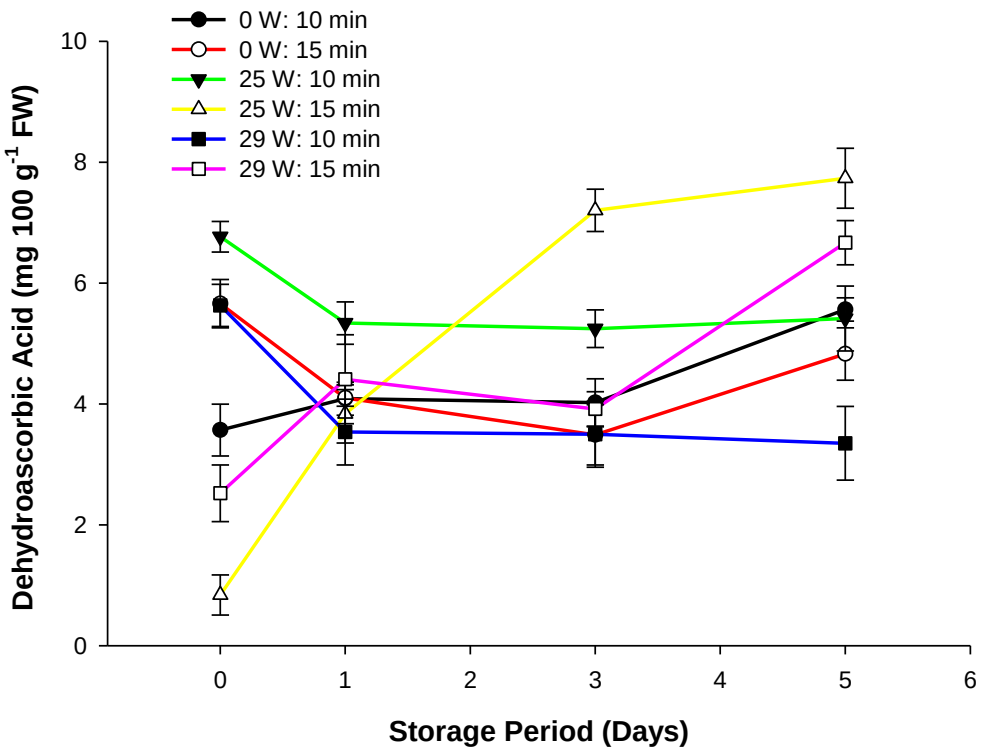


Figure 7.4 Dehydroascrobic acid concentration in ultrasound treated fresh-cut pineapple at different power inputs and times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replications.

Ascorbate is a highly abundant, powerful and water soluble metabolite which plays an important in plant stress physiology and growth and development. It can help to minimise the cellular damage caused by ROS in plants by directly scavenging singlet oxygen, $O_2^{\bullet-}$ and $OH^{\bullet}$ and indirectly eliminating H_2O_2 via APX (Blokhina et al., 2003; Conklin, 2001). The ability of apoplastic ascorbate to react enzymatically and non-enzymatically with a wide range of ROS makes ascorbate as the main ROS detoxifying compound in the aqueous state (Blokhina et al., 2003; Davey et al., 2000). Conklin and Barth (2004) also estimated that approximately 10% of ascorbate pool is localised within the apoplastic space which makes apoplastic ascorbate as the first line of defence in plants to govern resistance to abiotic stress.

Increased ascorbate concentration in ultrasound treated fresh-cut pineapple indicates the possibility of simultaneous biosynthesis of ascorbate in the tissue to

acclimatise to increase production rate of intracellular ROS. The higher concentration of ascorbate is essential to increase the capacity of fresh-cut pineapple to cope with oxidative stress resulted from extreme physical and sonochemical reactions produced during sonication. Free radicals such as $O_2^{\bullet-}$ and H_2O_2 are produced during sonolysis of water. Nonetheless, the increase of ascorbate was coincided with the accumulation of ROS in fresh-cut pineapples. The ascorbate concentration was affected by the concentration of H_2O_2 ($r = 0.4982$, $P<0.001$, Appendix B.25) and production rate of $O_2^{\bullet-}$ ($r = 0.7362$, $P<0.001$, Appendix B.26). Hence, results suggest that the possible role of ROS to induce ascorbate biosynthesis when subjected to oxidative stress.

The increased in the ascorbate concentration was similar to the findings of Cao et al. (2010a) who reported that the ascorbate concentration of ultrasound treated strawberry was 11% higher than control at the end of storage. It was also found that the concentration of ascorbate in ultrasound treated peach fruit was 2% higher than control after stored for 6 days at 20 ^{o}C (Yang et al., 2011). Ultrasound treatment operated at higher power input (100 W) was increased the ascorbate concentration of plum fruit following 30 days of storage at 4 ^{o}C (Chen & Zhu, 2011).

The application of several postharvest treatment which can impose oxidative stress was also found to increase the ascorbate concentration of fresh produce. Wu et al. (2012a) reported that the concentration of ascorbate in high-pressure argon treated fresh-cut pineapple was higher than that of control throughout the storage. Similarly, the ascorbate concentration of fresh-cut mango treated with pulsed light applied at total fluence of 8 J cm^{-2} was 71.6% higher than control after 2 days of storage at 6 ^{o}C, the (Charles et al., 2013). Aguayo et al. (2006) also demonstrated that cyclic exposure to 4 µl L^{-1} ozone gas resulted in higher ascorbate concentration in sliced tomato throughout 15 days of cold storage. Similar increment in the concentration of ascorbate was reported in ozone treated fresh-cut papaya (Yeoh et

al., 2014) and lettuce (Beltrán et al., 2005), short term nitrogen treated fresh-cut water chestnut (You et al., 2012) and highly pressurised fresh-cut bell peppers (Meng et al., 2012).

Although the activity of APX was increased on day 0, 1 and 3, respectively, the changes did not reduced the concentration of ascorbate in ultrasound treated samples. It can be deduced that the production and capacity of regeneration of ascorbate may have surpassed the oxidation of ascorbate by APX to eliminate the accumulation of H_2O_2. Consequently, the lower concentration of DHA in fresh-cut pineapple treated at 25 and 29 W of ultrasound for 15 min on day 0 and 1 was coincided with the transient increase in the enzymatic activity of MDHAR and DHAR. At the end of storage, the higher DHA concentration in samples treated at 25 and 29 W of ultrasound for 15 min may be due to a decline in regeneration capacity and high rate of $O_2^{\bullet -}$ production. Singh and Singh (2013) suggest that the accumulation of DHA in fresh produce during cold storage may indicate the failure of the antioxidative system to regenerate ascorbate due to the shift in equilibrium towards more oxidised state in plant tissues.

7.3.3 Total antioxidant activity

FRAP assay measures the reducing potential of an antioxidant by the reduction of ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to blue coloured ferrous tripyridyltriazine complex (Fe^{2+}-TPTZ) through the mechanism of SET. As shown in Figure 7.5, the initial antioxidant capacity of fresh-cut pineapple as estimated by FRAP was in the approximately 28 mmol Fe (II) g^{-1} FW. Immediately after exposed to ultrasound treatment at 25 W for 10 and 15 min, respectively, the total antioxidant capacity of fresh-cut pineapple was 9 and 13% higher than dH_2O controls. Similarly, the total antioxidant capacity of fresh-cut pineapple was increased after ultrasound treatment at 29 W of ultrasound for 10 and 15 min, respectively. Regardless of treatment

applied, the antioxidant capacity of fresh-cut pineapple as measured by FRAP was decreased gradually throughout cold storage. On day 5 of storage, antioxidant activity of samples treated at 29 W of ultrasound was 12% lower than dH_2O control.

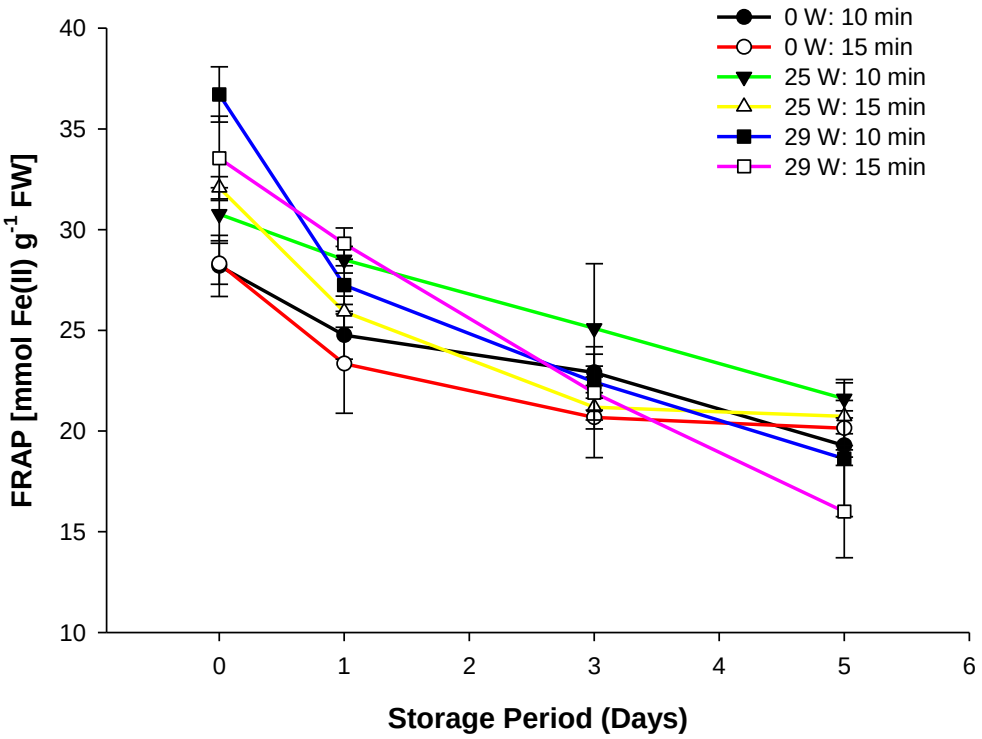


Figure 7.5 Antioxidant activity (measured by FRAP) of fresh-cut pineapple treated at different power inputs and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Both DPPH and ABTS assays are based on the measurement of scavenging ability of antioxidants towards $DPPH^{\bullet}$ and long life anion $ABTS^{\bullet+}$ radicals. The neutralisation of these two indicator radicals by direct reduction or radical quenching via SET or HAT, respectively, can be used to estimate the total antioxidant capacity of various fruits and vegetables (Prior et al., 2005). The effect of ultrasound at different power and treatment time on the total antioxidant activity of fresh-cut pineapple as measured by DPPH and ABTS assays is shown in Figure 7.6 – 7.7. The initial free radical scavenging activity determined by DPPH and ABTS was approximately 72 mM TE g^{-1} FW and 64 mM TE g^{-1} FW, respectively. The total

antioxidant capacity as measured by DPPH and ABTS assays of dH_2O controls was decreasing progressively throughout cold storage.

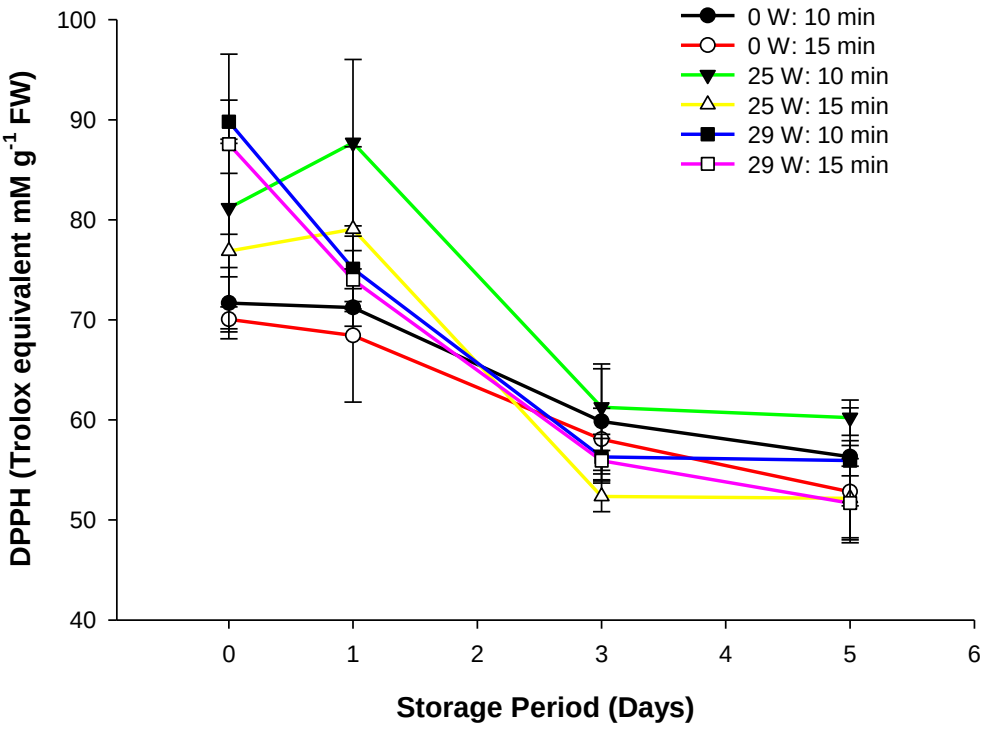


Figure 7.6 Antioxidant activity (measured by DPPH) of fresh-cut pineapple treated at different power inputs and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH.Vertical bars represent the SD of three replicates.

The highest total antioxidant activity (88 mM TE g^{-1} FW in DPPH and 84 mM TE g^{-1} FW in ABTS) was recorded in fresh-cut pineapple after exposed to ultrasound treatment at 29 W for 10 min. Similarly, increased in the total antioxidant activity was observed in samples treated at 29 W: 15 min, 25 W: 10 min and 25 W: 15 min.

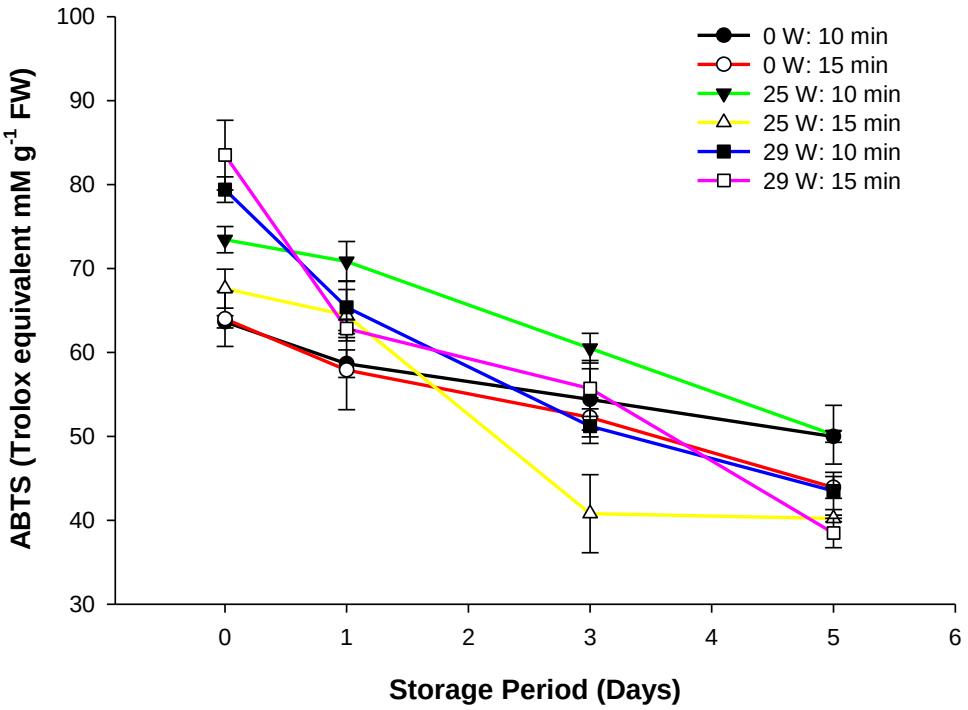


Figure 7.7 Antioxidant activity (measured by ABTS) of fresh-cut pineapple treated at different power inputs and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH.Vertical bars represent the SD of three replicates.

On day 1, it was observed that the antioxidant capacity of samples treated at 25 W for 10 min was higher than those treated at 29 W of ultrasound. Also, the antioxidant capacity of those treated at 25 W for 10 min as measured by DPPH and ABTS assays was 23.1 and 20.8% higher than dH_2O control after 1 day of storage. Meanwhile after 5 days, no effect on total antioxidant activity of ultrasound treatment was detected.

The antioxidant capacity of redox molecules in foods can be defined as their ability to scavenge free radicals and electrophiles in a biological system (Floegel et al., 2011; Queiroz et al., 2011). Due the complex nature of phytochemicals and multiple form of free radicals and oxidants that can be found in a biological system, antioxidants may respond in different manners to distinctive free radicals or other oxidant sources (Prior et al., 2005). In general, the ability of antioxidants to quench

free radicals is normally due to the primary mechanisms of single electron transfer (SET) and hydrogen atom transfer (HAT) (Ozgen et al., 2006; Prior et al., 2005). Studies have suggested that synergistic effect between antioxidants which acts in multiple reaction and mechanisms as well as different phase of localisation to quench the oxidant sources could exist within a biological system (Du et al., 2009; Floegel et al., 2011). The isolation of a particular antioxidant compound or sole dependence on an antioxidant assay would not reflect the total antioxidant capacity in fruits and vegetables (Leong & Shui, 2002; Tyug et al., 2010). Therefore, FRAP, DPPH and ABTS assays were employed in the present study to estimate the total antioxidant capacity of fresh-cut pineapple.

Several workers have also demonstrated that the use of postharvest treatment that impose oxidative stress can induce the antioxidant capacities of fresh commodities. Du et al. (2012) demonstrated that the application of UV-B irradiation at a total energy dose of 141 mJ cm^{-2} resulted in higher total antioxidant capacity of fresh-cut carrot that control after 3 days of storage at 15 ^{o}C. UV-C treatment at the dosage ranged from 1.5 – 15 kJ m^{-2} was also found to enhance the antioxidant capacity of fresh-cut broccoli through 19 days of storage at 5 ^{o}C (Martínez-Hernández et al., 2011).

Changes in the total phenolic concentration were positively correlated with total antioxidant activity of fresh-cut pineapple as measured by FRAP ($r = 0.7280$, $P<0.001$, Appendix B.30), DPPH ($r = 0.6758$, $P<0.001$, Appendix B.31) and ABTS ($r = 0.7368$, $P<0.001$, Appendix B.32) assays. However, no correlation was found between ascorbate concentration and total antioxidant activity of fresh-cut pineapple. Results suggest that polyphenols are the main antioxidants contributed to the total antioxidant capacity of fresh-cut pineapple.

In conclusion, ultrasound treatment regulates the activity of PAL inducing the production of protective secondary metabolites such as phenolic compounds as indicated by higher total phenolic concentrations under stress. Also, the induction of MDHAR and DHAR activity in ultrasound treated fresh-cut pineapple may regulate the regeneration capacity of ascorbate and hence resulted in higher total ascorbate concentration in comparison to control. The increment of total antioxidant capacity in ultrasound treated fresh-cut pineapple may have induced intrinsic plant defence system and resulted in lower microbial count during cold storage. However, consumers purchase intention and demands are often affected by organoleptic and physical characteristics of fruits and vegetables. Therefore, it will be of interest to determine the effect of ultrasound treatment on the physico-chemical and physiological behaviour of fresh-cut pineapple.

8 EFFECT OF ULTRASOUND ON THE PHYSICO-CHEMICAL AND PHYSIOLOGICAL BEHAVIOUR OF FRESH-CUT PINEAPPLE

8.1 Introduction

Processing of pineapple is often time and labour consuming due to its large size, thick inedible peel and large crown (Azarakhsh et al., 2014b; Wu et al. 2012b). Therefore, fresh-cut pineapple has commercial advantages in terms of weight reduction for lower transportation cost and convenience of consumptions among consumers.

The storage life of fresh-cut pineapple is often limited due to microbial spoilage and increase of respiration rate during storage (Marrero & Kader, 2006). The proliferation of anaerobes microorganism can enhance the production of volatile organic compounds which affects the sensory quality of fruit (Zhang et al., 2014). Besides microbial growth, several physico-chemical changes such as enzymatic and non-enzymatic browning, juice leakage, softening, and off-flavour development can be triggered due to the liberation of cellular concentrations at the site of wounding (González-guilar et al., 2004; Mantilla et al., 2013). Marrero and Kader (2006) suggested that the marketability of fresh-cut pineapple following storage for 5 days at 10 °C was unacceptable as signaled by a sharp increase in respiratory activity beyond the threshold limit.

Studies have been focused on the use of chemical preservatives and additives such as ascorbic acid and edible coating materials to maintain the physico-chemical properties of fresh-cut pineapple. For instance, González-Aguilar et al. (2004) showed that the application of antibrowning agents (N-acetylcysteine, ascorbic acid and isoascorbic acid) on fresh-cut pineapple reduced firmness loss, juice leakage, rate of respiration and browning index when compared with that of the controls following storage for 14 days at 10 °C. Although the application of 0.56% gellan based edible

coatings did not inhibit the growth of spoilage microorganisms, the firmness of fresh-cut pineapple was maintained by slowing down the rate of respiration and weight loss on day 8 of storage at 5 °C (Azarakhsh et al., 2014a). Similarly, the combination of 2% calcium lactate with 2% cassava starch (Bierhals et al., 2011), 0.3% lemongrass oil with 1.29% sodium alginate (Azarakhsh et al., 2014b) and multilayered antimicrobial coatings (Mantilla et al., 2013) maintained the overall quality of fresh-cut pineapple throughout cold storage.

However, the formulation of edible coatings, which is often added with glycerol, calcium chloride and ascorbic acid, may have negatively affected the taste and aroma of coated products and hindered consumers' acceptance towards this technology. Therefore, considerable interest should also be focused on the use of physical treatments to enhance the storage life and physico-chemical properties of fresh-cut pineapple.

In Chapter 4, it was reported that ultrasound treatment suppressed the growth of microorganisms of fresh-cut pineapple. Although the reduction of microbial growth is in agreement with the antimicrobial mechanisms of ultrasound involving the generation of localised hot spots which is characterised by extreme temperature (5000 K) and high pressure (1000 atm) and micro-mechanical shocks due to the collapse of cavitation bubbles (Kentish & Feng, 2014Soria & Villamiel, 2010), limited reports are available on the effect of ultrasound on the physico-chemical properties of fresh or fresh-cut produce. Aday et al. (2013) reported that the firmness and colour of strawberry treated at 30 and 60 W of ultrasound was better maintained than control following storage for 4 weeks at 4 °C. Similarly, the firmness of strawberry treated at 250 W for 10 min was 28.9% higher than that of control at the end of cold storage (Cao et al., 2010a). Therefore, this study was aimed to study the effect

ultrasound treatment on the physico-chemical and respiration rate of fresh-cut pineapple during cold storage.

8.2 Materials and methods

The fresh-cut pineapple cubes used in this study were prepared as described in Section 3.2. Ultrasound treatment at the power output of 25 and 29 W and exposure time of 10 and 15 min were used in this study. Treatments were applied as described in Section 3.3. The fruits were then air dried at room temperature (25 ± 1 ^{o}C) and packed in a polystyrene container (9.0 x 9.0 x 4.0 cm). The boxes were randomly stored at 7 ^{o}C and 80 ± 5% relative humidity (RH). Pineapple cubes were sampled for analysis on day 0, 1, 3 and 5. Destructive analyses which involved the measurement of firmness, soluble solid concentration, titratable acidity, pH and major sugars, three replications of three fruits were sampled on each observation days. Meanwhile, three replications of 9 fruits were used throughout the experiment to determine juice leakage, colour, and respiration rate. The data were subjected to three way analysis of variance (ANOVA) and treatment means were compared using Duncan Multiple Range Test at P=0.05.

8.2.1 Determination of physical quality

8.2.1.1 Firmness

The firmness of fresh-cut pineapple was determined by measuring the maximum force required to penetrate a depth of 10 mm in the fruit on each sampling day using an Instron Universal Testing Machine with a 2.0 mm stainless steel cylindrical probe, single-column model interfaced with Bluehill® 2 software. The machine was set for maximum compression with a speed of 10 mm s^{-1}. Measurements were taken at the top, middle and end of the pineapple cubes and the mean of the three readings was expressed in Newton (N).

8.2.1.2 Juice leakage

Juice leakage from the pineapple cubes was measured as described by Montero-Calderón et al. (2008). Juice leakage was assayed by tilting the containers packed with 9 fruits at a 20^{o} angle for 5 min and accumulated juice was recovered with a 5 ml syringe. Results were expressed as liquid volume recovered per 100 g of fresh-cut pineapple in the container.

8.2.1.3 Colour

The colour of pineapple cubes was determined using Miniscan XE Plus portable colorimeter, which was standardised first using a black and white tile with values of X = 79.0, Y = 83.9 and Z = 87.9. Values were recorded as lightness (L^{*}) with values range from 100 (white) to 0 (black), chroma value (C^{*}) = $(a^{*} + b^{*})^{½}$ which represented the hypotenuse of a right angle triangle with values ranging from 0 = least intense to 60 = most intense, and hue angle (h^{o}) was the angle of tangent^{-1} b^{*}/a^{*}, where 0^{o} = red purple, 90^{o} = yellow, 180^{o} = bluish green and 270^{o} = blue. Measurements were taken at the top, middle and end of the pineapple cubes and the mean was calculated based on the three readings.

8.2.2 Determination of chemical quality

8.2.2.1 Soluble solid concentration (SSC)

SSC was analysed according to the method as described by Ranggana (1997). Total soluble solid (^{o}Brix) was determined using a hand held Palette Digital Refractometer. Briefly, 10 g of pineapple cubes was homogenised using a kitchen blender with 40 ml of dH_2O. The mixture was filtered through muslin cloth and a drop of filtrate was placed on the prism glass of the refractometer to obtain the reading. The refractometer was calibrated with dH_2O before used. The readings were multiplied by the dilution factor to obtain the original SSC (%) of pineapple as shown below:

SSC (%) = (°Brix) reading x dilution factor

8.2.2.2 Titratable acidity (TA)

TA of pineapple was analysed using titration method as described by Ranggana (1997). 10 g of flesh tissue was homogenised in a blender with 40 ml of dH_2O and the mixture was filtered through muslin cloth. 5 ml of filtrate was added with two drops of 0.1% (v/v) phenolphthalein as a pH indicator and titrated against 0.1 N sodium hydroxide (NaOH) to a pink endpoint. The results were expressed as g of citric acid per 100 g of fresh weight by using the formula as below:

$$\text{Citric acid (g 100 g}^{-1}\text{ FW)} = \frac{\text{titre value (ml)} \times \text{normality of NaOH (N)} \times 0.064 \times \text{total volume of extract (ml)}}{\text{aliquot taken for estimation (ml)} \times \text{weight of sample (g)}} \times 100$$

8.2.2.3 Sugars

Extraction of fructose, glucose and sucrose was carried out as described by Hong et al. (2013) with some modifications. Briefly, 1 g of pineapple cubes was homogenised with 2.5 ml of dH_2O in a mortar and pestle. After incubated at 80 °C for 15 min, the homogenates was centrifuged at 10,000 x g for 15 min at 25 °C. The pellet fraction was redissolved in 2.5 ml of dH_2O and recentrifuged at 10,000 x g for 15 min at 25 °C. The two clear supernatants were vortex mixed and made up to 5 ml. The supernatant was filtered through a nylon membrane filter with 0.45 µM pore size, loaded into 2 ml glass injection vial and analysed immediately with high performance liquid chromatography (HPLC).

The concentration of individual sugars (fructose, glucose or fructose) was analysed on a HPLC system using the ZORBAX Carbohydrates Analysis Column. 20 µl of the extract was injected using an autosampler. The mobile phase was eluted isocratically using acetonitrile and water in a ratio of 75:25 (v/v) which was membrane filtered with regenerated cellulose filter membrane (0.45 µm pore size)

and degassed before analysis. Flow rate was mobile phase was set to 1.2 ml min^{-1}, column and refractive index detector (RID) temperature were set at 30 ^{o}C.

RID signals of samples (Appendix A.10) were identified by comparing its retention time with that of external standard of fructose (Appendix A.11), glucose (Appendix A.12) and sucrose (Appendix A.13), respectively. A standard curve for fructose (R^2 = 0.9990; Appendix A.14), glucose (R^2 = 0.9992; Appendix A.15) and sucrose (R^2 = 0.9952; Appendix A.16) was established, respectively, by using 20 µl of different concentrations of fructose, glucose and sucrose, respectively. Briefly, 100 mg of fructose, glucose and sucrose was dissolved individually in 5 ml of dH_2O to obtain a final concentration of 20 mg ml^{-1}. The stock solution of each individual sugars was diluted to 1, 2, 4, 6, 8, 10, 15 and 20 mg ml^{-1}, respectively. The concentration of each individual sugars was compared against standards and expressed as mg g^{-1} FW.

8.2.3 Gaseous exchange analysis

8.2.3.1 Determination of respiration rate

The respiration rate and ethylene production of fresh-cut pineapple were carried out as described by Ong et al. (2013) with some modifications. 9 pineapple cubes were placed in 9.0 cm x 9.0 cm x 4.0 cm (width x length x height) air tight containers. The lid of the containers was modified and equipped with a rubber septum. After incubated at room temperature for 1 h, 1 ml of headspace sample was withdrawn with a gas tight syringe through the septum and CO_2 analysis was carried out on a gas chromatography equipped with a thermal conductivity detector (TCD) using a 1.2 m long stainless steel packed column (Porapak R, mesh size 80/100). Helium was used as a carrier gas at the flow rate of 19 ml min^{-1}. Temperature for packed injector, oven and detector was set at 150, 60 and 200 ^{o}C, respectively. 1 ml of 1% CO_2 in nitrogen

was used as the external standard for calibration (Appendix A.17). The production rate of CO_2 was expressed as ml kg^{-1} h^{-1}.

8.2.4 Sensory evaluation of fresh-cut pineapple

Based on the microbial evaluation and quality as well as physiological changes, the control samples and ultrasound treated fresh-cut pineapple were further analysed for sensorial quality. Sensory evaluation was not carried out after 5 days of cold storage due to the rapid proliferation of microorganisms beyond the acceptance limit (>6 log CFU g^{-1}) suggested by IFST, although the storage life of ultrasound treated fresh-cut pineapple can be extended up to 5 days of storage based on microbial and appearance qualities. Also, due to the rapid depletion of sugar concentrations and high respiration rate of fresh-cut pineapple treated at 29 W for 10 and 15 min, respectively, sensory evaluation was not carried out for those ultrasound parameters.

A 9-point hedonic scale liking test was carried out as described by (Azarakhsh et al., 2014b) to determine the sensory characteristics of pineapple cubes after 3 days of storage. Twenty individuals aged between 20 and 50 years old were recruited among students and staff in the Faculty of Science, the University of Nottingham Malaysia Campus. The proportion of male to female assessors was equal. For sensory testing, pineapple cubes were exposed to 25 W of ultrasound for 10 and 15 min, respectively. As controls, pineapple cubes were only dipped in dH_2O for 10 and 15. Samples were air dried before placing in a 9.0 mm x 9.0 mm x 4.0 mm (width x length x height) polystyrene containers and stored for 3 days at 7 ^oC. The samples were codified with three-digit number codes and the order of sample presentation was randomised among assessors. Assessors were provided with water and plain crackers as palate cleansers between samples. They were required to evaluate the appearance, colour, odour, taste, texture and overall acceptability of the samples.

Feedbacks from the assessors were recorded on the scoresheet (Appendix A.19) provided.

8.3 Results and discussion

8.3.1 Firmness and juice leakage

Firmness is one of the important parameters relates to fruit ripening and senescence as the change in firmness may promote the growth of microorganisms and affect the mouth feel of fresh commodities (Toivonen & Brummell, 2008). The effect of ultrasound waves at different power and treatment time on the firmness and juice leakage of fresh-cut pineapple is shown in Figure 8.1 – 8.2, respectively. Regardless of treatment applied, the firmness of fresh-cut pineapple was decreased gradually throughout storage. On day 5, firmness of samples washed with distilled water for 10 min was reduced from 2.26 to 1.70 N which retained 75% of initial firmness. Similarly, fresh-cut pineapple treated at 25 W: 15 min, 29 W: 10 min and 29 W: 15 min retained 87, 91 and 88% of initial firmness, respectively. Although the highest retention of firmness (98%) was observed in fruit treated at 25 W of power ultrasound for 10 min, no changes was found between control and treated samples. Results suggest that the decreased in firmness was mainly attributed by storage day.

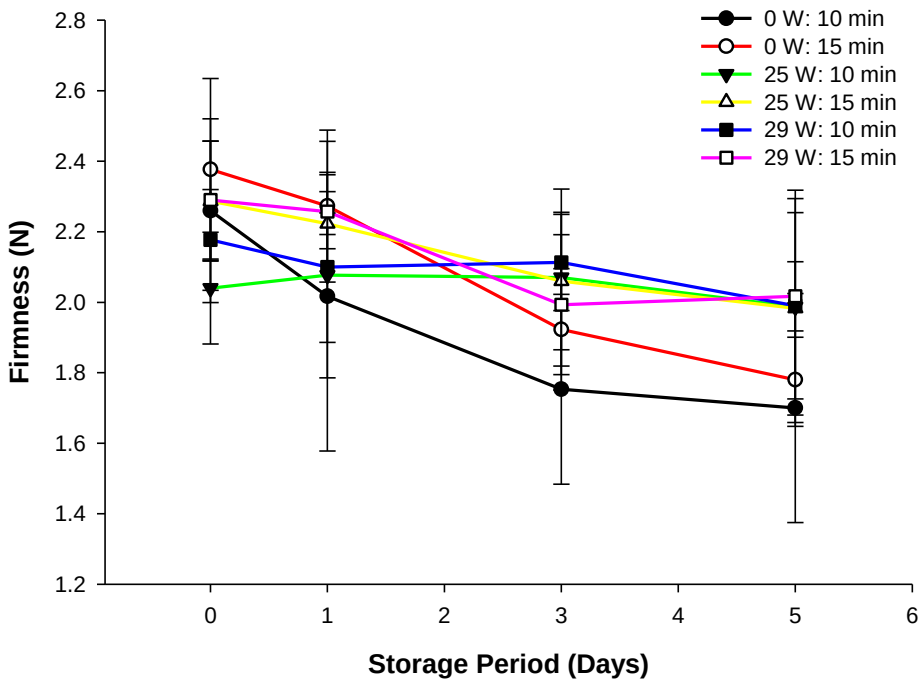


Figure 8.1 Firmness of fresh-cut pineapple following ultrasound treatment at different power inputs and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

The result of this study support those of Yang et al. (2011) who reported that there was no changes observed in the firmness between control and ultrasound treated (40 kHz, 350 W, 10 min) peach fruit following storage for 6 days at 20 °C. Although the firmness of lettuce treated with 40 kHz of ultrasound operated at 30 W L^{-1} for 5 min was higher than control, no changes were observed after 7 days of storage at 4 °C (Sagong et al., 2011). The application of 30 W of ultrasound for 5 – 10 min also retained the firmness better in strawberry than controls following 4 weeks of storage at 4 °C (Aday et al., 2013). Research conducted by Cao, Hu and Pang (2010) also reported that the firmness of strawberry treated at 250 W of ultrasonic wave for 10 min retained 75% of initial firmness at the end of storage period.

Textural changes in fresh or fresh-cut commodities during storage is due to cell wall modifications which involve the loss of neutral sugars from side chains,

solubilisation and depolymerisation of the polysaccharides of primary cell wall (Goulao & Oliveira, 2008; Toivonen & Brummell, 2008). Cell wall polysaccharides undergo enzymatic hydrolysis due to the *de novo* synthesis of various CWDEs such as PME, PG, ß-galactosidase and cellulose (Alexander & Grierson, 2002). Besides the activity of CWDEs, loss of cellular integrity as a result of juice leakage can cause tissue softening as well (Montero-Calderón et al., 2008).

The large standard deviation as shown in Figure 8.1 in the measurement of texture was mostly due to the lack of internal structure uniformity in the pineapple (Bierhals et al., 2011). Hajare et al. (2006) suggested that texture measurement is unsuitable to be taken at the edge of pineapple as the tissues may have lost their integrity due to the removal of 'eyes' on the surface.

Increase in juice leakage decrease the firmness of fresh-cut pineapple throughout the storage. Regardless of treatment applied, the volume of juice that leaked from fresh-cut pineapple increased throughout storage. Sharp increase of juice leakage in ultrasound treated fruit at 29 W for 15 min was recorded on day 3 and 5, respectively. Although the volume of juice accumulated inside the container between control and ultrasound treated showed no differences at the end of storage, juice leakage of ultrasound treated samples at 29 W for 15 min was 25% higher than that of control.

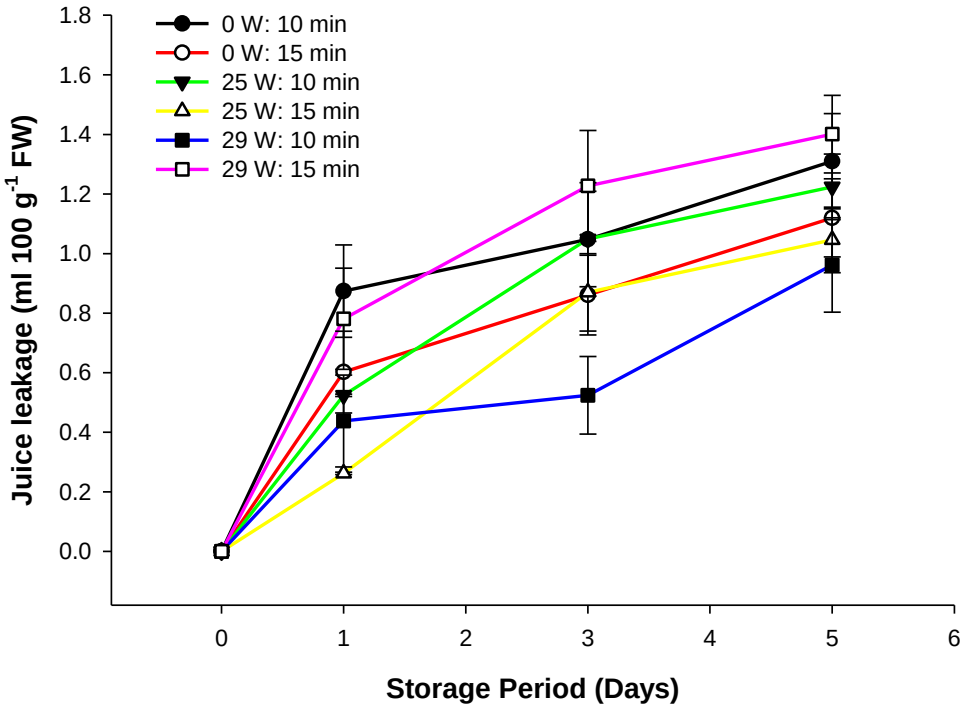


Figure 8.2 Juice leakage of fresh-cut pineapple following ultrasound treatment at different power inputs and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Ultrasound does not suppress juice leakage in fresh-cut pineapple during cold storage. The rapid loss of water from ultrasound treated fruit may be due to the loss of cell wall rigidity and intracellular adhesion upon exposure to high pressure generated by the implosion of cavitation bubbles. Transmission electron microscopy analysis conducted by Wu et al. (2012a) demonstrated the plasmalemma of cells was broken and separated from the cell wall in fresh-cut pineapple treated with high pressure argon. Disruption of cell permeability due to high pressure in fruits and vegetables enhances the movement of water and metabolites in the cell and hence results in cellular leakage.

8.3.2 Colour (L, C* and h°)

Changes in colour parameters L, C* and h° of fresh-cut pineapple are shown in Figure 8.3 – 8.5. Regardless of treatment applied, the L, C* and h° values of samples were decreased gradually throughout 5 days of storage at 7 °C. After storage for 5 days, fruit treated at 25 W for 10 min retained 97, 75 and 98% of initial L, C* and h° values. Similarly, retention of L, C* and h° values in samples treated with distilled water for 10 min were 96, 77 and 99%, respectively when compared to that of initial values. Also, those treated at 25 W: 10 min, 29 W: 10 min and 29 W: 15 min showed no changes in colour parameters with their respective control at the end of storage.

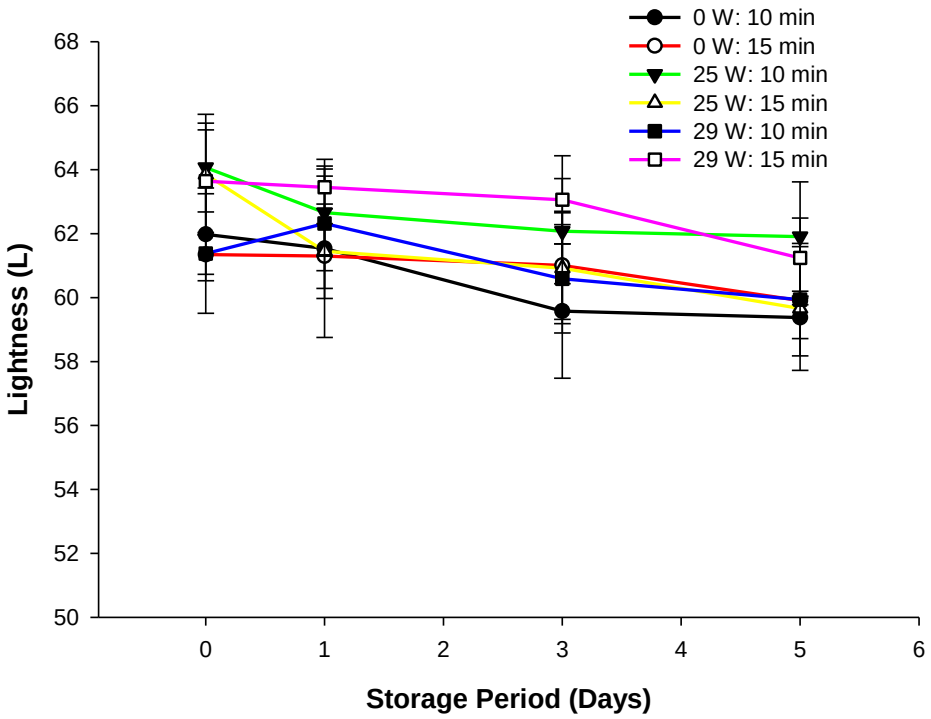


Figure 8.3 Lightness (L) of fresh-cut pineapple treated at different ultrasound power inputs and exposure times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

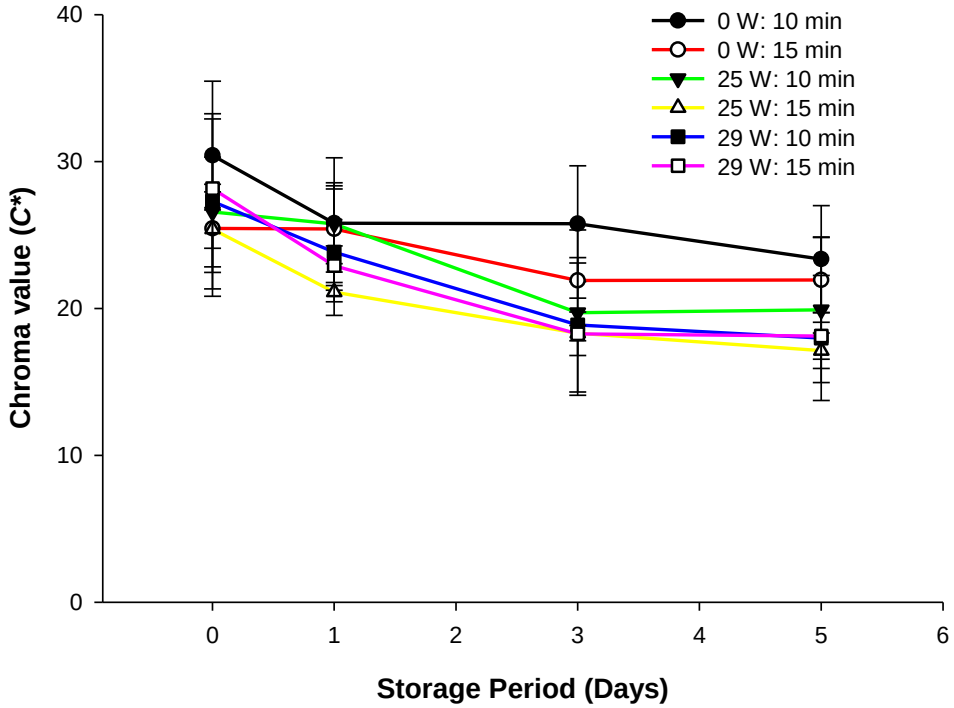


Figure 8.4 Chroma value of fresh-cut pineapple treated at different ultrasound power inputs and exposure times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Similarly, Aday et al. (2013) reported that there was no differences between untreated and ultrasound treated strawberry at 90 W during storage. The results obtained in this study were in accordance with ultrasound treated strawberry and lettuce at 30 W L^{-1} for 10 min which showed no changes in L value when compared to that of control at the end of storage (Birmpa et al., 2013). Sagong et al. (2011) also showed that the changes in the L, a* and b* values of fresh lettuce treated at 40 kHz of ultrasound and power of 30 W L^{-1} for 5 min was comparable to that of control after 7 days of storage at 4 °C.

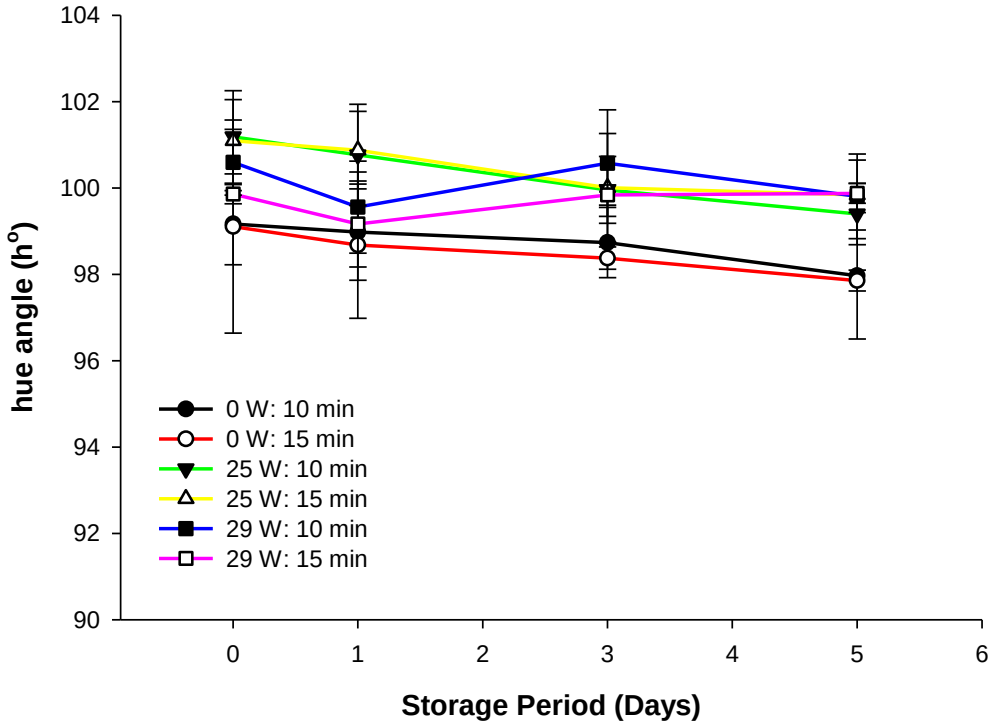


Figure 8.5 Hue angle (h°) of fresh-cut pineapple treated at different ultrasound power inputs and exposure times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

In this study, no visible browning appearance was observed in fresh-cut pineapple during storage. However, study conducted by Wu et al. (2012a) showed that high pressure treatment (above 2.5 MPa) changed the L and b* value of fresh-cut pineapple due to enzymatic browning. Authors suggested that the decompartmentalisation of POD and PPO in high pressure processed samples accelerated enzymatic browning. Although it is hypothesised that the mechanisms of ultrasound involving the generation of free radicals and high pressure would have resulted in browning symptoms on fresh-cut pineapple. The presence of high ascorbate concentration due to the high regeneration capacity ascorbate regulated by the enzyme MDHAR and DHAR may have prevented the oxidation of phenols and inhibited enzymatic browning in this study.

8.3.3 Soluble solid concentration (SSC)

The effect of ultrasound at different power and treatment time on SSC of fresh-cut pineapple is shown in Figure 8.6. SSC of samples washed with distilled water for 10 and 15 min reached a peak on day 1 and decreased gradually throughout storage. The increase in SSC in control and ultrasound treated samples on day 1 and 3, respectively, may be due to the enzymatic activity of sucrose-phosphate synthase and invertase which catalyse the hydrolysis of sucrose to non-reducing sugars (Asghari & Aghdam, 2010).

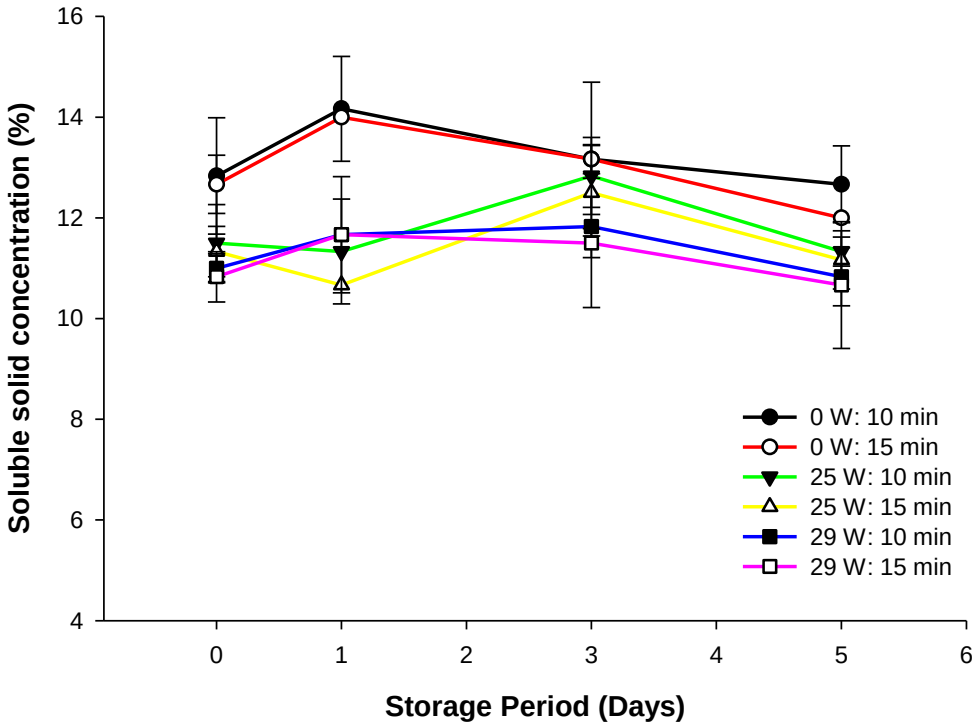


Figure 8.6 Soluble solid concentration of fresh-cut pineapple treated at different ultrasound power inputs and exposure times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Upon exposure to 29 W of ultrasound for 10 and 15 min, SSC was reduced by 2%, respectively. SSC of fresh-cut pineapple was affected by the power input of ultrasound as increased in power inputs to 29 W resulted in the lowest SSC following 5 days of storage.

Similarly, Aday et al. (2013) also reported that the increment in ultrasound power input to 90 W resulted in lower total soluble solid concentration of strawberry when compared with samples treated at 30 or 60 W. Contradictorily, studies showed that the total soluble concentration in cherry tomatoes (Ding et al., 2015) and peach fruit (Yang et al., 2011) was not affected by ultrasound treatment.

Low concentrations of SSC in fresh-cut pineapple treated at 29 W of ultrasound was coincided with high juice leakage following storage on day 5. Results suggest that the high reduction of SSC in ultrasound treated samples may be due the disruption of cell wall structures caused by high pressure and strong shear forces generated by ultrasound which enhances water and metabolites loss from the cells (Aday et al., 2013).

8.3.3.1 Titratable acidity (TA) and pH

The effect of ultrasound at different power and treatment time on the TA and pH of fresh-cut pineapple is shown in Figure 8.7 – 8.8, respectively. Regardless of treatment applied, pH of control and ultrasound treated samples was decreased gradually throughout storage. Meanwhile, TA of control and treated fresh-cut pineapple increased during the 5 days of storage. Following ultrasound treatment at 25 W for 10 min, no changes in TA and pH was observed when compared with samples treated with distilled water for 10 and 15 min.

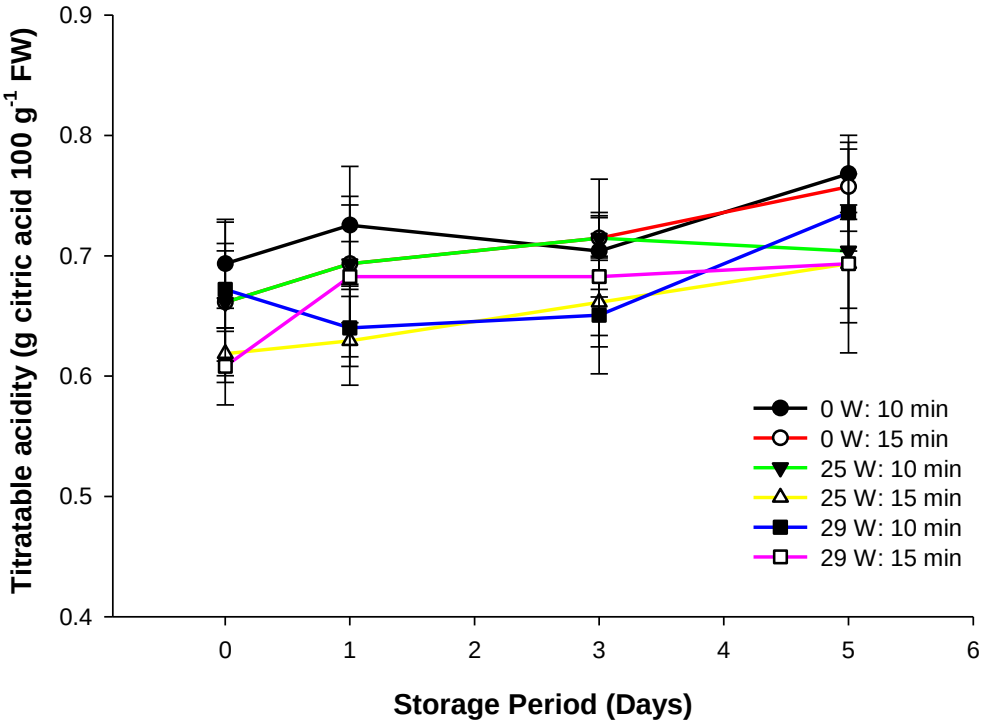


Figure 8.7 Titratable acidity of fresh-cut pineapple treated at different ultrasound power inputs and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Several studies operated at higher ultrasound power inputs also showed no changes in TA and pH during storage. Ding et al. (2015) reported that no changes in the pH of ultrasound treated cherry tomato when compared with control. Similarly, research conducted by Yang et al. (2011) also reported ultrasound treatment (40 kHz, 350 W, 10 min) had no effect on the TA of peach fruit following 6 days of storage at 20 °C. Aday et al. (2013) showed that the pH value of ultrasound treated strawberry showed no differences when compared with controls after 4 weeks of storage at 4 °C.

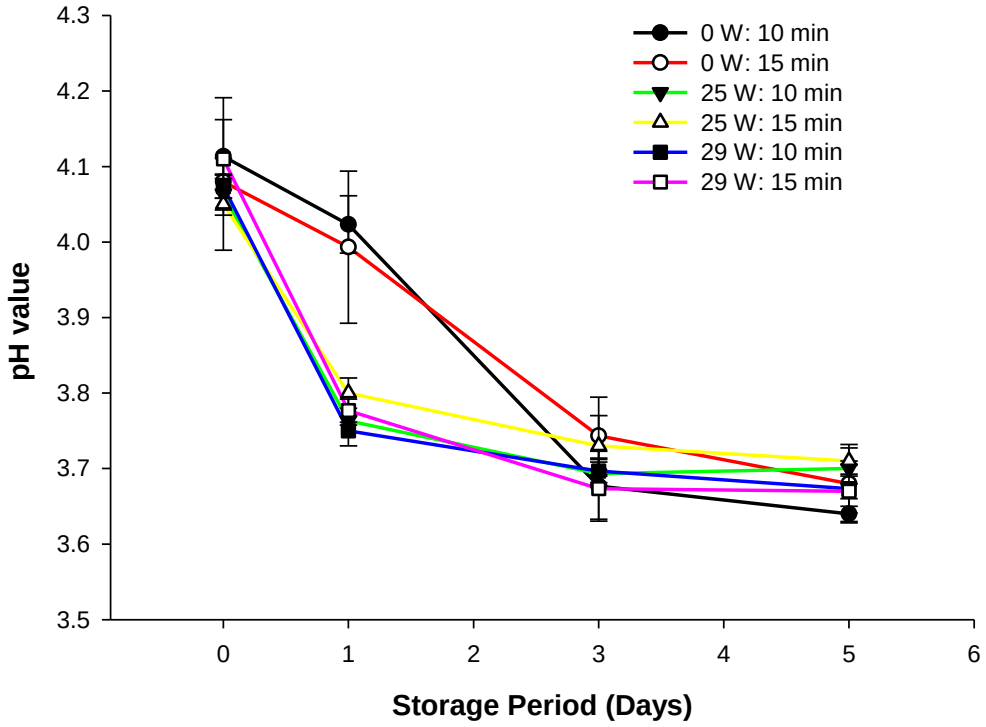


Figure 8.8 pH of fresh-cut pineapple treated at different ultrasound power inputs and treatment times during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

8.3.4 Sugars

The effect of ultrasound at different power and treatment time on the sucrose, fructose and glucose of fresh-cut pineapple is shown in Figure 8.9 – 8.11. Regardless of treatment applied, the concentration of sucrose gradually decreased throughout 5 days of storage at 7 °C. After ultrasound treatment at 29 W for 15 min, concentration of sucrose was decreased by 10.4% when compared to those washed with distilled water for 15 min. Higher power inputs and treatment times reduced the sucrose concentration at the end of storage. The highest reduction in sucrose concentration was observed in samples treated at 29 W of ultrasound for 15 min and followed by those treated at 29 W: 10 min and 25 W: 15 min. No differences was observed between sucrose concentration of control and samples treated at 25 W for 10 min.

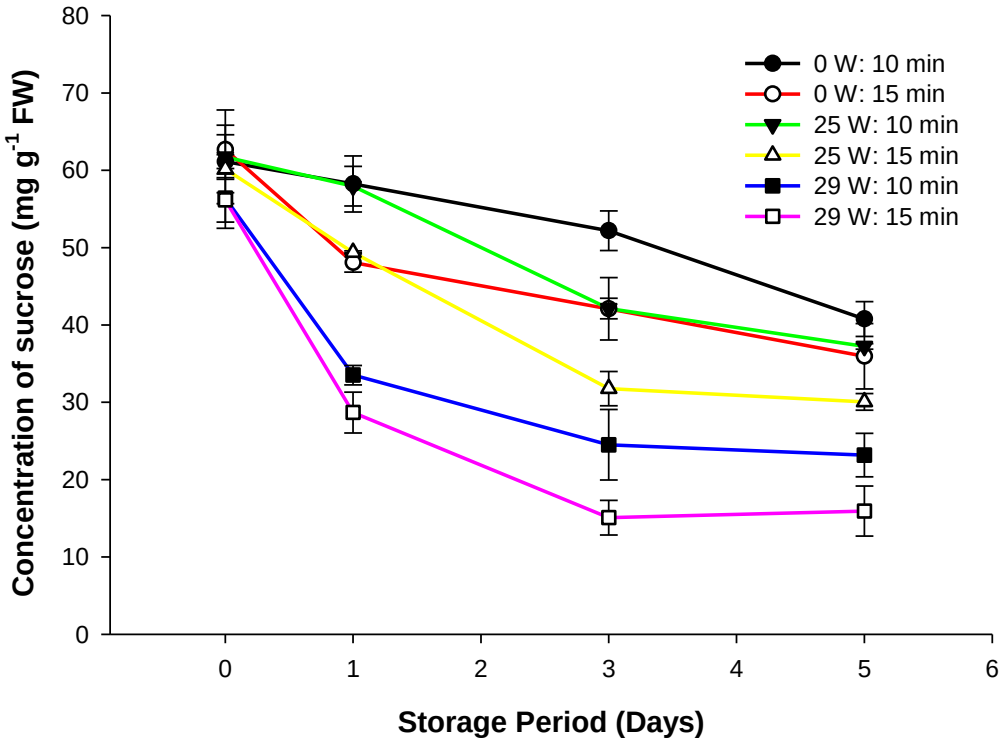


Figure 8.9 Sucrose concentration in fresh-cut pineapple following ultrasound treatment at different power and exposure time during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Meanwhile, the concentration of fructose and glucose increased throughout cold storage. On day 5 of storage, the fructose and glucose concentration showed no changes between samples treated in distilled water and those treated at 25 W of ultrasound for 10 min, respectively. However, concentration of fructose and glucose was reduced by 46 and 43%, respectively, when the power input and treatment time were increased to 29 W and 15 min. Increased SSC was positively correlated with the concentration of fructose ($r = 0.3100$, $P<0.01$, Appendix B.51) and glucose ($r = 0.4203$, $p<0.001$, Appendix B.52).

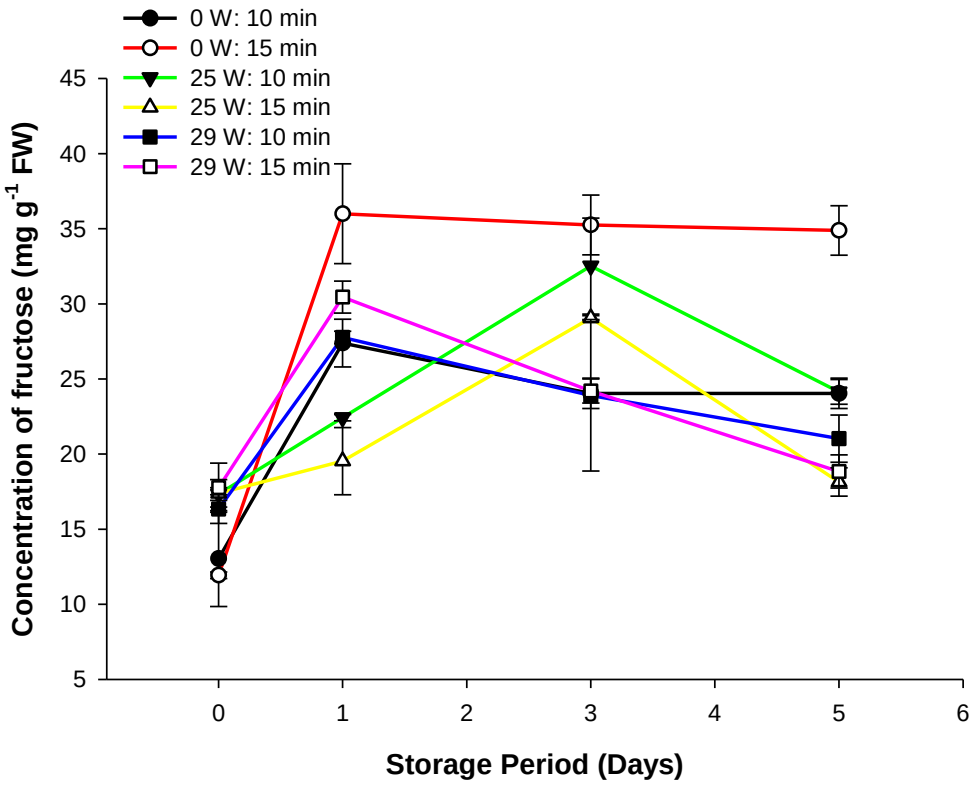


Figure 8.10 Fructose concentration in fresh-cut pineapple following ultrasound treatment at different power and exposure time during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

Similarly, after storage for 24 days at 25 °C, the concentration of sucrose, fructose and glucose of pineapple fruit was 32, 56 and 69% lowered than those stored at 6 °C (Hong et al., 2013). The high reduction of sucrose, fructose and glucose concentration in samples treated at 29 W for 15 min may be related to the conversion of carbon skeleton to synthesise antioxidant compounds such as ascorbic acid in response to oxidative stress (Davey et al., 2000; Interdonato et al., 2011) generated by ultrasound. Also, sugars may be used up due to higher respiratory activity in ultrasound treated samples.

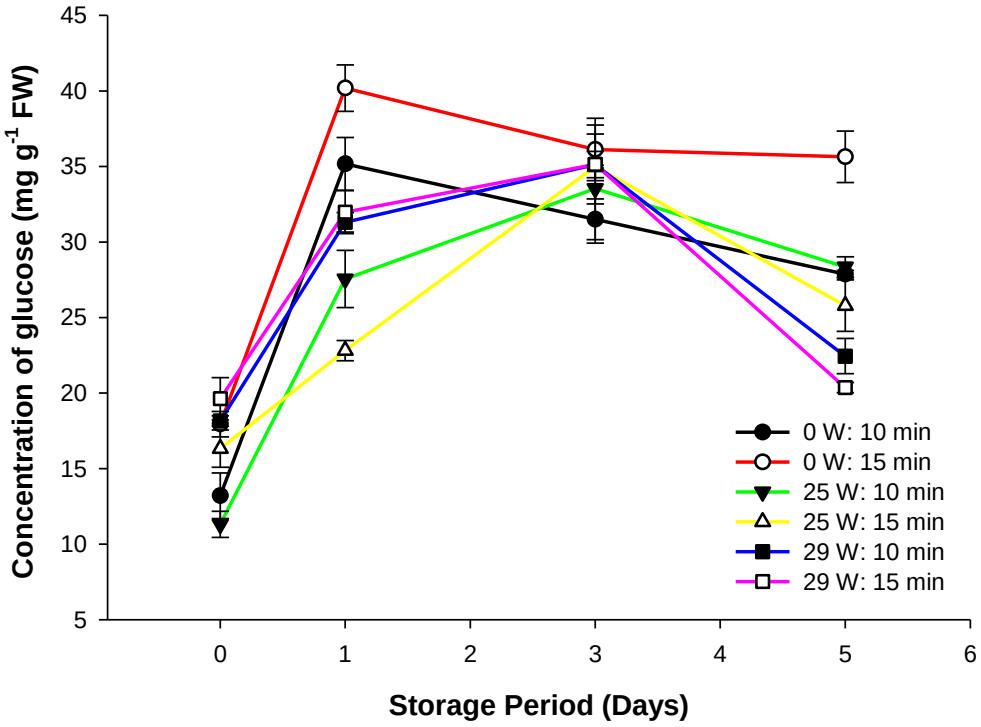


Figure 8.11 Glucose concentration in fresh-cut pineapple following ultrasound treatment at different power and exposure time during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

8.3.5 Gaseous exchange analysis

Respiration rate is a good indicator of metabolic activity in fresh-cut produce as higher respiration rate often results in lower storage life (Waghmare et al., 2013). The changes in the respiration rate of fresh-cut pineapple treated at different power and exposure time following storage for 5 days at 7 °C are shown in Figure 8.12.

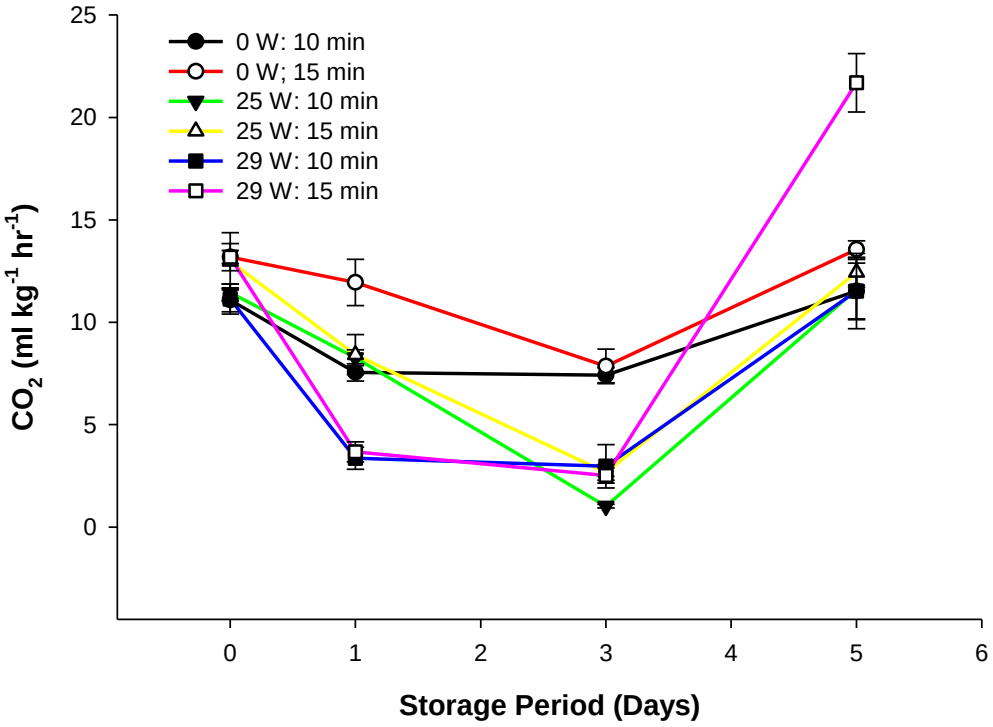


Figure 8.12 Production rate of CO_2 in ultrasound treated fresh-cut pineapple at different power and exposure time during 5 days of storage at 7 °C and 80 ± 5% RH. Vertical bars represent the SD of three replicates.

As shown in Figure 8.12, the rate of CO_2 production was gradually declined until an equilibrium was achieved on day 3 of storage, and rapidly increased, reaching the highest concentration at the end of storage. On day 5, no difference was observed between control and samples treated at 25 and 29 W for 10 min, respectively. However, increased power input and treatment time to 29 W and 15 min had resulted in the highest respiration rate (22 ml kg^{-1} hr^{-1}) in fresh-cut pineapple.

A high initial production rate of CO_2 in fresh-cut pineapple is due to wounding caused by various mechanical operations that increase the surface area of cut fruit and allow rapid diffusion of oxygen into the internal cells (Finnegan et al., 2013; Waghmare & Annapure, 2013). The production of wound-induced enzymes and the liberation of cellular substrates in damaged tissue can lead to the production of volatile long chain aldehydes which increase the respiratory activity in fresh-cut pineapple (Soliva-Fortuny & Martín-Belloso, 2003; Vandekinderen et al., 2008). Marrero and Kader (2006) reported that the respiration rate of fresh-cut pineapple was at least two times higher than of whole fruit. Benitez et al. (2014) showed that the respiration rate of fresh-cut pineapple was much higher initially and decreased gradually on subsequent storage until an equilibrium value was achieved on day 3. Several studies also have reported data on the similar trends to equilibrium in different cultivars of fresh-cut pineapple (Azarakhsh et al., 2014a; Finnegan et al., 2013).

Regardless of treatment applied, a rapid increase in the respiration rate of fresh-cut pineapple at the end of storage (day 5) may be due to the proliferation of microorganisms on the cut surface and general deterioration of tissue due to senescence (Aguayo et al., 2004a; Silveira et al., 2011). The growth of anaerobes such as lactic acid bacteria and yeast and mould can break down glucose and lead to the accumulation of CO_2 in the headspace of container (Wills, 2007). Also, oxidative stress induced production of intracellular ROS can stimulate the activity of H^+-ATP which leads to higher production rate of CO_2 (Wang et al., 2004).

8.3.6 Sensory evaluation of fresh-cut pineapple

As shown in Figure 8.13, ultrasound treated and dH_2O dipped fresh-cut pineapple were remained satisfactory as evidenced by sensory score higher than 5 following 3 days of storage at 7 ^oC. The mean of the evaluated attributes was mostly fell between

'like slightly' and 'like moderately' on the hedonic scale for all tested samples. In this study, no difference was observed between ultrasound treated samples and dH_2O controls in all of the tested sensory attributes (appearance, colour, odour, taste and texture) after 3 days of cold storage.

Quality of fresh-cut fruits and vegetables which involves a combination of parameters such as appearance, texture, flavours and freshness is often judged and determined by consumer at the time of purchase (Rico et al., 2007; Rojas-Graü et al., 2009b). At the market level, fresh-cut products that are above the acceptance limit under standardised storage condition has to be corresponded to consumer's satisfaction in terms of texture and flavour (Rico et al., 2007). Therefore, it is necessary to determine the effect of novel and potential postharvest treatments on the sensory and organoleptic properties of fresh-cut fruits and vegetables. The findings of this study suggest that the application of ultrasound treatment at 25 W may be acceptable by consumers as the sensory attributes of ultrasound treated samples resembled those of dH_2O controls.

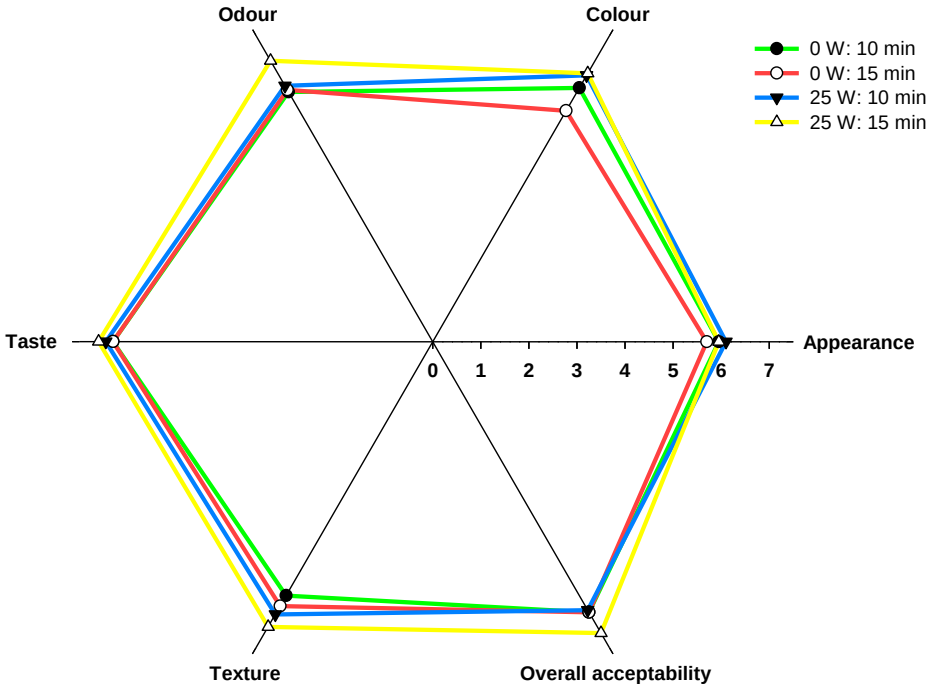


Figure 8.13 Sensory characteristics of fresh-cut pineapple treated at different ultrasound power inputs and treatment times after 3 days of storage at 7 °C and 80 ± 5% RH.

Physical attributes such as texture, flavour and aroma are the most noticeable changes used by consumers to judge the quality of fresh-cut products. Besides ensuring food safety and improving the antioxidant capacity, ultrasound treatment at 25 W for 10 min did not resulted in changes on physico-chemical properties and physiological behaviour of fresh-cut pineapple when compared to control during storage. Meanwhile, increment in ultrasound power input to 29 W and exposure time to 15 min adversely affected the physiochemical properties and metabolic activity of fresh-cut pineapple as demonstrated by higher volume of juice leakage and respiratory activity as well as lower sugar concentration during cold storage.

9 CONCLUSION

The main aim of this study was to investigate the effect of ultrasound treatment at different power inputs and treatment times on the storage life of fresh-cut pineapple. Increased in power inputs and treatment times suppressed the number of mesophilic, lactic acid bacteria and yeast and mould and achieved food safety in accordance to the bacteriological standards specified in Malaysia Food Act 1983 and Food Regulations 1985. Ultrasound treatments induced higher concentration of H_2O_2 and $O_2^{\bullet-}$ which was concomitant with higher PAL, APX, MDHAR and DHAR as well as total antioxidant activities. Thus, it was hypothesised that the regulation of intracellular H_2O_2 and $O_2^{\bullet-}$ concentration elicited the production of secondary metabolites such as phenolic compounds to confer better resistance against spoilage microorganisms. No differences were observed on physiochemical and production rate of CO_2 between samples treated at 25 W for 10 min and controls. The results suggest that the application of hormesis dosage of ultrasound treatment at 25 W for 10 min has the potential to suppress the growth of microorganisms and improve the antioxidant potential of fresh-cut pineapple without negatively affecting its organoleptic properties.

However, ultrasound treatment was unable to inhibit the proliferation of microorganisms as shown by the rapid growth of yeast and mould populations during subsequent storage. Hence, the storage life of ultrasound treated fresh-cut pineapple was only extended by 2 days. Overall, a 2 log reduction in the population of mesophilic, lactic acid bacteria and yeast and mould was achieved. This low reduction in the number of these microorganisms may be due to the application of low ultrasound power inputs when compared with other literature studies. Therefore, in order to further investigate the antimicrobial effect of ultrasound, power inputs in the range of 0 – 200 W should be adopted in future studies.

Although the increased of H_2O_2 and $O_2^{\bullet-}$ concentration concomitant with the induction of PAL, APX, MDHAR, DHAR and total antioxidant activity, these experiments and analyses have to be conducted concurrently to validate the hypothesis. It would be advisable to perform a large scale commercial trial by utilising the ideal parameters to study the correlations between ROS and enzymatic or antioxidant activity. Even though the antimicrobial mechanisms of ultrasound can suppress the growth of spoilage microorganisms on the uneven surface of various fresh-cut products, a long term solution has to be delivered to address the issue related to the increase in water bath temperature during sonication.

It also has been hypothesised that the generation of free radicals (H_2O_2 and OH^-) in the water due to the implosion of cavitation bubbles can suppress the growth of microorganisms and result in changes in the intracellular ROS concentration, enzymes and total antioxidant activity. However, the presence of these free radicals during sonication was not measured in this study. Therefore, measurements have to be taken to quantify the concentration of these free radicals which may cause the reduction in the number of microorganisms as well as elicitation of plant defence responses. Finally, the effect of the increased of H_2O_2 and $O_2^{\bullet-}$ concentration in the ultrasound treated fruits on consumers' health remains unknown. Therefore, the lethal concentration of ROS on normal human cell lines should be identified.

REFERENCES

Abadias, M., Usall, J., Oliveira, M., Alegre, I., & Viñas, I. (2008). Efficacy of neutral electrolyzed water (NEW) for reducing microbial contamination on minimally-processed vegetables. *International Journal of Food Microbiology*, *123*, 151–158.

Aday, M. S., & Caner, C. (2014). Individual and combined effects of ultrasound, ozone and chlorine dioxide on strawberry storage life. *LWT - Food Science and Technology*, *57*, 344–351.

Aday, M. S., Temizkan, R., Büyükcan, M. B., & Caner, C. (2013). An innovative technique for extending shelf life of strawberry: Ultrasound. *LWT - Food Science and Technology*, *52*, 93–101.

Adekunte, A. O., Tiwari, B. K., Cullen, P. J., Scannell, A. G. M., & O'Donnell, C. P. (2010). Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chemistry*, *122*, 500–507.

Agar, I. T., Massantini, R., Hess-Pierce, B., & Kader, A. A. (1999). Postharvest CO_2 and ethylene production and quality maintenance of fresh-cut kiwifruit slices. *Journal of Food Science*, *64*, 433–440.

Aghdam, M. S., Asghari, M., Farmani, B., Mohayeji, M., & Moradbeygi, H. (2012). Impact of postharvest brassinosteroids treatment on PAL activity in tomato fruit in response to chilling stress. *Scientia Horticulturae*, *144*, 116–120.

Aguayo, E., Escalona, V., & Artés, F. (2004a). Metabolic behavior and quality changes of whole and fresh processed melon. *Journal of Food Science*, *69*, SNQ148–SNQ155.

Aguayo, E., Escalona, V., & Artés, F. (2004b). Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. *European Food Research and Technology*, *219*, 492–499.

Aguayo, E., Escalona, V., & Artés, F. (2006). Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. *Postharvest Biology and Technology*, *39*, 169–177.

Aguayo, E., Escalona, V., Silveira, A. C., & Artés, F. (2014). Quality of tomato slices disinfected with ozonated water. *Food Science and Technology International*, *20*, 227–35.

Ajlouni, S., Sibrani, H., Premier, R., & Tomkins, B. (2006). Ultrasonication and fresh produce (Cos lettuce) preservation. *Journal of Food Science*, *71*, M62–M68.

Akbas, M. Y., & Olmez, H. (2007). Effectiveness of organic acid, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. *Journal of the Science of Food and Agriculture*, *87*, 2609–2616.

Alegria, C., Pinheiro, J., Duthoit, M., Gonçalves, E. M., Moldão-Martins, M., & Abreu, M. (2012). Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress

(heat shock and UV-C irradiation) pre-treatments. *LWT - Food Science and Technology*, *48*, 197–203.

Alegria, C., Pinheiro, J., Gonçalves, E. M., Fernandes, I., Moldão, M., & Abreu, M. (2009). Quality attributes of shredded carrot (*Daucus carota* L. cv. Nantes) as affected by alternative decontamination processes to chlorine. *Innovative Food Science and Emerging Technologies*, *10*, 61–69.

Alexander, L., & Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany*, *53*, 2039–2055.

Alexandre, E. M. C., Brandão, T. R. S., & Silva, C. L. M. (2012). Assessment of the impact of hydrogen peroxide solutions on microbial loads and quality factors of red bell peppers, strawberries and watercress. *Food Control*, *27*, 362–368.

Alexandre, E. M. C., Brandão, T. R. S., & Silva, C. L. M. (2013). Impact of non-thermal technologies and sanitizer solutions on microbial load reduction and quality factor retention of frozen red bell peppers. *Innovative Food Science and Emerging Technologies*, *17*, 99–105.

Alexandre, E. M. C., Santos-Pedro, D. M., Brandão, T. R. S., & Silva, C. L. M. (2011a). Influence of aqueous ozone, blanching and combined treatments on microbial load of red bell peppers, strawberries and watercress. *Journal of Food Engineering*, *105*, 277–282.

Alexandre, E. M. C., Santos-Pedro, D. M., Brandão, T. R. S., & Silva, C. L. M. (2011b). Study on thermosonication and ultraviolet radiation processes as an alternative to blanching for some fruits and vegetables. *Food and Bioprocess Technology*, *4*, 1012–1019.

Allende, A., McEvoy, J. L., Luo, Y., Artes, F., & Wang, C. Y. (2006). Effectiveness of two-sided UV-C treatments in inhibiting natural microflora and extending the shelf-life of fresh-cut "Red Oak Leaf" lettuce. *Food Microbiology*, *23*, 241–249.

Allende, A., Selma, M. V., López-Gálvez, F., Villaescusa, R., & Gil, M. I. (2008). Role of commercial sanitizers and washing systems on epiphytic microorganisms and sensory quality of fresh-cut escarole and lettuce. *Postharvest Biology and Technology*, *49*, 155–163.

Alothman, M., Bhat, R., & Karim, A. A. (2009). UV radiation-induced changes of antioxidant capacity of fresh-cut tropical fruits. *Innovative Food Science and Emerging Technologies*, *10*, 512–516.

Alothman, M., Kaur, B., Fazilah, A., Bhat, R., & Karim, A. A. (2010). Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits. *Innovative Food Science and Emerging Technologies*, *11*, 666–671.

Alphas, H., & Bozoglu, F. (2000). The combined effect of high hydrostatic prerssure, heat and bacteriocins on inactivation of foodborne pathogens in milk and orange juice. *World Journal of Microbiology and Biotechnology*, *16*, 387–392.

Alvarez, M. V., Ponce, A. G., & Moreira, M. D. R. (2013). Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli. *LWT - Food Science and Technology*, *50*, 78–87.

Amaral, R. D. A., Benedetti, B. C., Pujola, M., Achaerandio, I., & Bachelli, M. L. B. (2015). Effect of ultrasound on quality of fresh-cut potatoes during refrigerated storage. *Food Engineering Reviews*, *7*, 176–184.

Amodio, M. L., Derossi, A., & Colelli, G. (2014). Modeling phenolic content during storage of cut fruit and vegetables: A consecutive reaction mechanism. *Journal of Food Engineering*, *140*, 1–8.

Andrade Cuvi, M. J., Vicente, A. R., Concellón, A., & Chaves, A. R. (2011). Changes in red pepper antioxidants as affected by UV-C treatments and storage at chilling temperatures. *LWT - Food Science and Technology*, *44*, 1666–1671.

Ansorena, M. R., Moreira, M. R., & Roura, S. I. (2014). Combined effect of ultrasound, mild heat shock and citric acid to retain greenness, nutritional and microbiological quality of fresh-cut broccoli (*Brassica oleracea* L.): An optimization study. *Postharvest Biology and Technology*, *94*, 1–13.

Antoniolli, L. R., Benedetti, B. C., Sigrist, J. M. M., & Silveira, N. F. D. A. (2007). Quality evaluation of fresh-cut 'Pérola' pineapple stored in controlled atmosphere. *Ciência E Tecnologia de Alimentos*, *27*, 530–534.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, *55*, 373–399.

Aquino-Bolaños, E. N., Cantwell, M. I., Peiser, G., & Mercado-Silva, E. (2000). Changes in the quality of fresh-cut jicama in relation to storage temperatures and controlled atmospheres. *Journal of Food Science: Sensory and Nutritive Qualities of Food*, *65*, 1238–1243.

Aquino-Bolaños, E. N., & Mercado-Silva, E. (2004). Effects of polyphenol oxidase and peroxidase activity, phenolics and lignin content on the browning of cut jicama. *Postharvest Biology and Technology*, *33*, 275–283.

Artés, F., Gomez, P. A., & Artes-Hernandez, F. (2007). Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. *Food Science and Technology International*, *13*, 177–188.

Artés, F., Gómez, P., Aguayo, E., Escalona, V., & Artés-Hernández, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology*, *51*, 287–296.

Artés-Hernández, F., Escalona, V. H., Robles, P. A., Martínez-Hernández, G. B., & Artés, F. (2009). Effect of UV-C radiation on quality of fresh-cut spinach leaves. *Journal of the Science of Food and Agriculture*, *89*, 414–421.

Artés-Hernández, F., Robles, P. A., Gómez, P. A., Tomás-Callejas, A., & Artés, F. (2010). Low UV-C illumination for keeping overall quality of fresh-cut watermelon. *Postharvest Biology and Technology*, *55*, 114–120.

Asghari, M., & Aghdam, M. S. (2010). Impact of salicylic acid on post-harvest physiology of horticultural crops. *Trends in Food Science and Technology*, *21*, 502–509.

Awad, T. S., Moharram, H. A., Shaltout, O. E., Asker, D., & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Research International*, *48*, 410–427.

Azarakhsh, N., Osman, A., Ghazali, H. M., Tan, C. P., & Mohd Adzahan, N. (2014a). Effects of gellan-based edible coating on the quality of fresh-cut pineapple during cold storage. *Food and Bioprocess Technology*, *7*, 2144–2151.

Azarakhsh, N., Osman, A., Ghazali, H. M., Tan, C. P., & Mohd Adzahan, N. (2014b). Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. *Postharvest Biology and Technology*, *88*, 1–7.

Back, K. H., Ha, J. W., & Kang, D. H. (2014). Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. *Food Control*, *44*, 78–85.

Barros, M., & Saltveit, M. E. (2013). Microbial growth in fresh-cut lettuce increases when wound-induced phenolic accumulation is suppressed. *Postharvest Biology and Technology*, *83*, 34–39.

Barry-Ryan, C., & O'Beirne, D. (1998). Quality and shelf-life of fresh cut carrot slices as affected by slicing method. *Journal of Food Science*, *63*, 851–856.

Bartholomew, D., Paull, R. E., & Rohrbach, K. (2003). *Pineapple: Botany, Production and Uses*. Oxon, UK: CABI Publishing.

Beirao-da-Costa, S., Moura-Guedes, M. C., Ferreira-Pinto, M. M., Empis, J., & Moldao-Martins, M. (2014). Alternative sanitizing methods to ensure safety and quality of fresh-cut kiwifruit. *Journal of Food Processing and Preservation*, *38*, 1–10.

Beltrán, D., Selma, M. V., Marín, A., & Gil, M. I. (2005). Ozonated water extends the shelf life of fresh-cut lettuce. *Journal of Agricultural and Food Chemistry*, *53*, 5654–5663.

Benítez, S., Achaerandio, I., Sepulcre, F., & Pujolà, M. (2013). Aloe vera based edible coatings improve the quality of fresh-cut "Hayward" kiwifruit. *Postharvest Biology and Technology*, *81*, 29–36.

Benitez, S., Soro, L., Achaerandio, I., Sepulcre, F., & Pujola, M. (2014). Combined effect of a low permeable film and edible coatings or calcium dips on the quality of fresh-cut pineapple. *Journal of Food Process Engineering*, *37*, 91–99.

Beuchat, L. R., & Ryu, J. H. (1997). Produce Handling and Processing Practices. *Emerging Infectious Diseases*, *3*, 459–465.

Bi, X., Wu, J., Zhang, Y., Xu, Z., & Liao, X. (2011). High pressure carbon dioxide treatment for fresh-cut carrot slices. *Innovative Food Science and Emerging Technologies*, *12*, 298–304.

Bierhals, V. S., Chiumarelli, M., & Hubinger, M. D. (2011). Effect of cassava starch coating on quality and shelf life of fresh-cut pineapple (*Ananas comosus* L. Merril cv "Pérola"). *Journal of Food Science*, *76*, 62–72.

Bilek, S. E., & Turantaş, F. (2013). Decontamination efficiency of high power ultrasound in the fruit and vegetable industry, a review. *International Journal of Food Microbiology*, *166*, 155–162.

Birmpa, A., Sfika, V., & Vantarakis, A. (2013). Ultraviolet light and Ultrasound as non-thermal treatments for the inactivation of microorganisms in fresh ready-to-eat foods. *International Journal of Food Microbiology*, *167*, 96–102.

Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany*, *91*, 179–194.

Cao, J., & Jiang, W. (2006). Induction of resistance in Yali pear (Pyrus bretschneideri Rehd.) fruit against postharvest diseases by acibenzolar-S-methyl sprays on trees during fruit growth. *Scientia Horticulturae*, *110*, 181–186.

Cao, S. F., Hu, Z. C., Pang, B., Wang, H., Xie, H. X., & Wu, F. (2010a). Effect of ultrasound treatment on fruit decay and quality maintenance in strawberry after harvest. *Food Control*, *21*, 529–532.

Cao, S. F., Hu, Z., & Pang, B. (2010b). Optimization of postharvest ultrasonic treatment of strawberry fruit. *Postharvest Biology and Technology*, *55*, 150–153.

Cárcel, J. A., García-Pérez, J. V., Benedito, J., & Mulet, A. (2012). Food process innovation through new technologies: Use of ultrasound. *Journal of Food Engineering*, *110*, 200–207.

Chan, Y. K., & Lee, H. K. (1996). Josapine: A new pineapple hybrid developed at MARDI. In *Second National Congress on Genetics* (pp. 217–220). Genetic Society of Malaysia.

Chandrapala, J., Oliver, C., Kentish, S., & Ashokkumar, M. (2012). Ultrasonics in food processing – Food quality assurance and food safety. *Trends in Food Science & Technology*, *26*, 88–98.

Charles, F., Vidal, V., Olive, F., Filgueiras, H., & Sallanon, H. (2013). Pulsed light treatment as new method to maintain physical and nutritional quality of fresh-cut mangoes. *Innovative Food Science and Emerging Technologies*, *18*, 190–195.

Chauhan, O. P., Raju, P. S., Ravi, N., Singh, A., & Bawa, A. S. (2011). Effectiveness of ozone in combination with controlled atmosphere on quality characteristics including lignification of carrot sticks. *Journal of Food Engineering*, *102*, 43–48.

Chemat, F., Zill-E-Huma, & Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, *18*, 813–835.

Chen, Z., & Zhu, C. (2011). Combined effects of aqueous chlorine dioxide and ultrasonic treatments on postharvest storage quality of plum fruit (*Prunus salicina* L.). *Postharvest Biology and Technology*, *61*, 117–123.

Chen, Z., Zhu, C., Zhang, Y., Niu, D., & Du, J. (2010). Effects of aqueous chlorine dioxide treatment on enzymatic browning and shelf-life of fresh-cut asparagus lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology*, *58*, 232–238.

Cheng, L. H., Soh, C. Y., Liew, S. C., & Teh, F. F. (2007). Effects of sonication and carbonation on guava juice quality. *Food Chemistry*, *104*, 1396–1401.

Chisari, M., Barbagallo, R. N., Spagna, G., & Artes, F. (2011). Improving the quality of fresh-cut melon through inactivation of degradative oxidase and pectinase enzymatic activities by UV-C treatment. *International Journal of Food Science and Technology*, *46*, 463–468.

Cisneros-Zevallos, L. (2003). The use of controlled postharvest abiotic stresses as a tool for enhancing the nutraceutical content and adding-value. *Journal of Food Science*, *68*, 1560–1565.

Cocetta, G., Baldassarre, V., Spinardi, A., & Ferrante, A. (2014). Effect of cutting on ascorbic acid oxidation and recycling in fresh-cut baby spinach (*Spinacia oleracea* L.) leaves. *Postharvest Biology and Technology*, *88*, 8–16.

Conklin, P. L. (2001). Recent advances in the role and biosynthesis of ascorbic acid in plants. *Plant, Cell and Environment*, *24*, 383–394.

Conklin, P. L., & Barth, C. (2004). Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant, Cell and Environment*, *27*, 959–970.

Cruz, R. M. S., Vieira, M. C., & Silva, C. L. M. (2006). Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). *Journal of Food Engineering*, *72*, 8–15.

Davey, M. W., Dekempeneer, E., & Keulemans, J. (2003). Rocket-powered high-performance liquid chromatographic analysis of plant ascorbate and glutathione. *Analytical Biochemistry*, *316*, 74–81.

Davey, M. W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I. J. J., Favell, D., & Fletcher, J. (2000). Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, *80*, 825–860.

Degl'Innocenti, E., Pardossi, A., Tognoni, F., & Guidi, L. (2007). Physiological basis of sensitivity to enzymatic browning in "lettuce", "escarole" and "rocket salad" when stored as fresh-cut products. *Food Chemistry*, *104*, 209–215.

Delaquis, P. J., Fukumoto, L. R., Toivonen, P. M. A., & Cliff, M. A. (2004). Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. *Postharvest Biology and Technology*, *31*, 81–91.

Deza-Durand, K. M., & Petersen, M. A. (2011). The effect of cutting direction on aroma compounds and respiration rate of fresh-cut iceberg lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology*, *61*, 83–90.

Ding, T., Ge, Z., Shi, J., Xu, Y. T., Jones, C. L., & Liu, D. H. (2015). Impact of slightly acidic electrolyzed water (SAEW) and ultrasound on microbial loads and quality of fresh fruits. *LWT - Food Science and Technology*, *60*, 1195–1199.

Djioua, T., Charles, F., Lopez-Lauri, F., Filgueiras, H., Coudret, A., Jr, M. F., Ducamp-Collin, M., & Sallanon, H. (2009). Improving the storage of fresh-cut mangoes (*Mangifera indica* L.) by hot water treatments. *Postharvest Biology and Technology*, *52*, 221–226.

Dobhal, S., Zhang, G., Gautam, D., Fletcher, J., & Ma, L. M. (2015). Uneven distribution of microorganisms on the surface of field-grown cantaloupes. *Food Control*, *47*, 185–189.

Du, G., Li, M., Ma, F., & Liang, D. (2009). Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. *Food Chemistry*, *113*, 557–562.

Du, W. X., Avena-Bustillos, R. J., Breksa, A. P., & McHugh, T. H. (2012). Effect of UV-B light and different cutting styles on antioxidant enhancement of commercial fresh-cut carrot products. *Food Chemistry*, *134*, 1862–1869.

Du, W. X., Avena-Bustillos, R. J., Breksa, A. P., & McHugh, T. H. (2014). UV-B light as a factor affecting total soluble phenolic contents of various whole and fresh-cut specialty crops. *Postharvest Biology and Technology*, *93*, 72–82.

Eichholz, I., Rohn, S., Gamm, A., Beesk, N., Herppich, W. B., Kroh, L. W., Ulrichs, C., & Huyskens-Keil, S. (2012). UV-B-mediated flavonoid synthesis in white asparagus (*Asparagus officinalis* L.). *Food Research International*, *48*, 196–201.

Elizaquível, P., Sánchez, G., Selma, M. V., & Aznar, R. (2012). Application of propidium monoazide-qPCR to evaluate the ultrasonic inactivation of *Escherichia coli* O157:H7 in fresh-cut vegetable wash water. *Food Microbiology*, *30*, 316–320.

Eltelib, H. A., Badejo, A. A., Fujikawa, Y., & Esaka, M. (2011). Gene expression of monodehydroascorbate reductase and dehydroascorbate reductase during fruit ripening and in response to environmental stresses in acerola (*Malpighia glabra*). *Journal of Plant Physiology*, *168*, 619–627.

Ercan, S. Ş., & Soysal, Ç. (2011). Effect of ultrasound and temperature on tomato peroxidase. *Ultrasonics Sonochemistry*, *18*, 689–695.

Erkan, M., Wang, S. Y., & Wang, C. Y. (2008). Effect of UV treatment on antioxidant capacity, antioxidant enzyme activity and decay in strawberry fruit. *Postharvest Biology and Technology*, *48*, 163–171.

European Commission. (2007). Agricultural commodity markets past developments. Retrieved October 12, 2015, from http://ec.europa.eu/agriculture/analysis/tradepol/worldmarkets/fruitveg/072007_en.pdf

FAO/WHO. (2004). Fruit and vegetables for health. Retrieved October 12, 2015, from http://www.who.int/dietphysicalactivity/publications/fruit_vegetables_report.pdf

FAOSTAT. (2015). Food and Agricultural commodities production/ Countries by commodity. Retrieved March 27, 2015, from http://faostat3.fao.org/browse/rankings/countries_by_commodity/E

Fava, J., Hodara, K., Nieto, A., Guerrero, S., Alzamora, S. M., & Castro, M. A. (2011). Structure (micro, ultra, nano), color and mechanical properties of *Vitis labrusca* L. (grape berry) fruits treated by hydrogen peroxide, UV-C irradiation and ultrasound. *Food Research International*, *44*, 2938–2948.

Federal Agricultural and Marketing Authority (FAMA). (2015). Grading standards and specification of fruits and vegetables. Retrieved August 10, 2015, from http://www.fama.gov.my/documents/10157/af9f2d40-c116-4a3e-8c8c-cc1c897bb039

Fernando, Y., Ng, H. H., & Yusoff, Y. (2014). Activities, motives and external factors influencing food safety management system adoption in Malaysia. *Food Control*, *41*, 69–75.

Finnegan, E., Mahajan, P. V., O'Connell, M., Francis, G. A., & O'Beirne, D. (2013). Modelling respiration in fresh-cut pineapple and prediction of gas permeability needs for optimal modified atmosphere packaging. *Postharvest Biology and Technology*, *79*, 47–53.

Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, *24*, 1043–1048.

Fonteles, T. V., Costa, M. G. M., de Jesus, A. L. T., de Miranda, M. R. A., Fernandes, F. A. N., & Rodrigues, S. (2012). Power ultrasound processing of cantaloupe melon juice: Effects on quality parameters. *Food Research International*, *48*, 41–48.

Food and Drug Administration (FDA). (2000a). CFR-Code of Federal Regulations Title 21. Retrieved August 9, 2015, from http://www.gpo.gov/fdsys/pkg/CFR-2000-title21-vol3/pdf/CFR-2000-title21-vol3-sec184-1366.pdf

Food and Drug Administration (FDA). (2000b). Kinetics of microbial inactivation for alternative food processing technologies -- high pressure processing. Retrieved August 5, 2015, from http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm101456.htm

Forghani, F., Rahman, S. M. E., Park, M. S., Park, J. H., Park, J., Song, K. Bin, & Oh, D. H. (2013). Ultrasonication enhanced low concentration electrolyzed water efficacy on bacteria inactivation and shelf life extension on lettuce. *Food Science and Biotechnology*, *22*, 131–136.

Forney, C. F., Song, J., Hildebrand, P. D., Fan, L., & McRae, K. B. (2007). Interactive effects of ozone and 1-methylcyclopropene on decay resistance and quality of stored carrots. *Postharvest Biology and Technology*, *45*, 341–348.

Foyer, C. H., & Noctor, G. (2005). Oxidant and antioxidant signalling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment*, *28*, 1056–1071.

Foyer, C. H., & Noctor, G. (2011). Ascorbate and glutathione: the heart of the redox hub. *Plant Physiology*, *155*, 2–18.

Franck, C., Baetens, M., Lammertyn, J., Verboven, P., Davey, M. W., & Nicolaï, B. M. (2003). Ascorbic acid concentration in cv. Conference pears during fruit development and postharvest storage. *Journal of Agricultural and Food Chemistry*, *51*, 4757–4763.

Garcia, A., Mount, J. R., & Davidson, P. M. (2003). Ozone and Chlorine Treatment of Fresh-cut Lettuce. *Journal of Food Science*, *68*, 2747–2751.

Ghidelli, C., Mateos, M., Rojas-Argudo, C., & Pérez-Gago, M. B. (2014). Extending the shelf life of fresh-cut eggplant with a soy protein-cysteine based edible coating and modified atmosphere packaging. *Postharvest Biology and Technology*, *95*, 81–87.

Gil, M. I., Aguayo, E., & Kader, A. A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food Chemistry*, *54*, 4284–4296.

Gil, M. I., Selma, M. V., López-Gálvez, F., & Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology*, *134*, 37–45.

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*, 909–930.

Gómez, F., Fernández, L., Gergoff, G., Guiamet, J. J., Chaves, A., & Bartoli, C. G. (2008). Heat shock increases mitochondrial H_2O_2 production and extends postharvest life of spinach leaves. *Postharvest Biology and Technology*, *49*, 229–234.

Gómez-López, V. M., Lannoo, A. S., Gil, M. I., & Allende, A. (2014). Minimum free chlorine residual level required for the inactivation of *Escherichia coli* O157: H7 and trihalomethane generation during dynamic washing of fresh-cut spinach. *Food Control*, *42*, 132–138.

González-Aguilar, G. A., Ruiz-Cruz, S., Cruz-Valenzuela, R., Rodríguez-Félix, A., & Wang, C. Y. (2004). Physiological and quality changes of fresh-cut pineapple treated with antibrowning agents. *LWT - Food Science and Technology*, *37*, 369–376.

González-Aguilar, G. A., Valenzuela-Soto, E., Lizardi-Mendoza, J., Goycoolea, F., Martínez-Téllez, M. A., Villegas-Ochoa, M. A., Monray-García, I. N., & Ayala-Zavala, J. F. (2009). Effect of chitosan coating in preventing deterioration and preserving the quality of fresh-cut papaya "Maradol." *Journal of the Science of Food and Agriculture*, *89*, 15–23.

Gonzalez-Aguilar, G. A., Villa-Rodriguez, J. A., Ayala-Zavala, J. F., & Yahia, E. M. (2010). Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments. *Trends in Food Science and Technology*, *21*, 475–482.

González-Aguilar, G. A., Villegas-Ochoa, M. A., Martínez-Téllez, M. A., Gardea, A. A., & Ayala-Zavala, J. F. (2007a). Improving antioxidant capacity of fresh-cut mangoes treated with UV-C. *Journal of Food Science*, *72*, 197–202.

González-Aguilar, G. A., Zavaleta-Gatica, R., & Tiznado-Hernández, M. E. (2007b). Improving postharvest quality of mango "Haden" by UV-C treatment. *Postharvest Biology and Technology*, *45*, 108–116.

Goulao, L. F., & Oliveira, C. M. (2008). Cell wall modifications during fruit ripening: when a fruit is not the fruit. *Trends in Food Science and Technology*, *19*, 4–25.

Graça, A., Abadias, M., Salazar, M., & Nunes, C. (2011). The use of electrolyzed water as a disinfectant for fresh-cut apples. *Postharvest Biology and Technology*, *61*, 172–177.

Graça, A., Salazar, M., Quintas, C., & Nunes, C. (2013). Low dose UV-C illumination as an eco-innovative disinfection system on fresh-cut apples. *Postharvest Biology and Technology*, *85*, 1–7.

Guan, W., Fan, X., & Yan, R. (2013). Effect of combination of ultraviolet light and hydrogen peroxide on inactivation of *Escherichia coli* O157: H7, native microbial loads, and quality of button mushrooms. *Food Control*, *34*, 554–559.

Guerrero-Beltran, J. A., & Barbosa-Cánovas, G. V. (2004). High hydrostatic pressure processing of peach puree with and without antibrowning agents. *Journal of Food Processing Preservation*, *28*, 69–85.

Guzel-Seydim, Z. B., Greene, A. K., & Seydim, A. C. (2004). Use of ozone in the food industry. *LWT - Food Science and Technology*, *37*, 453–460.

Hajare, S. N., Dhokane, V. S., Shashidhar, R., Saroj, S., Sharma, A., & Bandekar, J. R. (2006). Radiation processing of fresh-cut pineaple (*Ananas comosus* Merr.): Effect on nutritional and sensory quality. *Journal of Food Science*, *71*, S501–505.

Hao, J., Li, H., Wan, Y., & Liu, H. (2015). Combined effect of acidic electrolyzed water (AcEW) and alkaline electrolyzed water (AlEW) on the microbial reduction of fresh-cut cilantro. *Food Control*, *50*, 699–704.

Heredia, J. B., & Cisneros-Zevallos, L. (2009). The effect of exogenous ethylene and methyl jasmonate on pal activity, phenolic profiles and antioxidant capacity of carrots (*Daucus carota*) under different wounding intensities. *Postharvest Biology and Technology*, *51*, 242–249.

Hinojosa, A., Silveira, A. C., Ospina, M., Char, C., Sáenz, C., & Escalona, V. H. (2013). Safety of ready-to-eat watercress using environmentally friendly sanitization methods. *Journal of Food Quality*, *36*, 66–76.

Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, *207*, 604–611.

Hodges, D. M., Lester, G. E., Munro, K. D., & Toivonen, P. M. A. (2004). Oxidative stress: Importance for postharvest quality. *HortScience*, *39*, 924–929.

Hodges, D. M., & Toivonen, P. M. A. (2008). Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. *Postharvest Biology and Technology*, *48*, 155–162.

Holvoet, K., De Keuckelaere, A., Sampers, I., Van Haute, S., Stals, A., & Uyttendaele, M. (2014). Quantitative study of cross-contamination with *Escherichia coli*, *E.coli* O157, MS2 phage and murine norovirus in a simulated fresh-cut lettuce wash process. *Food Control*, *37*, 218–227.

Hong, K., Xu, H., Wang, J., Zhang, L., Hu, H., Jia, Z., Gu, H., He, Q., & Gong, D. (2013). Quality changes and internal browning developments of summer pineapple fruit during storage at different temperatures. *Scientia Horticulturae*, *151*, 68–74.

Hossain, M. A., & Rahman, S. M. M. (2011). Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Research International*, *44*, 672–676.

Hricova, D., Stephan, R., & Zweifel, C. (2008). Electrolyzed water and its application in the food industry. *Journal of Food Protection*, *71*, 1934–1947.

Hu, K., Wang, Q., Hu, L. Y., Gao, S. P., Wu, J., Li, Y. H., Zheng, J., Han, Y., & Zhang, H. (2014). Hydrogen sulfide prolongs postharvest storage of fresh-cut pears (Pyrus pyrifolia) by alleviation of oxidative damage and inhibition of fungal growth. *PLoS ONE*, *9*, 1–9.

Huang, T. S., Xu, C., Walker, K., West, P., Zhang, S., & Weese, J. (2006). Decontamination efficacy of combined chlorine dioxide with ultrasonication on apples and lettuce. *Journal of Food Science*, *71*, 134–139.

Huang, Y. R., Hung, Y. C., Hsu, S. Y., Huang, Y. W., & Hwang, D. F. (2008). Application of electrolyzed water in the food industry. *Food Control*, *19*, 329–345.

Huang, Y., Ye, M., & Chen, H. (2012). Efficacy of washing with hydrogen peroxide followed by aerosolized antimicrobials as a novel sanitizing process to inactivate *Escherichia coli* O157:H7 on baby spinach. *International Journal of Food Microbiology*, *153*, 306–313.

Hussain, P. R., Omeera, A., Suradkar, P. P., & Dar, M. A. (2014). Effect of combination treatment of gamma irradiation and ascorbic acid on physicochemical and microbial quality of fresh-cut eggplant (*Solanum melongena* L.). *Radiation Physics and Chemistry*, *103*, 131–141.

Ikeura, H., Kobayashi, F., & Tamaki, M. (2011). Removal of residual pesticide, fenitrothion, in vegetables by using ozone microbubbles generated by different methods. *Journal of Food Engineering*, *103*, 345–349.

Interdonato, R., Rosa, M., Nieva, C. B., González, J. A., Hilal, M., & Prado, F. E. (2011). Effects of low UV-B doses on the accumulation of UV-B absorbing compounds and total phenolics and carbohydrate metabolism in the peel of harvested lemons. *Environmental and Experimental Botany*, *70*, 204–211.

Jang, J. H., & Moon, K. D. (2011). Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. *Food Chemistry*, *124*, 444–449.

Jimenez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M., & Mullineaux, P. (2002). Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta*, *214*, 751–758.

Joyce, E., Phull, S. S., Lorimer, J. P., & Mason, T. J. (2003). The development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power and sonication time on cultured Bacillus species. *Ultrasonics Sonochemistry*, *10*, 315–318.

Jung, L. S., Lee, S. H., Kim, S., & Ahn, J. (2013). Effect of high hydrostatic pressure on the quality-related properties of carrot and spinach. *Food Science and Biotechnology*, *22*, 189–195.

Jung, L.-S., Lee, S. H., Kim, S., Cho, Y., & Ahn, J. (2014). Effect of high-pressure post-packaging pasteurization on microbiological quality of ready-to-use vegetables. *Journal of Food Processing and Preservation*, *38*, 406–412.

Kaewtathip, T., & Charoenrein, S. (2012). Changes in volatile aroma compounds of pineapple (*Ananas comosus*) during freezing and thawing. *International Journal of Food Science and Technology*, *47*, 985–990.

Karaca, H., Walse, S. S., & Smilanick, J. L. (2012). Effect of continuous 0.3 µl/L gaseous ozone exposure on fungicide residues on table grape berries. *Postharvest Biology and Technology*, *64*, 154–159.

Karakurt, Y., & Huber, D. J. (2003). Activities of several membrane and cell-wall hydrolases, ethylene biosynthetic enzymes, and cell wall polyuronide degradation during low-temperature storage of intact and fresh-cut papaya (*Carica papaya*) fruit. *Postharvest Biology and Technology*, *28*, 219–229.

Karakurt, Y., & Huber, D. J. (2007). Characterization of wound-regulated cDNAs and their expression in fresh-cut and intact papaya fruit during low-temperature storage. *Postharvest Biology and Technology*, *44*, 179–183.

Kentish, S., & Feng, H. (2014). Applications of power ultrasound in food processing. *Annual Review of Food Science and Technology*, *5*, 263–84.

Ketnawa, S., Chaiwut, P., & Rawdkuen, S. (2012). Pineapple wastes: A potential source for bromelain extraction. *Food and Bioproducts Processing*, *90*, 385–391.

Ketteringham, L., Gausseres, R., James, S. J., & James, C. (2006). Application of aqueous ozone for treating pre-cut green peppers (*Capsicum annuum* L.). *Journal of Food Engineering*, *76*, 104–111.

Khadre, M. A., Yousef, A. E., & Kim, J. G. (2001). Microbiological aspects of ozone applications in food: A review. *Journal of Food Science*, *66*, 1242–1252.

Kiang, W. S., Bhat, R., Rosma, A., & Cheng, L. H. (2013). Effects of thermosonication on the fate of *Escherichia coli* O157: H7 and *Salmonella* Enteritidis in mango juice. *Letters in Applied Microbiology*, *56*, 251–257.

Kim, H. J., Fonseca, J. M., Kubota, C., & Choi, J. H. (2007). Effect of hydrogen peroxide on quality of fresh-cut tomato. *Journal of Food Science*, *72*, 4–8.

Kim, Y. H., Jeong, S. G., Back, K. H., Park, K. H., Chung, M. S., & Kang, D. H. (2013). Effect of various conditions on inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in fresh-cut lettuce using ultraviolet radiation. *International Journal of Food Microbiology*, *166*, 349–355.

Klaiber, R. G., Baur, S., Wolf, G., Hammes, W. P., & Carle, R. (2005). Quality of fresh-cut carrots as affected by warm water washing and chlorination. *Innovative Food Science and Emerging Technologies*, *6*, 351–362.

Krebbers, B., Matser, A. M., Hoogerwerf, S. W., Moezelaar, R., Tomassen, M., & van den Berg, R. W. (2003). Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Science & Emerging Technologies*, *4*, 377–385.

Kwak, T. Y., Kim, N. H., & Rhee, M. S. (2011). Response surface methodology-based optimization of decontamination conditions for *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh-cut celery using thermoultrasound and calcium propionate. *International Journal of Food Microbiology*, *150*, 128–135.

Lagnika, C., Zhang, M., & Mothibe, K. J. (2013). Effects of ultrasound and high pressure argon on properties of white mushrooms (*Agaricus bisporus*) during postharvest storage. *Postharvest Biology and Technology*, *82*, 87–94.

Lavinas, F. C., Miguel, M. A. L., Lopes, M. L. M., & Valente Mesquita, V. L. (2008). Effect of high hydrostatic pressure on cashew apple (*Anacardium occidentale* L.) juice preservation. *Journal of Food Science*, *73*, M273–M277.

Leceta, I., Molinaro, S., Guerrero, P., Kerry, J. P., & Caba, K. De. (2015). Quality attributes of map packaged ready-to-eat baby carrots by using chitosan-based coatings. *Postharvest Biology and Technology*, *100*, 142–150.

Lemoine, M. L., Civello, P. M., Chaves, A. R., & Martínez, G. A. (2008). Effect of combined treatment with hot air and UV-C on senescence and quality parameters of fresh-cut broccoli (*Brassica oleracea* L. var. Italica). *Postharvest Biology and Technology*, *48*, 15–21.

Lemoine, M. L., Civello, P. M., Chaves, A. R., & Martínez, G. A. (2010). Influence of a combined hot air and UV-C treatment on quality parameters of fresh-cut broccoli florets at 0°C. *International Journal of Food Science and Technology*, *45*, 1212–1218.

Leong, L. P., & Shui, G. (2002). An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chemistry*, *76*, 69–75.

Li, J., Yan, J., Wang, J., Zhao, Y., Cao, J., & Jiang, W. (2010). Effects of chitosan coating on oxidative stress in bruised Yali pears (*Pyrus bretschneideri* Rehd.). *International Journal of Food Science and Technology*, *45*, 2149–2154.

Li, Y., & Wu, C. (2013). Enhanced inactivation of Salmonella Typhimurium from blueberries by combinations of sodium dodecyl sulfate with organic acids or hydrogen peroxide. *Food Research International*, *54*, 1553–1559.

Liu, F., Wang, Y., Bi, X., Guo, X., Fu, S., & Liao, X. (2013a). Comparison of microbial Inactivation and rheological characteristics of mango pulp after high hydrostatic pressure treatment and high temperature short time treatment. *Food and Bioprocess Technology*, *6*, 2675–2684.

Liu, H., Jiang, W., Bi, Y., & Luo, Y. (2005). Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. *Postharvest Biology and Technology*, *35*, 263–269.

Liu, J., Wu, Y. C., Kan, J., Wang, Y., & Jin, C. H. (2013b). Changes in reactive oxygen species production and antioxidant enzyme activity of Agaricus bisporus harvested at different stages of maturity. *Journal of the Science of Food and Agriculture*, *93*, 2201–2206.

Locato, V., Cimini, S., & Gara, L. De. (2013). Strategies to increase vitamin C in plants: from plant defense perspective to food biofortification. *Frontiers in Plant Science*, *4*, 152.

López-Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J. A., Selma, M. V., & Gil, M. I. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology*, *55*, 53–60. h

López-Rubira, V., Conesa, A., Allende, A., & Artés, F. (2005). Shelf life and overall quality of fresh-cut pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology*, *37*, 174–185.

Lu, X., Sun, D., Li, Y., Shi, W., & Sun, G. (2011). Pre- and post-harvest salicylic acid treatments alleviate internal browning and maintain quality of winter pineapple fruit. *Scientia Horticulturae*, *130*, 97–101.

Luna, M. C., Tudela, J. a., Martínez-Sánchez, A., Allende, A., Marín, A., & Gil, M. I. (2012). Long-term deficit and excess of irrigation influences quality and browning related enzymes and phenolic metabolism of fresh-cut iceberg lettuce (*Lactuca sativa* L.). *Postharvest Biology and Technology*, *73*, 37–45.

Luo, H., Jiang, L. I., Bao, Y., Wang, L., & Yu, Z. (2013a). Effect of chitosan/nana-chitosan composite coating on browning and lignification of fresh-cut *Zizania latifolia*. *Journal of Food Quality*, *36*, 426–431.

Luo, Y. G., Nou, X. W., Millner, P., Zhou, B., Shen, C. L., Yang, Y., Wu, Y., Wang, Q., Feng, H., & Shelton, D. (2012). A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy against pathogen survival and cross-contamination during produce wash. *International Journal of Food Microbiology*, *158*, 133–139.

Luo, Y., Zhou, Y., & Zeng, K. (2013b). Effect of *Pichia membranaefacien*s on ROS metabolism and postharvest disease control in citrus fruit. *Crop Protection*, *53*, 96–102.

Maghoumi, M., Gómez, P. A., Artés-Hernández, F., Mostofi, Y., Zamani, Z., & Artés, F. (2013). Hot water, UV-C and superatmospheric oxygen packaging as hurdle techniques for maintaining overall quality of fresh-cut pomegranate arils. *Journal of the Science of Food and Agriculture*, *93*, 1162–1168.

Maitland, J. E., Boyer, R. R., Eifert, J. D., & Williams, R. C. (2011). High hydrostatic pressure processing reduces *Salmonella enterica* serovars in diced and whole tomatoes. *International Journal of Food Microbiology*, *149*, 113–117.

Malaysian Agricultural Research and Development Institute (MARDI). (1996). Josapine. Retrieved August 17, 2015, from http://www.mardi.gov.my/c/document_library/get_file?uuid=1c492fae-5e74-451a-80eb-7d6e5636a591&groupId=10138

Malaysian Pineapple Board Industry (MPBI). (2015). Cultivar. Retrieved August 17, 2015, from http://www.mpib.gov.my/en/web/guest/kultivar1

Mañas, P., & Pagán, R. (2005). Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology*, *98*, 1387–1399.

Mantilla, N., Castell-Perez, M. E., Gomes, C., & Moreira, R. G. (2013). Multilayered antimicrobial edible coating and its effect on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*). *LWT - Food Science and Technology*, *51*, 37–43.

Marrero, A., & Kader, A. A. (2006). Optimal temperature and modified atmosphere for keeping quality of fresh-cut pineapples. *Postharvest Biology and Technology*, *39*, 163–168.

Martin, H., & Maris, P. (2012). Synergism between hydrogen peroxide and seventeen acids against six bacterial strains. *Journal of Applied Microbiology*, *113*, 578–590.

Martínez-Hernández, G. B., Artés-Hernández, F., Gómez, P. A., Formica, A. C., & Artés, F. (2013). Combination of electrolysed water, UV-C and superatmospheric O_2 packaging for improving fresh-cut broccoli quality. *Postharvest Biology and Technology*, *76*, 125–134.

Martínez-Hernández, G. B., Gómez, P. A., Pradas, I., Artés, F., & Artés-Hernández, F. (2011). Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi® broccoli. *Postharvest Biology and Technology*, *62*, 327–337.

Martínez-Hernández, G. B., Huertas, J., Navarro-Rico, J., Gomez, P. A., Artes, F., Palop, A., & Artes-Hernandez, F. (2015a). Inactivation kinetics of foodborne pathogens by UV-C radiation and its subsequent growth in fresh-cut kailan-hybrid broccoli. *Food Microbiology*, *46*, 263–271.

Martínez-Hernández, G. B., Navarro-Rico, J., Gómez, P. A., Otón, M., Artés, F., & Artés-Hernández, F. (2015b). Combined sustainable sanitising treatments to reduce *Escherichia coli* and *Salmonella* Enteritidis growth on fresh-cut kailan-hybrid broccoli. *Food Control*, *47*, 312–317.

Martínez-Solano, J. R., Sánchez-Bel, P., Egea, I., Olmos, E., Hellin, E., & Romojaro, F. (2005). Electron beam ionization induced oxidative enzymatic activities in pepper (*Capsicum annuum* L.), associated with ultrastructure cellular damages. *Journal of Agricultural and Food Chemistry*, *53*, 8593–8599.

Martiñon, M. E., Moreira, R. G., Castell-Perez, M. E., & Gomes, C. (2014). Development of a multilayered antimicrobial edible coating for shelf-life extension of fresh-cut cantaloupe (*Cucumis melo* L.) stored at 4°C. *LWT - Food Science and Technology*, *56*, 341–350.

Maurer, H. R. (2001). Bromelain: biochemistry, pharmacology and medical use. *Cellular and Molecular Life Sciences*, *58*, 1234–1245.

Maya-Meraz, I. O., Espino-Díaz, M., Molina-Corral, F. J., González-Aguilar, G. a., Jacobo-Cuellar, J. L., Sepulveda, D. R., & Olivas, G. I. (2014). Production of volatiles in fresh-cut apple: Effect of applying alginate coatings containing linoleic acid or isoleucine. *Journal of Food Science*, *79*, C2185–C2191.

Meng, X., Zhang, M., & Adhikari, B. (2012). Extending shelf-life of fresh-cut green peppers using pressurized argon treatment. *Postharvest Biology and Technology*, *71*, 13–20.

Millan-Sango, D., Mcelhatton, A., & Valdramidis, V. P. (2015). Determination of the efficacy of ultrasound in combination with essential oil of oregano for the decontamination of *Escherichia coli* on inoculated lettuce leaves. *Food Research International*, *67*, 145–154.

Ministry of Health Malaysia (MOH). (2016). Food Act 1983 (Act 281) & Regulations. Selangor, Malaysia: International Law Book Services, Malaysia.

Mishra, B. B., Gautam, S., & Sharma, A. (2012). Browning of fresh-cut eggplant: Impact of cutting and storage. *Postharvest Biology and Technology*, *67*, 44–51.

Mitra, S. K. (1997). *Postharvest Physiology and Storage of Tropical and Subtropical Fruits*. Oxon, UK: CABI Publishing.

Mittler, R., Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science*, *9*, 490–498.

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G., Tognetti, V. B., Vandepoele, K., Gollery, M., Shulaev, V., & Van Breusegem, F. (2011). ROS signaling: The new wave? *Trends in Plant Science*, *16*, 300–309.

Moerman, F. (2005). High hydrostatic pressure inactivation of vegetative microorganisms, aerobic and anaerobic spores in pork Marengo, a low acidic particulate food product. *Meat Science*, *69*, 225–232.

Montero-Calderón, M., Rojas-Graü, M. A., & Martín-Belloso, O. (2008). Effect of packaging conditions on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*). *Postharvest Biology and Technology*, *50*, 182–189.

Moreira, M. D. R., Roura, S. I., & Ponce, A. (2011). Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. *LWT - Food Science and Technology*, *44*, 2335–2341.

Mújica-Paz, H., Valdez-Fragoso, A., Samson, C. T., Welti-Chanes, J., & Torres, A. (2011). High-pressure processing technologies for the pasteurization and sterilization of foods. *Food and Bioprocess Technology*, *4*, 969–985.

Mukhopadhyay, S., & Ramaswamy, R. (2012). Application of emerging technologies to control *Salmonella* in foods: A review. *Food Research International*, *45*(2), 666–677.

Nadas, A., Olmo, M., & Garcia, J. M. (2003). Growth of Botrytis cinerea and strawberry quality in ozone enriched atmospheres. *Journal of Food Science*, *68*, 1798–1802.

Navarro-Rico, J., Artés-Hernández, F., Gómez, P. A., Núñez-Sánchez, M. Á., Artés, F., & Martínez-Hernández, G. B. (2014). Neutral and acidic electrolysed water

kept microbial quality and health promoting compounds of fresh-cut broccoli throughout shelf life. *Innovative Food Science and Emerging Technologies*, *21*, 74–81.

Novillo, P., Salvador, A., Magalhaes, T., & Besada, C. (2014). Deastringency treatment with CO_2 induces oxidative stress in persimmon fruit. *Postharvest Biology and Technology*, *92*, 16–22.

Nyathi, Y., & Baker, A. (2006). Plant peroxisomes as a source of signalling molecules. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1763*, 1478–1495.

O'Connor-Shaw, R. E., Roberts, R., Ford, A. L., & Nottingham, S. M. (1994). Shelf life of fresh-cut honeydew, kiwifruit, papaya, pineapple and cantaloupe.

O'Donnell, C. P., Tiwari, B. K., Bourke, P., & Cullen, P. J. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in Food Science and Technology*, *21*, 358–367.

Ojeda, G. A., Sgroppo, S. C., & Zaritzky, N. E. (2014). Application of edible coatings in fresh-cut sweet potatoes (*Ipomoea batatas* L.) to prevent enzymatic browning. *International Journal of Food Science and Technology*, *49*, 876–883.

Olaimat, A. N., & Holley, R. A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, *32*, 1–19.

Ölmez, H., & Akbas, M. Y. (2009). Optimization of ozone treatment of fresh-cut green leaf lettuce. *Journal of Food Engineering*, *90*, 487–494.

Ölmez, H., & Kretzschmar, U. (2009). Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT - Food Science and Technology*, *42*, 686–693.

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., Munuera, I. P., Fiszman, S., & Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. *Postharvest Biology and Technology*, *57*, 139–148.

Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Using polysaccharide-based edible coatings to enhance quality and antioxidant properties of fresh-cut melon. *LWT - Food Science and Technology*, *41*, 1862–

Ong, M. K., Forney, C. F., Alderson, P. G., & Ali, A. (2013). Postharvest profile of a Solo variety "Frangi" during ripening at ambient temperature. *Scientia Horticulturae*, *160*, 12–19.

Ortega, V. G., Ramírez, J. A., Velázquez, G., Tovar, B., Mata, M., & Montalvo, E. (2013). Effect of high hydrostatic pressure on antioxidant content of "Ataulfo" mango during postharvest maturation. *Food Science and Technology*, *33*, 561–568.

Ozgen, M., Reese, R. N., Tulio, A. Z., Scheerens, J. C., & Miller, A. R. (2006). Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1- picrylhydrazyl (DPPH) methods. *Journal of Agricultural and Food Chemistry*, *54*, 1151–1157.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008 - Volume 19, Number 3—March 2013 - Emerging Infectious Disease journal - CDC. *Emerging Infectious Diseases*, *19*, 407–415.

Patist, A., & Bates, D. (2008). Ultrasonic innovations in the food industry: From the laboratory to commercial production. *Innovative Food Science and Emerging Technologies*, *9*, 147–154.

Paull, R. E., & Duarte, O. (2011). *Crop Production Science in Horticulture, Volume 20: Tropical Fruits, Volume 1* (2nd ed). Oxfordshire, UK: CABI Publishing.

Peng, L., Yang, S., Li, Q., Jiang, Y., & Joyce, D. C. (2008). Hydrogen peroxide treatments inhibit the browning of fresh-cut Chinese water chestnut. *Postharvest Biology and Technology*, *47*, 260–266.

Pingret, D., Fabiano-Tixier, A. S., & Chemat, F. (2013). Degradation during application of ultrasound in food processing: A review. *Food Control*, *31*, 593–606.

Pinto, L., Ippolito, A., & Baruzzi, F. (2015). Control of spoiler *Pseudomonas spp*. on fresh cut vegetables by neutral electrolyzed water. *Food Microbiology*, *50*, 102–108.

Piyasena, P., Mohareb, E., & McKellar, R. C. (2003). Inactivation of microbes using ultrasound: A review. *International Journal of Food Microbiology*, *87*, 207–216.

Posada-Izquierdo, G. D., Pérez-Rodríguez, F., López-Gálvez, F., Allende, A., Selma, M. V., Gil, M. I., & Zurera, G. (2013). Modelling growth of *Escherichia col*i O157:H7 in fresh-cut lettuce submitted to commercial process conditions: Chlorine washing and modified atmosphere packaging. *Food Microbiology*, *33*, 131–138.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*, 4290–4302.

Pui, C. F., Wong, W. C., Chai, L. C., Nillian, E., Ghazali, F. M., Cheah, Y. K., Nakaguchi, Y., Nishibuchi, M., & Radu, S. (2011). Simultaneous detection of *Salmonella spp*., *Salmonella* Typhi and *Salmonella* Typhimurium in sliced fruits using multiplex PCR. *Food Control*, *22*, 337–342.

Queiroz, C., Lopes, M. L. M., Fialho, E., & Valente-Mesquita, V. L. (2011). Changes in bioactive compounds and antioxidant capacity of fresh-cut cashew apple. *Food Research International*, *44*, 1459–1462.

Rahman, S. M. E., Jin, Y. G., & Oh, D. H. (2011). Combination treatment of alkaline electrolyzed water and citric acid with mild heat to ensure microbial safety, shelf-life and sensory quality of shredded carrots. *Food Microbiology*, *28*, 484–491.

Ramos, B., Miller, F. a., Brandão, T. R. S., Teixeira, P., & Silva, C. L. M. (2013). Fresh fruits and vegetables - An overview on applied methodologies to improve its quality and safety. *Innovative Food Science and Emerging Technologies*, *20*, 1–15.

Ramos-villarroel, A. Y., Aron-maftei, N., Martín-belloso, O., & Soliva-fortuny, R. (2012). Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology*, *69*, 32–39.

Ranggana. (1997). *Manual Analysis of Fruit and Vegetable Products*. New Delhi: Tata McGraw- Hill Publish. Comp. Ltd.

Ren, Y., Wang, Y., Bi, Y., Ge, Y., Wang, Y., Fan, C., Li, D., & Deng, H. (2012). Postharvest BTH treatment induced disease resistance and enhanced reactive oxygen species metabolism in muskmelon (*Cucumis melo* L.) fruit. *European Food Research and Technology*, *234*, 963–971.

Rico, D., Martín-Diana, a. B., Barat, J. M., & Barry-Ryan, C. (2007). Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science and Technology*, *18*, 373–386.

Rico, D., Martín-Diana, A. B., Barry-Ryan, C., Frías, J. M., Henehan, G. T. M., & Barat, J. M. (2008). Use of neutral electrolysed water (EW) for quality maintenance and shelf-life extension of fresh-cut lettuce. *Innovative Food Science and Emerging Technologies*, *9*, 37–48.

Rithmanee, T., & Intipunya, P. (2012). Effects of high power ultrasonic pretreatment on physicochemical quality and enzymatic activities of dried longan. *Journal of Agricultural Science*, *4*, 299–306.

Rivera-Lopez, J., Vazques-Ortiz, F. A., Ayala-Zavala, J. F., Sotelo-Mundo, R. R., & Gonzalez-Aguilar, G. A. (2005). Cutting shape and storage temperature affect overall quality of fresh-cut Papaya cv . " Maradol ." *Journal of Food Science*, *70*, 482–489.

Rojas-Graü, M. A., Oms-Oliu, G., Soliva-Fortuny, R., & Martín-Belloso, O. (2009a). The use of packaging techniques to maintain freshness in fresh-cut fruits and vegetables: A review. *International Journal of Food Science and Technology*, *44*, 875–889.

Rojas-Graü, M. A., Soliva-Fortuny, R., & Martín-Belloso, O. (2009b). Edible coatings to incorporate active ingredients to fresh-cut fruits: a review. *Trends in Food Science and Technology*, *20*, 438–447.

Ross, A. I. V, Griffiths, M. W., Mittal, G. S., & Deeth, H. C. (2003). Combining nonthermal technologies to control foodborne microorganisms. *International Journal of Food Microbiology*, *89*, 125–138.

Saftner, R. A., Abbott, J. . A., Bhagwat, A. A., & Vinyard, B. T. (2002). Quality measurement of intact and fresh-cut slices of Fuji , Granny Smith , Pink Lady , and GoldRush Apples. *Journal of Food Science*, *70*, S317–S324.

Sagong, H. G., Cheon, H. L., Kim, S. O., Lee, S. Y., Park, K. H., Chung, M. S., Choi, Y. J., & Kang, D. H. (2013). Combined effects of ultrasound and surfactants to reduce *Bacillus cereus* spores on lettuce and carrots. *International Journal of Food Microbiology*, *160*, 367–372.

Sagong, H. G., Lee, S. Y., Chang, P. S., Heu, S., Ryu, S., Choi, Y. J., & Kang, D. H. (2011). Combined effect of ultrasound and organic acids to reduce *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh lettuce. *International Journal of Food Microbiology*, *145*, 287–292.

Salgado, S. P., Pearlstein, A. J., Luo, Y., & Feng, H. (2014a). Quality of Iceberg (*Lactuca sativa* L.) and Romaine (*L. sativa* L. var. longifolial) lettuce treated by combinations of sanitizer, surfactant, and ultrasound. *LWT - Food Science and Technology*, *56*, 261–268.

Salgado, S. P., Pearlstein, A. J., Luo, Y., Park, H. K., & Feng, H. (2014b). Whole-head washing, prior to cutting, provides sanitization advantages for fresh-cut Iceberg lettuce (*Latuca sativ*a L.). *International Journal of Food Microbiology*, *179*, 18–23.

Salleh-Mack, S. Z., & Roberts, J. S. (2007). Ultrasound pasteurization: The effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922. *Ultrasonics Sonochemistry*, *14*, 323–329. 4

Sango, D. M., Abela, D., Mcelhatton, A., & Valdramidis, V. P. (2014). Assisted ultrasound applications for the production of safe foods. *Journal of Applied Microbiology*, *116*, 1067–1083.

Sangsuwan, J., Rattanapanone, N., & Rachtanapun, P. (2008). Effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple. *Postharvest Biology and Technology*, *49*, 403–410.

São José, J. F. B., Andrade, N. J., Ramos, A. M., Vanetti, M. C. D., Stringheta, P. S., & Chaves, J. B. P. (2014). Decontamination by ultrasound application in fresh fruits and vegetables. *Food Control*, *45*, 36–50.

São José, J. F. B., & Dantas Vanetti, M. C. (2012). Effect of ultrasound and commercial sanitizers in removing natural contaminants and *Salmonella enterica* Typhimurium on cherry tomatoes. *Food Control*, *24*, 95–99.

Sauter, C., Emin, M. A., Schuchmann, H. P., & Tavman, S. (2008). Influence of hydrostatic pressure and sound amplitude on the ultrasound induced dispersion and de-agglomeration of nanoparticles. *Ultrasonics Sonochemistry*, *15*, 517–523.

Schenk, M., García Loredo, A., Raffellini, S., Alzamora, S. M., & Guerrero, S. (2012). The effect of UV-C in combination with H_2O_2 treatments on microbial response and quality parameters of fresh cut pear slices. *International Journal of Food Science and Technology*, *47*, 1842–1851.

Seymour, I. J., Burfoot, D., Smith, R. L., Cox, L. A., & Lockwood, a. (2002). Ultrasound decontamination of fresh-cut fruits and vegetables. *International Journal of Food Science and Technology*, *37*, 547–557.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., & Yoshimura, K. (2002). Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany*, *53*, 1305–1319.

Siboza, X. I., Bertling, I., & Odindo, A. O. (2014). Salicylic acid and methyl jasmonate improve chilling tolerance in cold-stored lemon fruit (*Citrus limon*). *Journal of Plant Physiology*, *171*, 1722–1731.

Silveira, A. C., Aguayo, E., & Artés, F. (2010). Emerging sanitizers and Clean Room packaging for improving the microbial quality of fresh-cut "Galia" melon. *Food Control*, *21*, 863–871.

Silveira, A. C., Aguayo, E., Chisari, M., & Artés, F. (2011). Calcium salts and heat treatment for quality retention of fresh-cut "Galia" melon. *Postharvest Biology and Technology*, *62*, 77–84.

Silveira, A. C., Conesa, A., Aguayo, E., & Artes, F. (2008). Alternative sanitizers to chlorine for use on fresh-cut "galia" (*Cucumis melo* var. catalupensis) melon. *Journal of Food Science*, *73*, 405–411.

Singh, S. P., & Singh, Z. (2013). Dynamics of enzymatic and non-enzymatic antioxidants in Japanese plums during storage at safe and lethal temperatures. *LWT - Food Science and Technology*, *50*, 562–568.

Singh, S. P., Singh, Z., & Swinny, E. E. (2012). Climacteric level during fruit ripening influences lipid peroxidation and enzymatic and non-enzymatic antioxidative systems in Japanese plums (*Prunus salicina* Lindell). *Postharvest Biology and Technology*, *65*, 22–32.

Siroli, L., Patrignani, F., Serrazanetti, D. I., Gardini, F., & Lanciotti, R. (2015). Innovative strategies based on the use of bio-control agents to improve the safety, shelf-life and quality of fresh-cut fruits and vegetables. *Trends in Food Science & Technology*, 1–12.

Smirnoff, N. (1996). The Function and metabolism of ascorbic acid in plants. *Annals of Botany*, *78*, 661–669.

Soliva-Fortuny, R. C., & Martín-Belloso, O. (2003). New advances in extending the shelf-life of fresh-cut fruits: A review. *Trends in Food Science and Technology*, *14*, 341–353.

Soon, J. M., Manning, L., Davies, W. P., & Baines, R. (2012). Fresh produce-associated outbreaks: a call for HACCP on farms? *British Food Journal*, *114*, 553–597.

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: A review. *Trends in Food Science and Technology*, *21*, 323–331.

Stevens, C., Khan, V. A., Lu, J. Y., Wilson, C. L., Chalutz, E., Droby, S., Kabwe, M. K., Haung, Z., Adeyeye, O., Pusey, L. P., & Tang, A. Y. A. (1999). Induced resistance of sweetpotato to *Fusarium* root rot by UV-C hormesis. *Crop Protection*, *18*, 463–470.

Surjadinata, B. B., & Cisneros-Zevallos, L. (2012). Biosynthesis of phenolic antioxidants in carrot tissue increases with wounding intensity. *Food Chemistry*, *134*, 615–624.

Taban, B. M., & Halkman, A. K. (2011). Do leafy green vegetables and their ready-to-eat [RTE] salads carry a risk of foodborne pathogens? *Anaerobe*, *17*, 286–287.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, *19*, 669–675.

Tian, S., Qin, G., & Li, B. (2013). Reactive oxygen species involved in regulating fruit senescence and fungal pathogenicity. *Plant Molecular Biology*, *82*, 593–602.

Toivonen, P. M. A. (2004). Postharvest storage procedures and oxidative stress. *Horticultural Science*, *39*, 938–942.

Toivonen, P. M. A., & Brummell, D. A. (2008). Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology*, *48*, 1–14.

Toivonen, P. M. A., Lu, C., Bach, S., & Delaquis, P. (2012). Modulation of wound-induced hydrogen peroxide and its influence on the fate of *Escherichia coli* O157:H7 in cut lettuce tissues. *Journal of Food Protection*, *75*, 2208–12.

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture*, *81*, 853–876.

Tomás-Callejas, A., Martínez-Hernández, G. B., Artés, F., & Artés-Hernández, F. (2011). Neutral and acidic electrolyzed water as emergent sanitizers for fresh-cut mizuna baby leaves. *Postharvest Biology and Technology*, *59*, 298–306.

Tomás-Callejas, A., Otón, M., Artés, F., & Artés-Hernández, F. (2012). Combined effect of UV-C pretreatment and high oxygen packaging for keeping the quality of fresh-cut Tatsoi baby leaves. *Innovative Food Science and Emerging Technologies*, *14*, 115–121.

Torres, R., Teixidó, N., Usall, J., Abadias, M., Mir, N., Larrigaudiere, C., & Viñas, I. (2011). Anti-oxidant activity of oranges after infection with the pathogen *Penicillium digitatum* or treatment with the biocontrol agent Pantoea agglomerans CPA-2. *Biological Control*, *57*, 103–109.

Torres, R., Valentines, M. C., Usall, J., Viñas, I., & Larrigaudiere, C. (2003). Possible involvement of hydrogen peroxide in the development of resistance mechanisms in "Golden Delicious" apple fruit. *Postharvest Biology and Technology*, *27*, 235–242.

Tsaniklidis, G., Delis, C., Nikoloudakis, N., Katinakis, P., & Aivalakis, G. (2014). Low temperature storage affects the ascorbic acid metabolism of cherry tomato fruits. *Plant Physiology and Biochemistry*, *84*, 149–157.

Tyug, T. S., Prasad, K. N., & Ismail, A. (2010). Antioxidant capacity, phenolics and isoflavones in soybean by-products. *Food Chemistry*, *123*, 583–589.

Tzortzakis, N., Singleton, I., & Barnes, J. (2008). Impact of low-level atmospheric ozone-enrichment on black spot and anthracnose rot of tomato fruit. *Postharvest Biology and Technology*, *47*, 1–9.

Ukuku, D. O. (2004). Effect of hydrogen peroxide treatment on microbial quality and appearance of whole and fresh-cut melons contaminated with Salmonella spp. *International Journal of Food Microbiology*, *95*, 137–146.

United States Department of Agriculture (USDA). (2015). National Nutrient Database for Standard Reference Release 28. Retrieved from http://ndb.nal.usda.gov/ndb/foods/show/2340?fgcd=&manu=&lfacet=&format=&count=&max=35&offset=&sort=&qlookup=pineapple+

Valero, M., Recrosio, N., Saura, D., Muñoz, N., Martí, N., & Lizama, V. (2007). Effects of ultrasonic treatments in orange juice processing. *Journal of Food Engineering*, *80*, 509–516.

Van Haute, S., Sampers, I., Holvoet, K., & Uyttendaelea, M. (2013). Physicochemical quality and chemical safety of chlorine as a reconditioning agent and wash water disinfectant for fresh-cut lettuce washing. *Applied and Environmental Microbiology*, *79*, 2850–2861.

Vandekinderen, I., Devlieghere, F., De Meulenaer, B., Veramme, K., Ragaert, P., & Van Camp, J. (2008). Impact of decontamination agents and a packaging delay

on the respiration rate of fresh-cut produce. *Postharvest Biology and Technology*, *49*, 277–282.

Vandekinderen, I., Van Camp, J., De Meulenaer, B., Veramme, K., Bernaert, N., Denon, Q., Ragaert, P., & Devlieghere, F. (2009). Moderate and high doses of sodium hypochlorite, neutral electrolyzed oxidizing water, peroxyacetic acid, and gaseous chlorine dioxide did not affect the nutritional and sensory qualities of fresh-cut iceberg lettuce (*Lactuca sativa* Var. capitata L.) after. *Journal of Agricultural and Food Chemistry*, *57*, 4195–4203.

Vogt, T. (2010). Phenylpropanoid biosynthesis. *Molecular Plant*, *3*, 2–20.

Waghmare, R. B., & Annapure, U. S. (2013). Combined effect of chemical treatment and/or modified atmosphere packaging (MAP) on quality of fresh-cut papaya. *Postharvest Biology and Technology*, *85*, 147–153.

Waghmare, R. B., Mahajan, P. V., & Annapure, U. S. (2013). Modelling the effect of time and temperature on respiration rate of selected fresh-cut produce. *Postharvest Biology and Technology*, *80*, 25–30.

Wang, H., Feng, H., & Luo, Y. (2004). Microbial reduction and storage quality of fresh-cut cilantro washed with acidic electrolyzed water and aqueous ozone. *Food Research International*, *37*, 949–956.

Wang, Q., Cao, Y., Zhou, L., Jiang, C.-Z., Feng, Y., & Wei, S. (2015). Effects of postharvest curing treatment on flesh colour and phenolic metabolism in fresh-cut potato products. *Food Chemistry*, *169*, 246–254.

Watada, A. E., & Qi, L. (1999). Quality of fresh-cut produce. *Postharvest Biology and Technology*, *15*, 201–205.

Wills, R. M. (2007). *Postharvest: An Introduction to the Physiology and Handling of Fruit, Vegetables and Ornamenals* (Ed. 5th). Australia: UNSW Press.

Wong, E., Vaillant, F., & Pérez, A. (2010). Osmosonication of blackberry juice: Impact on selected pathogens, spoilage microorganisms, and main quality parameters. *Journal of Food Science*, *75*, 468–474.

Wu, Z. S., Zhang, M., & Adhikari, B. (2012a). Application of high pressure argon treatment to maintain quality of fresh-cut pineapples during cold storage. *Journal of Food Engineering*, *110*, 395–404.

Wu, Z. S., Zhang, M., & Adhikari, B. (2013). Effects of high pressure argon and xenon mixed treatment on wound healing and resistance against the growth of *Escherichia coli* or *Saccharomyces cerevisiae* in fresh-cut apples and pineapples. *Food Control*, *30*, 265–271.

Wu, Z. S., Zhang, M., & Wang, S. J. (2012b). Effects of high-pressure argon and nitrogen treatments on respiration, browning and antioxidant potential of fresh-cut pineapples during shelf life. *Journal of the Science of Food and Agriculture*, *92*, 2250–2259.

Wulfkuehler, S., Kurfiss, L., Kammerer, D. R., Weiss, A., Schmidt, H., & Carle, R. (2013). Impact of different washing procedures on quality of fresh-cut iceberg lettuce (*Lactuca sativa* var. capitata L.) and endive (*Cichorium endivia* L.). *European Food Research and Technology*, *236*, 229–241.

Xia, H., Wang, Q., Liao, Y., Xu, X., Baxter, S. M., Slone, R. V., Wu, S., Swift, G., & Westmoreland, D. G. (2002). Polymerization rate and mechanism of ultrasonically initiated emulsion polymerization of n-butyl acrylate. *Ultrasonics Sonochemistry*, *9*, 151–158.

Yang, Y., Luo, Y., Millner, P., Shelton, D., & Nou, X. (2012). Enhanced chlorine efficacy against bacterial pathogens in wash solution with high organic loads. *Journal of Food Processing and Preservation*, *36*, 560–566.

Yang, Z., Cao, S., Cai, Y., & Zheng, Y. (2011). Combination of salicylic acid and ultrasound to control postharvest blue mold caused by *Penicillium expansum* in peach fruit. *Innovative Food Science and Emerging Technologies*, *12*, 310–314.

Yang, Z., Cao, S., Su, X., & Jiang, Y. (2014). Respiratory activity and mitochondrial membrane associated with fruit senescence in postharvest peaches in response to UV-C treatment. *Food Chemistry*, *161*, 16–21.

Yeoh, W. K., Ali, A., & Forney, C. F. (2014). Effects of ozone on major antioxidants and microbial populations of fresh-cut papaya. *Postharvest Biology and Technology*, *89*, 56–58.

Yoon, J. H., Bae, Y. M., Jung, K. S., Heu, S., & Lee, S. Y. (2013). Combined effect of calcium oxide and sonication to reduce foodborne pathogens on fresh produce. *Food Science and Biotechnology*, *22*, 275–278.

You, Y., Jiang, Y., Sun, J., Liu, H., Song, L., & Duan, X. (2012). Effects of short-term anoxia treatment on browning of fresh-cut Chinese water chestnut in relation to antioxidant activity. *Food Chemistry*, *132*, 1191–1196.

Zeng, K., Deng, Y., Ming, J., & Deng, L. (2010). Induction of disease resistance and ROS metabolism in navel oranges by chitosan. *Scientia Horticulturae*, *126*, 223–228.

Zhan, L., Hu, J., Lim, L. T., Pang, L., Li, Y., & Shao, J. (2013). Light exposure inhibiting tissue browning and improving antioxidant capacity of fresh-cut celery (*Apium graveolens* var. dulce). *Food Chemistry*, *141*, 2473–2478.

Zhang, B. Y., Samapundo, S., Pothakos, V., de Baenst, I., Sürengil, G., Noseda, B., & Devlieghere, F. (2013a). Effect of atmospheres combining high oxygen and carbon dioxide levels on microbial spoilage and sensory quality of fresh-cut pineapple. *Postharvest Biology and Technology*, *86*, 73–84.

Zhang, B. Y., Samapundo, S., Rademaker, M., Noseda, B., Denon, Q., de Baenst, I., Surengil, G., De Baets, B., & Devlieghere, F. (2014). Effect of initial headspace oxygen level on growth and volatile metabolite production by the specific

spoilage microorganisms of fresh-cut pineapple. *LWT - Food Science and Technology*, *55*, 224–231.

Zhang, C., Chen, K., & Wang, G. (2013b). Combination of the biocontrol yeast Cryptococcus laurentii with UV-C treatment for control of postharvest diseases of tomato fruit. *BioControl*, *58,* 269–281.

Zhang, L., Lu, Z., Yu, Z., & Gao, X. (2005). Preservation of fresh-cut celery by treatment of ozonated water. *Food Control*, *16*, 279–283.

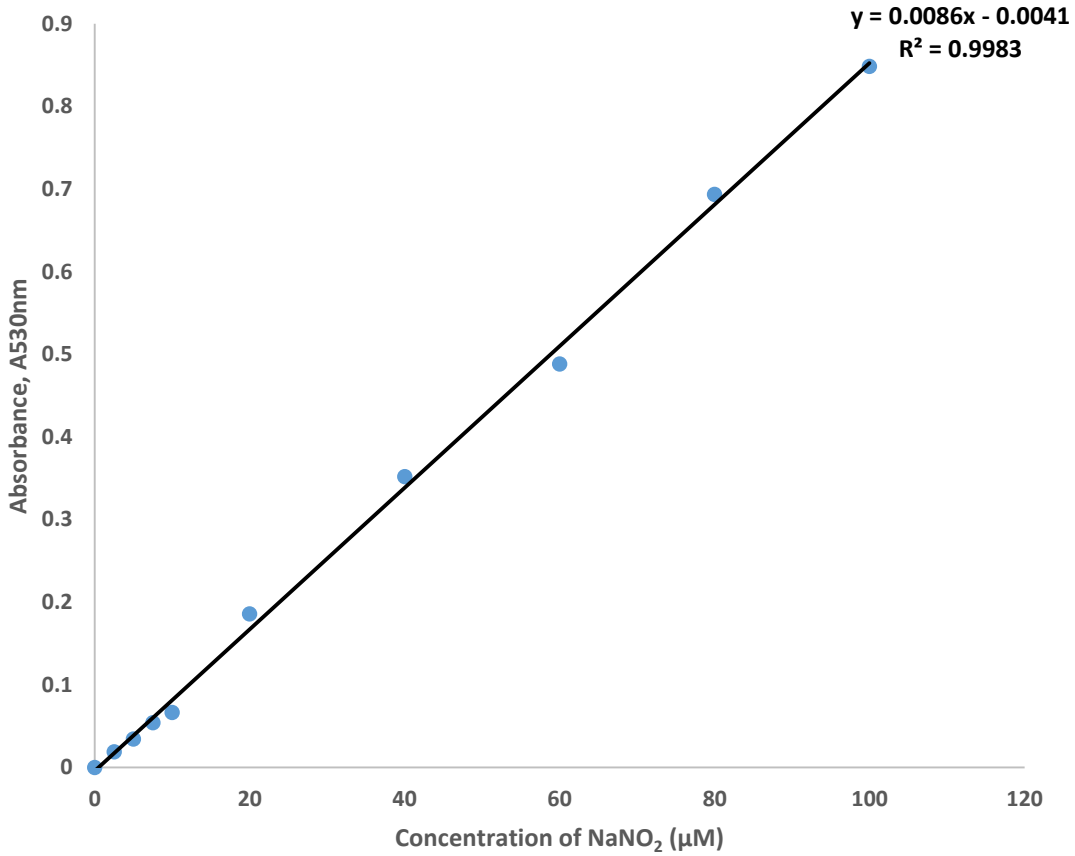
Zhou, B., Feng, H., & Luo, Y. (2009). Ultrasound Enhanced Sanitizer Efficacy in Reduction of *Escherichia coli* O157:H7 Population on Spinach Leaves. *Journal of Food Science*, *74*, M308–M313.

Zhou, B., Feng, H., & Pearlstein, A. J. (2012). Continuous-flow ultrasonic washing system for fresh produce surface decontamination. *Innovative Food Science and Emerging Technologies*, *16*, 427–435.

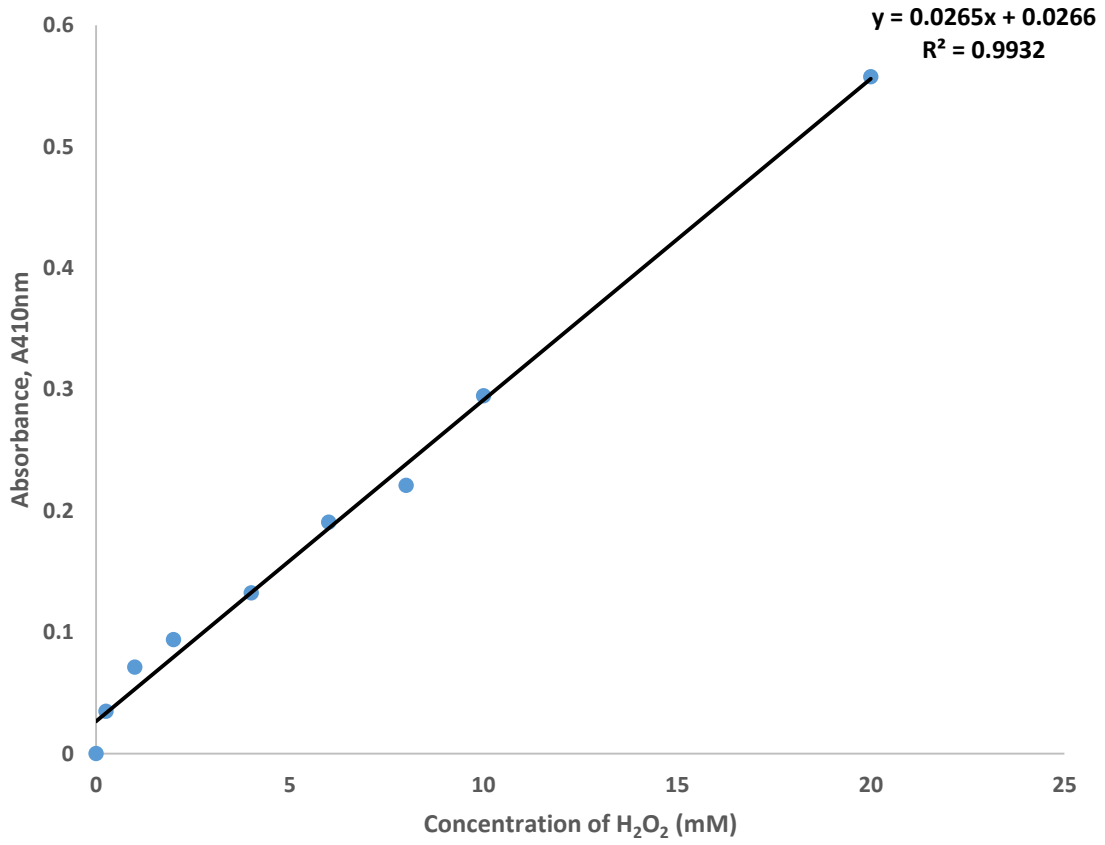
Zhu, S., Sun, L., Liu, M., & Zhou, J. (2008). Effect of nitric oxide on reactive oxygen species and antioxidant enzymes in kiwifruit during storage. *Journal of the Science of Food and Agriculture*, *10*, 103.

APPENDIX A

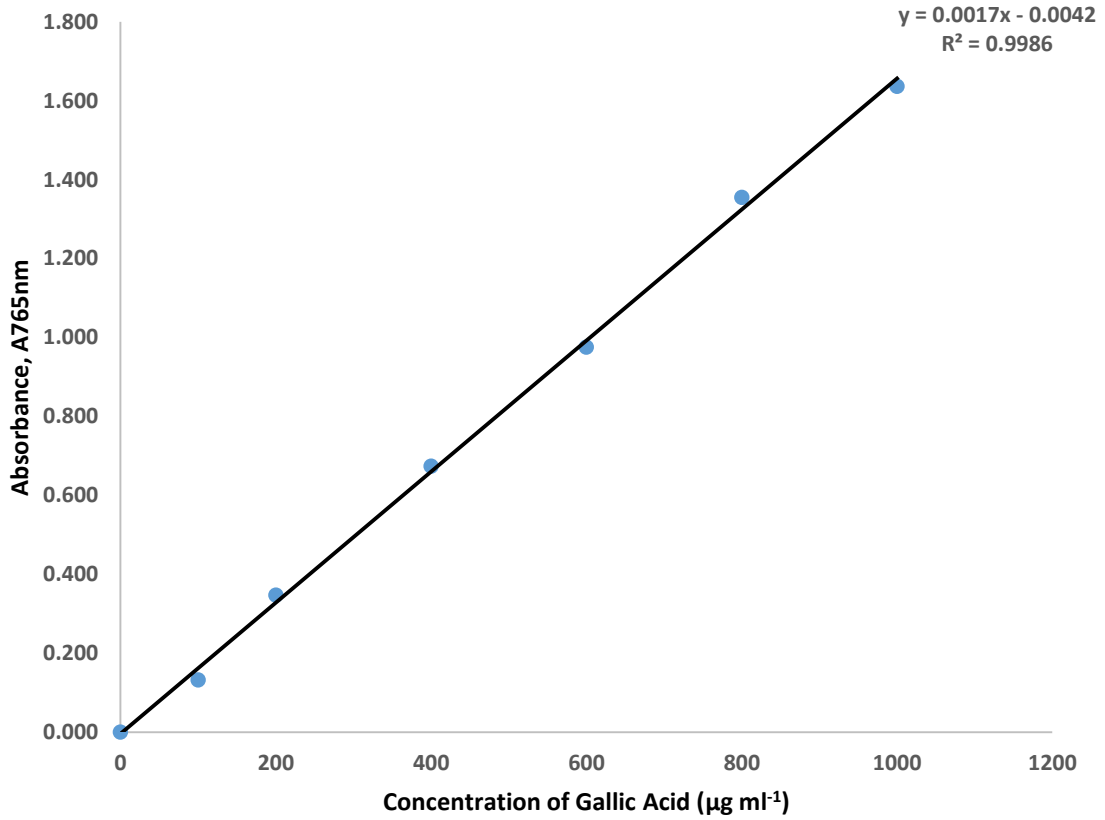
Appendix A.1 Standard curve for $NaNO_2$ (0 – 100 µM) at A530nm



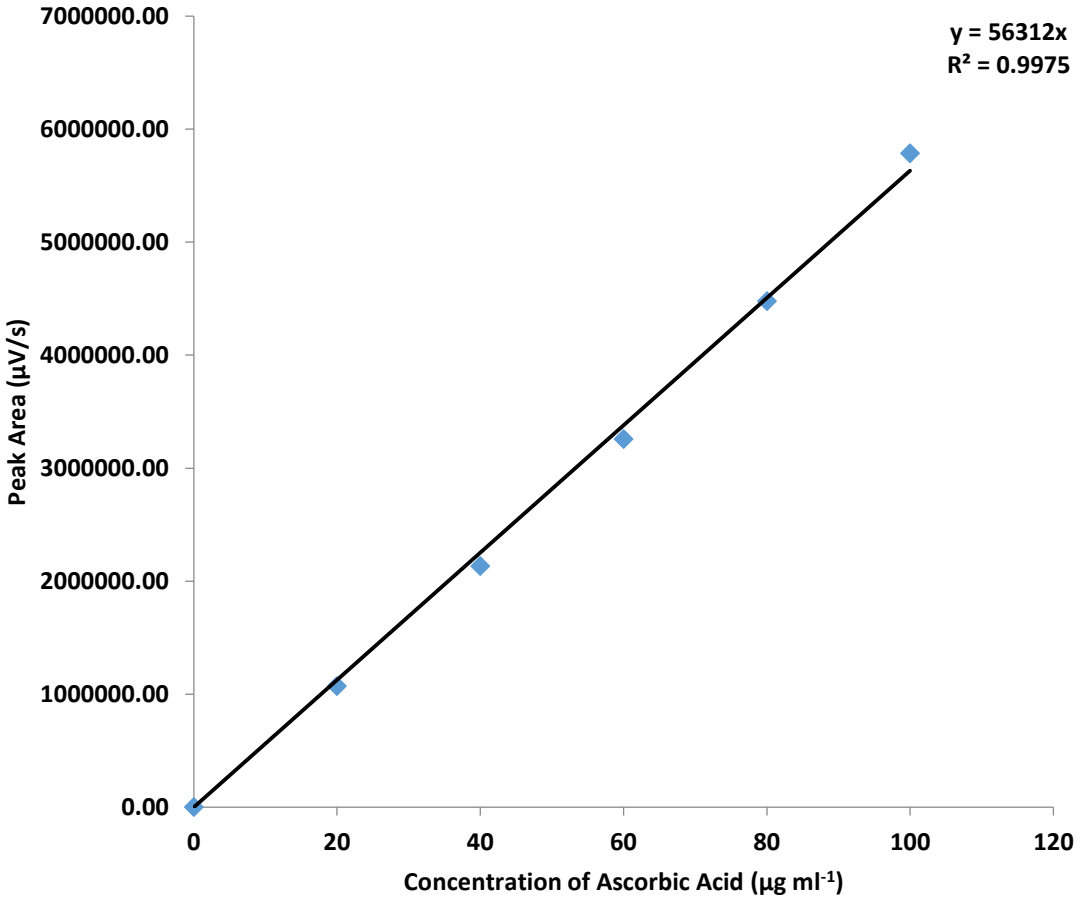
Appendix A.2 Standard curve for H_2O_2 (0 – 20 mM) at A410nm



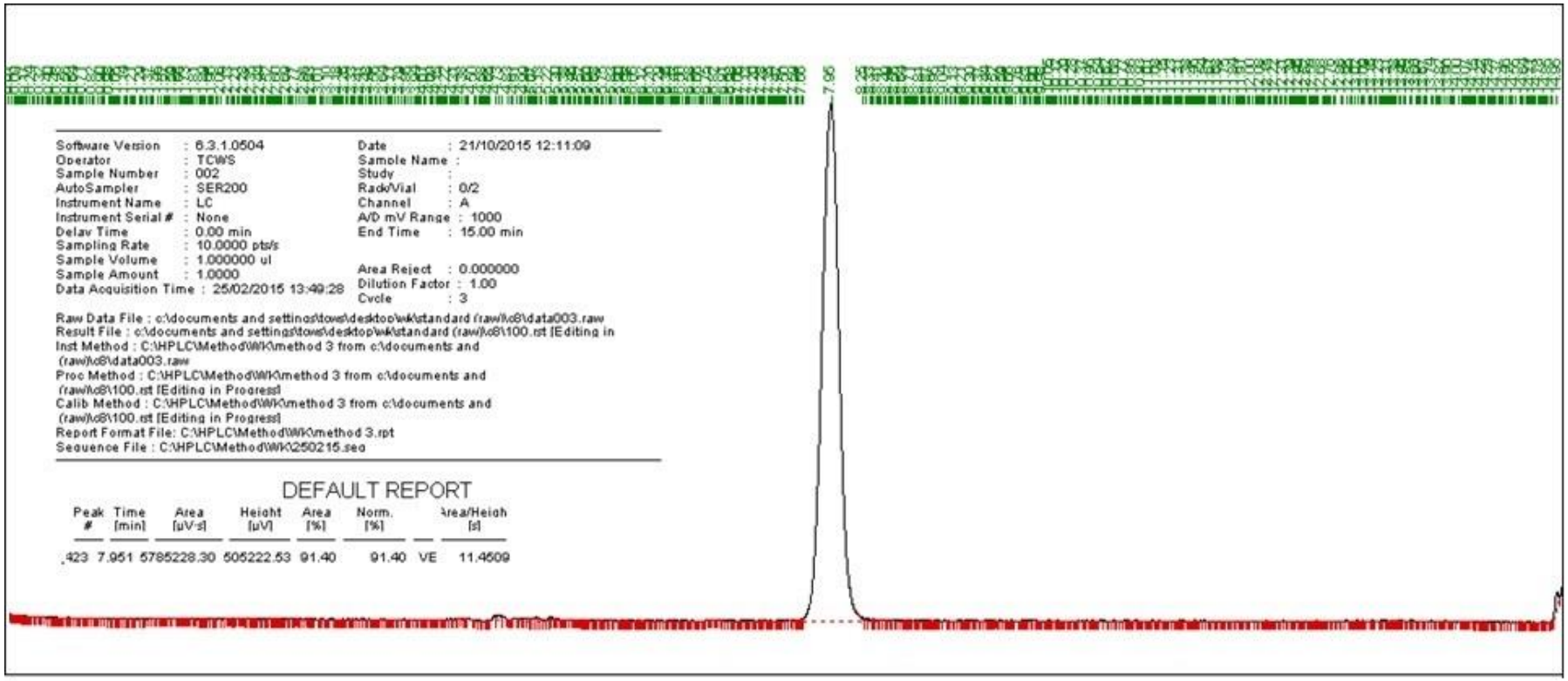
Appendix A.3 Standard curve for Folin-Ciocalteu assay using gallic acid (0 – 1000 µg ml^{-1}) as standard at the absorbance reading of 765 nm



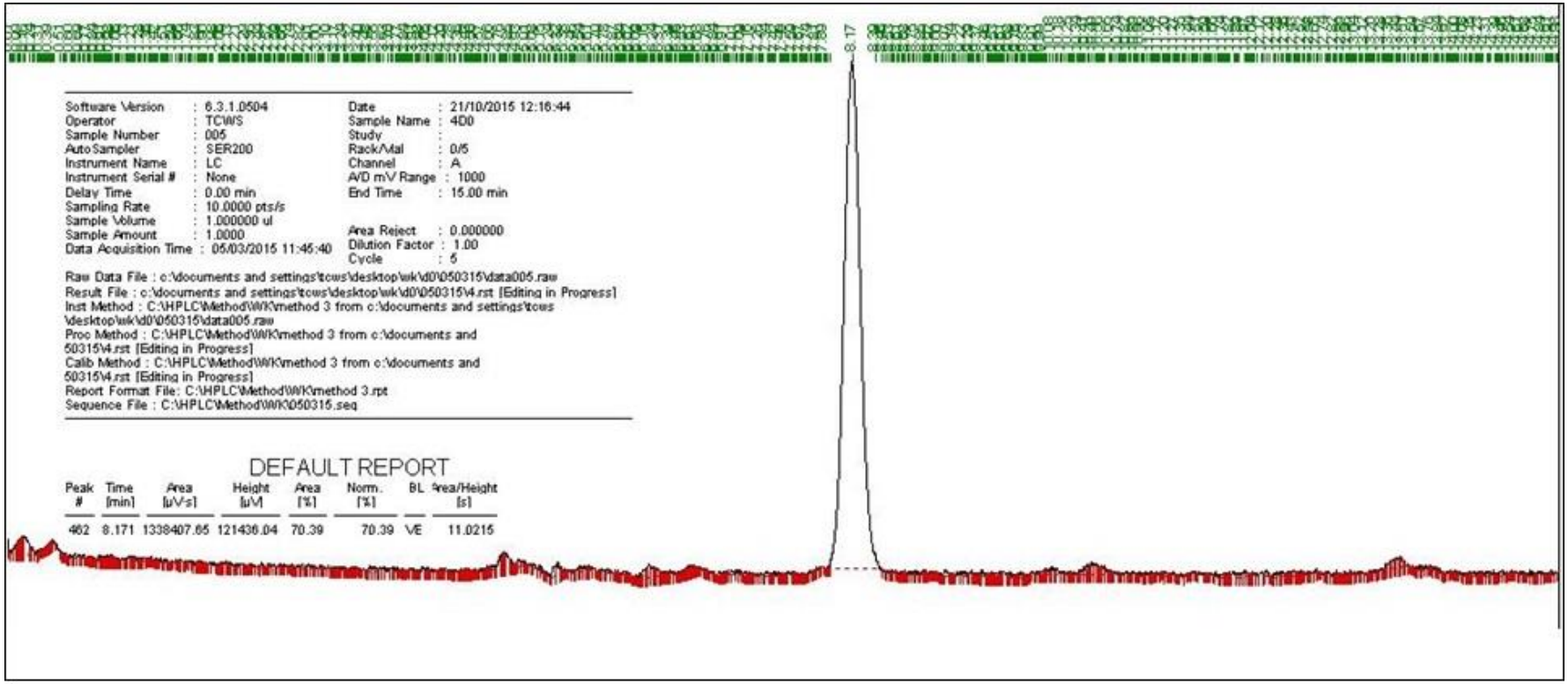
Appendix A.4 Standard curve for ascorbic acid (0 – 100 µg ml^{-1}) at the absorbance reading of 242 nm



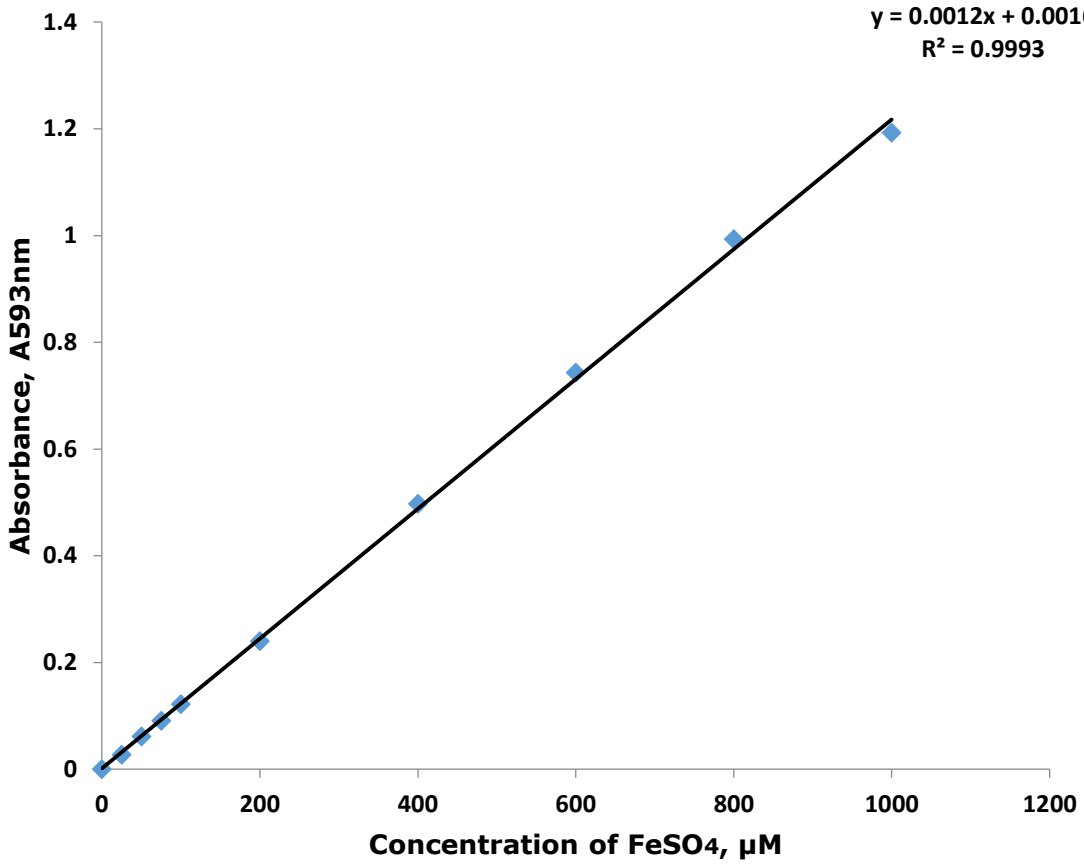
Appendix A.5 Chromatogram report for L-ascorbic acid as standard



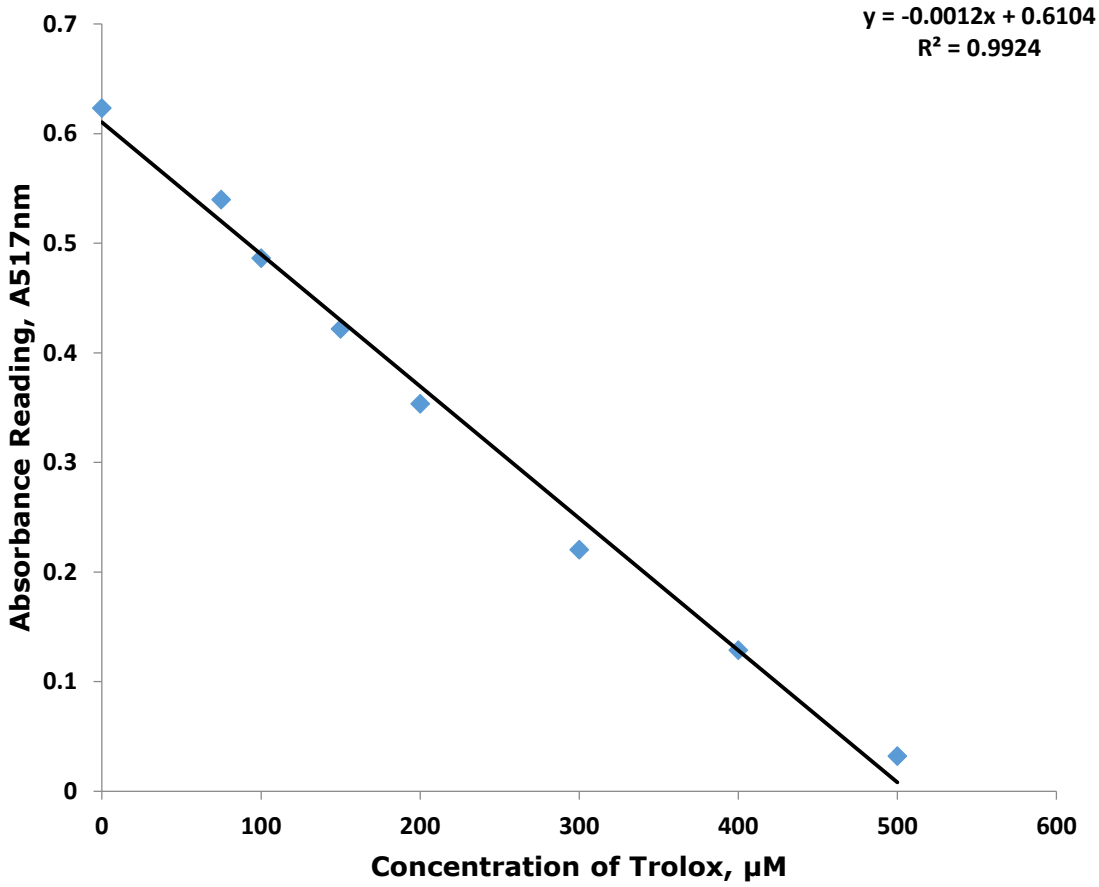
Appendix A.6 Chromatogram report of ascorbic acid analysis for sample



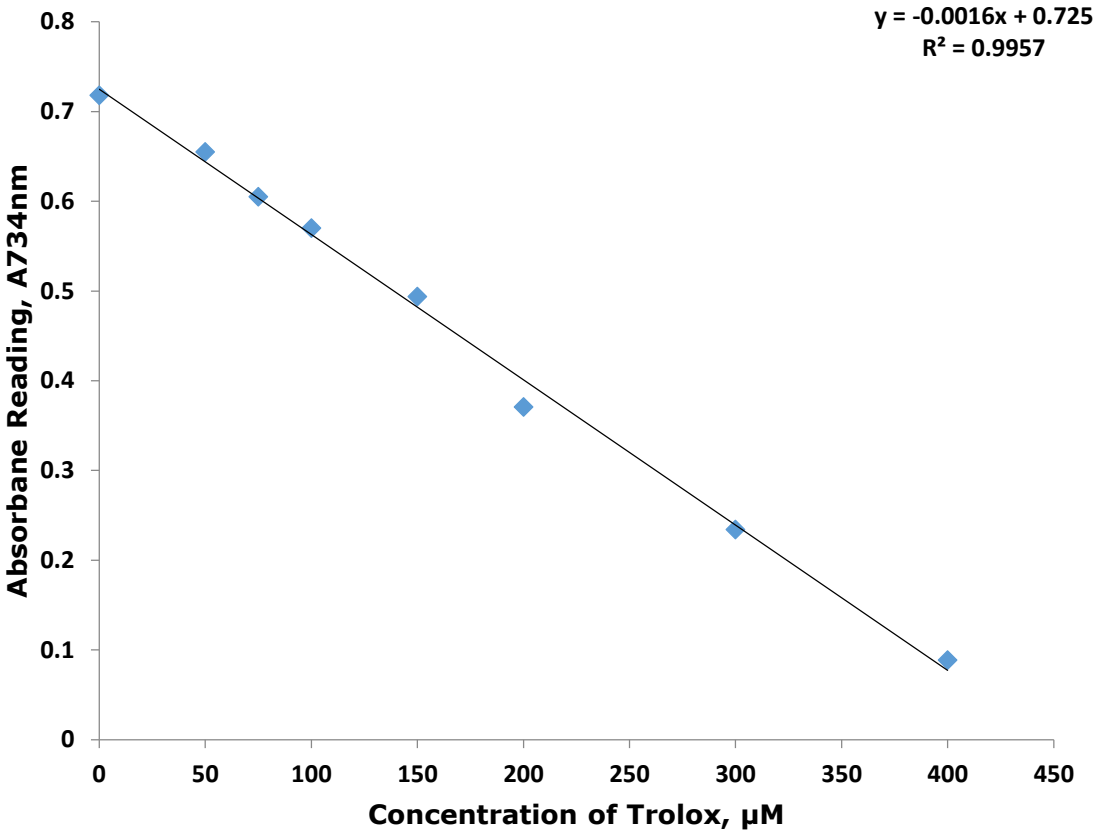
Appendix A.7 Standard curve for FRAP assay using $FeSO_4$ (0 – 1000 µM) as standard at the absorbance reading of 593 nm



Appendix A.8 Standard curve for DPPH assay using trolox (0 – 500 µM) as standard at the absorbance reading of 517 nm



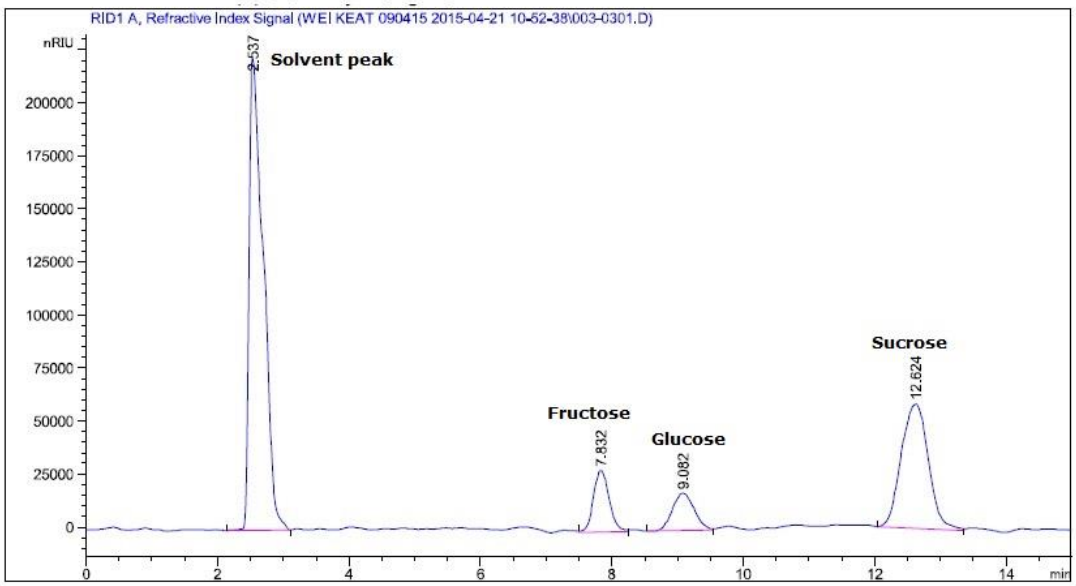
Appendix A.9 Standard curve for ABTS assay using trolox (0 – 400 μM) as standard at the absorbance reading of 734 nm



Appendix A.10 Chromatogram report of sugar analysis for sample

Sample Name: 3

```
======================================================================
Acq. Operator   : GHJ                          Seq. Line :   3
Acq. Instrument : Instrument 1                  Location : Vial 3
Injection Date  : 21/4/2015 12:34:52 PM            Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-21 10-52-38\WEI KEAT (RID).M
Last changed    : 21/4/2015 10:52:38 AM by GHJ
Analysis Method : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-21 10-52-38\003-0301.D\DA.M (WEI
                  KEAT (RID).M, From Data File)
Last changed    : 21/4/2015 10:52:38 AM by GHJ
Additional Info : Peak(s) manually integrated
```



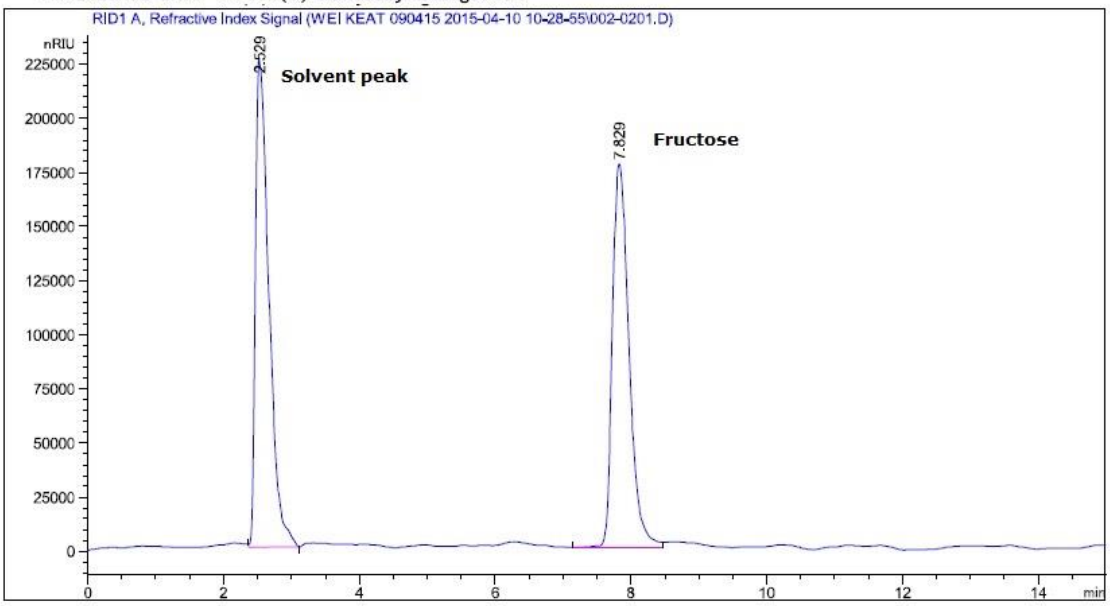
Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	2.537	BB	0.2120	3.34333e6	2.22569e5	56.3639
2	7.832	VV	0.2647	4.86159e5	2.89952e4	8.1960
3	9.082	BV	0.3661	4.11086e5	1.75507e4	6.9304
4	12.624	BV	0.4681	1.69110e6	5.86580e4	28.5097

Appendix A.11 Chromatogram report of fructose (standard)

Sample Name: Fructose

```
==================================================================
Acq. Operator   : GHJ                          Seq. Line :   2
Acq. Instrument : Instrument 1                  Location : Vial 2
Injection Date  : 10/4/2015 10:47:17 AM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-10 10-28-55\WEI KEAT (RID).M
Last changed    : 10/4/2015 10:28:37 AM by GHJ
Analysis Method : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-10 10-28-55\002-0201.D\DA.M (WEI
                  KEAT (RID).M, From Data File)
Last changed    : 10/4/2015 10:28:37 AM by GHJ
Additional Info : Peak(s) manually integrated
```



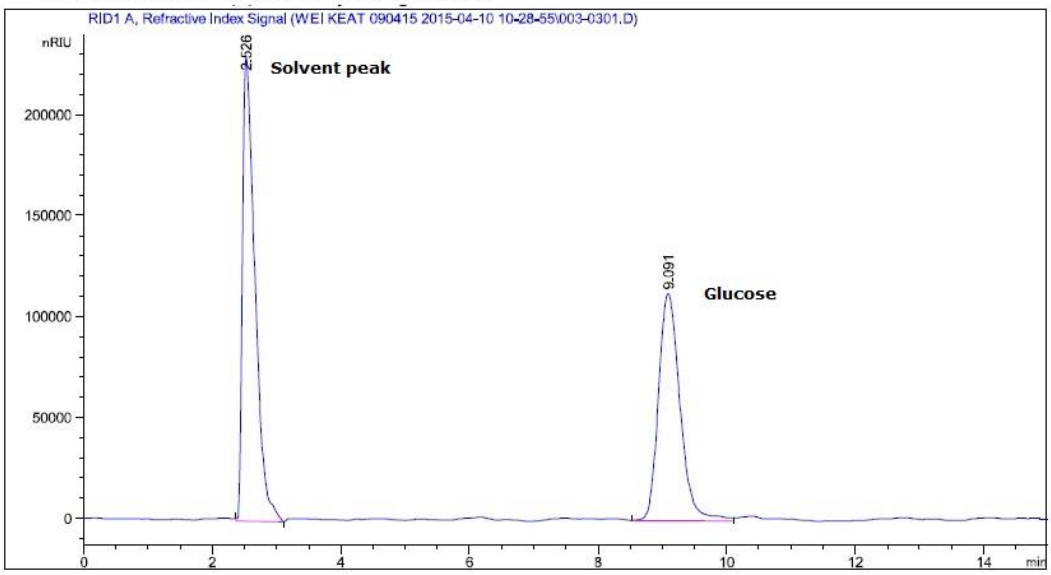
Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	2.530	VB	0.2169	3.15930e6	2.26228e5	57.1974
2	12.628	VB	0.4809	2.36421e6	7.99981e4	42.8026

Appendix A.12 chromatogram report of glucose (standard)

Sample Name: Glucose

```
======================================================================
Acq. Operator   : GHJ                          Seq. Line :   3
Acq. Instrument : Instrument 1                  Location : Vial 3
Injection Date  : 10/4/2015 11:04:05 AM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-10 10-28-55\WEI KEAT (RID).M
Last changed    : 10/4/2015 10:28:37 AM by GHJ
Analysis Method : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-10 10-28-55\003-0301.D\DA.M (WEI
                  KEAT (RID).M, From Data File)
Last changed    : 10/4/2015 10:28:37 AM by GHJ
Additional Info : Peak(s) manually integrated
```



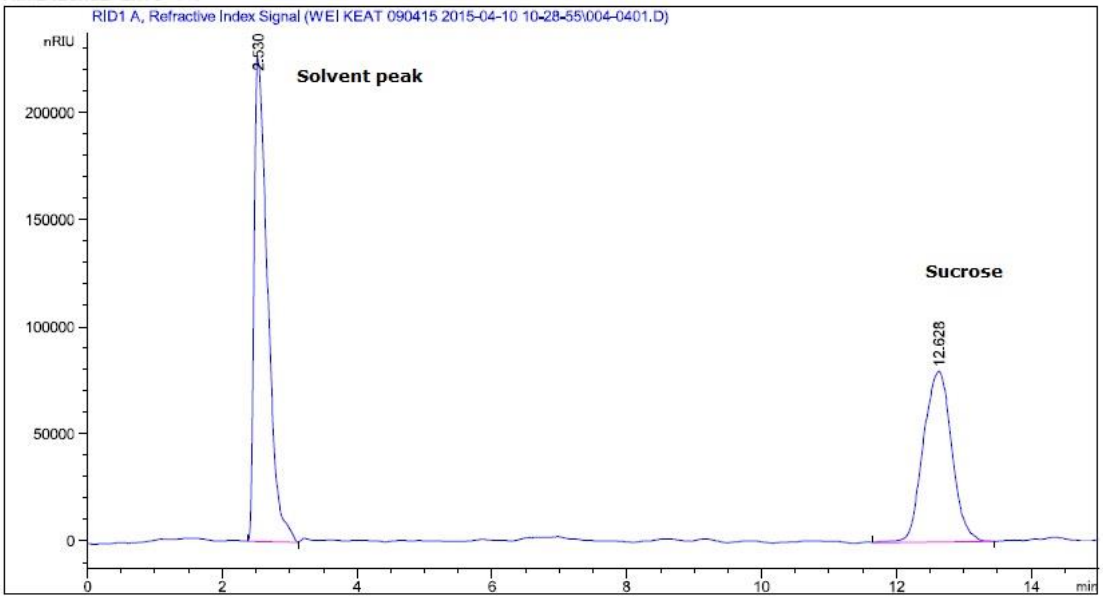
Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	2.526	VB	0.1961	3.09221e6	2.29521e5	54.0545
2	9.091	BV	0.3631	2.62833e6	1.12562e5	45.9455

Appendix A.13 Chromatogram report of sucrose (standard)

Sample Name: Sucrose

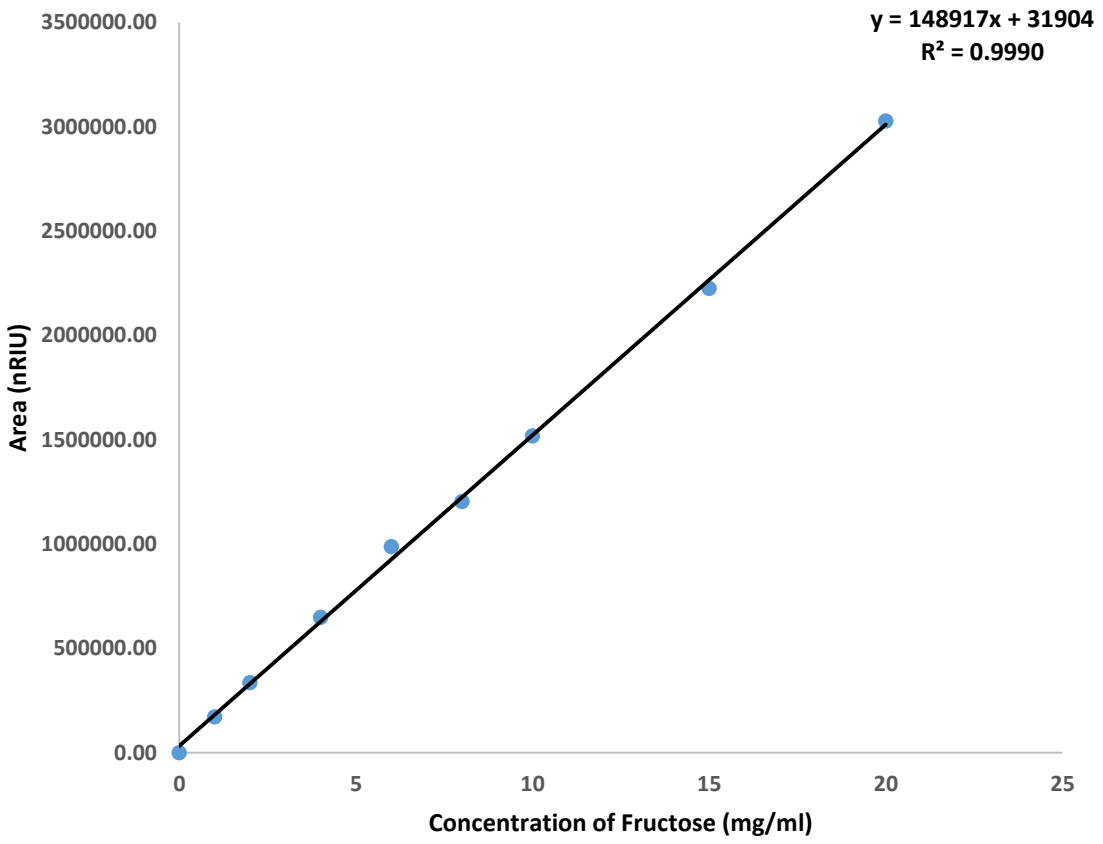
```
==================================================================
Acq. Operator   : GHJ                          Seq. Line :   4
Acq. Instrument : Instrument 1                  Location : Vial 4
Injection Date  : 10/4/2015 11:20:48 AM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-10 10-28-55\WEI KEAT (RID).M
Last changed    : 10/4/2015 10:28:37 AM by GHJ
Analysis Method : C:\CHEM32\1\DATA\WEI KEAT 090415 2015-04-10 10-28-55\004-0401.D\DA.M (WEI
                  KEAT (RID).M, From Data File)
Last changed    : 10/4/2015 10:28:37 AM by GHJ
Additional Info : Peak(s) manually integrated
```



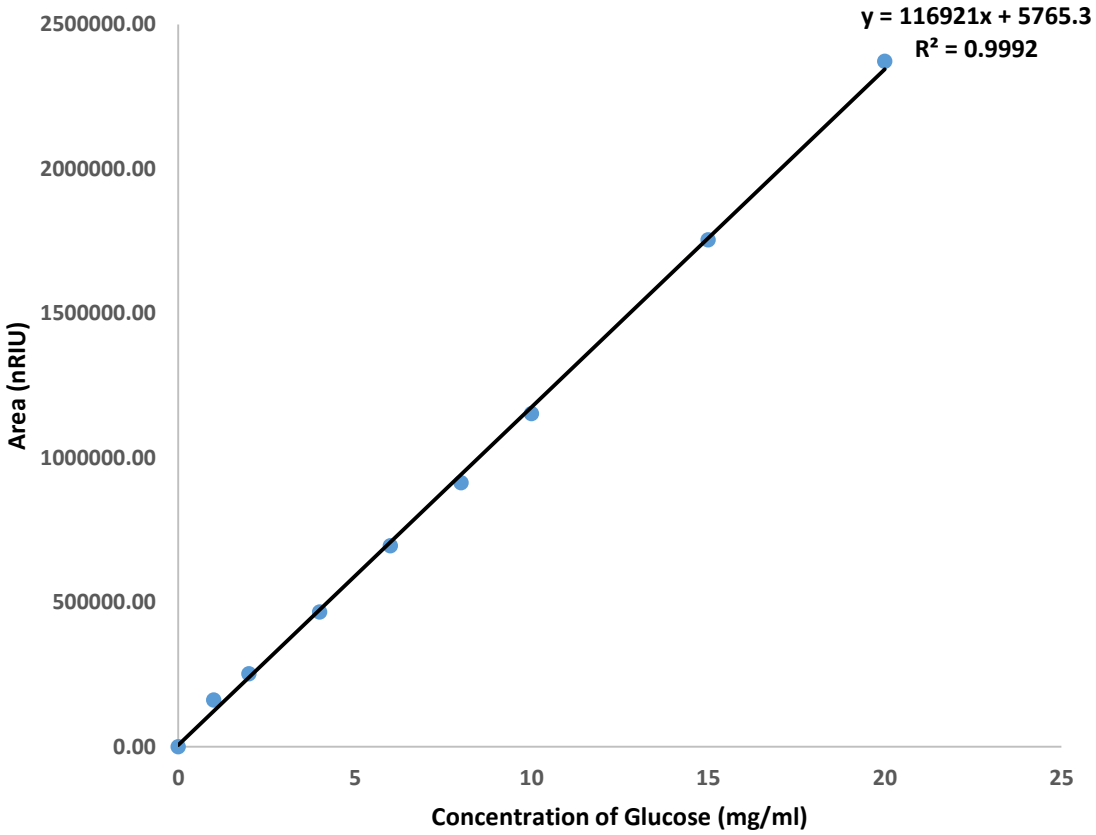
Signal 1: RID1 A, Refractive Index Signal

Peak #	RetTime [min]	Type	Width [min]	Area [nRIU*s]	Height [nRIU]	Area %
1	2.530	VB	0.2169	3.15930e6	2.26228e5	57.1974
2	12.628	VB	0.4809	2.36421e6	7.99981e4	42.8026

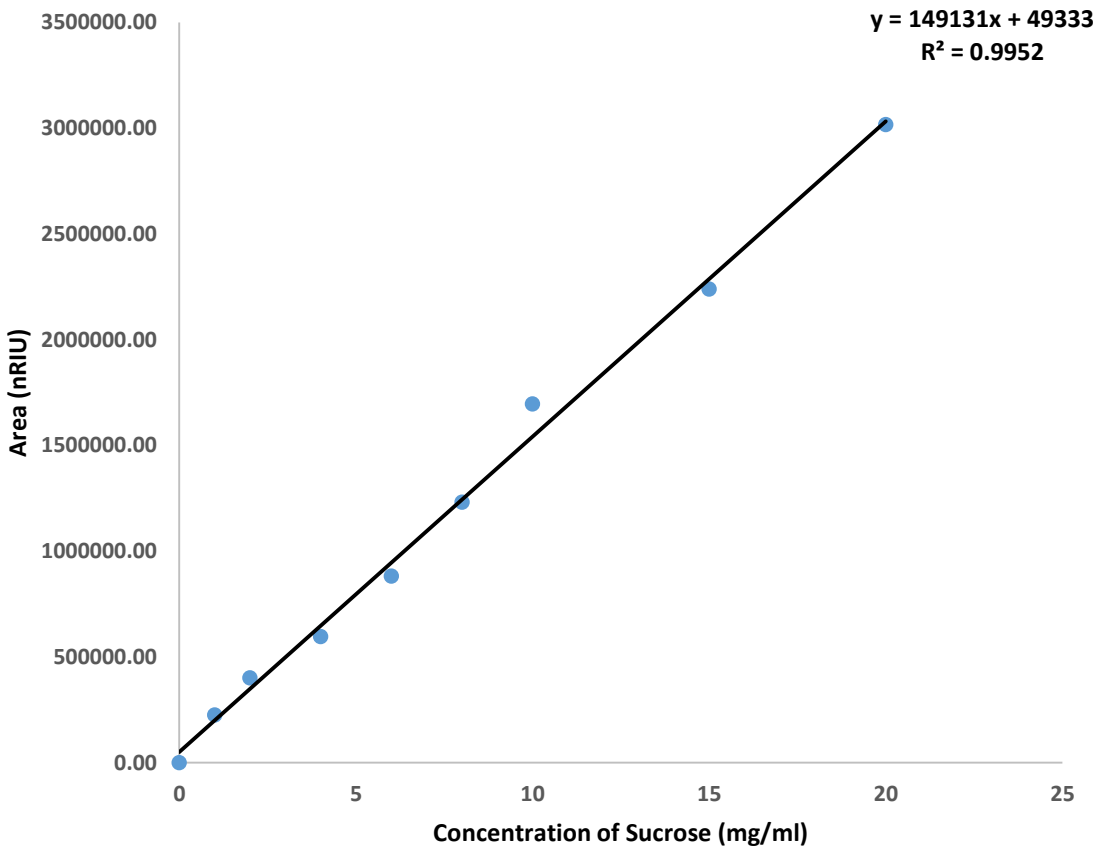
Appendix A.14 Standard curve of fructose (0 – 20 mg ml^{-1})



Appendix A.15 Standard curve of glucose (0 – 20 mg ml^{-1})



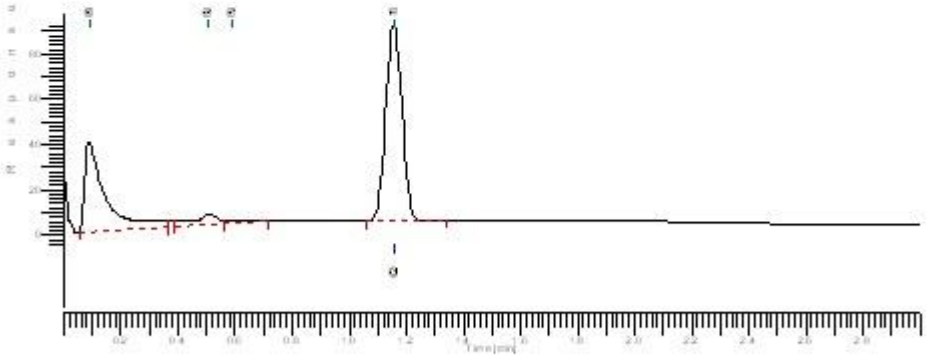
Appendix A.16 Standard curve of sucrose (0 – 20 mg ml^{-1})



Appendix A.17 Chromatogram report of 1% CO_2 (standard)

Software Version	: 6.3.1.0504	Date	: 10/21/2015 11:46:29 AM
Operator	: TCWS	Sample Name	:
Sample Number	: 001	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: Clarus 500	Channel	: B
Instrument Serial #	: 650N5072604	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 3.00 min
Sampling Rate	: 3.1250 pts/s		
Sample Volume	: 1.000000 ul	Area Reject	: 10000.000000
Sample Amount	: 1.0000	Dilution Factor	: 1.00
Data Acquisition Time	: 6/27/2015 10:00:58 AM	Cycle	: 1

Raw Data File : C:\Documents and Settings\TCWS\Desktop\WK\270615\CO2\datb001.raw
Result File : c:\documents and settings\tcws\desktop\wk\270615\co2\co2 std 1%.rst [Editing in Progress]
Inst Method : C:\GC\Method\CO2 from C:\Documents and Settings\TCWS\Desktop\WK\270615\CO2\datb001.raw
Proc Method : C:\GC\Method\CO2 from c:\documents and settings\tcws\desktop\wk\270615\co2\co2 std [Editing in Progress]
Calib Method : C:\GC\Method\CO2 from c:\documents and settings\tcws\desktop\wk\270615\co2\co2 std 1%.rst [Editing in Progress]
Report Format File: C:\GC\Method\CO2.rpt
Sequence File : C:\GC\Method\CO2.seq



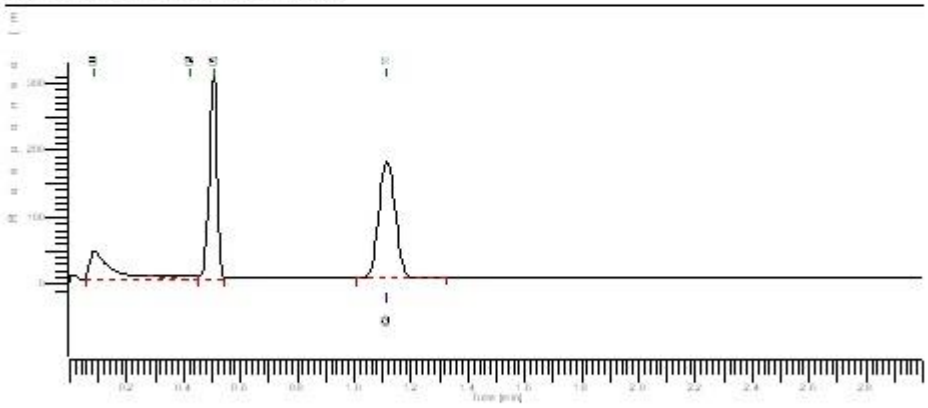
GC Analysis Report

Peak #	Time [min]	Component Name	Area [uV*sec]	Height [uV]	Area [%]
1	0.091		202402.89	39168.26	35.12
2	0.503		28638.97	4426.05	4.97
4	1.156	CO2	345278.89	87266.36	59.91

Appendix A.18 Chromatogram report of CO_2 analysis for sample

Software Version	: 6.3.1.0504	Date	: 10/21/2015 11:51:31 AM
Operator	: TCWS	Sample Name	:
Sample Number	: 006	Study	:
AutoSampler	: NONE	Rack/Vial	: 0/0
Instrument Name	: Clarus 500	Channel	: B
Instrument Serial #	: None	A/D mV Range	: 1000
Delay Time	: 0.00 min	End Time	: 3.00 min
Sampling Rate	: 3.1250 pts/s		
Sample Volume	: 1.000000 ul		
Sample Amount	: 1.0000	Area Reject	: 10000.000000
Data Acquisition Time	: 7/12/2015 12:03:17 PM	Dilution Factor	: 1.00
		Cycle	: 6

Raw Data File : C:\Documents and Settings\TCWS\Desktop\WK\120715\datb006.raw
Result File : c:\documents and settings\tcws\desktop\wk\120715\datb006.rst [Editing in Progress]
Inst Method : C:\GC\Method\CO2 from C:\Documents and Settings\TCWS\Desktop\WK\120715\datb006.raw
Proc Method : C:\GC\Method\CO2 from c:\documents and settings\tcws\desktop\wk\120715\datb006.rst
[Editing in Progress]
Calib Method : C:\GC\Method\CO2 from c:\documents and settings\tcws\desktop\wk\120715\datb006.rst
[Editing in Progress]
Report Format File: C:\GC\Method\CO2.rpt
Sequence File : C:\GC\Method\CO2.seq



GC Analysis Report

Peak #	Time [min]	Component Name	Area [uV*sec]	Height [uV]	Area [%]
1	0.085		222939.11	41973.93	14.29
2	0.421		33829.17	5906.36	2.17
3	0.506		593142.28	308246.60	38.01
4	1.114	CO2	710530.51	174515.97	45.53

Appendix A.19 Scoresheet of 9-point hedonic scale liking test

Instructions:

Name: Product:

Panelist No: Date:

1. You are presented with three digits coded samples of fresh-cut pineapple. Evaluate its appearance, colour, odour, taste, texture and overall acceptability based on the scale given below, which best describe your feelings.

Score/Rating	Standard Hedonic Scale
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

2. Please rinse your mouth with water and plain crackers before testing the next samples

	Coded Samples			
	233	**544**	**388**	**799**
Appearance				
Colour				
Odour				
Taste				
Texture				
Overall acceptability				

APPENDIX B

Appendix B.1 Analysis of variance for different ultrasound power input and treatment time on total microbial count of fresh-cut pineapple stored at 7 °C for 7 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	3	32.87	10.96	85.07	<.001
Treatment_Time (T)	2	1.14	0.57	4.44	0.014
Storage_Day (S)	4	172.09	43.02	334.06	<.001
P x T	6	2.04	0.34	2.64	0.019
P x S	12	9.63	0.80	6.23	<.001
T x S	8	1.85	0.23	1.79	0.085
P x T x S	24	7.74	0.32	2.5	<.001
Residual	120	15.45	0.13		
Total	179	242.81			

Appendix B.2 Analysis of variance for different ultrasound power input and treatment time on lactic acid bacteria count of fresh-cut pineapple stored at 7 °C for 7 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	3	40.98	13.66	116.58	<.001
Treatment_Time (T)	2	2.54	1.27	10.86	<.001
Storage_Day (S)	4	202.69	50.67	432.43	<.001
P x T	6	3.76	0.63	5.35	<.001
P x S	12	7.48	0.62	5.32	<.001
T x S	8	1.44	0.18	1.54	0.150
P x T x S	24	3.29	0.14	1.17	0.284
Residual	120	14.06	0.12		
Total	179	276.25			

Appendix B.3 Analysis of variance for different ultrasound power input and treatment time on yeast and mould count of fresh-cut pineapple stored at 7 °C for 7 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	3	21.93	7.31	87.46	<.001
Treatment_Time (T)	2	0.43	0.21	2.56	0.082
Storage_Day (S)	4	346.72	86.68	1036.96	<.001
P x T	6	1.64	0.27	3.28	0.005
P x S	12	13.42	1.12	13.38	<.001
T x S	8	0.97	0.12	1.46	0.180
P x T x S	24	2.49	0.10	1.24	0.221
Residual	120	10.03	0.08		
Total	179	397.63			

Appendix B.4 Analysis of variance for different ultrasound power input and treatment time on O_2^- production of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	187.84	93.92	33.79	<.001
Treatment_Time (T)	1	1.00	1.00	0.36	0.552
Storage_Day (S)	3	84.24	28.08	10.1	<.001
P x T	2	4.80	2.40	0.86	0.428
P x S	6	75.82	12.64	4.55	0.001
T x S	3	2.98	0.99	0.36	0.784
P x T x S	6	2.64	0.44	0.16	0.986
Residual	48	133.43	2.78		
Total	71	492.74			

Appendix B.5 Analysis of variance for different ultrasound power input and treatment time on H_2O_2 content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	1882.61	941.3	28.93	<.001
Treatment_Time (T)	1	12.28	12.28	0.38	0.542
Storage_Day (S)	3	3092.45	1030.82	31.69	<.001
P x T	2	147.94	73.97	2.27	0.114
P x S	6	677.94	112.99	3.47	0.006
T x S	3	434.11	144.7	4.45	0.008
P x T x S	6	230.06	38.34	1.18	0.334
Residual	48	1561.55	32.53		
Total	71	8038.93			

Appendix B.6 Regression analysis between H_2O_2 content and total microbial count of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	23.16	23.16	48.89	<.001
Residual	70	33.16	0.47		
Total	71	56.32	0.79		

Appendix B.7 Regression analysis between H_2O_2 content and lactic acid bacteria count of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	27.30	13.65	22.71	<.001
Residual	70	41.47	0.60		
Total	71	68.77	0.97		

Appendix B.8 Regression analysis between H_2O_2 content and yeast and mould count of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	24.5	24.50	38.46	<.001
Residual	70	44.59	0.64		
Total	71	69.09	0.97		

Appendix B.9 Analysis of variance for different ultrasound power input and treatment time on MDA content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	20.28	10.14	0.58	0.565
Treatment_Time (T)	1	20.35	20.35	1.16	0.287
Storage_Day (S)	3	2307.69	769.23	43.85	<.001
P x T	2	24.1	12.05	0.69	0.508
P x S	6	392.08	65.35	3.72	0.004
T x S	3	8.29	2.76	0.16	0.924
P x T x S	6	82.26	13.71	0.78	0.589
Residual	48	842.07	17.54		
Total	71	3697.11			

Appendix B.10 Analysis of variance for different ultrasound power input and treatment time on PAL activity of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	8.92	4.46	60.2	<.001
Treatment_Time (T)	1	3.04	3.04	41.08	<.001
Storage_Day (S)	3	53.82	17.94	242.2	<.001
P x T	2	0.52	0.26	3.5	0.038
P x S	6	17.65	2.94	39.71	<.001
T x S	3	2.35	0.78	10.59	<.001
P x T x S	6	2.39	0.40	5.38	<.001
Residual	48	3.56	0.07		
Total	71	92.25			

Appendix B.11 Analysis of variance for different ultrasound power input and treatment time on PPO activity of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	57.15	28.57	593.37	<.001
Treatment_Time (T)	1	2.94	2.94	61	<.001
Storage_Day (S)	3	26.54	8.85	183.73	<.001
P x T	2	2.43	1.22	25.24	<.001
P x S	6	4.48	0.75	15.51	<.001
T x S	3	1.04	0.35	7.22	<.001
P x T x S	6	9.25	1.54	32	<.001
Residual	48	2.31	0.05		
Total	71	106.14			

Appendix B.12 Analysis of variance for different ultrasound power input and treatment time on POD activity of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	7.56	3.78	43.08	<.001
Treatment_Time (T)	1	1.32	1.32	15.09	<.001
Storage_Day (S)	3	53.17	17.72	202.12	<.001
P x T	2	0.25	0.13	1.45	0.244
P x S	6	23.70	3.95	45.04	<.001
T x S	3	2.76	0.92	10.48	<.001
P x T x S	6	2.77	0.46	5.26	<.001
Residual	48	4.21	0.09		
Total	71	95.73			

Appendix B.13 Analysis of variance for different ultrasound power input and treatment time on APX activity of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	13472.91	6736.45	709.21	<.001
Treatment_Time (T)	1	141.85	141.85	14.93	<.001
Storage_Day (S)	3	40295.79	13431.93	1414.11	<.001
P x T	2	391.03	195.51	20.58	<.001
P x S	6	3042.00	507.00	53.38	<.001
T x S	3	573.92	191.31	20.14	<.001
P x T x S	6	1253.68	208.95	22	<.001
Residual	48	455.93	9.50		
Total	71	59627.10			

Appendix B.14 Regression analysis between APX and H_2O_2 content of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	34999.00	34999.50	99.48	<.001
Residual	70	24628.00	351.80		
Total	71	59627.00	839.80		

Appendix B.15 Analysis of variance for different ultrasound power input and treatment time on MDHAR activity of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	15671.60	7835.80	4085.51	<.001
Treatment_Time (T)	1	975.56	975.56	508.65	<.001
Storage_Day (S)	3	14486.85	4828.95	2517.77	<.001
P x T	2	2720.49	1360.24	709.22	<.001
P x S	6	6775.58	1129.26	588.79	<.001
T x S	3	24.54	8.18	4.26	0.010
P x T x S	6	420.04	70.01	36.5	<.001
Residual	48	92.06	1.92		
Total	71	41166.71			

Appendix B.16 Regression analysis between MDHAR and H_2O_2 content of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	18851.00	18850.60	59.13	<.001
Residual	70	22316.00	318.80		
Total	71	41167.00	579.80		

Appendix B.17 Analysis of variance for different ultrasound power input and treatment time on DHAR activity of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	642.96	321.48	173.04	<.001
Treatment_Time (T)	1	704.16	704.16	379.02	<.001
Storage_Day (S)	3	44922.45	14974.15	8060.01	<.001
P x T	2	81.99	40.99	22.06	<.001
P x S	6	756.09	126.02	67.83	<.001
T x S	3	262.09	87.36	47.02	<.001
P x T x S	6	152.13	25.35	13.65	<.001
Residual	48	89.18	1.86		
Total	71	47611.04			

Appendix B.18 Regression analysis between DHAR and H_2O_2 content of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	18463.00	18462.90	44.34	<.001
Residual	70	29148.00	416.40		
Total	71	47611.00	670.60		

Appendix B.19 Regression analysis between PAL and H_2O_2 content of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	21.95	21.95	21.86	<.001
Residual	70	70.3	1.00		
Total	71	92.25	1.30		

Appendix B.20 Analysis of variance for different ultrasound power input and treatment time on total phenolic content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	564.41	282.21	8.07	<.001
Treatment_Time (T)	1	60.69	60.69	1.73	0.194
Storage_Day (S)	3	6710	2236.67	63.94	<.001
P x T	2	13.14	6.57	0.19	0.829
P x S	6	1834.84	305.81	8.74	<.001
T x S	3	118.21	39.4	1.13	0.348
P x T x S	6	220.37	36.73	1.05	0.405
Residual	48	1679.09	34.98		
Total	71	11200.76			

Appendix B.21 Regression analysis between PAL and total phenolic content of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	6348.00	6347.7	91.56	<.001
Residual	70	4853.00	69.33		
Total	71	11201.00	157.76		

Appendix B.22 Analysis of variance for different ultrasound power input and treatment time on total ascorbate content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	206.44	103.22	832.31	<.001
Treatment_Time (T)	1	6.01	6.01	48.43	<.001
Storage_Day (S)	3	137.34	45.78	369.15	<.001
P x T	2	2.66	1.33	10.72	<.001
P x S	6	79.38	13.23	106.69	<.001
T x S	3	31.36	10.45	84.3	<.001
P x T x S	6	6.06	1.01	8.14	<.001
Residual	48	5.95	0.12		
Total	71	475.20			

Appendix B.23 Analysis of variance for different ultrasound power input and treatment time on AA content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	164.26	82.13	927	<.001
Treatment_Time (T)	1	4.67	4.67	52.75	<.001
Storage_Day (S)	3	181.90	60.63	684.36	<.001
P x T	2	8.28	4.14	46.75	<.001
P x S	6	57.18	9.53	107.57	<.001
T x S	3	8.98	2.99	33.78	<.001
P x T x S	6	53.07	8.85	99.84	<.001
Residual	48	4.25	0.09		
Total	71	482.60			

Appendix B.24 Analysis of variance for different ultrasound power input and treatment time on DHA content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	16.45	8.23	44.68	<.001
Treatment_Time (T)	1	0.08	0.08	0.45	0.504
Storage_Day (S)	3	16.59	5.53	30.03	<.001
P x T	2	4.76	2.38	12.93	<.001
P x S	6	21.52	3.59	19.48	<.001
T x S	3	45.10	15.03	81.66	<.001
P x T x S	6	66.57	11.10	60.27	<.001
Residual	48	8.84	0.18		
Total	71	179.91			

Appendix B.25 Regression analysis between total ascorbate content and H_2O_2 content of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	117.90	117.93	23.11	<.001
Residual	70	357.30	5.10		
Total	71	475.20	6.69		

Appendix B.26 Regression analysis between total ascorbate content and O_2- production of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	257.60	257.57	82.84	<.001
Residual	70	217.60	3.11		
Total	71	475.20	6.69		

Appendix B.27 Analysis of variance for different ultrasound power input and treatment time on total antioxidant capacity of fresh-cut pineapple stored at 7 °C for 5 days as measured by FRAP assay.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	82.90	41.45	16.35	<.001
Treatment_Time (T)	1	21.20	21.20	8.36	0.006
Storage_Day (S)	3	1515.98	505.33	199.34	<.001
P x T	2	2.17	1.09	0.43	0.654
P x S	6	168.33	28.05	11.07	<.001
T x S	3	8.13	2.71	1.07	0.371
P x T x S	6	49.16	8.19	3.23	0.010
Residual	48	121.68	2.54		
Total	71	1969.55			

Appendix B.28 Analysis of variance for different ultrasound power input and treatment time on total antioxidant capacity of fresh-cut pineapple stored at 7 °C for 5 days as measured by DPPH assay.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	406.85	203.42	8.65	<.001
Treatment_Time (T)	1	282.67	282.67	12.02	0.001
Storage_Day (S)	3	8614.24	2871.41	122.14	<.001
P x T	2	111.42	55.71	2.37	0.104
P x S	6	1158.96	193.16	8.22	<.001
T x S	3	14.98	4.99	0.21	0.887
P x T x S	6	21.89	3.65	0.16	0.987
Residual	48	1128.42	23.51		
Total	71	11739.42			

Appendix B.29 Analysis of variance for different ultrasound power input and treatment time on total antioxidant capacity of fresh-cut pineapple stored at 7 °C for 5 days as measured by ABTS assay.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	241.47	120.74	16.94	<.001
Treatment_Time (T)	1	306.21	306.21	42.96	<.001
Storage_Day (S)	3	7904.16	2634.72	369.64	<.001
P x T	2	381.28	190.64	26.75	<.001
P x S	6	1120.25	186.71	26.19	<.001
T x S	3	114.43	38.15	5.35	0.003
P x T x S	6	209.47	34.91	4.9	<.001
Residual	48	342.13	7.13		
Total	71	10619.40			

Appendix B.30 Regression analysis between total phenolic content and total antioxidant capacity (FRAP assay) of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	1043.90	1043.88	78.94	<.001
Residual	70	925.70	13.22		
Total	71	1969.50	27.74		

Appendix B.31 Regression analysis between total phenolic content and total antioxidant capacity (DPPH assay) of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	5362.00	5362.10	58.86	<.001
Residual	70	6377.00	91.10		
Total	71	11739.00	165.34		

Appendix B.32 Regression analysis between total phenolic content and total antioxidant capacity (ABTS assay) of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	5764.00	5764.45	83.11	<.001
Residual	70	4855.00	69.36		
Total	71	10619.00	149.57		

Appendix B.33 Regression analysis between ascorbate content and total antioxidant capacity (FRAP assay) of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	2.00	2.21	0.08	0.78
Residual	70	1967.00	28.10		
Total	71	1970.00	27.74		

Appendix B.34 Regression analysis between ascorbate content and total antioxidant capacity (DPPH assay) of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	102.00	101.60	0.61	0.437
Residual	70	11638.00	166.30		
Total	71	11739.00	165.30		

Appendix B.35 Regression analysis between ascorbate content and total antioxidant capacity (ABTS assay) of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	1.00	0.70	0.00	0.946
Residual	70	10619.00	151.70		
Total	71	10619.00	149.60		

Appendix B.36 Analysis of variance for different ultrasound power input and treatment time on firmness of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	0.15	0.07	1.38	0.261
Treatment_Time (T)	1	0.17	0.17	3.24	0.078
Storage_Day (S)	3	1.24	0.41	7.71	<.001
P x T	2	0.04	0.02	0.35	0.707
P x S	6	0.38	0.06	1.19	0.328
T x S	3	0.10	0.03	0.64	0.595
P x T x S	6	0.06	0.01	0.19	0.979
Residual	48	2.57	0.05		
Total	71	4.72			

Appendix B.37 Analysis of variance for different ultrasound power input and treatment time on juice leakage of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	0.13	0.07	1.21	0.306
Treatment_Time (T)	1	0.01	0.01	0.11	0.744
Storage_Day (S)	3	14.07	4.69	85.39	<.001
P x T	2	1.12	0.56	10.22	<.001
P x S	6	0.28	0.05	0.84	0.543
T x S	3	0.07	0.02	0.43	0.730
P x T x S	6	0.42	0.07	1.28	0.283
Residual	48	2.64	0.05		
Total	71	18.74			

Appendix B.38 Regression analysis between firmness and juice leakage in fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	1.39	1.39	29.32	<.001
Residual	70	3.33	0.05		
Total	71	4.72	0.07		

Appendix B.39 Analysis of variance for different ultrasound power input and treatment time on L value of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	25.46	12.73	1.47	0.240
Treatment_Time (T)	1	1.45	1.45	0.17	0.684
Storage_Day (S)	3	58.41	19.47	2.25	0.095
P x T	2	26.97	13.49	1.56	0.221
P x S	6	9.39	1.57	0.18	0.981
T x S	3	3.43	1.14	0.13	0.941
P x T x S	6	5.43	0.91	0.1	0.996
Residual	48	415.72	8.66		
Total	71	546.26			

Appendix B.40 Analysis of variance for different ultrasound power input and treatment time on C* value of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	161.56	80.78	6.1	0.004
Treatment_Time (T)	1	56.16	56.16	4.24	0.045
Storage_Day (S)	3	648.39	216.13	16.33	<.001
P x T	2	24.04	12.02	0.91	0.410
P x S	6	50.64	8.44	0.64	0.699
T x S	3	1.15	0.38	0.03	0.993
P x T x S	6	33.41	5.57	0.42	0.862
Residual	48	635.19	13.23		
Total	71	1610.55			

Appendix B.41 Analysis of variance for different ultrasound power input and treatment time on hue angle of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	40.86	20.43	18.76	<.001
Treatment_Time (T)	1	0.57	0.57	0.52	0.475
Storage_Day (S)	3	9.90	3.30	3.03	0.038
P x T	2	1.00	0.50	0.46	0.633
P x S	6	7.05	1.17	1.08	0.389
T x S	3	0.63	0.21	0.19	0.900
P x T x S	6	0.36	0.06	0.06	0.999
Residual	48	52.27	1.09		
Total	71	112.63			

Appendix B.42 Analysis of variance for different ultrasound power input and treatment time on soluble solid concentration (SSC) of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	46.53	23.26	37.22	<.001
Treatment_Time (T)	1	0.68	0.68	1.09	0.302
Storage_Day (S)	3	12.81	4.27	6.83	<.001
P x T	2	0.11	0.06	0.09	0.915
P x S	6	10.69	1.78	2.85	0.019
T x S	3	0.40	0.13	0.21	0.886
P x T x S	6	0.72	0.12	0.19	0.977
Residual	48	30.00	0.63		
Total	71	101.94			

Appendix B.43 Analysis of variance for different ultrasound power input and treatment time on titratable acidity of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	0.03	0.02	7.72	0.001
Treatment_Time (T)	1	0.01	0.01	4.56	0.038
Storage_Day (S)	3	0.05	0.02	8.47	<.001
P x T	2	0.00	0.00	1.01	0.370
P x S	6	0.00	0.00	0.3	0.934
T x S	3	0.00	0.00	0.73	0.541
P x T x S	6	0.01	0.00	1.09	0.381
Residual	48	0.09	0.00		
Total	71	0.21			

Appendix B.44 Analysis of variance for different ultrasound power input and treatment time on pH of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Power (P)	2	0.06	0.03	18.58	<.001
Treatment_Time (T)	1	0.00	0.00	1.75	0.192
Storage_Day (S)	3	1.85	0.62	383.67	<.001
P x T	2	0.00	0.00	0.07	0.930
P x S	6	0.18	0.03	18.49	<.001
T x S	3	0.00	0.00	0.43	0.732
P x T x S	6	0.02	0.00	1.59	0.172
Residual	48	0.08	0.00		
Total	71	2.19			

Appendix B.45 Regression analysis between titratable acidity and pH of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	0.03	0.03	10.08	0.002
Residual	70	0.18	0.00		
Total	71	0.21	0.00		

Appendix B.46 Regression analysis between pH and lactic acid bacteria counts of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	19.58	19.58	27.86	<.001
Residual	70	49.19	0.70		
Total	71	68.77	0.97		

Appendix B.47 Regression analysis between pH and yeast and mould counts of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	22.00	22.00	32.71	<.001
Residual	70	47.09	0.67		
Total	71	69.09	0.97		

Appendix B.48 Analysis of variance for different ultrasound power input and treatment time on sucrose content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	875.69	437.84	47.58	<.001
Treatment_Time (T)	1	325.37	325.37	35.36	<.001
Storage_Day (S)	3	9427.94	3142.65	341.53	<.001
P x T	2	71.49	35.75	3.88	0.027
P x S	6	5087.31	847.89	92.14	<.001
T x S	3	307.92	102.64	11.15	<.001
P x T x S	6	92.36	15.39	1.67	0.148
Residual	48	441.68	9.20		
Total	71	16629.76			

Appendix B.49 Analysis of variance for different ultrasound power input and treatment time on fructose content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	171.36	85.68	24.17	<.001
Treatment_Time (T)	1	47.21	47.21	13.32	<.001
Storage_Day (S)	3	1759.42	586.47	165.44	<.001
P x T	2	339.15	169.58	47.84	<.001
P x S	6	772.56	128.76	36.32	<.001
T x S	3	23.94	7.98	2.25	0.094
P x T x S	6	173.26	28.88	8.15	<.001
Residual	48	170.16	3.55		
Total	71	3457.06			

Appendix B.50 Analysis of variance for different ultrasound power input and treatment time on glucose content of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	262.87	131.44	58.73	<.001
Treatment_Time (T)	1	57.00	57.00	25.47	<.001
Storage_Day (S)	3	3493.97	1164.66	520.36	<.001
P x T	2	127.40	63.70	28.46	<.001
P x S	6	613.32	102.22	45.67	<.001
T x S	3	29.67	9.89	4.42	0.008
P x T x S	6	74.90	12.48	5.58	<.001
Residual	48	107.43	2.24		
Total	71	4766.56			

Appendix B.51 Regression analysis between fructose and SSC of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	332	332.3	7.44	0.008
Residual	70	3125	44.64		
Total	71	3457	48.69		

Appendix B.52 Regression analysis between glucose and SSC of fresh-cut pineapple stored at 7 °C for 5 days.

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	1	842	842.14	15.02	<.001
Residual	70	3924	56.06		
Total	71	4767	67.13		

Appendix B.53 Analysis of variance for different ultrasound power input and treatment time on respiration rate of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	2	54.03	27.02	34.08	<.001
Treatment_Time (T)	1	61.35	61.35	77.38	<.001
Storage_Day (S)	3	1071.42	357.14	450.45	<.001
P x T	2	23.90	11.95	15.07	<.001
P x S	6	266.22	44.37	55.96	<.001
T x S	3	40.26	13.42	16.93	<.001
P x T x S	6	86.95	14.49	18.28	<.001
Residual	48	38.06	0.79		
Total	71	1642.19			

Appendix B.54 Analysis of variance for different ultrasound power input and treatment time on sensory evaluation (appearance) of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	1	0.80	0.80	0.35	0.554
Treatment_Time (T)	1	0.80	0.80	0.35	0.554
P x T	1	0.05	0.05	0.02	0.882
Residual	76	171.90	2.26		
Total	79	173.55			

Appendix B.55 Analysis of variance for different ultrasound power input and treatment time on sensory evaluation (colour) of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	1	7.20	7.20	3.33	0.072
Treatment_Time (T)	1	1.25	1.25	0.58	0.450
P x T	1	1.80	1.80	0.83	0.365
Residual	76	164.50	2.16		
Total	79	174.75			

Appendix B.56 Analysis of variance for different ultrasound power input and treatment time on sensory evaluation (odour) of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	1	3.61	3.61	1.70	0.196
Treatment_Time (T)	1	2.11	2.11	1.00	0.322
P x T	1	1.51	1.51	0.71	0.401
Residual	76	161.25	2.12		
Total	79	168.49			

Appendix B.57 Analysis of variance for different ultrasound power input and treatment time on sensory evaluation (taste) of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	1	1.01	1.01	0.45	0.502
Treatment_Time (T)	1	0.11	0.11	0.05	0.823
P x T	1	0.11	0.11	0.05	0.823
Residual	76	169.25	2.23		
Total	79	170.49			

Appendix B.58 Analysis of variance for different ultrasound power input and treatment time on sensory evaluation (texture) of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	1	4.51	4.51	2.26	0.137
Treatment_Time (T)	1	1.51	1.51	0.76	0.387
P x T	1	0.01	0.01	0.01	0.937
Residual	76	151.85	2.00		
Total	79	157.89			

Appendix B.59 Analysis of variance for different ultrasound power input and treatment time on sensory evaluation (overall acceptability) of fresh-cut pineapple stored at 7 °C for 5 days.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Power (P)	1	1.01	1.01	0.58	0.449
Treatment_Time (T)	1	1.51	1.51	0.86	0.355
P x T	1	1.51	1.51	0.86	0.355
Residual	76	132.95	1.75		
Total	79	136.99			