

**PROTEOMIC AND QUALITY ANALYSIS OF UV-C AND  
THERMAL TREATED CUT CHOKANAN MANGO  
(*MANGIFERA INDICA* L.) AND JOSEPHINE PINEAPPLE  
(*ANANAS COMOSUS* L. MERR.)**

**DOMINIC SOLOMAN A/L GEORGE**

**THESIS SUBMITTED IN FULFILMENT OF THE  
REQUIREMENT FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY**

**FACULTY OF SCIENCE  
UNIVERSITY OF MALAYA  
KUALA LUMPUR**

**2016**

# UNIVERSITI MALAYA

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## ABSTRACT

The increasing demand for high quality fruit products, fresh-cut and the likes that meet safety standards has spurred the development of treatments such as ultraviolet-C (UV-C) light and thermal treatment. In this study, fresh cut Chokanan mangoes and Josephine pineapples were subjected to UV-C treatment (for 15, 30 and 60 minutes at  $25 \pm 1$  °C) and thermal treatment (at  $70 \pm 1$  °C for 5, 10 and 20 minutes). The proteomic changes in the fruits following UV-C and thermal treatments as well as the effects of the treatments on the quality characteristics (physicochemical, nutritional, microbial, sensorial and shelf-life) were evaluated and compared with untreated fruits (control).

UV-C and thermal treatments resulted in significant changes in the expression of several proteins that are known to play important roles in various metabolic processes. Spots that were differentially expressed following UV-C and thermal treatment were identified through LCMS-MS and classified according to functional groups. They were, in the order, stress and defence, energy and metabolism, cell structure and finally ripening and senescence. Several allergenic proteins were found to be reduced in expression following UV-C and thermal treatment which could prove to be invaluable in reducing allergic reactions in patients.

As for the quality attributes, no significant changes were observed in pH, total soluble solids and titratable acidity after UV-C and thermal treatment. UV-C treated fruits showed significant improvement in selected quality parameters, which included a significant enhancement in polyphenols (up to 7%), DPPH scavenging activity (up to 4 %) and total antioxidant capacity (up to 17%) when compared to the control. Thermal treatment on the other hand resulted in a decrease in these quality parameters. In

addition, significant reduction in microbial load was observed in both UV-C and thermally treated fruits with UV-C achieving higher microbial inactivation. Both UV-C and thermal treatment successfully extended shelf-life of fresh cut fruits to a maximum of 15 days.

Sensory attributes (appearance, aroma, texture, and taste) were evaluated by 90 panellists using a hedonic scale, and results showed that UV-C treated fruit samples were preferred more than thermally treated fruit samples. The sensory evaluation verified that UV-C treatment for 30 minutes was the most promising treatment compared to the other treatments.

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## ABSTRAK

Peningkatan permintaan terhadap produk buah-buahan berkualiti tinggi seperti buah-buahan potong segar dan seumpamanya yang memenuhi piawaian keselamatan telah merangsang pembangunan rawatan seperti rawatan ultraviolet-C (UV-C) dan rawatan therma. Dalam kajian ini, mangga chokanan dan nanas Josephine potong segar dirawat melalui kaedah UV-C (untuk 15, 30 dan 60 minit pada  $25 \pm 1^\circ\text{C}$ ) dan kaedah therma (pada  $70 \pm 1^\circ\text{C}$  selama 5, 10 dan 20 minit). Perubahan proteomik serta kesan-kesan rawatan tersebut ke atas ciri-ciri kualiti (fizikokimia, pemakanan, mikrob, deria dan jangka-hayat) dalam buah-buahan yang dirawat dengan kaedah UV-C dan therma telah dinilai dan dibandingkan dengan buah-buahan yang tidak dirawat (kawalan).

Rawatan UV-C dan therma menyebabkan perubahan ketara kepada beberapa protein yang telah dikenalpasti untuk memainkan peranan penting dalam pelbagai proses metabolik. Protein yang mengalami perubahan akibat rawatan UV-C dan rawatan therma telah dikenalpasti melalui LCMS-MS dan dikelaskan mengikut kumpulan berfungsi masing-masing. Protein-protein adalah mengikut turutan berikut: tekanan dan pertahanan, tenaga dan metabolisme, struktur sel dan akhirnya protein yang terlibat dalam proses pemasakan dan senesi buah-buahan. Beberapa protein yang boleh menyebabkan alahan turut mengalami perubahan berikutan rawatan UV-C dan rawatan therma. Fenomena ini berpotensi untuk mengurangkan reaksi alahan pada pesakit yang mempunyai alahan terhadap buah-buahan ini.

Bagi ciri-ciri kualiti, tiada perubahan ketara diperhatikan dalam pH, jumlah pepejal larut dan keasidan tertitrat selepas rawatan UV-C dan rawatan therma. Rawatan UV-C berjaya meningkatkan beberapa parameter kualiti. Berikutan rawatan UV-C, peningkatan ketara

dalam polifenol (sebanyak 7%), aktiviti DPPH (sebanyak 4%) dan jumlah kapasiti antioksidasi (sebanyak 17%) berbanding dengan kawalan telah diperhatikan. Rawatan terma sebaliknya menyebabkan penurunan dalam parameter kualiti. Di samping itu, pengurangan yang ketara dalam jumlah mikrob diperhatikan selepas rawatan UV-C dan terma. Rawatan UV-C didapati lebih berkesan dalam pengurangan jumlah mikrob berbanding dengan rawatan terma. Rawatan UV-C dan terma berjaya mempertingkatkan jangka-hayat buah-buahan potong segar sehingga 15 hari.

Sifat-sifat deria (penampilan, aroma, tekstur, dan rasa) telah dinilai oleh 90 ahli panel menggunakan skala hedonik, dan keputusan menunjukkan bahawa sampel buah-buahan yang dirawat menggunakan kaedah UV-C lebih diterima berbanding sampel buah-buahan yang dirawat melalui kaedah terma. Penilaian deria mengesahkan bahawa rawatan UV-C selama 30 minit merupakan rawatan yang paling sesuai berbanding rawatan-rawatan yang lain.

## ACKNOWLEDGEMENT

“And we know that in all things God works for the good of those who love him, who have been called according to his purpose.” Romans 8:28.

I thank the Lord for His never ending blessings upon my life up to this very day.

First and foremost, I would like to express my gratitude to my supervisor, Associate Professor Dr. Chandran Somasundram for his concordant support, unceasing patience, constructive feedback and suggestions in making this thesis possible. Secondly, my gratitude to Dr. Zuliana Razali for her valuable advice and opinions while supervising this thesis.

I would also like to thank the Postharvest Biotechnology Laboratory members, namely Dr. Vicknesha, Kelvin, Dr. Rebecca, Nadiah, Avinash, Hasvin, Chew Weng and Mr. Doraisamy for their support and encouragement throughout my postgraduate candidature. I would like to thank them for making my years of candidature a lot less stressful and even fun by their words of encouragement and helping hands. My postgraduate study at the postharvest lab has truly been a memorable one.

A big thank you to my loving family: parents, Mr. George Xavier and Margaret William; brothers, Christopher Raj, Daniel Anand and Jeremiah Philips; and sister-in-law, Annie Sundari. Thank you for supporting me in embarking on this journey of postgraduate study. Your loving and unceasing support throughout the years is invaluable to me. My deepest appreciation for your prayers, unconditional love and words of wisdom and encouragement all these years.

Last but not least, to my fiancée, Diane Sunira Daniel, thank you for your unwavering love and support. Your unfaltering support and encouragement has been pivotal in driving me towards the completion of this thesis.

**PROTEOMIC AND QUALITY ANALYSIS OF UV-C AND THERMAL  
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## LIST OF ABBREVIATIONS

µg	Microgram
µl	Microliters
AAE	Ascorbic acid equivalent
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	Analysis of variance
APC	Aerobic plate count
BSA	Bovine serum albumin
CC	Coliform count
CFU	Colony-forming units
DCPIP	2,6-dichlorophenol-indophenol
DPPH	1,1-di-phenyl-2-picrylhydrazyl
FAMA	Federal Agricultural Marketing Authority
FAOSTAT	Statistics Division of the Food and Agriculture Organisation of the United Nations
FDA	Food and Drug Administration
GAE	Gallic acid equivalent
Ha	Hectares
HHP	High hydrostatic pressure
HMF	5-hydroxymethyl furfural
HSD	Honestly significant difference
IR	Ionizing radiation
kGy	Kilogray
kHz	Kilohertz
LCMS/MS	Liquid Chromatography Tandem Mass Spectrometry
ml	Mililiters
MPa	Megapascal
MT	Metric tonnes
NEBI	Non-enzymatic browning index
nm	Nanometers
PEF	Pulsed electric field
ROS	Reactive oxygen species
RT	Room temperature

SD	Standard deviation
SDS	Sodium Dodecyl Sulphate
SDW	Sterile distilled water
TA	Titrateable acidity
TAC	Total antioxidant capacity
TCA	Trichloroacetic acid
TPC	Total polyphenol content
TSS	Total soluble solids
UPLC	Ultra High Performance Liquid Chromatography
UV-C	Ultraviolet-c
V	Volt
W	Watt
YMC	Yeast and mould count

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## CHAPTER 1: GENERAL INTRODUCTION

Over the last decade, the world production of tropical fruits has been recorded to increase by more than 5 % annually. Ninety eight percent of the total production of tropical fruits is contributed by developing countries. Asia accounts for 70 % of this total, making it the major fruit producing region in the world. Mangoes and pineapples are the two major fruits produced among the tropical fruits worldwide. The production of these fruits in Malaysia is estimated to be at 75.1 thousand MT for mangoes and 23.3 million MT for pineapples in 2012. A high production of these fruits is a result of the considerable high total area for cultivation of up to 15.3 thousand Ha for mangoes and 15.6 thousand Ha for pineapples in the same year (FAOSTAT, 2014). The commercialization of mangoes and pineapples in Malaysia focuses on several popular domestic cultivars. The Chokanan mango is regarded as one of the most popular mango cultivars in Malaysia while the Josephine pineapple is among the most popular pineapple cultivars. The demand and production of these fruits has been on the rise in recent years (FAOSTAT, 2014).

The increasing demand for fruits is due to the increase in fruit consumption which is attributed to increased general health consciousness and the growing interest in the role of food such as fruits in maintaining and improving human well-being. The benefits of fruit consumption have been related to reduced risks of developing diseases (Lui *et al.*, 2000). The increased demand for fruits is also met by an increased demand for readily available food which has led to the demand of fresh cut as well as fresh-like fruit products. However, the major limitation of the industry is the limited shelf-life of fruits due to deterioration brought about by microbial growth or physiological disorders (Das, 2010). The cases of illnesses caused by the consumption of contaminated food have been on the rise according to the Centre for Disease Control and Prevention (1996) and

hence, there is a need for processing technologies for safer food products. Apart from diseases caused by contaminating microbes, consumption of fruits such as mangoes and pineapples has been reported to cause allergic reactions in certain consumers (Hedge & Venkatesh, 2007; Reindl *et al.*, 2002).

Food processing technologies with the efficacy to address the issue of food deterioration due to microbial spoilage and physiological disorders will pave the way for further advancement of the industry. At present, the most common and preferred treatment is the thermal or heat treatment. Heat treatment is known to be effective in conferring shelf-life extension through the inactivation of microorganism and enzymes that causes spoilage. However, the disadvantage of this treatment can be seen in the adverse effects it causes in the food product with regards to their nutritional values and sensory attributes such as colour, aroma and taste (Rawson *et al.*, 2011).

Non-thermal treatment has been gaining popularity over the recent years due to its ability to confer shelf-life extension while ensuring minimal deleterious effects to the quality of the food product (Morris *et al.*, 2007). One of the most common and emerging non-thermal treatment is the UV-C light treatment. The UV-C light with a peak emission of 254nm has been reported to be successful in the extension of shelf-life of food products through its mechanism of action on microorganism which eventually leads to cell death of the microorganisms (Guerrero-Beltran & Barbosa-Canovas, 2004). The efficacy of UV-C treatment in reducing microbial load has been reported in several studies up to date (Yaun, 2004; Fonseca & Rushing, 2006). Some of the advantages of using UV-C treatment are that it does not leave residues as compared to other chemical treatments and it does not require extensive safety equipments (Yaun *et al.*, 2004).

In this study, the Chokanan mango and Josephine pineapple will be subjected to UV-C and thermal treatment (70°C) as a standalone. Processing technologies for the fresh cut industry has been predominantly focused on the efficacy in reducing microbial loads and less attention has been given to product quality maintenance as well as the efficacy of these technologies in removing or reducing harmful substances. Besides reducing microbial loads, thermal treatment has been reported to affect proteins through denaturation while UV-C has been reported to damage proteins through photolysis (Nowak-Wegrzyn, 2007; Chan *et al.*, 2006). Various proteins are known to be responsible for physiological changes in fruits which results in desirable or undesirable characteristics. Therefore, a holistic approach is needed to fully understand the overall effects of these treatments on the fruits both on a molecular level as well as on the overall quality.

In this study, the Chokanan mango and Josephine pineapple was selected as they are the two most lucrative fruits among tropical fruits with total output of 38% and 21% out of the total cultivation of tropical fruits (Mohamed *et al.*, 2011). Thermal and UV-C treatment may have different effects on these fruits due to the differing natures of these fruits. Mango is classified as a climacteric fruit as its ripening is accompanied by a distinct increase in respiratory rate, which is generally associated with elevated ethylene production (Hoffman & Yang, 1980). Pineapple on the other hand is a representative of the non-climacteric fruits which are classified as fruits that do not exhibit increases in ethylene and respiration, but rather undergo a gradual decline in respiration during ripening (Knee *et al.*, 1977). Apart from that, mangoes are fruits from tropical fruiting trees, with a soft peel (exocarp) and are representatives of the stone fruits (Stern, 1997). Pineapples on the other hand, are fruits from tropical plants with a hard peel and are representatives of the multiple fruits (Spjut & Thieret, 1987). These differences may

result in different outcomes in the fruits following postharvest treatments. The effects of these treatments as a standalone on a molecular level of fruits as well as the quality of the fruits as a whole will be a boon for the industry with regards to implementation of better technologies with advantages such as quality maintenance and shelf life extension.

In order to understand the different aspects of the effects of these treatments on the Chokanan mango and Josephine pineapple, analysis will comprise of both proteomic and quality analysis. This study will therefore expand our understanding on the proteins that are affected by these postharvest treatments as well as their effects on the quality and shelf-life of the Chokanan mango and Josephine pineapple which will lead to better processing technologies of postharvest crops in the future.

Hence, this study aims to answer the following questions:

- 1) Does UV-C and thermal treatment affect the total proteome of the Chokanan mango and Josephine pineapple?
- 2) What proteins/groups of proteins are affected by these treatments?
- 3) Do these treatments affect the Chokanan mango and Josephine pineapple differently?
- 4) Do these treatments affect the quality and shelf-life of the Chokanan mango and Josephine pineapple?

Correspondingly, the objectives of this study are:

- 1) To investigate the effects of UVC and thermal-treatment on the proteome of the Chokanan mango and Josephine pineapple.
- 2) To identify proteins significantly affected by these treatments.
- 3) To compare proteins affected by UVC-treated as well as thermal-treated Chokanan mango and Josephine pineapple.
- 4) To assess the effects of these treatments on the quality of the Chokanan mango and Josephine pineapple, which include:
  - i) Physicochemical analysis
  - ii) Antioxidant analysis
  - iii) Microbial inactivation analysis
  - iv) Sensory analysis
  - v) Shelf-life studies



## CHAPTER 2: LITERATURE REVIEW

### 2.1 Introduction to Tropical Fruits

Tropical fruits are mostly cultivated in countries with warm climates which are completely intolerable to frost. They come in different shapes, sizes, textures and flavours. Besides being cultivated for export, tropical fruits are also consumed raw during the in season period in tropical countries. Some of the tropical fruits that have been consistently exported by developing countries include mangoes, pineapple, bananas, lychees as well as a variety of melons. The production of tropical fruits by developing countries makes up 98% of the global tropical fruit production (Kader & Yahia, 2011). Among the various tropical fruits cultivated, mangoes account for 38 % of the global tropical fruit output followed by pineapple (21 %), papayas (14%) and avocados (4 %). The remaining global output of tropical fruits is contributed by minor tropical fruits, which include lychees, durian, rambutan, guava and passion fruit (Mohamed *et al.*, 2011).

#### 2.1.1 Mango (*Mangifera indica*)

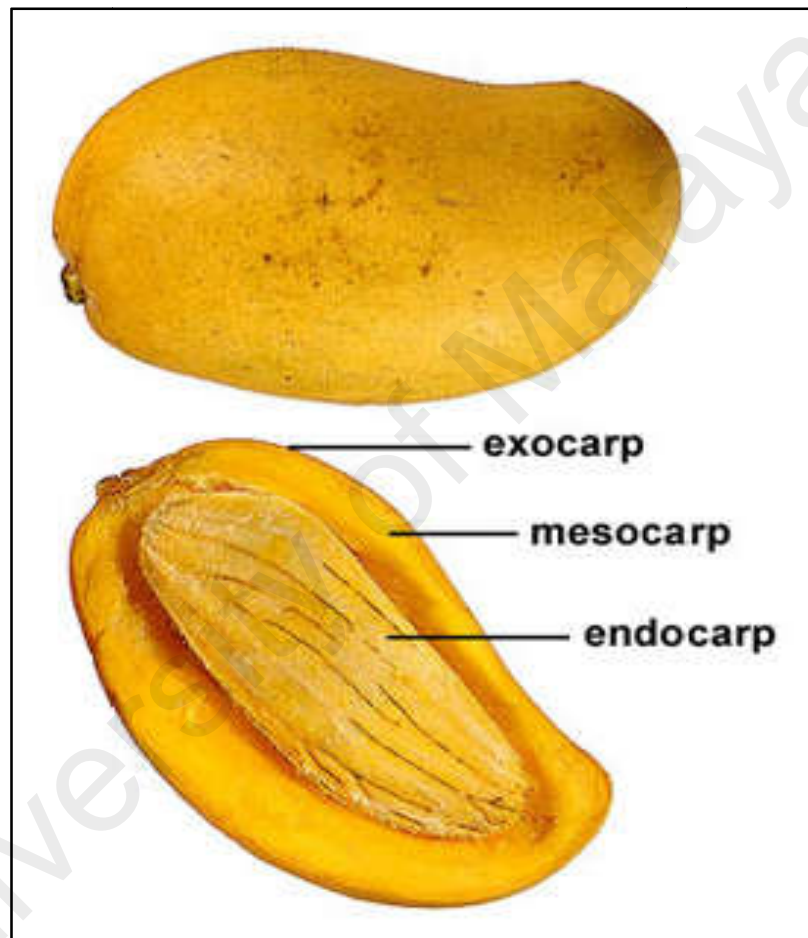
The mango (*Mangifera indica* L.) is one of the most celebrated tropical fruits, which belongs to the family Anacardiaceae which is known for encompassing several highly poisonous plants. The *Mangifera* genus is comprised of 69 species, out of which the common mango tree (*Mangifera indica*) is the most popular. Out of these 69 species, approximately 27 species produce fleshy fruits that are edible with the common mango (*Mangifera indica*) being the most popular (Mukherjee & Litz, 2009). The nomenclature of mango is as listed (Singh, 1960):

Kingdom	:	Plantae (Plants)
Subkingdom	:	Tracheobionta (Vascular plants)
Superdivision	:	Spermatophyta (Seed plants)
Division	:	Magnoliophyta (Flowering plants)
Class	:	Magnoliopsida (Dicotyledons)
Subclass	:	Rosidae
Order	:	Sapindales
Family	:	Anacardiaceae (Sumac family)
Genus	:	<i>Mangifera</i> L. (Mango)
Species	:	<i>Mangifera indica</i> L. (Mango)

Mangoes originated from southern Asia especially eastern India, Burma and the Andaman Island. Its cultivation has since spread to areas such as Eastern Africa, Eastern Asia as well as to Malaysia (Mitra & Baldwin, 1997). At present, the common mango is cultivated around the world and is revered as one of the most famous tropical fruits (Morton, 1987). The production of mangoes in Malaysia is mainly focused in peninsular Malaysia which includes states such as Kedah, Perak, Negeri Sembilan, Melaka and Perlis (Department of Agriculture, 2009).

The mango fruit is known as a drupe or a stone fruit whereby an outer fleshy part (skin and flesh) surrounds a shell (the pit, stone, or pyrene) with a seed inside (Stern, 1997). It grows from the end of a long, string-like stem to form the fruit (Figure 2.1). Mango fruits may vary greatly in shape, size, weight and colour based on its cultivar. They are normally 2 to 9 inches long and are kidney shaped, ovate, elongated and nearly round. The fruit weight ranges from 50 gram to over 2 kilograms (Mukherjee & Litz, 2009). The outer most layer of the fruit (exocarp) is the leathery skin, which is normally waxy and smooth and turns pale green, yellow or yellow marked with red according to

corresponding cultivars (Figure 2.1). The exocarp is inedible and contains saps that may cause irritation in some people. The mesocarp or the flesh of a mango is the edible part of the fruit, which is made up of fibres emitting from the husk of the large kidney-shaped seed (endocarp) (Figure 2.1). The flavour and taste of the mango flesh is pleasant and it is high in sugars and acid giving it a distinct flavour (Bally, 2006).



**Figure 2.1: Fruit structure of a ripe mango (*Mangifera indica*).**

(Source: Armstrong, 2011)

Mangoes possess many health benefits and are rich in vitamins, minerals, pre-biotic dietary fibres as well as many antioxidant compounds. It has also been found to confer protection against several cancers which include breast, colon, leukaemia and prostate cancers by recent studies. The protection against colon and breast cancers is said to be offered by polyphenolic anti-oxidant compounds (Rocha *et al.*, 2007). In addition,

mangoes are also known to be a great source of flavanoids like alpha-carotene, beta-carotene and beta-cryptoxanthin as well as Vitamin-A, which is vital for maintenance of healthy mucous and skin. It is also a good source of potassium which aids in controlling blood pressure and heart rate. Furthermore, mangoes are also rich in vitamins such as vitamin B6, C and E which are important vitamins for health and wellbeing (Rodeiro *et al.*, 2006). Vitamin-C plays a role in developing resistance against infectious agents as well as scavenging harmful free radicals while vitamin B6 can prevent strokes and coronary artery disease by controlling homocystiene levels within the blood (Pardo-Andreu *et al.*, 2006). Mangoes also possess a considerable amount of copper, which is a cofactor for many enzymes for their optimal functions. Copper has also been linked with red blood cell production in the human body. Last but not least, the mango peel also possesses several nutritional benefits which include antioxidants, polyphenols and carotenoids (Mahattanatawee *et al.*, 2006). A serving of 100 g of fresh mango contains nutritional values as shown in Table 2.1.

**Table 2.1: Nutritional value of fresh mango fruit (100 g).**

(Source: USDA National Nutrient database)

<b>Principle</b>	<b>Nutrient Value</b>	<b>Percentage of RDA</b>
Energy	70 Kcal	3.5%
Carbohydrates	17 g	13%
Protein	0.5 g	1%
Total Fat	0.27 g	1%
Cholesterol	0 mg	0%
Dietary Fiber	1.80 g	4.5%
<b>Vitamins</b>		
Folates	14 µg	3.5%
Niacin	0.584 mg	3.5%
Pantothenic acid	0.160 mg	1%
Pyridoxine (vit B-6)	0.134 mg	10%
Riboflavin	0.057 mg	4%
Thiamin	0.058 mg	5%
Vitamin C	27.7 mg	46%
Vitamin A	765 IU	25.5%
Vitamin E	1.12 mg	7.5%
Vitamin K	4.2 µg	3.5%
<b>Electrolytes</b>		
Sodium	2 mg	0%
Potassium	156 mg	3%
<b>Minerals</b>		
Calcium	10 mg	1%
Copper	0.110 mg	12%
Iron	0.13 mg	1.5%
Magnesium	9 mg	2%
Manganese	0.027 mg	1%
Zinc	0.04 mg	0%

\*Percent Daily Values are based on a 2,000 calorie diet.

To date, 800 mango cultivars have been successfully named. Mango cultivars are distinguished based on differences in geological characteristics, climate, harvest period and marketing season of each mango growing country. Therefore, different countries have different cultivars characterised as major cultivars due to commercial values (Pandey, 1986; Nakosone & Paull, 1998). There are approximately 216 mango cultivars in Malaysia; however, only a handful are recommended for commercial cultivation, which include Chokanan, *Harumanis*, *Mas Muda*, Maha 65, Sala and Siam Panjang (Agri-food Business Development Centre, 2010).

Mangoes are economically important crops especially to the Asian region. Approximately 72% of global mango production is mainly from Asia which contributes about 30.2 million metric tonnes (MT) to the international market. Top mango producers in the region include India, China, Thailand, Indonesia and Philippines. Globally, mangoes are grown in 94 countries which contribute to the estimated worldwide production of 42.1 million MT in 2012. As of 2014, the total area of mango cultivation worldwide is approximately 5.2 million Ha (FAOSTAT, 2014). Accordingly, the production of mangoes in Malaysia has been experiencing an increasing trend in recent years. The production of mango in Malaysia increased from 67.7 thousand MT in 2011 to 75.1 thousand MT in 2012, recording an increase of up to 11% with the total area of cultivation increasing from 14.5 thousand Ha to 15.3 thousand Ha in the same period of time, recording an increase of up to 5.5% (FAOSTAT, 2014). Furthermore, the commercialization of domestic mango cultivars, especially in Malaysia, has reached the worldwide market with exports to countries such as Singapore, Brunei and Hong Kong (Agri-food Business Development Centre, 2010). In Malaysia, Chokanan mango (MA224) is one of the most popular cultivars grown for local consumption as well as for export. The Chokanan mango is also regarded as the

“honey mango” as a result of its succulent sweet taste and demands for this cultivar is on the rise due to its exotic flavour, vibrant colour, distinctive taste, pleasant aroma and, most importantly, its nutritional properties (Arauz, 2000; Agri-food Business Development Centre, 2010).



**Figure 2.2: Ripe Chokanan mango**

### **2.1.2 Pineapple (*Ananas comosus*)**

The pineapple (*Ananas comosus*) belongs to the family Bromeliaceae which is comprised of approximately 2000 species. The pineapple is the most economically important species out of the 2000 species which puts it alongside bananas as one of the important fruiting monocots. The pineapple is also the leading member of the Bromeliaceae family which comprises mostly of epiphytic and ornamental species. Following is the Nomenclature of pineapple (National Plant Data Center, 2000):

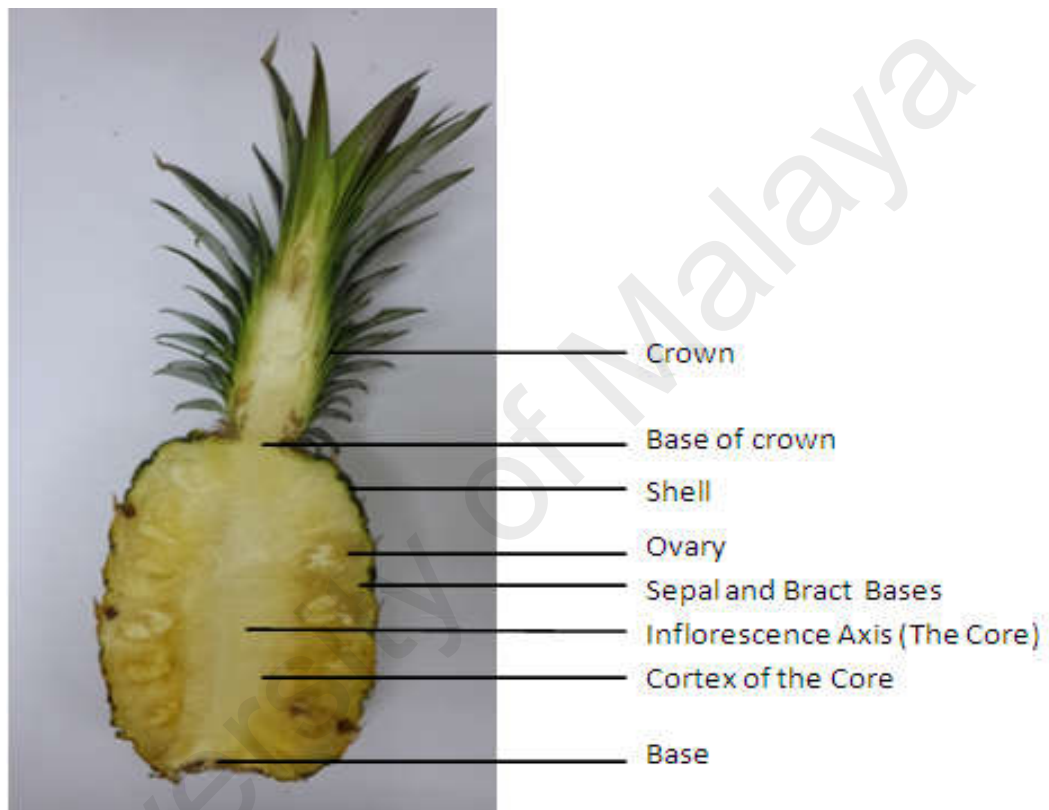
Kingdom	:	Plantae (Plants)
Subkingdom	:	Viridaeplantae (Green plants)
Division	:	Tracheophyta (Vascular plants)
Subdivision	:	Spermatophytina (Spermatophytes)
Class	:	Liliopsida
Subclass	:	Lilianaes (Monocotyledons)
Order	:	Poales
Family	:	Bromeliaceae
Genus	:	<i>Ananas Mill.</i> (Pineapple)
Species	:	<i>Ananas comosus</i> L. (Pineapple)

The pineapple was known to have originated from South America and domesticated by the Tupi-Guarani Indians from *Ananas comosus* var. *ananassoides*. Its cultivation then spread northward to the North Andes, Antilles and Central America (Bertoni, 1919). Not long after the discovery of pineapples, it was found to be in various foreign countries by accident or through introduction of the species to foreign land. Currently, it is revered as the second most commercial tropical fruit crop in the world only behind mango (Chan, 1995). According to the Malaysian Pineapple Industrial Board (MPIB), there were an estimated 2,016 pineapple growers in Malaysia, who are predominantly located in the state of Johor (MPIB, 2010).

The pineapple fruit is known to develop from multiple small fruits (fruitlets) that fuse together to form an oval to cylindrical shaped fruit referred to as collective fruits. It is a fleshy and juicy fruit with a stem serving as a fibrous core. The outer most layer of the fruit (waxy rind) is dark green initially and changes to yellow, orange-yellow or reddish as it matures and ripens. A pineapple fruit can grow up to 12 inches long and weigh up to 10 pounds or more (Morton, 1987). The significant structures of pineapples are



illustrated in Figure 2.3. The pineapple is also well known for its vibrant tropical flavour as well as its various nutritional and health benefits. It contains a considerable amount of calcium, potassium, fibre and vitamin C. Pineapples possess potential anti-inflammatory and digestive benefits, antioxidant protection and immune support as well as protection against macular degeneration (Joy, 2010).



**Figure 2.3: Morphological structure of pineapples (*Ananas comosus*).**

(Source: George *et al.*, 2015)

Pineapple contains no saturated fats or cholesterol but contains minerals and vitamins which are essential for wellbeing. It is also a rich source of fibres which include both soluble and insoluble dietary fibre. Pineapples are also rich sources of antioxidant vitamins such as vitamin C, which helps the human body develop resistance against infectious agents, protect against the scurvy disease and aid in the scavenging of harmful free radicals in the human body. Vitamin C is also often linked to an overall

boost of the human's immune system (Smith, 2003). Besides vitamin C, vitamin A and beta-carotene are also present in the flesh of pineapples which play roles including protection against cancers and maintenance of healthy skin and vision. Apart from that, it is also rich in B-complex vitamins such as thiamin, folates, pyridoxine, riboflavin and minerals, namely, potassium, manganese and copper, which are also vital for optimum functions of various enzymes for health and wellbeing (Hassan *et al.*, 2011). Over the recent years, pineapples have been gaining popularity for the proteolytic enzyme bromelain which has been reported to have anti-clotting, anti-inflammatory and anti-cancer properties (Juhasz *et al.*, 2008; Gläser & Hilberg, 2006). A serving of 100 g of fresh pineapple contains nutritional values as shown in Table 2.2.

University of Malaya

**Table 2.2: Nutritional value of fresh pineapple fruit (100 g).**

(Source: USDA National Nutrient database)

<b>Principle</b>	<b>Nutrient Value</b>	<b>Percentage of RDA</b>
Energy	50 Kcal	2.5%
Carbohydrates	13.52 g	10%
Protein	0.54 g	1%
Total Fat	0.12 g	<1%
Cholesterol	0 mg	0%
Dietary Fiber	1.40 g	4%
<b>Vitamins</b>		
Folates	18 µg	4.5%
Niacin	0.500 mg	4%
Pyridoxine	0.112 mg	9%
Riboflavin	0.018 mg	1.5%
Thiamin	0.079 mg	6.5%
Vitamin A	58 IU	2%
Vitamin C	47.8 mg	80%
Vitamin E	0.02 mg	<1%
Vitamin K	0.07 µg	0.5%
<b>Electrolytes</b>		
Sodium	1 mg	0%
Potassium	109 mg	2.5%
<b>Minerals</b>		
Calcium	13 mg	1.3%
Copper	0.110 mg	12%
Iron	0.29 mg	3.5%
Magnesium	12 mg	3%
Manganese	0.927 mg	40%
Phosphorus	8 mg	1%
Selenium	0.1 µg	<1%
Zinc	0.12 mg	1%

\*Percent Daily Values are based on a 2,000 calorie diet.

The worldwide production of pineapples was estimated to be at 22.7 million tonnes in 2011, (FAOSTAT, 2014) which is more than of what it was in 1987, highlighting the ever increasing importance of this fruit crop. Fifty percent of the total worldwide production of pineapples is contributed by Asian countries which include the Philippines, Thailand, Indonesia, India and Malaysia. The global total area of pineapple cultivation was recorded to be at 9.9 million Ha in 2012 with a total production of 23.3 million MT. Production of pineapples worldwide has been increasing as compared to previous years. The total production of pineapples worldwide in 2011 was recorded to be at 21.9 million MT and 20.3 million MT in 2010. Accordingly, the total area of pineapple cultivation in Malaysia increased from 14.9 thousand Ha in 2011 to 15.6 thousand Ha in 2012 and the total production increased from 3 million MT in 2011 to 3.3 million MT in 2012 (FAOSTAT, 2014). Among the different varieties of pineapples, the Smooth Cayenne is the most imported worldwide (Smith, 2003).

Currently, there are more than 100 cultivars of pineapples but these cultivars are grouped in four main classes (Smooth Cayenne, Red Spanish, Queen and Abacaxi), although there are considerable variations in the cultivars within each class (Morton, 1987). In Malaysia, the most cultivated cultivars include Johor (Spanish), Moris (Queen), Sarawak (Cayenne), Gandol (N19), N36, Josapine and Maspine (FAMA, 2004). Among all the cultivars grown in Malaysia, the Josapine pineapple is one of the most popular in the country which is grown for both local consumption and for export. The Josapine pineapple is a hybrid of the Johor and Sarawak pineapple, and hence the acronym JOSAPINE (**JO**hor and **SAR**awak **PINE**apple). The Josapine pineapple is also referred to as Josephine, Josaphine and Josa by the local farmers and consumers (Chan & Lee, 1996). It is known for its petite size as compared to other varieties, vibrant skin colour when it is ripe and its considerably high sugar content (Yuris, 2014). This variety

is also described as having a good sugar-acid balance and flavour (Bartolome *et al.*, 1994).



**Figure 2.4: Ripe Josephine pineapple**

## **2.2 Fresh-Cut Products**

Fresh-cut products can be defined as fruits or vegetables that have been trimmed, peeled and/or cut into usable products and subsequently packaged. Fresh-cut products serve to offer consumers with convenience while maintaining freshness, flavour and high nutrition of fresh produce such as vegetables and fruits (IFPA, 2001). In recent years,

sensational growth has been observed in the market for chilled fresh-cut produce which is predominantly attributed to the increased consumers' demand of fresh, healthy, convenient, preservative free food that are safe and nutritious at the same time (Gonzalez-Aguilar *et al.*, 2011).

Fresh-cut produce currently available can be divided into four main groups which include fresh-cut tropical fruits, fresh-cut salads, fresh-cut vegetables and fresh-cut herbs. Fresh-cut tropical fruits include mangoes, pineapples, melons, cantaloupe, watermelon, mangosteen, rambutan, jackfruit, pomelo, papaya, grapefruit, and fruit mixes. Fresh-cut salads include salad mixes and shredded leafy vegetables. Fresh-cut vegetables on the other hand include those for cooking such as peeled baby carrots, baby corn, broccoli and cauliflower florets, cut celery stalks, shredded cabbage, cut asparagus, stir-fry mixes and cut sweet potatoes while fresh-cut herbs include common herbs used for cooking (IFPA, 2001).



**Figure 2.5: Fresh cut-fruits A: Fresh-cut mango, B: Fresh-cut pineapple.**

The growth of the fresh-cut industry has been noticed around the world with marked growth areas that include the European Union, United States and in the Asian region. Europe's leading fresh-cut market is currently attributed to United Kingdom in terms of value followed by Italy while other countries like Netherlands, Switzerland and Spain also show exponential growth in the fresh-cut produce sector. An exponential growth in the fresh-cut produce sector of United States was also realized with an increase in sales value from US\$3.3 billion in 1999 to US\$15.5 billion in 2007 recording a marked increase (Cook, 2009). Similarly, the fresh-cut produce sector in Asia has also shown a steady growth over the years (Kim, 2007). With the ever increasing demand for ready-to-eat-products in Asia, the market for fresh-cut products in the region is predicted to show a steady increasing trend (Sa-nguanpuag *et al.* 2007).

### **2.2.1 Fresh-Cut Products in Developing Countries**

Current consumers' profile in developing countries of increased consumption of readily available food as compared to cooked food has led to higher demand for fresh-cut produce. Ready to eat foods such as fresh-cut fruits are preferred over whole fruits due to the large size of whole fruits as well as the additional task of peeling it before it is fit for consumption. These issues are acted upon by introducing products in a convenient and ready to eat form with minimal waste through fresh-cut processing. Demands for ready-to-eat food products have led to an increased production and sales of packaged fresh-cut fruits by cottage vendors in the present years. At present, the supply of fresh cut fruits and vegetables in developing countries are contributed mostly by small vendors and cottage industries. However, the increasing demand in developing countries for fresh-like products needs new innovative approaches in order to provide constant supply of fresh-cut tropical fruits and vegetables (Kim, 2007).

Currently, there are several challenges that are faced by the fresh-cut industry in marketing fresh-cut produce with the major challenge being the production of fresh produce that is of high quality, safe and with a satisfactory shelf-life. Among the other challenges of marketing fresh-cut produce are the needs to preserve product quality through the marketing chain and maintain cold chain and proper logistics, the fragile nature of certain fruits and the inadequacy of equipment such as processing facilities and refrigerated storage. Besides that, there is a lack of research conducted in order to preserve tropical fresh-cut produce quality due to the lack of technology for setting up required processing plants (Kim, 2007). On the other hand, some of the major challenges faced from a consumer's perspective include the need to meet the quality, taste and price expectation of consumers as well as product presentation to appeal to consumers. Emerging technologies for preservation of fresh-cut produce should



therefore be able to address most, if not all, of the challenges currently faced by the industry (Gil *et al.*, 2006).

### **2.3 Food Quality**

The quality of a food product can be classified as a combination of various characteristics that determines the value of that particular food product to consumers. The general expectations of consumers are for produce to be free of defects and in fresh or good condition, which is determined by the general appearances, sensory quality, flavour and nutritional quality (Watada & Qi, 2000; Kader, 2002). External appearances are the first characteristic that consumers often use in distinguishing the quality of a food product. However, the eating experience, which includes the flavour, aroma, taste and texture of a food product, is the characteristic that leads to subsequent purchases. Nutritional quality and the safety of a food commodity will further increase the likelihood of acceptability and purchases by consumers. Food processing of fresh-cut commodities should therefore be of good quality to ensure consumer acceptability (Watada & Qi, 2000).

The major components of food quality are comprised of external, internal and hidden attributes. External attributes involve visual appearance of a product at first encounter which can be classified as appearance quality and texture quality. Internal attributes involves attributes when the food is consumed which include taste, aroma and mouth feel or texture and can be classified as flavour and eating quality, while hidden attributes is simply the safety and nutritional quality of a product (Watada & Qi, 2000).

Appearance of a product is closely related to size, shape, colour and cleanliness of the surface. Furthermore, it is also important for a product to be free of external defects

such as shrivelling, bruising, wilting, colour changes, physical disorders and tissue softening to be regarded as having good appearance (Kader, 2002). The eating quality of a food commodity is a combination of texture quality and flavour quality. Texture quality for external attributes involves the firmness and hardness of a food commodity while for internal attributes; it involves juiciness, crispness, tenderness, mealiness, toughness and crispness of a food commodity. Flavour quality is closely related to both the aroma and taste of a food product. It has been reported that flavour quality is one of the most important qualities as it ensures consumer satisfaction (Ragaert *et al.*, 2004).

Nutritional quality of food commodities are often determined based on the content of nutrients such as vitamins, minerals, flavanoids, carotenoids and phenolic compounds. The potential of these nutrients in lowering the risk of cancers, heart disease and various other illnesses increases the acceptability of products that contains such nutrients. Last but not least, the safety of a food product is determined by the absence of biological, chemical and physical hazards. Biological hazards involve the presence of pathogenic microorganisms, with the potential of causing human illnesses after consumption, in a particular food commodity. Chemical hazards, on the other hand, involve toxic elements such as arsenic, zinc, cadmium and lead, chemical products such as water disinfectants and pesticides as well as natural substances such as allergens, alkaloids, enzyme inhibitors and mycotoxins. Finally, physical hazards include the presence of glass particles, wood, hair, metals and stones which may have been introduced by accident while producing the food product (Kader, 2002).

## 2.4 Food Spoilage

Fresh-cut produce, inclusive of both fresh-cut fruits and vegetables, are in demand due to convenience and the health benefits of its consumption. Inappropriate processing techniques and storage conditions of these food commodities however, may contribute to deterioration and spoilage. Deterioration and spoilage of fresh-cut produce can be caused by factors such as intrinsic physiological and biochemical changes of the food, enzymatic activities as well as microorganisms (Odumeru, 2012).

Fresh food commodities like fruits and vegetables continue to respire after detachment from the plant or tree. Minimal processing such as cutting is perceived to increase respiration rates in fruits and vegetables due to increased ethylene production. An increased rate of respiration consequently results in detrimental effects which include loss of water, a reduced level of carbohydrates, organic acids and vitamin as well as negative effects on the aroma and flavour. Fresh cut processing has also been said to cause changes in several aspects which include colour changes, flavour changes, texture change and nutritional changes. These changes are often undesirable and are characterized as quality loss of the food commodity (Ragaert *et al.*, 2004).

Colour changes are one of the most common physiological effects of fresh-cut processing that leads to quality loss in fresh produce. Colour changes are often observed as darkening or browning of the surface which results from oxidation by the enzyme polyphenol oxidase (McEvily *et al.*, 1992). Factors such as abundance of the active PPO enzyme, pH, oxygen and temperature available to the tissues and amount of phenolic compounds determine the intensity of browning in a particular fresh produce (Kader, 2002). The flavour of a food product can be perceived as its sweetness, astringency, acidity and bitterness. These attributes of flavour are often lost in fresh-cut fruits due to

increased respiration as well as enzymatic activities. As for changes in nutritional values, it has been reported that fresh-cut fruits and vegetables do not undergo nutrient loss easily and more often than not, it appears spoilt visually before nutrient loss actually happens (Gil *et al.*, 2006). Changes of texture in fresh-cut fruits are caused by loss of water as well as enzymatic activities in fresh-cut produce. Water loss leads to wilting and shrivelling and therefore an eventual loss of its firm texture and crispness (Kader, 2002).

Microbiological spoilage in fruit products are classified as fermentative spoilage and are brought about by wilting, lactic acid bacteria and yeast (Heard, 2000). Yeast and moulds are said to thrive in low pH causing spoilage of the food commodity (Raybaudi-Massilia *et al.*, 2009). There has been an increase in the number of cases of illness caused by consumption of contaminated food which is a concern that should be addressed. Food borne diseases are highly related to pathogenic pathogens which include the likes of *Escherichia coli* O157:H7, *Campylobacter* spp., *Salmonella* spp. and *Listeria monocytogenes*. Severe forms of food borne illness such as haemolytic uremic syndrome (HUS) and salmonellosis requires hospitalization. Haemolytic uremic syndrome (HUS) is caused by *E. coli* O157:H7 and causes symptoms which include abdominal cramps, kidney failure, and could cause death. Salmonellosis on the other hand is caused by *Salmonella* spp. and its symptoms include abdominal pain, nausea, diarrhoea and fever (Odumeru, 2012).

Apart from bacteria, several yeast and moulds could cause spoilage in food products. Yeast that commonly cause food spoilage include *Candida* spp., *Saccharomyces* spp., *Zygosaccharomyces* spp., and *Debaryomyces* spp. As for moulds known to cause food spoilage, they include *Aspergillus* spp., *Penicillium* spp., *Eurotium* spp., *Cladosporium*

spp., *Alternaria* spp., *Botrytis* spp. and *Paecilomyces* spp. (Bates *et al.*, 2001; Raybaudi-Massilia *et al.*, 2009).

## **2.5 Food Processing and Quality**

One of the main goals of the food industry at present is to extend the shelf-life (period during which a food remains fit for consumption) by preservation techniques hence, allowing time for distribution, storage and sales. These preservation techniques achieve their goal either by inhibiting microbiological growth or biochemical changes in a particular fruit. At present, there are several food preservation technologies for controlling microbial growth and biochemical changes, some of which may cause significant organoleptic changes in food products following processing. Current demand of consumers are for food with minimal synthetic additives or those that have undergone minimal changes following processing. An ideal food processing technique, therefore, should prevent microbiological and biochemical changes, whilst assuring safety and maintaining quality of the food. In that light, the emergence of ‘minimal’ processing techniques which have limited impact on a food’s quality as a whole is a major boost for the development of the food industry (Fellows, 2000). The food processing technologies comprises of two groups, namely thermal and non-thermal processing (Bates *et al.*, 2001).

### **2.5.1 Thermal Processing**

Thermal processing can be defined as any form of food processing that applies heat in order to achieve a common goal of microorganism destruction. It includes pasteurization, sterilization, and conventional methods such as cooking, baking roasting and blanching (Roeck *et al.*, 2009). Conventional thermal treatment can bring about both desirable (protein coagulation, starch swelling, textural softening, aroma

enhancement and inactivation of microorganisms and enzymes causing spoilage) and undesirable changes (loss of vitamins and minerals, loss of fresh appearance, flavour and texture) in food. The mechanism of action of traditional thermal processing depends on heat generation outside the product and its eventual transfer into the product via conduction and convection mechanisms (Pereira & Vicente, 2010).

One of the most commonly practiced thermal treatments in the food industry is pasteurization which can be carried out in two ways namely, low temperature long time (LTLT) and high temperature short time (HTST). LTLT or batch pasteurization is conducted at 63 °C for 30 min for the preservation of milk while HTST or flash pasteurization is conducted from a range of 90 °C to 95 °C for 15 to 60 seconds for fruit juice preservations (David, 1996). Thermal processing has been proven to be successful in the destruction of microorganisms in fruit juices by numerous studies (Shao *et al.*, 2007; Kuo-Chiang, 2008; Derek *et al.*, 2010; Igual *et al.*, 2010; Timmermans *et al.*, 2011). Some of the predominant benefits of thermal processing include inactivation of food-borne pathogens, natural toxins or other detrimental constituents, degradation of allergenic proteins, extension of shelf-life, improved digestibility and bioavailability of nutrients, enhanced functional properties, including augmented antioxidants and other defence reactivity or increased antimicrobial effectiveness.

Although thermal treatment with high temperatures may be successful in the preservation of liquid food products such as milk and fruit juices, it is inappropriate for other food products such as fruits and vegetables. In the fresh produce industry, thermal treatments are carried out at lower temperatures to minimize quality loss in fruits. The time and temperature variables for thermal treatment of fruit depends on the fruit and the cultivar. Treatment conditions should be selected appropriately to avoid over-

processing. Besides that, under-processing may not completely inactivate microorganism growth, thus resulting in spoilage (Rawson *et al.*, 2011; Osaili, 2012). Thermal processing in fruits and vegetables can be carried out through hot water dips and sprays, vapour heat and hot air from temperatures ranging from as low as 35 °C to as high as 70 °C with varying times depending on the nature of fruits (Lurie & Pedreschi, 2014).

The efficacy of thermal treatment in inactivating microorganisms is due to the adverse effects caused by heat on microbes which leads to damage of organic molecules (nucleic acids and proteins) required for the proper functioning of cells (Santhirasegaram *et al.*, 2014). Relatively high temperatures are required for successful inactivation of spoilage causing microorganisms which may in turn cause adverse affect on the quality of food products in terms of nutritional value and sensory attributes, such as colour and flavour (Rawson *et al.*, 2011). Therefore, appropriate temperature and length of treatment is crucial to ensure that proper inactivation of spoilage causing microbial and enzyme denaturation is achieved, whilst having minimal deleterious effects on the quality of fruits. Thermal (Heat) treatments has been successfully used for preservation of fruit crops which include apple slices and mango cubes with improved texture (Kim *et al.*, 1994; Banjongsinsiri *et al.* 2004; Trindade *et al.*, 2003). Heat treatment has also been reported to prevent wound-induced browning of iceberg-lettuce (Loaiza-Velarde *et al.*, 1997; Loaiza-Velarde & Saltveit, 2001; Saltveit, 2000). Furthermore, heat treatment has also been reported to increase the effectiveness of other preservation techniques such as CaCl<sub>2</sub> in fresh-cut fruits (Garcia & Barret, 2002).

## **2.5.2 Non-Thermal Processing**

The growing interest of consumers for food products that are high in nutritional content and with a fresh appearance has brought about the development of innovative non-thermal food preservation methods. Non-thermal processing techniques have gained popularity in recent years as a result of its capacity to confer shelf-life extension, enhance safety of food while preserving organoleptic and nutritional qualities. Furthermore, these preservation methods are energy efficient and capacitate better retention of quality as compared to their conventional thermal processing counterparts. The most common non-thermal processing methods in practice for minimal processing of food at present include high hydrostatic pressure, ionizing radiation, ozone, chemical treatments, modified atmosphere packaging and ultraviolet light irradiation (Morris *et al.*, 2007). The classification of these treatments as 'non-thermal' is attributed to the elimination of the use of high temperature to achieve microbial inactivation. However, treatments such as high pressure and pulsed electric field may involve heat due to generation of internal energy (Pereira & Vicente, 2010).

### **2.5.2.1 High hydrostatic pressure**

High hydrostatic pressure (HHP) or ultra-high pressure is a cold pasteurization technique that involves the application of pressure to food and ranges from 100 to 1000 MPa. Current HHP techniques are performed by the application of pressures between 300 and 700 MPa for durations ranging from a millisecond pulse to over 20 minutes in either a batch or semi-continuous system. In this processing technique, solid or liquid food products are loaded into steel vessels filled with hydraulic fluid and pressurized either through direct or indirect compression (Morris *et al.*, 2007; Lopez-Gomez *et al.*, 2009). Unlike thermal processing, the efficacy of HHP processing is not determined by



product size or shape as uniform pressure is often transmitted throughout the product till isostatic equilibrium is reached (Oms-Oliu *et al.*, 2012b).

HHP is perceived to inactivate microbial cells by breaking non-covalent bonds and damaging the cell membrane. It also has the capability of causing protein denaturation which eventually results in deactivation of enzymes and microbes that can cause spoilage of food (Morris *et al.*, 2007; Lopez-Gomez *et al.*, 2009). Some of the advantages of this processing technique include minimal alteration in nutritional and sensory quality attributed to its constrained effects on covalent bonds (Oey *et al.*, 2008; Ferrari *et al.*, 2010). Better quality retention has been reported in HHP treated food as compared to conventional thermal processing in previous reports (Bull *et al.*, 2004; Polydera *et al.*, 2005).

#### **2.5.2.2 Ionizing radiation**

Ionizing radiation (IR) can be achieved through means of ionizing radiation sources such as high-energy electrons, X-rays (machine generated) and gamma rays (from radioisotopic source cobalt-60 or cesium-137). According to the World Health Organization, exposure of food products up to 10 kGy does not involve any effects to nutritional content, microbiological problems or toxicological hazards in food (Lopez-Gomez *et al.*, 2009; Sorour *et al.*, 2014). IR achieves microbial inactivation by damaging microbial DNA leading to termination of cellular and reproductive functions. Furthermore, in food commodities like fruit juices, IR has been linked to indirectly promoting the formation of radiolytic products (free radicals) which have the capacity to inactivate spoilage microorganism (Sorour *et al.*, 2014). The use of IR with low doses is ideal for minimal food processing as it causes minimal modification of nutritional and organoleptic qualities of food (Morris *et al.*, 2007). Higher doses of those exceeding 10

kGy however, have been reported to cause adverse effects to quality with regards to total phenolics (Villavicencio *et al.*, 2000). A 2 kGy dose of gamma rays is sufficient to achieve microbial inactivation as reported by Alighourchi *et al.*, (2008).

### **2.5.2.3 Ozone**

Ozone (triatomic allotrope of oxygen) is a water-soluble gas that has been characterised to have high biocidal properties with strong oxidizing capacity as well as high penetrability and reactivity. However, this gas is unstable under ambient conditions. The Food and Drug Administration (FDA) approved the use of ozone for food preservation purposes through direct additive to food by giving it a generally recognized as safe (GRAS) status. The generation of ozone involves the reaction of free oxygen radicals with diatomic oxygen via the electric discharge method or ultraviolet radiation. Currently, solid foods can be treated with Ozone by gaseous treatment or simply by washing with ozonated water (Sorour *et al.*, 2014; Rawson *et al.*, 2011). The recommended concentration of ozone for wash water and flume water is 0.5 to 4 ppm and 0.1 ppm respectively. The mechanism of action of ozone in microbial inactivation is by oxidation of microbial cells as well as disruption of cell membrane leading to cell lysis of microbes. The successful biocidal action of ozone treatment in food processing has been reported in orange and apple juices (Patil *et al.*, 2009; Steenstrup & Floros, 2004).

### **2.5.2.4 Chemical treatment**

Chemical treatments are currently being employed mostly for solids in the food industry. Chemical treatments are administered to food with the purpose of extending shelf-life by delaying physical decay processes caused by microbial or enzymatic activity. The efficiency of chemical methods is reliant upon the inhibition of specific

reactions that could cause undesirable changes in food products. Current chemical techniques practiced by the food processing industry include acidification, reducing agents, application of edible coatings and natural antimicrobials. Acidification involves dipping of products in mildly acidic food grade solutions of ascorbic, acetic, fumaric, citric, phosphoric or tartaric acid, which have been proven to be effective in maintaining quality and reducing microbial spoilage of food products (Gould, 1996). Reducing agents often used by the food processing industry for food preservation are ascorbic acid or the erythrostate isomer of ascorbic acid in a solution with concentrations of 1  $\mu$ M. Common edible coatings currently in use for successful preservation of food by the food industry include sodium caseinate and stearic acid (Olivas & Barbosa-Cánovas, 2005). Typical examples of antimicrobial compounds include lactoperoxidase (milk), lysozyme (egg white, figs), saponins and flavonoids (herbs and spices), bacteriocins (lactic acid bacteria) and chitosan (shrimp shells) which exhibit substantial antimicrobial characteristics in both solid and liquid media (Devlieghere *et al.*, 2004). Determination of suitable compound for preservation of a particular food is dependent on the nature of the food (Devlieghere *et al.*, 2002).

#### **2.5.2.5 Modified atmosphere packaging**

Modified atmosphere (MA) can be simply defined as atmospheres that differ in composition from that of normal air which is generated passively or actively. In the food industry, food products are packed in film bags or plastic containers with modified atmosphere within the package for preservation purposes. The modified atmosphere packaging (MAP) technique achieves its goal of food preservation by means of modifying the atmosphere surrounding a food product via gas flushing, vacuum or controlled permeability of the package. This MA in turn controls the enzymatic, biochemical and microbial actions of the food product decreasing degradations or decay

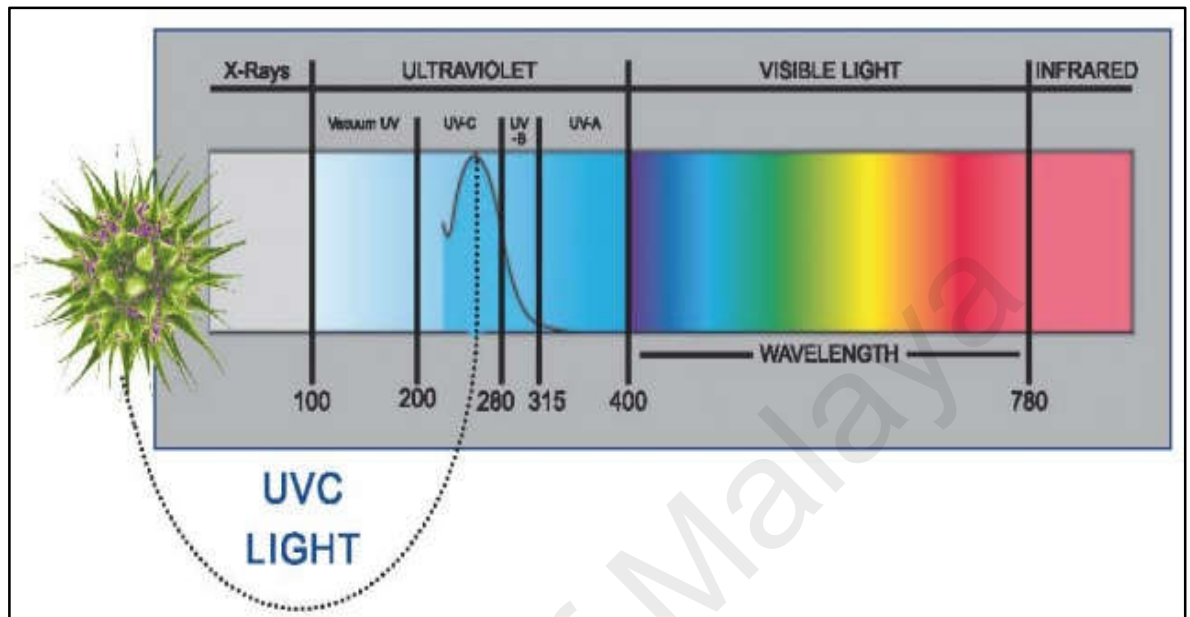
that might occur. A passive modified atmosphere is achieved by the respiration of the food product (minimally processed fruits for example) within the package combined with the permeability of the package (Church & Parsons, 1995).

An active atmosphere, on the other hand, can be achieved by creating an initial atmosphere through flushing with a particular gas or mixture of gases; or by the additives such as oxygen scavengers into the packaging. Low oxygen and high CO<sub>2</sub> concentrations are the general MA composition which serves to reduce ethylene production and respiration rates in fresh products like minimally processed fruits and vegetables, as well as to inhibit microbial growth and retard enzymatic activity. The success of a MAP design is dependent on the respiration rate and respiration quotient (Al-Ati & Hotchkiss, 2002). Furthermore, the knowledge of produce respiration characteristics, headspace volumes as well as the choice of appropriate polymer films with suitable permeability and appropriate film area is essential for successful design of MAP. MAP has been reported to be successful in the preservation of food products over the years (El-Kazzaz *et al.*, 1983; Carlin *et al.*, 1990; Beaudry, 1993)

#### **2.5.2.6 Ultraviolet-C light treatment**

Ultraviolet light or UV light is a type of radiation that is invisible to human eye. UV light is an invisible part of the electromagnetic spectrum which can be classified into four wavelength ranges: UV-A (315 to 400 nm), UV-B (280 to 315 nm), UV-C (200 – 280) and vacuum-UV (100 to 200 nm) (Figure 2.6). These different ranges of UV light function differently from one another. UV-A causes changes in the human skin which is often referred to as tanning, UV-B on the other hand causes skin burning which may lead to skin cancer, UV-C exhibits germicidal properties with a peak germicidal effect being achieved at the wavelength of 254 nm, inactivating microorganisms while the

vacuum-UV which can only be transmitted in a vacuum is absorbed by almost any substance (Gomez-Lopez *et al.*, 2012; Ribeiro *et al.*, 2012).



**Figure 2.6: The electromagnetic spectrum**

(Source: agtuv.com, 2014)

UV-C light has the capacity to exhibit germicidal effects on microorganisms such as viruses, bacteria, yeasts and moulds by causing damage to the microorganism's nucleic acid by forming covalent bonds between certain adjacent bases in the DNA (Tran & Farid, 2004). These bonds in turn prevent the DNA from being unzipped for replication rendering the organism unable to reproduce and could even cause death of the organism when it attempts to replicate (Guerrero-Beltran & Barbosa-Canovas, 2004). Viruses and bacteria are more susceptible to UV-C irradiation compared to yeast and mould due to their considerable non-complex structure. The passage of UV-C light through microorganism is also perceived to be influenced by cell wall thickness and size of a particular microorganism (Miller *et al.*, 1999). According to Miller *et al.*, (1999) yeast and mould have higher resistance to UV-C light due to their DNA strand containing lesser pyrimidine bases making it less likely for formation of cross link.

The use of UV-C light for its germicidal effects has been observed in disinfection of air, sterilization of liquids, as well as to combat spoilage caused by enzymatic and microbial activity in a variety of food commodities which include milk, fresh-cut fruits and vegetables, fruit juices, ciders, eggs and other fresh products. Some of the key advantages of UV-C treatment for food preservation include low maintenance, simple and cost effective (Falguera *et al.*, 2011; Guerrero-Beltran & Barbosa-Canovas, 2004). The potential application of this treatment as a preservation technique in the food industry has been halted by consumers' misconception about this processing technology. In fact, the use of UV-C radiation does not leave chemical residues as compared to other physical treatments such as chemical treatments (Yaun *et al.*, 2004). The consumption of UV-C irradiated food products by humans is therefore safe (Environmental Protection Agency, 1999).

There are several sources of UV light such as the continuous UV low-pressure and medium-pressure mercury lamps, excimer and broadband pulse lamps. Out of these, the use of low pressure mercury lamp is approved by the FDA for food preservation by the food industry (Koutchma *et al.*, 2009). The effectiveness of UV-C processing is determined by factors such as the absorbance of medium, thickness of medium, exposure time and the UV dose delivered. Thus, UV-C processing of different food commodities has to be optimized accordingly for successful preservation of the respective commodities (Guerrero-Beltran & Barbosa-Canovas, 2004; Koutchma *et al.*, 2004).

The use of UV-C treatment has been reported to be successful in both solid and liquid food commodities. A successful reduction of microbial count has been reported in apple and orange juice (Walkling-Ribeiro *et al.*, 2008; Pala & Toklucu, 2013). UV-C

treatment was also reported to reduce postharvest decay in onions, sweet potatoes, carrots, tomatoes, strawberry, apples, peaches, lemon fruits and table grape (Lu *et al.*, 1987; Stevens *et al.*, 1999; Mercier & Arul, 1993; Liu *et al.*, 1993b; Maharaj, 1995; Marquenie *et al.*, 2002; Wilson *et al.*, 1997; Stevens *et al.*, 1998; Ben-Yehoshua *et al.*, 1992). Currently, there has also been a rise in the use of UV-C treatment for preservation of fresh produce. Successful shelf-life extension has been reported in fresh-cut commodities such as melons, tomatoes, lettuce, watermelon and apple (Manzocco, *et al.*, 2011; Antunes *et al.*, 2013; Allende & Artes, 2003; Fonseca & Rushing, 2006; Gómez *et al.*, 2010).

## **2.6 Food Allergy**

Another issue that is related to food safety includes food allergy. Food allergy has become a concern amongst mankind in the recent years. Individuals allergic to certain foods have to practice abstinence towards these foods. No treatment or cure is currently available for food allergies and hence, the only means to remain symptom free is to avoid consumption of foods that might trigger an allergic reaction. This brings about the burden of reading ingredient labels on every packaged food product purchased to ensure safety. Although this might be a demanding and time consuming task, serious attention has to be given to this issue as eating certain foods can be life threatening to those with food allergies (National Institute of Allergy and Infectious Diseases, 2010).

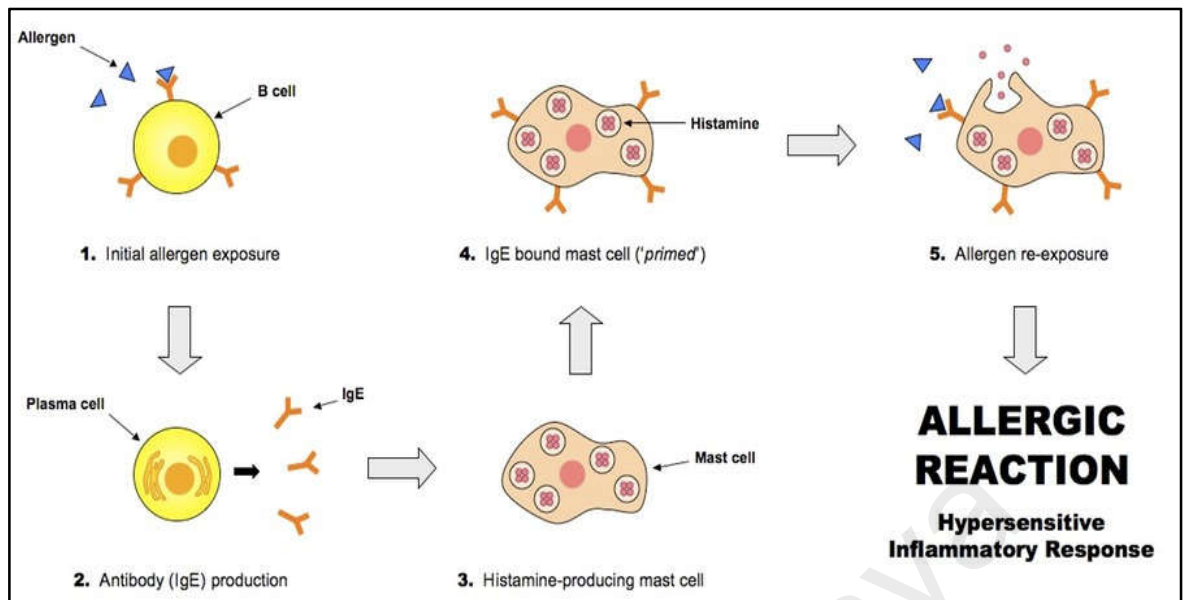
Food allergies are a significant public health issue worldwide and concerns about this form of allergy is ever growing. Food allergy in itself can be caused by many different forms of food which affect different age groups in different ways. The most common food allergies are to eggs (mostly in children), fish (older children and

adults), milk (mostly in children), peanuts (people of all ages), shellfish (people of all ages), Soy (mostly in children), tree nuts (people of all ages) and wheat (mostly in children). These foods, often referred to as the “Big 8”, are responsible for vast majority of IgE-mediated food allergies worldwide (FAO, 1995). Unlike the “big 8”, fruit and vegetable allergy is generally more common in older children and young adults. Most people with a fruit or vegetable allergy tend to continue to be allergic as they grow older. An adverse reaction to food may or may not be due to an allergic reaction. These responses are referred to simply as food sensitivities (Taylor & Hefle, 2002).

### **2.6.1 Food hypersensitivity**

Food hypersensitivity is defined as “an abnormal immunological response to a particular food or food component, usually a naturally occurring protein” and has been viewed as an emerging public health problem (Bush & Hefle, 1996; Taylor & Hefle, 2002). The most important mechanism involved in food hypersensitivities is the IgE-mediated hypersensitivity (immediate hypersensitivity reaction) which specifically recognizes certain allergenic proteins in foods followed by the cell-mediated hypersensitivity (delayed hypersensitivity reaction) which requires an interaction between a particular food antigen and sensitized T lymphocytes (Taylor & Hefle, 2006).





**Figure 2.7: Overview of an allergic reaction (Hypersensitive inflammatory response)**

(Source: Taylor & Hefle, 2006)

IgE antibodies play a primary biological role in protective immunity, principally in response to parasitic infection. However, in atopic individuals, non-parasitic, innocuous, antigens are capable of invigorating inappropriate IgE responses which is often referred to as Type I hypersensitivity reactions (Goldsby *et al.*, 2003). A food allergy that is mediated by the antibody (IgE) is referred to as a true food allergy. Symptoms of an IgE mediated hypersensitivity often develop shortly after the exposure to the offending food which ranges from mild to severe, and may include skin rashes, hives, itching, swelling of the lips, throat, and other body parts, as well as, nasal congestion, trouble breathing, diarrhoea, vomiting, dizziness, and fainting. In addition severe reactions to foods could lead to anaphylactic shocks (FDA, 2009).

## 2.6.2 Allergens in Fruits

Fruit allergy is a form of food allergy that makes up 10 % all food related allergies. Allergic reactions to fruits are often mild and localized at the mouth causing hives, itch

and swelling. This form of manifestation in food allergy is referred to as an oral allergy syndrome and is most frequently associated with consumption of fresh fruits and vegetables (Pastorello & Ortolani, 1997). It is common for an individual with oral allergy syndrome to be sensitized to one or more pollens and experience adverse reactions to proteins in specific fresh fruits and vegetables that cross-react with the pollen allergens (Calkoven *et al.*, 1987; Ebner *et al.*, 1995; van Ree & Aalberse, 1993). The offending particle in fruits that is responsible for causing an allergic reaction are referred to as an allergen which is almost always a protein (Calkoven *et al.*, 1987).

A considerable amount of fruit allergies are attributed to the proteins called profilins. Profilins can be found in pollens of trees, weed and grass as well as in many fruits and vegetables which is why the incidence of cross reactions between a pollen and fruit allergy is common. Allergic reactions following consumption of fruits and vegetables are more commonly related to symptoms of an Oral Allergy syndrome (British Allergy Foundation, 2014). Some of the fruits and vegetables that have been observed to result in an oral allergy syndrome in susceptible individuals are represented by Table 2.3.

**Table 2.3: Fruit and vegetables commonly involved in Oral Allergy Syndrome.**

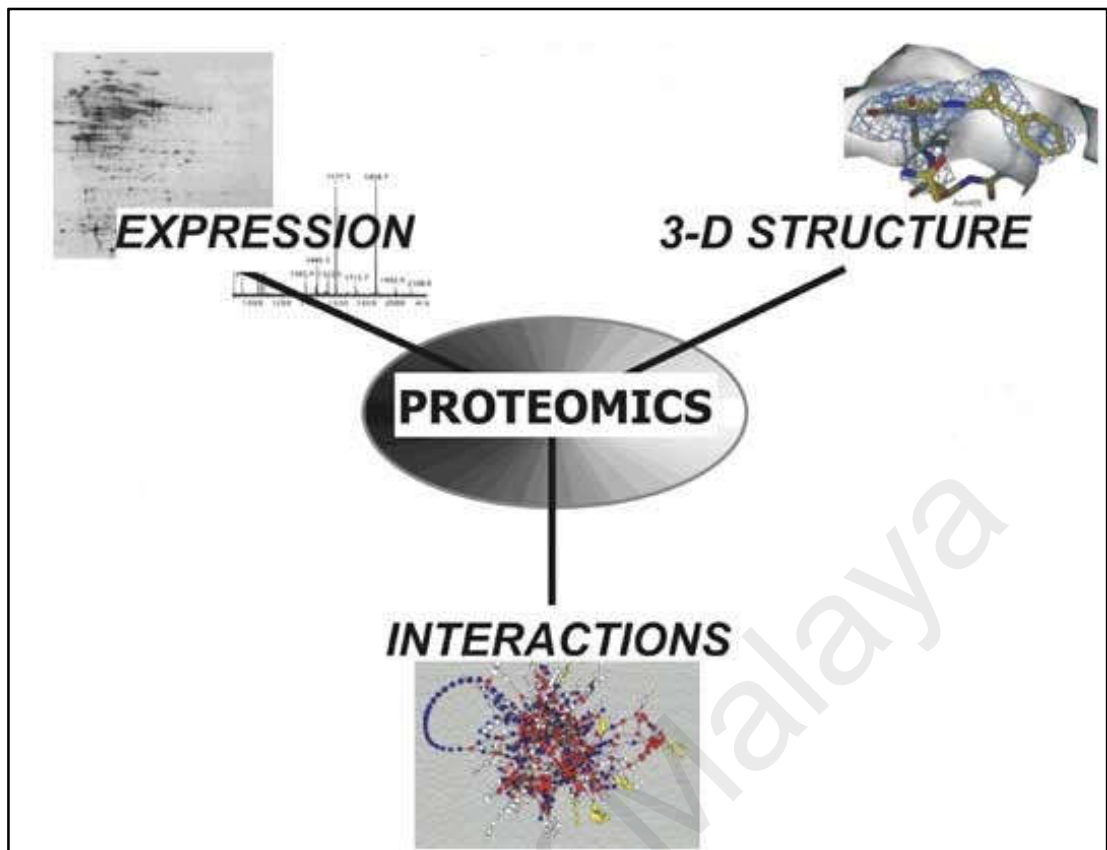
(Source: British Allergy Foundation, 2014)

Fruits	Vegetables
<p>Apple, Peach Pear, Plum, Cherry, Nectarine, Apricot, Kiwi, Strawberry, Orange, Melon, Watermelon, Mango and Pineapple</p>	<p>Carrot, Celery, Potatoes, Tomatoes, Swiss chard, Beans and peas, Mange tout, Bean sprouts, Parsley, Fennel, Cucumber, Peppers and Courgettes</p>

## 2.7 Proteomics

Proteomics is a field of molecular biology which comprises of the study of proteins with regards to their functions and structure (Anderson & Anderson, 1998). Proteins as we know it are integral parts of living organisms whereby they facilitate metabolic pathways of cells in organisms (James, 1997). Exemplary proteomic studies involve evaluating the differential expression of proteins as affected by different conditions. The first proteomic studies was recorded in 1975 via the mapping of proteins from *E. coli* (O'Farrell, 1975), guinea pig (Scheele, 1975) and mouse (Klose, 1975) with the introduction of two-dimensional gel electrophoresis technology. Proteomic studies can be divided into 3 groups which are structural proteomics, expression proteomics and functional proteomics (Graves & Haystead, 2002).

Expression proteomics involves quantitative evaluation of protein expression between samples with differing variables. This branch of proteomics allows the discrimination of proteins or groups of proteins that are affected by a particular condition. Expression proteomics involves technologies such as 2 dimensional electrophoresis and mass spectrometry. Structural proteomics involves mapping out the structure of protein complexes as well as specific cellular organelle which is useful in identification of the functions of new genes, identifying binding sites of drugs on proteins as well as protein interactions with each other. Technologies related to structural proteomics include nuclear magnetic resonance spectroscopy and x-ray crystallography. Finally, functional proteomics involves the determination of protein functions via protein-protein interaction characterization. Functional proteomics is also used as a tool to demonstrate protein assembly in larger complexes (Graves & Haystead, 2002). The major directions of proteomics are illustrated by Figure 2.8.



**Figure 2.8: Major proteomics directions.**

(Source: Malmstrom *et al.*, 2007)

### 2.7.1 Role of Proteomics in Food Technology

Food products are complex biological mixtures which are host to numerous proteins playing various roles in different biological processes. Food processing may bring about changes in these proteins resulting in changes in different attributes of the food such as overall quality and safety. Proteomics technology is beneficial for studies related to improvement of food processing techniques as it permits observation of the protein composition and changes in a particular food during the food production process. The use of technologies such as two dimensional electrophoresis coupled with mass spectrometry allows such observation. In food technology, the use of proteomics can be predominantly seen in optimization of processing techniques and assessment of quality of the final product. Proteomics is also beneficial for determination of food safety with

regards to biological and microbial safety as well as the safety of genetically modified foods (Gasó-Sokac *et al.*, 2010).

Food products undergo both desirable and undesirable changes in characteristics during the manufacturing process and proteins are predominantly responsible for these characteristics. For instance, the protein composition and content has been associated with the physicochemical properties, which include thermal conductivity and vapour pressure, viscosity, sensory properties as well as nutritional properties of food products such as milk and cereal based products. Several proteins have been identified to play important roles in optimal characteristics of different food products. Optimal characteristics in wheat flour derived products have been reported to be determined by gluten proteins while casein was found to be vital for optimal characteristics in milk products. However, foods such as meat, fruits and vegetables are known to bear a more complex protein composition. Physicochemical properties during processing of such food commodities therefore may be dependent on one or more highly abundant protein (Lametsch & Bendixen, 2002; Willis, 2007). Proteomic studies have reported that organoleptic characteristics such as texture are highly associated to structural proteins such as actin, desmin, tubulin and myosin (Zapata, 2009) while colour in meat colour development are associated with oxidative metabolism enzymes (Sayd, 2006). The effects of food processing on the quality of food products which include colour, texture and nutritional value can be as a result of protein degradation and a series of complex chemical reaction known as the Maillard reaction (Zhang *et al.*, 2009). Proteomic analysis therefore acts as a tool in aiding the optimization of food processing and validation of its products (Gasó-Sokac *et al.*, 2010).

Proteomics has also been used extensively in the detection of allergens for food safety and is referred to as ‘allergenomics’ (Yagami *et al.*, 2004). Allergen detection in food through proteomics involves analysis of proteomes through the means of 2D electrophoresis, immune blotting and high-resolution mass spectrometry (Chassaigne *et al.*, 2009). Successful detection of allergens via analysis of plant proteome has been reported in maize (Fasoli *et al.*, 2009), sesame seeds (Navuluri *et al.*, 2006) and wheat flour (Asero, 2005). Furthermore, quantitative proteomic analysis has also been used to observe the difference in the expression of allergens in different conditions. For example, through proteomic analysis, a reduced expression of peanut allergen was observed in genetically engineered peanut (Chassaigne *et al.*, 2009).

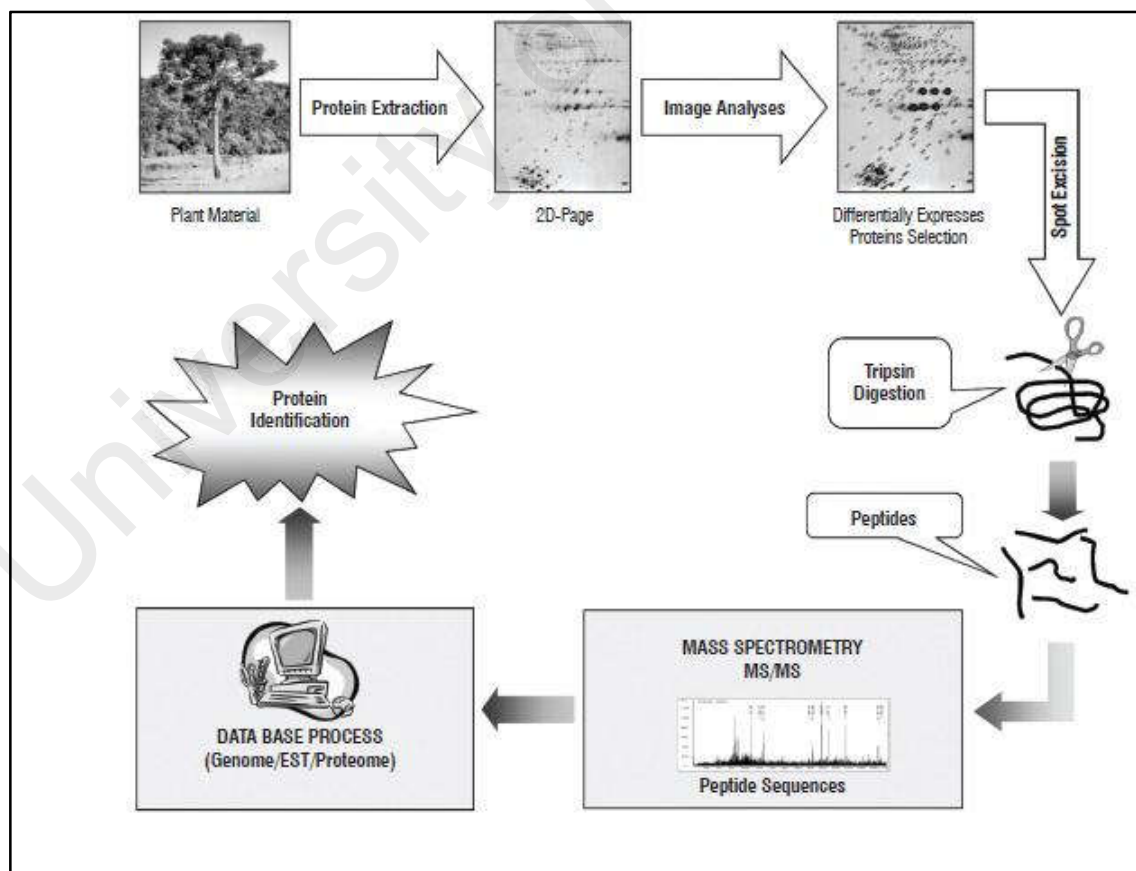
Currently, the use of proteomic techniques in food technology and biotechnology has been on the rise for development and optimization of new processes as well as for evaluation of raw materials, quality control and safety evaluation of final products. Recent advances of proteomics could be exploited for the greater good of food science in detection of food contaminants and allergens as well as the safety of genetically modified foods (Carbonaro, 2004).

### **2.7.2 Technologies Utilized in Proteomics**

Different technologies, from fields such as molecular biology, biochemistry, physiology, statistics and bioinformatics among others, working in tandem are required to properly conduct a proteomic study. For these studies, the crucial steps are the separation and identification of complex protein mixtures. Separation of complex mixtures is often performed via electrophoresis. The term electrophoresis was coined by Michaelis in 1990 and was performed for the first time in 1937. Electrophoresis achieves separation of molecules through separation based on their molecular weight,

conformation and electrical charges in a porous matrix and appropriate buffers under a continuous electric field (Alfenas, 1998).

Of the technologies currently in use, many were established before the rise of proteomics. A clear example of this is electrophoresis. Nonetheless, the advancement of protein sequencing technology through mass spectrometry heralded the emergence and development of proteomics (Tyers & Mann, 2003). Proteomics is studied using techniques such as two-dimensional electrophoresis in polyacrylamide gel (2D PAGE) followed by mass spectrometry (MS) (Figure 2.9). Recently, newer methods, such as the association of ionization and chromatographic methods have been developed to increase detection sensitivity (Anderson & Anderson, 1996). A typical proteomic study using different technologies is as illustrated below:



**Figure 2.9: Processes involved in proteomics studies.**

(Source: Gris and Baldoni, 2013)

The competence of the use of proteomics as a reliable tool for identification of important proteins as well as their functionality and the roles that they play in various biological processes cannot be over stated (Palma *et al.*, 2011). Differential proteomics permits one to discriminate the specific protein or proteins affected in certain physiological condition (Nogueira *et al.*, 2012). The use of proteomics has been documented in those that include the study of fruit development and ripening, stress resistance, differences between cultivars, postharvest conditions and physiological disorders (Faurobert *et al.*, 2007; Rocco *et al.*, 2006; Lee *et al.*, 2009a; Yun *et al.*, 2010; Pedreschi *et al.*, 2009).

University of Malaya



## CHAPTER 3: EFFECTS OF UV-C TREATMENT ON THE PROTEOME OF CHOKANAN MANGO (*Mangifera indica* L. cv. Chokanan) AND JOSEPHINE PINEAPPLE (*Ananas comosus* L. Merr. Josephine)

### 3.1 Introduction

Ultraviolet-C light treatment, which is free from chemical residues, has emerged as an effective postharvest treatment and is more feasible for commercial application as a postharvest treatment of fruits. Based on previous reports, UV-C treatments have been used for maintaining the quality as well as to reduce postharvest losses in fruits (Civello *et al.*, 2006; Shama, 2007). Fruits that were exposed to UV-C were found to exhibit delayed ripening (Liu *et al.*, 1993b), senescence (Costa *et al.*, 2006; Allende *et al.*, 2006) as well as reduced spoilage (Stevens *et al.*, 1998; Baka *et al.*, 1999). Several researches have reported on the immediate effects of UV-C treatment on the quality of fruits while the underlying changes at a proteomic level that occurs as a result of UV-C irradiation remains unclear (Shen *et al.*, 2013; Li *et al.*, 2014; Vincente *et al.*, 2005; Rodoni *et al.*, 2012; Bal, 2012).

To date, publications on the global analysis of the effect of postharvest UV-C on fruits like mango and pineapple quality deterioration remains scarce and hence, detailed molecular information at the protein regulation level following postharvest UV-C treatment of fruits remains unclear. Proteomics has emerged as a highly reliable tool for the screening of a large set of proteins which eventually enables the identification of important proteins as well as their functionality and the roles that they play in various biological processes (Palma *et al.*, 2011). This leads to an enhanced understanding of the molecular basis of fruit quality and facilitates the improvement of postharvest treatments for the extension of shelf-life. Despite the complexity of an organism's proteome, the use of differential proteomics enables one to discriminate the specific

proteins affected in a certain physiological condition (Nogueira *et al.*, 2012). To date, proteomics has been exploited to study fruit development and ripening, differences between cultivars, stress resistance, postharvest conditions and physiological disorders (Faurobert *et al.*, 2007; Rocco *et al.*, 2006; Lee *et al.*, 2009a; Yun *et al.*, 2010; Pedreschi *et al.*, 2009). In this study, two-dimensional electrophoresis and mass spectrometry were carried out to evaluate the effects of UV-C treatment on the proteome of mangoes and pineapples.

Two-dimensional gel electrophoresis or 2-DE is a method that has been extensively used for the analysis of complex protein mixtures extracted from various biological samples. It is a two step technique used to separate proteins according to two independent properties. It separates proteins according to their isoelectric points (pI) in the first-dimension which is known as isoelectric focusing (IEF) and in the second-dimension, proteins are further separated according to their molecular weights (MW) which is known as SDS-polyacrylamide gel electrophoresis (SDS-PAGE). In this way, complex mixtures consisting of thousands of different proteins can be resolved in the form of protein spots and the relative amount of each protein can be determined (Haleem *et al.*, 2007). Individual protein spots can then be identified via mass spectrometry.

Mass spectrometry is a method that is used for the characterization of proteins. Currently, there are two primary methods that can be used for ionization of whole proteins which include the matrix-assisted laser desorption/ionization (MALDI) as well as the electrospray ionization (ESI). The protein identification process involves, first: the enzymatic digestion of proteins into smaller peptides using a protease such as trypsin, followed by the introduction of these peptides into the mass spectrometer and

identification via peptide mass fingerprinting or tandem mass spectrometry (Sunia *et al.*, 2002). The identification of individual proteins allows identification of their functionality and the roles that they play in various biological processes.

## **3.2 Materials and Methods**

### **3.2.1 Sample Preparation**

Mature mango (*Mangifera indica* L. cv. Chokanan) and pineapple (*Ananas comosus* L. Merr. Josephine) fruits free from external defects were harvested from a mango plantation in Kangar, Perlis and a pineapple plantation in Batu Pahat, Johor. The fruits were rinsed under running water, dipped in a 5% benomyl solution for 1 minute, rinsed again with water, air dried and left to ripen at room temperature ( $25 \pm 2$  °C) in a respiration chamber aerated humidified air (95% relative humidity) at 0.5 L per hour 9 l/h). Fruits were peeled, cleaned and thinly sliced (5 mm) with the aid of a dial thickness gauge (Mitutoyo MI 7305, Kawasaki, Japan). The fruits were cut into uniform discs (diameter of 2 cm) with a borer (Figure 3.1 and 3.2). The uniform sample discs were used for subsequent food processing.



**Figure 3.1: Chokanan mango sample discs placed on petri dishes for treatment.**



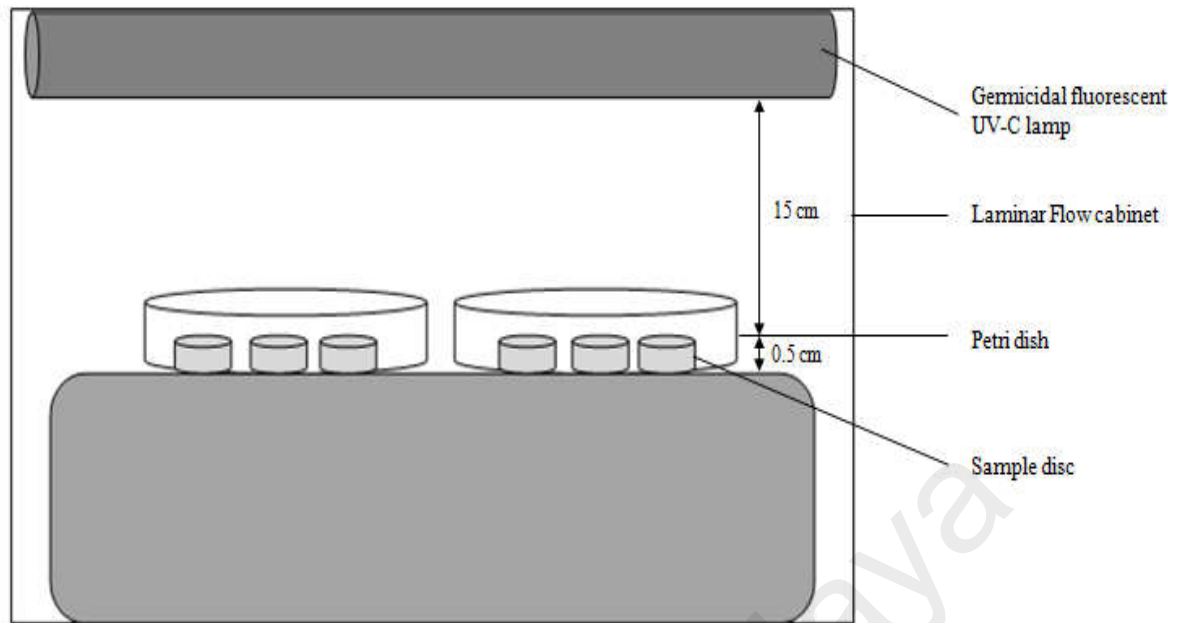
**Figure 3.2: Josephine pineapple sample discs placed on petri dishes for treatment.**

### 3.2.2 UV-C Treatment

Eleven to twelve fruit disc samples were placed on sterilized Petri dishes (15 x 100 mm; 10 mm depth) and exposed to a UV light (germicidal fluorescent lamp with a peak emission of 254 nm, Biological Safety Cabinet Class II, 240 V, 50 Hz, 10A) (Figure 3.3). The samples were irradiated for 15, 30 and 60 minutes. The UV lamp was situated 15 cm above the surface of the fruit discs during the experiment; it was turned on 30 min prior to the treatment to stabilize the emission. The mean of UV radiation dose received by each sample disc is 3.525 J/m<sup>2</sup> (digital radiometer) (Keyser *et al.*, 2008). Sample discs not exposed to UV-C radiation were treated as controls. Nine biological replicates (n=9) were used for each of the different durations of UV treatment.



**Figure 3.3: UV-C lamp in a laminar flow.**



**Figure 3.4: Schematic diagram of UV-C treatment of sample discs.**

### 3.2.3 Extraction of Total Protein

Treated and non-treated mango and pineapple (Section 3.2.2) were ground to a fine powder in liquid nitrogen with a mortar and pestle. Total proteins were extracted by adding 6 g of pulp powder to 8 mL of a modified Kanellis *et al.* (1989) protein extraction buffer containing 50 mM Tris-HCl, 0.5 M NaCl, 10 mM 2-mercaptoethanol, 10  $\mu$ M leupeptin, 1 mM DTT, 1 mM EDTA, 10 % glycerol and 0.5 % triton X-100 (tissue to volume ratio = 3:4). The mixture was then vortexed thoroughly, left on ice for 10 minutes and then centrifuged at 25,000 x g for 30 minutes at 4 °C in a Sorvall RC5C refrigerated centrifuge. Upon centrifugation, the pellet was discarded and the supernatant was collected by filtering through one layer of miracloth and saved as crude protein extracts.

### **3.2.4 Total Protein Estimation**

#### **3.2.4.1 Preparation of reagent**

##### Bradford reagent

*20 mg Coomassie Blue G250 (Nalgene) was dissolved in 10 ml of SDW. 10 ml of 95% ethanol and 20 ml of 85% phosphoric acid were added and made up to 200 ml with SDW. The solution was filtered with Whatman filter paper (No. 1) and stored in an amber schott bottle at 4°C.*

##### Bovine serum albumin stock solution

*10 mg of bovine serum albumin was dissolved in 10 ml of SDW.*

#### **3.2.4.2 Protein estimation**

The protein concentration in the pulp extracts was estimated using the Bradford (1976) method with Bovine Serum Albumin (BSA) as a standard. Varying amounts of BSA solution ranging from 10-100 µg (table 1) were pipetted into individual test tubes to set up three replicates each of five different protein standards. SDW was pipetted into each test tube to make up to a final volume of 100 µL for all standards. Samples were diluted by a factor of 2 by diluting 50 µL of samples with 50 µL of SDW. Protein samples were substituted with SDW for blank. 5 ml of Bradford reagent was pipetted into each test tube and mixed using a vortex mixer. Absorbance was measured at 595 nm using blank as reference. An absorbance vs µg of protein curve was plotted for each amount of BSA. The amounts of total protein in each sample unknowns were determined from the graph.

**Table 3.1: Typical amounts of BSA for standard curve.**

$\mu\text{L}$ protein (BSA)	$\mu\text{L}$ BSA solution	$\mu\text{L}$ SDW
20	20	80
40	40	60
60	60	40
80	80	20
100	100	0

### **3.2.5 Protein Extraction for Two Dimensional Gel Electrophoresis (2-DE)**

#### **3.2.5.1 Preparation of reagents**

##### Buffered Phenol

*100 ml of phenol solution was placed into a schott amber bottle and heated in a water bath at 65 °C. An equal volume of 0.5 M Tris·Cl (pH 8.0) was added to the phenol and mixed for 15 minutes. The mixture was then returned to the 65 °C water bath and the two phases were allowed to separate. The top layer of the two phases was siphoned off and discarded. The procedure as stated above was repeated twice. An equal volume of 0.1 M Tris·Cl (pH 8.0) was then added to the phenol and mixed for 15 minutes, returned to the 65 °C water bath and the phases were allowed to separate. The top layer was siphoned off and discarded. The extractions with 0.1 M Tris·Cl (pH 8.0) was repeated until the aqueous phase was ~pH 7.8. A 1 cm layer of 0.1 M Tris·Cl (pH 8.0) was left over the phenol. The buffer saturated phenol was stored at 4 °C for protein extraction.*

##### 1 M Potassium chloride (KCl)

*3.775 g of potassium chloride was dissolved in 20 ml of SDW. The solution was made up to 50 ml with SDW.*

##### 10% dithiothreitol (DTT)

*1 g of dithiothreitol was dissolved in 500  $\mu\text{l}$  of SDW. The solution was made up to 1 ml with SDW.*

##### 100mM ammonium acetate in methanol

*0.385 g of ammonium acetate was dissolved in 40 ml of methanol. The solution was made up to 50 ml with methanol.*



#### 0.5 M EDTA pH 8.0

*186.1 g of disodium ethylenediamine tetraacetate•2H<sub>2</sub>O was dissolved in 800 ml of SDW. The pH of the solution was adjusted to 8 with NaOH and made up to 1 L with SDW. The solution was filtered through a 0.5 micron filter and autoclaved.*

#### 1 M Tris-HCl pH 8.3

*6.055 g of tris-base was dissolved in 40 ml of SDW and the pH adjusted to 8.3 with concentrated HCl. The solution was made up to a final volume of 50ml with SDW.*

#### Rinsing solution

*1 g of dithiothreitol was dissolved in 1.8 ml of SDW and made up to 50 ml with acetone. The solution was stored at -20°C until used.*

#### Leupeptin working solution

*0.95 mg of leupeptin hemisulfate salt was dissolved in SDW and made up to 2ml with SDW. The stock solution was stored at -20°C until used.*

#### Extraction buffer

*1ml of 1M Tris-Hcl pH 8.3 stock solution, 100 µL of 0.5 M EDTA stock solution, 1 ml of 1M KCl, 1 ml of 10% DTT solution, 3 grams of sucrose and 0.1 ml of leupeptin working solution were added to a falcon tube and made up to 10 ml with SDW.*

#### Lysis buffer

*4.2 grams of urea and 1.5 grams of thiourea was dissolved in 8 ml of SDW and made up to 9.5 ml with SDW. 400 mg of CHAPS was then dissolved in the solution. The solution was stored as aliquots of 750 µL at -20°C. Prior to use, 8.3µL of IPG buffer pH 4-7 and 83 µL of 10% DTT solution was added to the lysis buffer.*

### **3.2.5.2 Protein extraction for 2-DE**

One gram of fresh sample tissue was transferred to a cooled mortar and pestle and the sample was ground to a fine powder with liquid nitrogen (N<sub>2</sub>). 150 mg of ground sample was transferred to a 2 ml eppendorf tube. 500 µL of extraction buffer was added and vortexed for 30 seconds. 500 µL of buffered phenol was then added and vortexed for a further 10 minutes at 4 °C. The sample mixture was then centrifuged for 3 minutes, 8000 rpm at 4°C and the phenolic phase (upper layer) was collected and

transferred to a new 2 ml eppendorf tube. 500  $\mu$ L of extraction buffer was added to the collected phenolic phase and vortexed briefly. The mixture was then centrifuged for 3 minutes, 8000 rpm at 4°C. The phenolic phase was once again collected and transferred into a new eppendorf tube and the proteins were precipitated overnight with 5 volumes of 100 mM ammonium acetate in methanol at -20 °C. Precipitated protein mixture was then centrifuged for 60 min, 13000 rpm, at 4°C. The supernatant was removed and the resulting pellet was rinsed in 2 ml of rinsing solution. The pellets were left in rinsing solution for 1 hour at -20 °C. After rinsing, the sample was centrifuged for 30 minutes, 13000 rpm at 4 °C. The supernatant was decanted and the pellet was rinsed again by adding 2 ml of rinsing solution and centrifuged for 30 minutes, 13000 rpm at 4 °C. The pellet was dried briefly before it was suspended in 100  $\mu$ L of freshly prepared lysis buffer. The samples were cleared by centrifugation for 30 minutes, 13000 rpm at 18 °C twice. The samples were transferred into new eppendorf tubes after each centrifugation. Clear supernatant were stored in aliquots at -80 °C. The protein concentration in the pulp extracts were quantified using the 2-D Quant Kit (GE Healthcare) according to manufactures protocol.

### **3.2.6 2-Dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (2D SDS-PAGE) Analysis**

#### **3.2.6.1 First dimension electrophoresis**

##### **(a) Preparation of reagents**

###### 1% bromophenol blue solution

*100 miligrams of bromophenol blue and 60 miligrams of Tris-base was dissolved in SDW and made up to 10 ml with SDW.*

## 1 M DTT

*1.55 grams of dithiothreitol was dissolved in SDW and made up to 10 ml with SDW.*

## Rehydration stock solution

*2.25 grams of urea, 0.762 grams of thiourea, 25 milligrams of CHAPS were dissolved in 3 ml of SDW. 0.5 ml of glycerol and 10  $\mu$ L of 1% bromophenol blue were added and the solution was made up to 5 ml with SDW.*

### **(b) Rehydration of isoelectrofocusing (IEF) strips**

The strip holder was washed prior to use with ddH<sub>2</sub>O followed adding a few drops of Ettan IPGphor Strip Holder Cleaning Solution, vigorous agitation with a tooth brush, rinsed again with ddH<sub>2</sub>O and thoroughly dried with lint-free tissue. Fresh rehydration buffer was prepared by thawing 3402  $\mu$ L of rehydration stock solution, adding 180  $\mu$ L of 1M DTT solution and 18  $\mu$ L of IPG buffer pH 4-7 and mixed well. 450  $\mu$ L of rehydration buffer was pipetted in to each channel. The protective cover was removed from the immobiline drystrip gel starting at the acidic (+) end. The strips were positioned into the channels containing rehydration buffer with the gel side down. Air bubbles were removed under the immobiline drystrip gels before each strip was overlaid with 3 ml of immobiline drystrip cover fluid to minimize evaporation and urea crystallization. The strips were allowed to rehydrate for a minimum of 12 hours.

### **(c) Isoelectrofocusing (IEF)**

Isoelectrofocusing was performed on an Ettan IPGphor 3 IEF system. Rehydrated strips were removed from strip holder and placed onto ceramic isoelectrofocusing tray with the gel side facing up and the acidic (+) and basic (-) ends of the gel corresponding to that of the IEF system. The ceramic tray was overlaid with 108 ml of immobiline drystrip cover fluid. Paper wicks were dampened with 150  $\mu$ L of ddH<sub>2</sub>O before it was placed on acidic and basic ends of each strip. The paper wicks were positioned on each

end of the IPG strips so that one end of the wick overlaps the end of the gel on the IPG strip. Electrodes were then assembled onto the system so that the electrodes are in contact with the wicks. Sample loading cups were then assembled on the ceramic plate closer to the anode electrode for sample loading. 35  $\mu\text{g}$  of each samples were loaded onto each drystrip. The different volumes of each samples corresponding to 35  $\mu\text{g}$  of protein were diluted with sample loading buffer (rehydration buffer) to a final volume of 150  $\mu\text{L}$ . The first dimension was run under the following running conditions:

**Table 3.2: Running conditions of 2D electrophoresis.**

Step voltage mode	Voltage (V)	Time (h:min)	kVh
1 step and hold	300	0:30	0.2
2 gradient	1000	0:30	0.3
3 gradient	5000	1:30	4.5
4 step and hold	5000	0:12-0:36	1.0-3.0
<b>Total</b>		2:42-3:06	6.0-8.0

### 3.2.6.2 Second dimension SDS-PAGE in ExcelGel gradient gels

#### (a) *Preparation of reagents*

##### 10% SDS stock solution

*10 grams of SDS (sigma) was dissolved in 80ml of SDW and made up to 100 ml with SDW.*

##### 1.5M Tris-HCl pH 8.8

*27.23 grams of Tris base was dissolved in 80 ml of SDW and the pH was adjusted to pH 8.8 with concentrated HCl. The solution was made up to 150 with SDW.*

##### SDS Equilibration buffer solution

*72.07 grams of urea was dissolved in 80 ml of SDW. 60 ml of glycerol, 40 ml of 10% SDS, 400  $\mu\text{L}$  of 1% bromophenol blue and 6.7 ml of Tris-HCl 1.5M pH 8.8 was added and the solution was made up to a final volume of 200 ml. The solution was dispensed, 40 ml per tube into falcon tubes and stored at -20 °C until use.*

### 10 X Laemmli SDS electrophoresis buffer

30.3 grams of Tris base, 144 grams of glycine, 10 grams of SDS were dissolved in 700 ml of SDW and the final volume was made up to 1 L with SDW.

### 10% Ammonium persulfate (APS)

0.3 grams ammonium persulfate was dissolved in 2 ml of SDW and made up to 3 ml with SDW.

### **(b) Casting of 12.5% resolving gel**

Polyacrylamide gels were casted on the same day that the isoelectrofocusing procedure was carried out and allowed to solidify overnight. Preparation of the gel mixture is as the following:

**Table 3.3: Gel mixture for 2-DE.**

Solution Component	Amount
Deionized water	143.1 ml
30% Acrylamide solution	112.5 ml
1.5 M Tris-Hcl, pH 8.8	4.5 ml
10% SDS	187.425 ml
10% APS	2.25 ml
TEMED	225 $\mu$ L
Total	450 ml

Glass plates were cleaned and dried thoroughly before assembly. Glass plates were assembled and placed into the Ettan DALTsix gel caster. 400 ml of freshly prepared SDS gel solution was then poured into the gel caster to make 6 gels. Each plate was then overlaid with 2 ml water saturated isobutanol, covered with aluminium foil and allowed to solidify overnight.

**(c) *Equilibration of immobiline drystrip***

IPG strips were placed in individual tubes with the support film toward the tube wall. Prior to use, 400 milligrams of dithiothreitol was added to 40 ml of SDS equilibration buffer solution for the first equilibration buffer while 1800 milligrams of iodoacetamide (IAA) was added to 40 ml of SDS equilibration buffer solution to make the second equilibration buffer. 6.5 ml of first equilibration buffer was added to each strip and the tubes were sealed and placed on a rocker for the equilibration process. The first equilibration was carried out for 15 minutes. Following the first equilibration, the first equilibration buffer solution was removed and 6.5 ml of the second equilibration buffer was added and allowed to equilibrate for a further 15 minutes. Following second equilibration, the strips were washed in 3 X Laemmli buffer (refer section 4.4.4.3) and placed between glass plates and pushed down with a plastic ruler until the strips were in contact with the surface of the slab gel. Molecular weight was applied to a sample application piece and covered with 50 $\mu$ L of agarose sealing solution. The application piece was then placed next to the acidic end of the IPG strip. Agarose sealing solution was melted in a microwave and allowed to cool. The IPG strips were then sealed in place by slowly pipetting the solution across the length of the IPG strips taking care not to introduce or trap bubbles. Agarose sealing solution was allowed a minimum of 1 minute to cool and solidify before the plates were placed into the electrophoresis system.

**(d) *Second dimension SDS-PAGE***

Second dimension electrophoresis was performed using the Ettan DALTsix electrophoresis unit. From the 10 X Laemmli buffer, 3 X and 1 X buffers were prepared according to the table below.

**Table 3.4: Buffer dilutions for 2-DE.**

	Amount of 10 X Laemmli Buffer	Amount of SDW	Total
1 X Laemmli buffer	500 ml	4500 ml	5 L
3 X Laemmli buffer	375 ml	875 ml	1.25 L

The anode assembly was first inserted into the tank and the tank was filled with 4.0 litres of 1X Laemmli SDS buffer. The circulator pump and the MultiTemp III were turned on and the temperature was set to 15°C. Prepared gels were then inserted into the unit and the upper buffer chamber was carefully slid over the gels. 1 X Laemmli buffer was added until the max line. 1 litre of 3 X Laemmli buffer was then filled in the upper chamber, the safety lid placed on the unit. The electrophoresis conditions were 2.5 W/gel for 30 minutes then 100 W total until the run is completed.

### **3.2.7 Staining Procedure**

#### **3.2.7.1 Preparation of reagent.**

##### Fixing Solution (30% Ethanol)

*75 ml of ethanol, 25ml of glacial acetic acid were added to SDW and made up to 250ml with SDW.*

##### Sensitizing Solution

*75 ml of ethanol, 10 ml of sodium thiosulphate (5% w/v), 17 g of sodium acetate was added to SDW and made up to 250 ml with SDW. 1.25 ml of glutardialdehyde (25% w/v) before use.*

##### Silver Solution

*25 ml of silver nitrate solution (2.5% w/v) was added to DW and made up to 250 ml with SDW.*

##### Developing Solution

*6.25 g of sodium carbonate was added to SDW and made up to 250 ml with SDW. 0.2 ml of formaldehyde (37% w/v) before use.*

### Stop Solution

*3.65 g of EDTA-Na<sub>2</sub>·2H<sub>2</sub>O was added to SDW and made up to 250 ml with SDW.*

### Preserving Solution

*75 ml of ethanol and 11.5 ml of glycerol (87% w/w) was added to SDW and made up to 250 ml with SDW.*

#### **3.2.7.2 Staining procedure**

Upon completion of 2D electrophoresis, gels were removed from glass plates and soaked in fixing solution overnight in a polyethylene (P.E) tray. Fixing procedures were carried out with gentle shaking on an orbital shaker (Stuart Scientific S01). The fixing solution was then removed and 250 ml of sensitizing solution was added to each gel and left shaking for 60 minutes. Following sensitization of gels, the sensitizing solution was removed and the gels were washed four times in 250 ml of distilled water for 15 minutes each time. Silver solution was then added to each gels and left shaking for a further 60 minutes. The gels were washed again twice in distilled water for one minute each time. Developing solution was then added to one gel at a time and left shaking for 4 minutes for development of spots. The gel was then transferred to stopping solution to halt further development of spots. Developing procedure was carried out for the remaining gels. All gels were left shaking in stopping solution for 60 minutes. For the purpose of preserving the gels, the gels were transferred to preserving solution and left to shake for 60 minutes. Finally, each gel was placed between wet cellophane sheets and left to dry overnight at room temperature.

#### **3.2.8 Image Acquisition and Data Analysis**

The silver-stained 2-DE gels were imaged by an ImageScanner III via the software LabScan 6.0 (GE Healthcare), and analyzed on the ImageMaster 2-DE Platinum



software Version 7.0 (GE Healthcare). Images were properly cropped and optimized, and then gel-to-gel matching of the standard protein maps was performed. The spot detection parameters were optimized by checking different protein spots in certain regions of the gel and then automatically detected, followed by visual inspection for removal or addition of undetected spots. Spot detection was refined by manual spot edition when needed. The spots that were present on all gels of one treatment or control based on the image analysis were identified as expressed protein spots. The abundance of each protein spot was estimated by the percentage volume (vol.%), that is, the spot volumes were normalized as a percentage of the total volume in all the spots present in the gel to correct the variability because of loading, gel staining, and destaining. The percentage volumes were used to designate the significant differentially abundant spots (at least two-fold increase/decrease and statistically significant as calculated by one-way ANOVA,  $P < 0.05$ ). Nine gels were used for each sample and the SD was calculated. Only those with reproducible and significant changes were considered to be proteins with differential abundance.

### **3.2.9 Protein In-Gel Digestion**

Spots that showed statistically significant changes (at  $P < 0.05$ ) and above a 2-fold threshold were excised from the gels and washed with double-distilled water and then transferred to sterilized Eppendorf tubes. Then, the protein spots were washed with  $25 \text{ mmol L}^{-1} \text{ NH}_4\text{HCO}_3$ , followed by dehydration with 50% (v/v) acetonitrile (ACN) in  $25 \text{ mmol L}^{-1} \text{ NH}_4\text{HCO}_3$ . The proteins therein were then reduced with  $10 \text{ mmol L}^{-1} \text{ DTT}$  in  $50 \text{ mmol L}^{-1} \text{ NH}_4\text{HCO}_3$  for 1 h at  $56^\circ\text{C}$  and alkylated in  $55 \text{ mmol L}^{-1}$  iodoacetamide in  $50 \text{ mmol L}^{-1} \text{ NH}_4\text{HCO}_3$  for 1 h at room temperature. The gel pieces were washed several times with  $50 \text{ mmol L}^{-1} \text{ NH}_4\text{HCO}_3$  followed by dehydration with ACN and finally dried in a vacuum centrifuge. The proteins were

digested overnight at 37 °C by addition of 15 mL of trypsin (Promega, USA, 12.5 ng mL<sup>-1</sup> in 25 mmol L<sup>-1</sup> NH<sub>4</sub>HCO<sub>3</sub>). The resulting peptides were extracted by washing the gel pieces with 0.1% trifluoroacetic acid in 67% ACN. The supernatants were gathered and stored at – 20 °C until analysis.

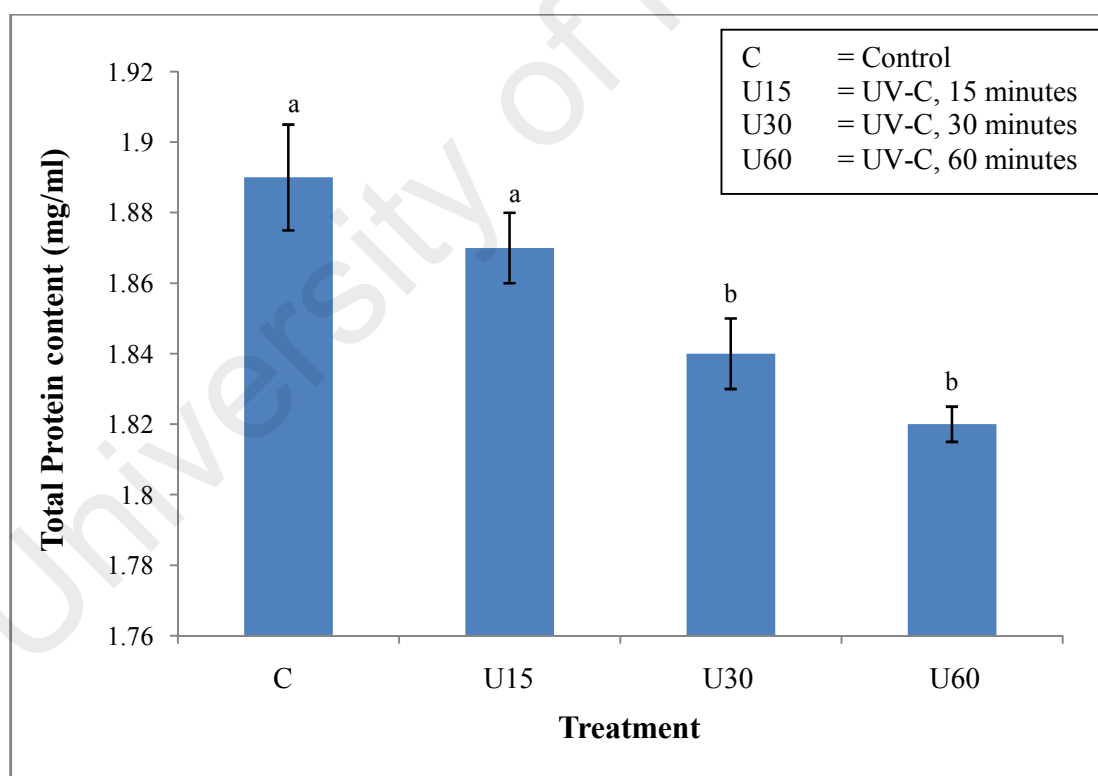
### **3.2.10 Protein Identification by MS and Database Search**

Peptides were analysed by electrospray ionization mass spectrometry using the Shimadzu Prominence nano HPLC system [Shimadzu] coupled to a 5600 Triple TOF mass spectrometer [ABSciex]. Tryptic peptides were loaded onto an Agilent Zorbax 300SB-C18, 3.5 µm [Agilent Technologies] and separated with a linear gradient of water/acetonitrile/0.1%formic acid (v/v). Spectra were analysed to identify proteins of interest using Mascot sequence matching software [Matrix Science] with the Ludwig NR database (non-identical sequences from GenBank CDS translations, PDB, Swiss-Prot, PIR, and PRF). Each spot was identified as the protein with the highest score and, consequently, the best match to its peptide sequence. For the peptides identified by MS, database searches (BLAST, National Center for Biotechnology Information, USA) were used to create protein sequence alignments. Searches were performed over the full molecular weight and pI range with no species restriction applied. Functional classification was determined by the primary biological function of the protein based on databases (NCBI/CDD, PantherDB, ExPASy—UniProt/ PROSITE, Gene Ontology, EMBL-EBI Interpro, KEGG) and on data available in the literature.

### 3.3 Result

#### 3.3.1 Total Protein Content

Figure 3.5 represents the changes of total protein in mangoes after the fruits were UV-C treated for 15, 30 and 60 minutes. As illustrated in Figure 3.5, there were no significant changes in the total protein content of mangoes following 15 minutes of UV-C treatment. However, UV-C treatments of 30 and 60 minutes resulted in decreases of total protein content. Total protein content in mangoes decreased from 1.89 mg/ml to 1.87 mg/ml following 30 minutes of treatment and further decreased to 1.84 mg/ml following 60 minutes of UV-C irradiation.

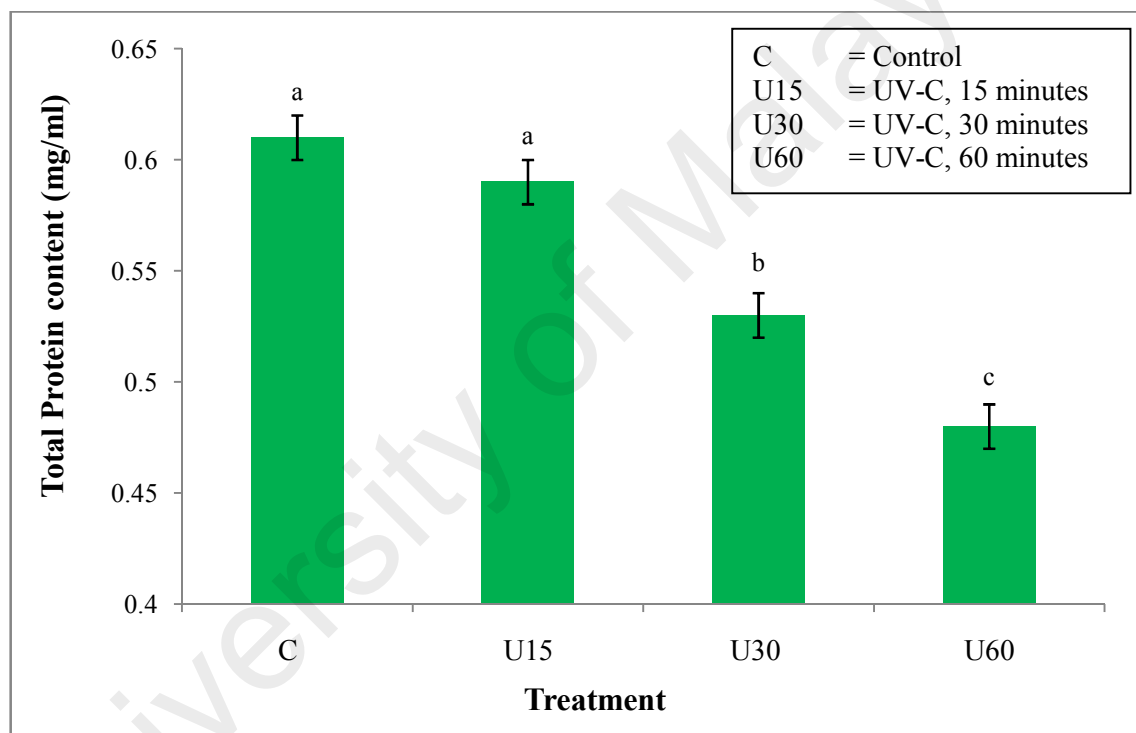


**Figure 3.5: Effects of UVC treatment on the total protein content of Chokanan mango.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different ( $p > 0.05$ ).

Figure 3.6 represents the changes of total protein in pineapples following UV-C treatment of 15, 30 and 60 minutes. As illustrated in Figure 3.6, there were no significant changes in the total protein content of pineapples following 15 minutes of UV-C treatment. However, UV-C treatments of 30 and 60 minutes resulted in decreases of total protein content. Total protein content in pineapples decreased from 0.61 mg/ml to 0.53 mg/ml following 30 minutes of treatment and further decreased to 0.48 mg/ml following 60 minutes of UV-C irradiation.



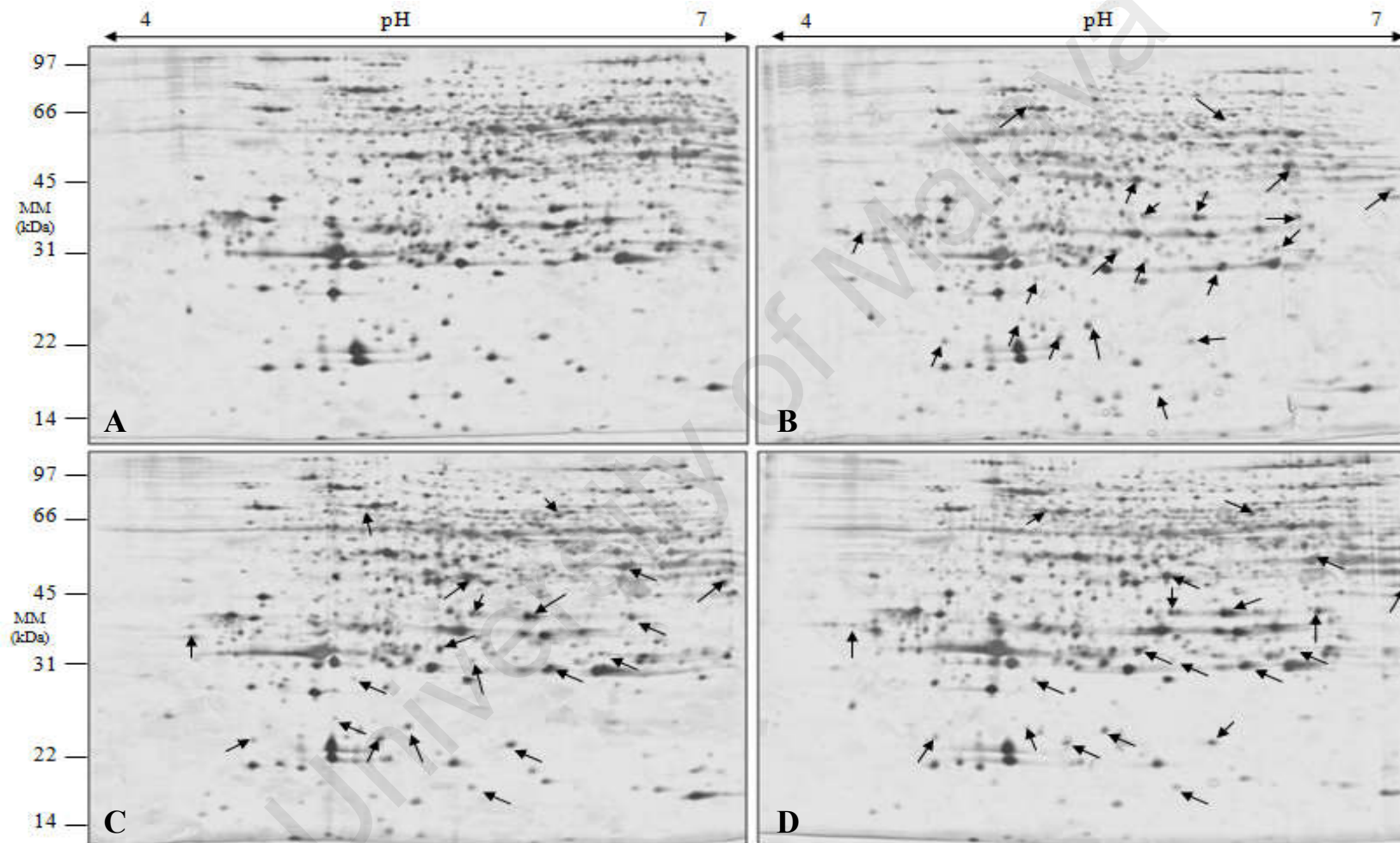
**Figure 3.6: Effects of UVC treatment on the total protein content of Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different ( $p > 0.05$ ).

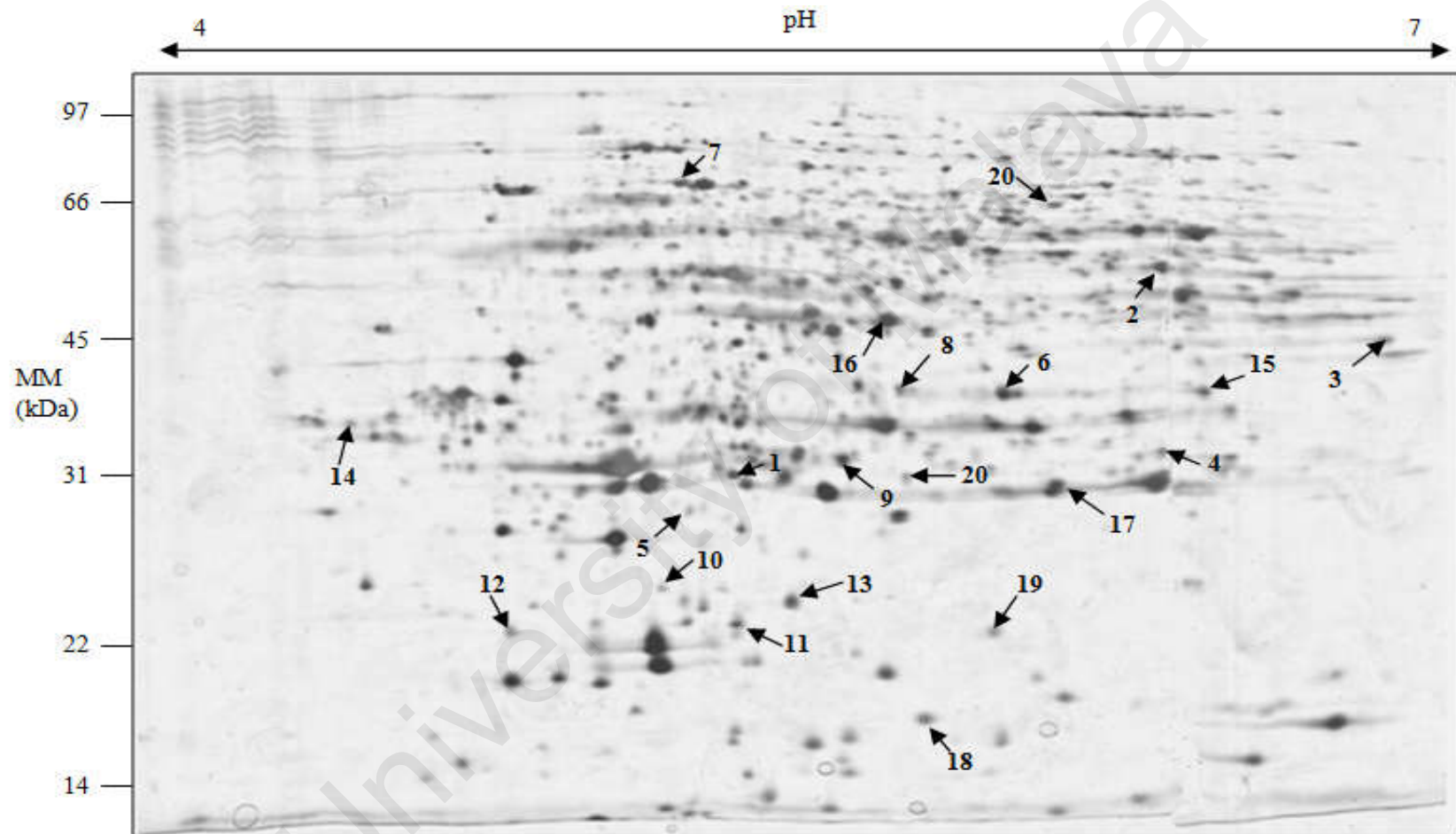
### 3.3.2 Effects of UV-C Treatment on the Proteome of Chokanan Mango

Results from the software guided comparative visual analysis of the representative 2-DE proteome profiles of Chokanan mango following UV-C treatment of 15, 30 and 60 minutes are represented by Figure 3.7. An average of 450 spots was detected in the gels while only 380 spots out of the 450 detected could be reproducibly detected. Spots detected were in the pH range of 4 to 7 and mainly in the molar mass range of 14 to 97 kDa. Gel analysis of the treated samples against controls via the ImageMaster 2-D Platinum software Version 7.0 (GE Healthcare) revealed 24 spots that were differentially abundant above the 2-fold threshold following UV-C treatment. Spots that were differentially abundant were excised, in gel digested via trypsin, and finally subjected to mass spectrometry. Out of the 24 spots that were differentially abundant, 20 spots were successfully identified using the Ludwig NR database (Figure 3.8).



**Figure 3.7: Representative spot maps of Chokanan mango A: control; B: UV-C irradiated for 15 minutes; C: UV-C irradiated for 30 minutes and D: UV-C irradiated for 60 minutes.**

<sup>1</sup>Arrows indicate spots that were significantly affected above the 2-fold threshold.



**Figure 3.8: Representative gel of Chokanan mango total proteome after 2-D electrophoresis and silver staining.**

<sup>1</sup>The numbers on top indicate the pH interval of isoelectric focusing (pH 4–7), and the numbers on the left show the position of the molecular mass markers (97 to 14.4 kDa).

<sup>2</sup>Spots differentially expressed above the 2-fold threshold are labelled accordingly.

The 20 differentially abundant spots that were successfully identified were divided into respective functional groups according to the homologies of the proteins and literatures (Table 3.5). Eight identified spots were found to correspond to sequences from *Mangifera indica* while the remaining 12 identified spots were found to be similar to sequences of other plants. Eight spots were found to be related to stress and defence based on their biological functions, nine spots were found to be related to energy and metabolism and 3 spots were found to be related to ripening and senescence namely. In addition, out of the 20 spots that were identified, several proteins that were known to cause allergic reactions in patients were also identified (Putative Allergen Pru P, Allergen Pyr c 3, Allergen Profilin 1 and Birch Pollen Allergen Bet V 1 Mutant).



**Table 3.5: Identity of spots affected by postharvest UV-C treatment in Chokanan mango.**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						UVC 15 mins	UVC 30 mins	UVC 60 mins
<b>Energy and Metabolism</b>								
1	6-phosphogluconolactonase [ <i>Mangifera indica</i> ] (Sanyal <i>et al.</i> , 2006b)	ABG23393	3	29886	5.16	1.95	2.17	2.34
2	Methionine gamma-lyase-like [ <i>Cicer arietinum</i> ] (NCBI citation)	XP_004502061	4	47989	6.28	2.12	2.87	3.43
3	Alcohol dehydrogenase 3 [ <i>Vitis vinifera</i> ] (Tesniere & Verries, 2000)	NP_001268071	2	41241	6.76	2.09	2.67	4.47
4	Sucrose synthase [ <i>Mangifera indica</i> ] (Li <i>et al.</i> , 2011c)	AEQ30069	4	30019	6.27	2.06	2.88	3.97
5	Neutral invertase [ <i>Mangifera indica</i> ] (Li <i>et al.</i> , 2011b)	AEQ30068	4	27812	5.13	4.12	4.88	5.35
6	Isoflavone reductase homolog [ <i>Solanum tuberosum</i> ] (NCBI citation)	XP_006353700	3	35258	5.82	4.07	4.38	5.41
7	ATP synthase beta subunit [ <i>Mangifera indica</i> ] (NCBI citation)	ADY90124	3	52594	5.04	3.79	4.37	5.58
8	Putative malate dehydrogenase [ <i>Mangifera indica</i> ] (NCBI citation)	XP_009312008	5	37408	5.49	2.22	3.05	3.55
9	Citrate synthase [ <i>Mangifera indica</i> ] (Li <i>et al.</i> , 2011a)	AEQ30066	3	30536	5.57	3.03	3.87	4.32

<sup>a</sup>Spot numbers corresponds to the 2-DE in Figure 3.8

<sup>b</sup>Experimental molecular weight

<sup>c</sup>Experimental pI value

<sup>d</sup>Fold change of protein abundance (decrease in abundance) as compared to controls following different durations of UV-C irradiation

**Table 3.5: Identity of spots affected by postharvest UV-C treatment in Chokanan mango (Continued).**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						UVC 15 mins	UVC 30 mins	UVC 60 mins
<b>Stress and Defence</b>								
10	Putative allergen Pru P [ <i>Prunus dulcis</i> x <i>Prunus Persica</i> ] (Chen <i>et al.</i> , 2008)	ACE80950	4	17437	5.10	2.12	2.24	2.36
11	Allergen Pyr c 3 [ <i>Pyrus communis</i> ] (Scheurer <i>et al.</i> , 2001)	Q9XF38	3	14064	5.19	2.01	2.16	2.33
12	Allergen profilin 1 [ <i>Mangifera indica</i> ] (Song <i>et al.</i> , 2006)	ABD62998.1	3	14070	4.89	1.98	2.15	2.43
13	Birch Pollen Allergen Bet V 1 Mutant [ <i>Betula pendula</i> ] (Gajhede <i>et al.</i> , 1996)	1QMR_A	2	17310	5.37	2.33	2.47	2.62
14	Thaumatin-like protein 1 [ <i>Solanum lycopersicum</i> ] (NCBI citation)	XP_004231024	2	36363	4.31	2.08	2.15	2.28
15	Polygalacturonase inhibiting protein [ <i>Prunus persica</i> ] (Wang <i>et al.</i> , 2010)	AEQ93253	3	36198	6.38	2.07	2.43	3.18
16	Superoxide dismutase [ <i>Zea mays</i> ] (Schnable <i>et al.</i> , 2009)	AFW86132	2	42866	5.63	2.11	2.56	3.38
17	RuBisCO complex protein [ <i>Glycine max</i> ] (Staswick <i>et al.</i> , 1994)	2019481A	7	31258	6.0	2.11	2.86	3.01
<b>Ripening and Senescence</b>								
18	l-aminocyclopropane-1-carboxylate oxidase [ <i>Mangifera indica</i> ] (Gupta <i>et al.</i> , 2004)	AAU06261	5	10573	5.60	2.16	2.38	2.59
19	Beta-1,3-glucanase [ <i>Mangifera indica</i> ] (Sanyal <i>et al.</i> , 2006a)	ABD16200	4	19507	5.77	1.96	2.16	2.22
20	Aminocyclopropane carboxylate synthase [ <i>Glycine max</i> ] (Liu <i>et al.</i> , 1993a)	2019442A	3	54730	5.84	2.56	3.19	3.87

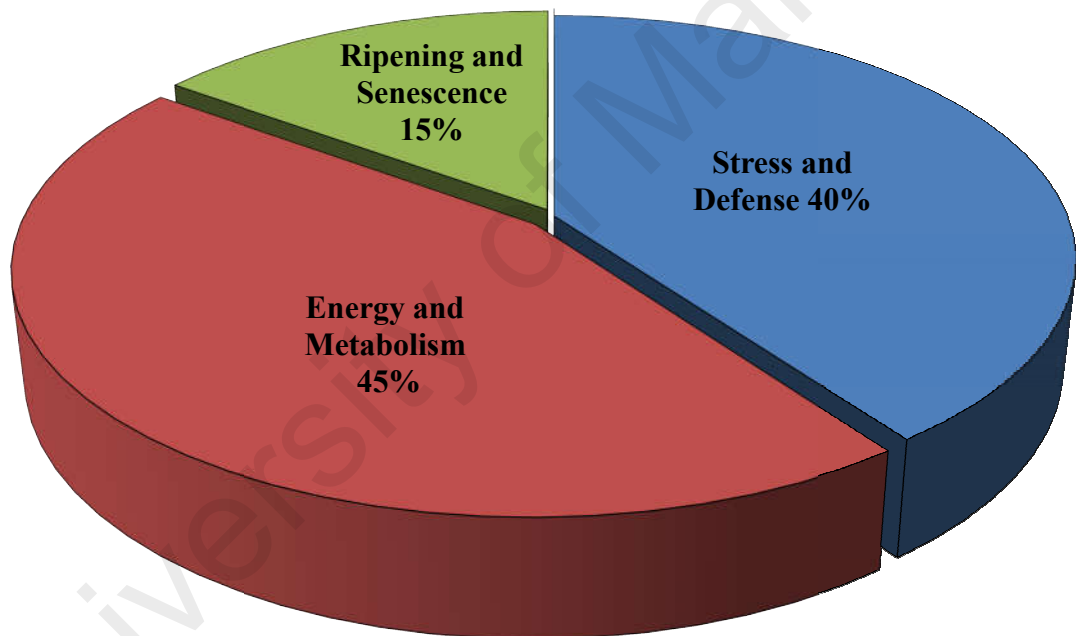
<sup>a</sup>Spot numbers corresponds to the 2-DE in Figure 3.8

<sup>b</sup>Experimental molecular weight

<sup>c</sup>Experimental pI value

<sup>d</sup>Fold change of protein abundance (decrease in abundance) as compared to controls following different durations of UV-C irradiation

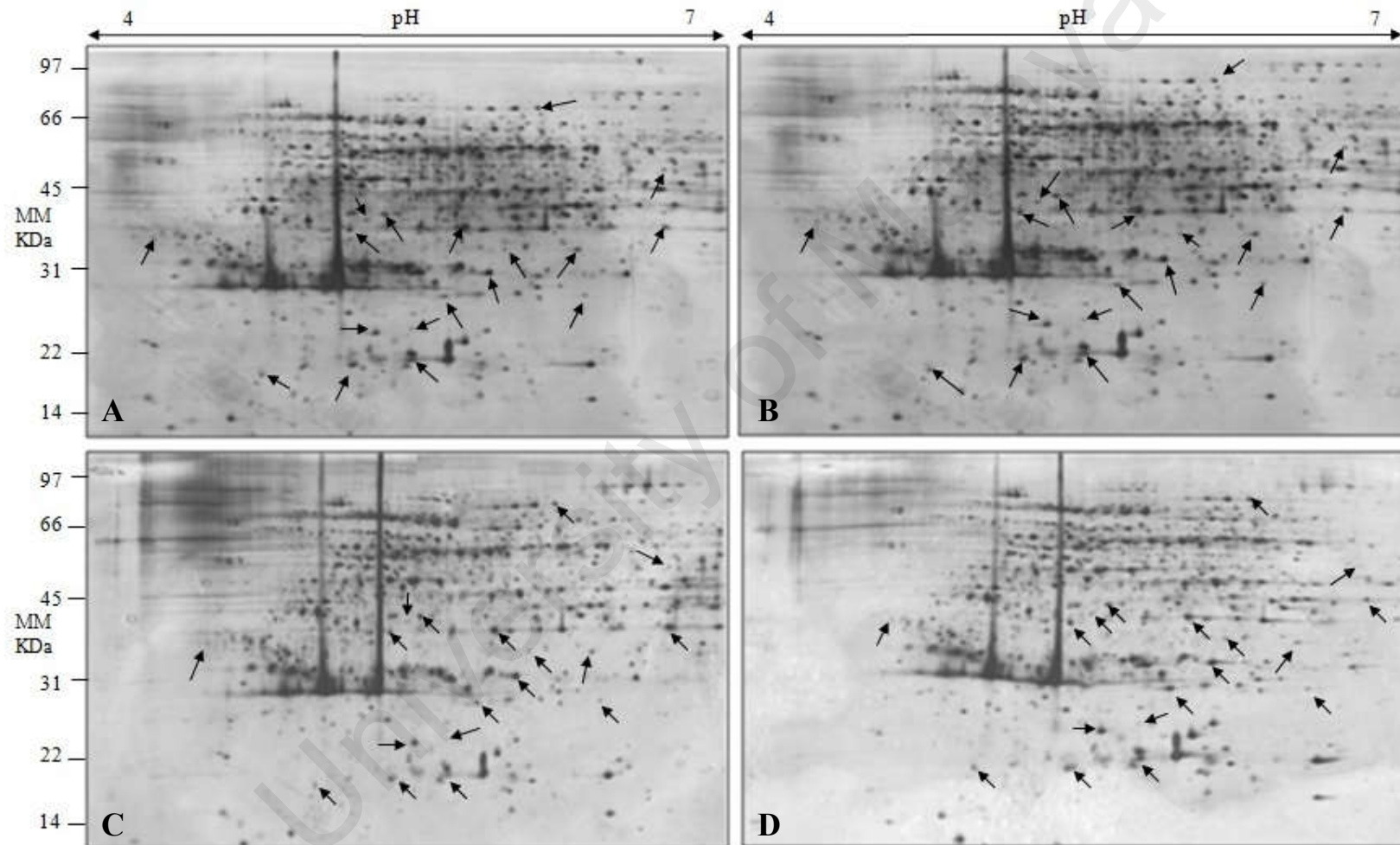
Proteins that were differentially abundant following UV-C treatments were found to be related mostly to energy and metabolism (45%), followed by stress and response (40%), and finally ripening and senescence (15%) (Figure 3.9). While the immediate effects of UV-C treatment on the quality of fruits are well documented, the effects of this treatment on a proteomic level remains scarce. The relation of the differentially abundant proteins to the deterioration of Chokanan mango is discussed in the following segments.



**Figure 3.9: Classification of proteins affected by UV-C treatment in Chokanan mango (identified by mass spectrometry analysis and grouped according to its functions).**

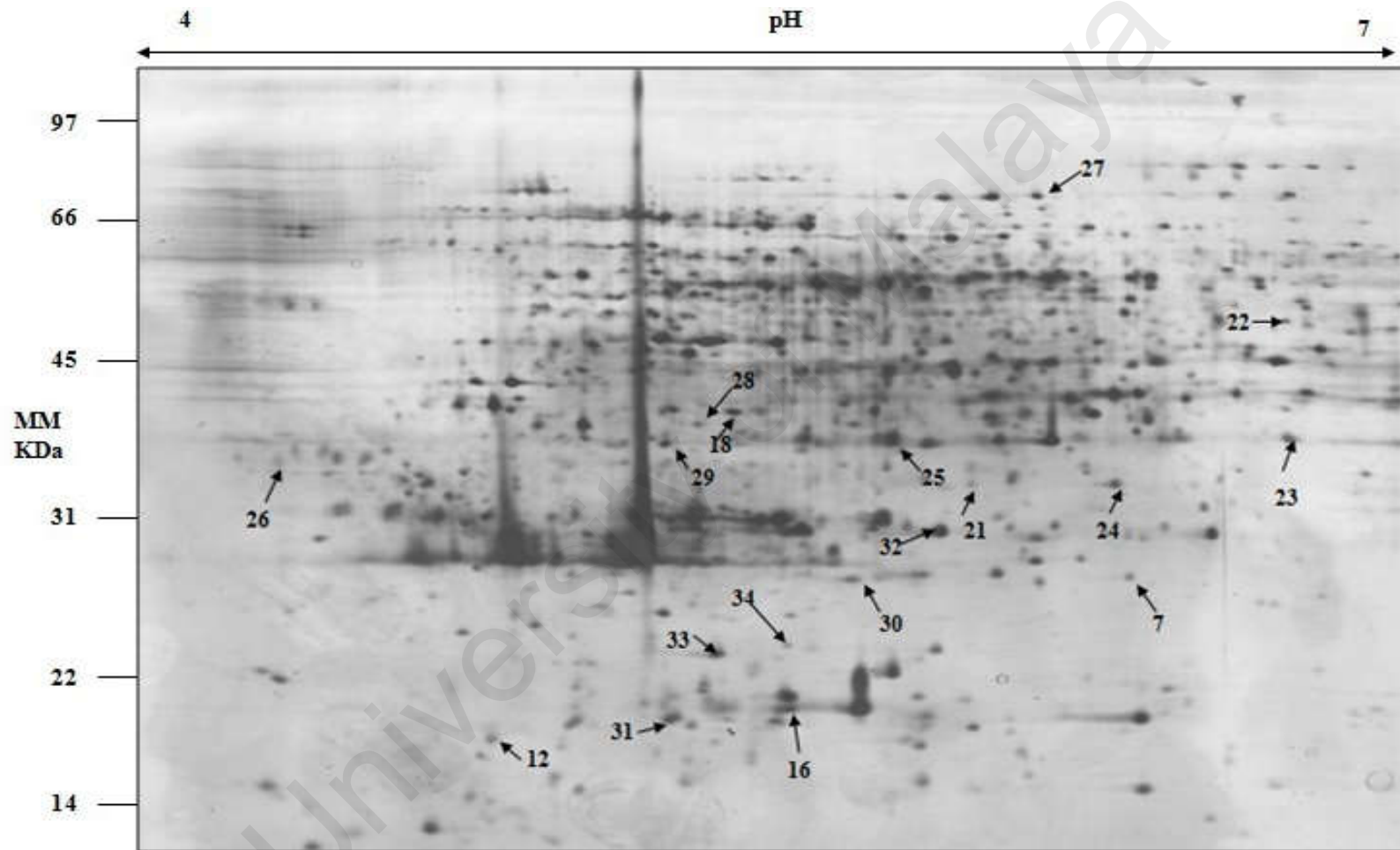
### **3.3.3 Effects of UV-C Treatment on the Proteome Changes Of Josephine Pineapple**

The representative image of the effects of UVC treatment (15, 30 and 60 minutes) on the proteome changes in pineapples is presented in Figure 3.10 and proteins spots that were significantly affected by this treatment is illustrated in Figure 3.11. An average of 460 protein spots were detected in the gels with only 400 well focused spots considered for analysis. Through quantitative analysis via the ImageMaster 2-D Platinum software Version 7.0 (GE Healthcare), 20 spots were found to be differentially abundant above the 2 fold threshold. These 20 spots were subjected to LC-MS/MS for peptide sequencing with only 2 spots not reaching the minimum requirements for proper identification resulting in 18 spots which were successfully identified.



**Figure 3.10: Representative spot maps of Josephine pineapple A: control; B: UVC irradiated for 15 minutes; C: UVC irradiated for 30 minutes and D: UVC irradiated for 60 minutes.**

<sup>1</sup>Arrows indicate spots that were significantly affected above the 2-fold threshold.



**Figure 3.11: Representative gel of Josephine Pineapple total proteome after 2-D electrophoresis and silver staining.**

<sup>1</sup>The numbers on the left indicates the position of the molecular mass markers (97 to 14 kDa) while the numbers on top shows the pH interval of isoelectric focusing (pH 4–7).

<sup>2</sup>Spots differentially expressed above the 2-fold threshold are labelled accordingly.

Figure 3.11 shows the functional classification of the proteins that were more abundant in the pineapple fruit before and after UVC irradiation. Classification was carried out according to their homologies as well as literatures (Table 3.6). Seven identified spots were found to correspond to sequences from *Ananas comosus* while the remaining 11 identified spots were found to be similar to sequences of other plants. Nine spots were found to be related to energy and metabolism based on their biological functions, 7 spots were found to be related to stress and defence and 2 spots were found to be related to ripening and senescence. In addition, out of the 18 spots that were identified, 2 proteins that were known to cause allergic reactions in patients were also identified (Allergen Ana c 1 and Ana c 2) which adds value to this current study.

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**Table 3.6: Identity of spots affected by postharvest UV-C treatment in Josephine pineapple.**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						UVC 15 mins	UVC 30 mins	UVC 60 mins
<b>Energy and Metabolism</b>								
7	ATP synthase [ <i>Eucalyptus globules</i> ] (Steane, 2005)	AAX21014	2	27524	6.03	2.09	2.22	2.44
21	Glyceraldehyde-3-phosphate dehydrogenase [ <i>Trypanosoma grayi</i> ] (Kelly <i>et al.</i> , 2014)	XP_009308756	6	37521	5.61	2.02	2.56	2.86
22	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [ <i>Ananas comosus</i> ] (Nashima <i>et al.</i> , 2014)	YP_009116350	4	53192	6.33	2.13	2.43	2.56
23	Methionine aminopeptidase 1 [ <i>Ananas comosus</i> ] (Fairbairn <i>et al.</i> , 2005)	ABB91774	6	43757	6.33	2.07	2.47	2.87
24	Gibberellin 2-beta-dioxygenase [ <i>Ananas comosus</i> ] (NCBI citation)	ACN30002	5	36223	5.95	2.21	2.55	2.93
25	Pyruvate dehydrogenase E1 beta subunit isoform [ <i>Zea mays</i> ] (Thelen <i>et al.</i> , 1999)	Q9ZQY2	4	41984	5.37	1.98	2.11	2.32
26	Phosphoglycerate kinase [ <i>Gossypium hirsutum</i> ] (Pang <i>et al.</i> , 2008)	ACJ11718.1	3	39900	4.14	2.10	2.32	2.54
27	D-3-phosphoglycerate dehydrogenase (GAPDH) [ <i>Ricinus communis</i> ] (NCBI citation)	XP_002518687.1	2	65100	5.74	1.99	2.19	2.35
28	Acid phosphatase 1 [ <i>Prunus dulcis</i> ] (Tuskan <i>et al.</i> , 2006)	B9H8Y0	7	43100	5.06	2.45	2.78	3.09

<sup>a</sup>Spot numbers corresponds to the 2-DE in Figure 3.11

<sup>b</sup>Experimental molecular weight

<sup>c</sup>Experimental pI value

<sup>d</sup>Fold change of protein abundance (decrease in abundance) as compared to controls following different durations of UV-C irradiation



**Table 3.6: Identity of spots affected by postharvest UV-C treatment in Josephine pineapple (Continued).**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						UVC 15 mins	UVC 30 mins	UVC 60 mins
<b>Stress and Defence</b>								
12	Profilin - Allergen Ana c 1 [ <i>Ananas comosus</i> ] (Reindl <i>et al.</i> , 2002)	Q94JN2.1	4	14229	4.71	2.22	2.87	3.22
16	Superoxide dismutase [ <i>Ananas comosus</i> ] (Lin <i>et al.</i> , 2000)	CAB60191	3	15176	5.29	1.98	2.55	3.02
29	Allergen Ana c 2 [ <i>Ananas comosus</i> ] (NCBI citation)	O23791.1	5	39055	5.00	2.33	2.77	3.18
30	Glutathione transferase [ <i>Carica papaya</i> ] (Lam & Bakar, 1999)	T09781	3	28538	5.43	2.44	2.73	3.24
31	Thioredoxin H-type (Trx-H) [ <i>Oryza sativa Japonica Group</i> ] (NCBI citation)	AAT44260	6	13177	4.96	2.37	2.68	2.99
32	Putative lactoylglutathione lyase [ <i>Malus Domestica</i> ] (NCBI citation)	MDP0000319112	2	35320	5.60	2.01	2.33	2.49
33	Mannose-binding lectin [ <i>Ananas comosus</i> ] (Neuteboom <i>et al.</i> , 2002)	AAM28277	3	17422	5.20	2.12	2.44	2.88
<b>Ripening and Senescence</b>								
18	1-Aminocyclopropane-1-carboxylate oxidase (ACC oxidase) [ <i>Musa acuminata</i> ] (NCBI citation)	Q6LC46 MUSAC	4	42836	5.18	2.05	2.39	3.07
34	Ripening-associated protein [ <i>Musa acuminata</i> ] (Clendennen & May, 1997)	O22322 MUSAC	4	21296	5.24	2.11	2.86	3.27

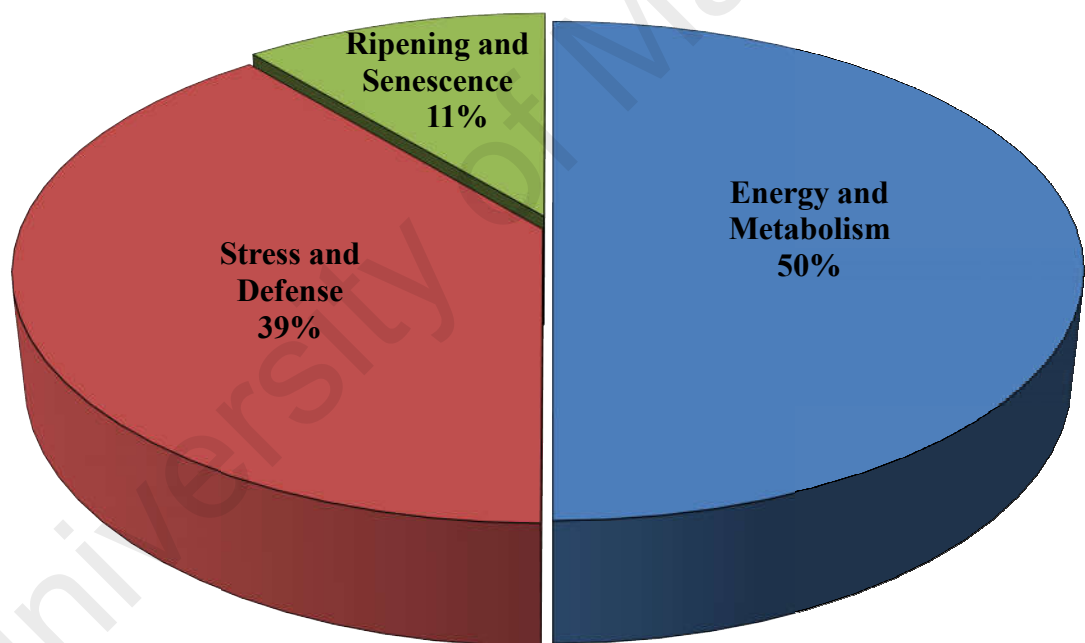
<sup>a</sup>Spot numbers corresponds to the 2-DE in Figure 3.11

<sup>b</sup>Experimental molecular weight

<sup>c</sup>Experimental pI value

<sup>d</sup>Fold change of protein abundance (decrease in abundance) as compared to controls following different durations of UV-C irradiation

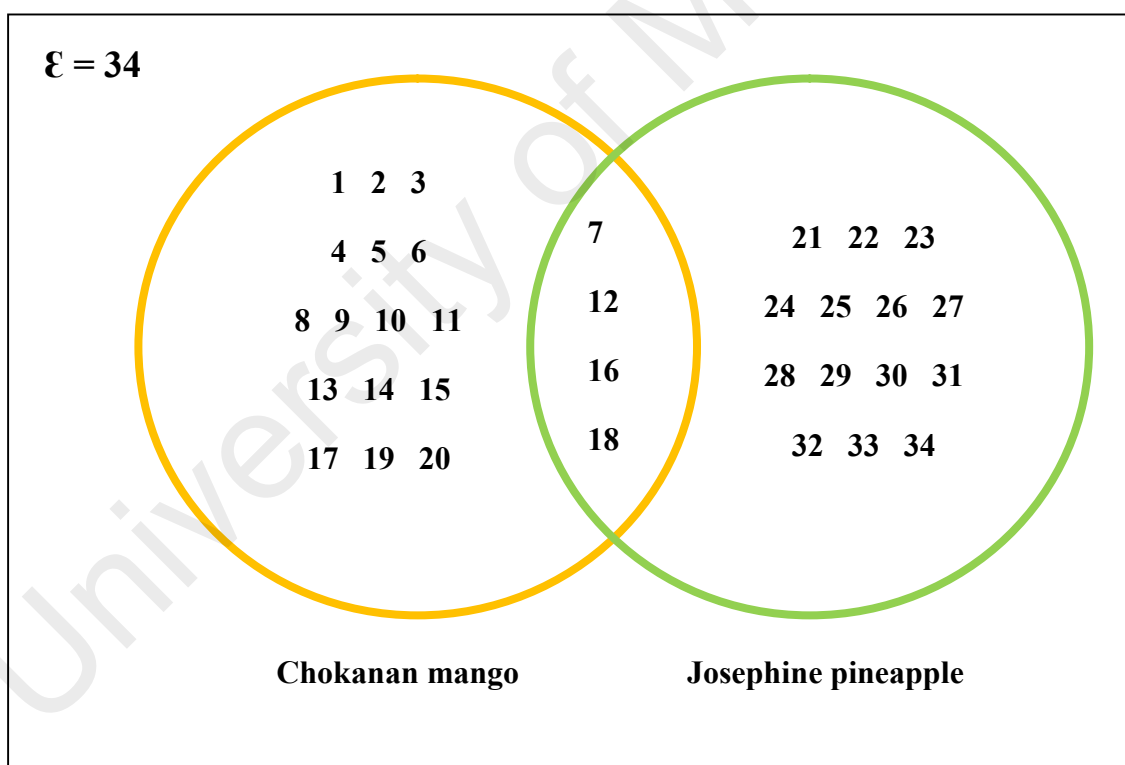
Proteins that were differentially abundant following UV-C treatments were found to be mostly related to energy and metabolism (50%), followed by stress and response (39%), and finally ripening and senescence (11%) (Figure 3.12). UVC has been exploited for quality maintenance of fruits as well as extension of shelf-life and the effects of this treatment on the physiological aspect have been well documented however, the effects of this treatment on a proteomic level remains scarce. In the following segments, the relation between this proteins and how they could relate to delayed fruit deterioration and shelf-life extension of pineapples are discussed.



**Figure 3.12: Classification of proteins affected by UV-C treatment in Josephine pineapple (identified by mass spectrometry analysis and grouped according to its functions).**

### 3.3.4 Effects of UV-C Treatment on the Proteome of Chokanan Mango and Josephine Pineapple

A total of 34 spots were found to be differentially abundant across both Chokanan mango and Josephine pineapple. Out of the 34 spots, 16 were differentially abundant only in mangoes, 14 were differentially abundant only in pineapples while 4 were found to be differentially abundant in both UV-C treated mango and pineapples namely ATP synthase (spot#7), profilin (spot#12), superoxide dismutase (spot#16) and 1-Aminocyclopropane-1-carboxylate oxidase (spot#18) (Figure 3.13). Proteins affected by UV-C treatment in both mango and pineapple were those that were from the energy and metabolism, stress and defence as well as ripening and senescence functional groups.



**Figure 3.13: Venn diagram of the number of identified proteins that differentially abundant in Chokanan mango and Josephine pineapple following UV-C treatment.**

<sup>1</sup>Numbers correspond to spot numbers in table 3.5 and 3.6.

### 3.4 Discussion

In order to understand the proteomic changes in fruits as a response to postharvest treatments such as UV-C irradiation, this study comprised of a comprehensive proteomic analysis comparing UV-C treated mango with untreated mango fruits (control) as well as UV-C treated pineapple with untreated pineapple fruits (control). Although it is understood that changes in fruits following postharvest treatments are associated with the disruption of various processes, the effects of these treatments on a proteomic level remains unclear. Proteins are known to play various roles in biological processes and an understanding of the proteins affected by UV-C treatment will provide an understanding of the molecular mechanism on how this treatment may delay deterioration in fruits leading to extension of shelf-life.

Following UV-C treatments, 9 protein spots were found to be related to energy and metabolism which were 6-phosphogluconolactonase, methionine gamma-lyase-like, alcohol dehydrogenase 3, sucrose synthase, neutral invertase, isoflavone reductase homolog, ATP synthase beta subunit, putative malate dehydrogenase and citrate synthase. The protein 6-phosphogluconolactonase is an enzyme responsible for the hydrolysis of 6-phosphogluconolactone to 6-phosphogluconate, which is the second step in the pentose phosphate pathway (Miclet *et al.*, 2001). Methionine gamma-lyase-like protein catalyses the reaction: L-methionine = methanethiol + NH<sub>3</sub> + 2-oxobutanoate (Tanaka *et al.*, 1977). Alcohol dehydrogenase 3 is an enzyme that catalyzes the 4<sup>th</sup> step in the metabolism of fructose before glycolysis. NAD<sup>+</sup> is regenerated along with a limited amount of ATP during the process (Strommer, 2011). Both the glycolytic and the pentose phosphate pathway have been reported to provide ATP for respiration burst in the increased energy demand during a climacteric phase (Piechulla, 1988). Our observation in this study suggests that a reduced abundance of

proteins involved in these pathways may reduce the rate of respiration in mangoes following treatment. A reduced rate of respiration may in turn translate to a delay in the deterioration process. Sucrose synthase has been reported to be involved in the catalysis of the cleavage of sucrose in the presence of uridine diphosphate (UDP) into UDP-glucose and fructose. Furthermore, it has also been reported that the enzyme sucrose synthase exhibits biochemical properties which allows it to function in the direction of both sucrose cleavage and synthesis (Déjardin *et al.*, 1997). Based on recent findings, neutral invertase is an enzyme that is involved in catalyzing the breakdown of sucrose into glucose and fructose which renders it as an essential protein in plants. Invertases are also known to be present in multiple locations as isoforms for the coordination of metabolic processes which further highlights the importance of this enzyme in plants (Martin *et al.*, 2013). Isoflavone reductase homologs are known to be involved in the biosynthesis of isoflavonoid phytoalexins. Several homologs are also known to catalyze distinct reductase reactions (Shoji *et al.*, 2002). ATP Synthase is made up of two main parts which are the F1 and Fo. The F1 is made up of five subunits: three alphas, three betas, one gamma, one delta, and one epsilon. The synthesis of ATP typically occurs in the beta subunit of the ATP synthase (Ko *et al.*, 2007). Besides its role in synthesis of ATPs, the ATP synthase beta subunit has been reported to be involved in plant cell death regulation (Chivasa *et al.*, 2011). The ability of UV-C treatments to reduce the abundance of this protein may therefore reduce cell death regulation which may translate to extended shelf-life in treated fruits. Malate dehydrogenase catalyzes the conversion of oxaloacetate and malate via the NAD/NADH coenzyme system. This reaction is pivotal in the malate/aspartate shuttle across the mitochondrial membrane and in the tricarboxylic acid cycle (Musrati *et al.*, 1998). The protein citrate synthase is an enzyme which is known for catalyzing the first reaction of the citric acid cycle which involves the condensation of acetyl-CoA and oxaloacetate to form citrate. Citrate

synthase has also been reported to be the rate determining enzyme in the citric acid cycle (Sienkiewicz-Porzucek *et al.*, 2008). As stated above, the proteins that were affected by UV-C treatment were found to play important roles in energy and metabolism with several proteins being rate determining enzymes in certain pathways. Although pre harvest metabolic processes which lead to changes in texture, pigmentation, taste and aroma of the fruits are highly beneficial (Aina, 1990), these changes which continue following harvest leading to undesirable changes which eventually results in deterioration and the short shelf-life of fruits (Joseph, 1990). A reduced abundance of these proteins following treatment may therefore result in extended shelf-life. Several postharvest treatments have been successful in the extension of shelf-life of mangoes through retardation of these metabolic changes (Jacobi *et al.*, 2001; Gonzalez-Aguilar *et al.*, 2001).

Besides proteins involved in energy and metabolism, UV-C irradiation of Chokanan mango affected 8 protein spots that were related to stress and defence namely putative allergen Pru P, allergen Pyr c 3, allergen profilin 1, birch Pollen Allergen Bet V 1 Mutant, thaumatin-like protein 1, polygalacturonase inhibiting protein, superoxide dismutase, RuBisCO complex protein were observed to be reduced in abundance. The allergen Pru P and Pyr c 3 are classified as lipid transfer proteins (LTP). Although the specific role of plant LTPs remains unclear it is likely that LTP's play a role in key processes of plant physiology (Carvalho & Gomes, 2007). Allergen profilin 1 and Celery Allergen Api G 1 are involved in actin polymerization while the birch Pollen Allergen Bet V 1 Mutant is known to have lipocalin protein like properties (Haarer & Brown, 2005; Jensen-Jarolim, 2014). These allergens have been reported to cause allergic reactions in those allergic to mangoes (Wellhausen *et al.*, 1996; Kinder *et al.*, 1999; Song *et al.*, 2008; Silva *et al.*, 2009). The ability of UV-C treatment to reduce the

abundance of allergenic proteins may prove to be invaluable in reducing or removing allergic reactions in patients. The effectiveness of several postharvest treatments on the stability of food allergens has been reported (Sathe *et al.*, 2005). However, it excludes the use of UV-C treatment as a potential treatment for reducing allergic reactions in patients. A reduced abundance of allergenic proteins following UV-C treatment may translate into reduced allergic reactions in patients which may pave the way for the advancement of new food processing technologies. Thaumatin-like proteins and polygalacturonase inhibiting proteins are proteins that have been reported to be synthesized by plants in response to fungal infections (Brandazza *et al.*, 2004). Polygalacturonase inhibiting proteins are capable of inhibiting fungal endopolygalacturonases (PGs) (Federici *et al.*, 2001). Superoxide dismutase plays an important role in a plants' defense mechanism whereby it constitutes the first line of defense against reactive O<sub>2</sub> species (ROS) (Alscher *et al.*, 2002). Reactive O<sub>2</sub> species (ROS) are produced in both unstressed and stressed cells which may lead to oxygen radical mediated toxicity. The function of superoxide dismutase is to catalyze the dismutation of superoxide into oxygen and hydrogen peroxide and therefore, it is vital for plant development in stressed conditions (Raychaudhuri & Deng, 2000). RuBisCO complex proteins are known as the predominant protein in photosynthesizing plant parts and the degradation of this protein has been related to senescence (Feller *et al.*, 2007). As described above, the various biological processes that these proteins are involved in may result in hastened deterioration of fruits and minimal shelf-life directly or indirectly. Based on our observations, the efficacy of UV-C treatment in reducing the abundance of proteins mentioned above is beneficial for extension of shelf-life in minimally processed mangoes as well as paving the way for further research related to allergens and allergic reactions.

UV-C treatment of mangoes was also found to affect 3 proteins that were related to ripening and senescence which are 1-aminocyclopropane-1-carboxylate oxidase, beta-1,3-glucanase and aminocyclopropane carboxylate synthase. Both aminocyclopropane carboxylate synthase and 1-aminocyclopropane-1-carboxylate oxidase has been reported to be involved in the biosynthesis of ethylene (Lui & Zhang, 2004; Bassan *et al.*, 2006). Aminocyclopropane carboxylate synthase catalyzes the conversion of S-adenosyl-L-methionine (SAM) into 1-aminocyclopropane-1-carboxylate (ACC) which is a direct precursor of ethylene (Liu & Zhang, 2004). On the other hand, the enzyme 1-aminocyclopropane-1-carboxylate oxidase which is a non-heme iron-containing enzyme is known to catalyze the final step in the biosynthesis of ethylene through its oxidation at the expense of O<sub>2</sub>, giving yield to ethylene (C<sub>2</sub>H<sub>4</sub>), CO<sub>2</sub> and water (Rocklin *et al.*, 2004). Ethylene is a plant hormone which is known to control various physiological processes in plants. Although these physiological processes are beneficial during ripening, it can induce negative effects during postharvest storage of fruits and vegetables, which include over-ripening, accelerated quality loss, increased susceptibility to pathogen and physiological disorders (Martinez *et al.*, 2007). A reduced abundance of 1-aminocyclopropane-1-carboxylate oxidase which is involved in the biosynthesis of ethylene may have led to the reduced adverse effects of ethylene and hence, an improved quality and extended shelf-life. The protein beta-1,3-glucanase is more commonly known for its antimicrobial activity in infected plants. However, there is strong evidence that this enzyme is also involved in diverse physiological and developmental processes in uninfected plants or fruits (Leubner-Metzger & Meins, 1999). Although these physiological changes are beneficial during fruit ripening, ongoing physiological changes may be detrimental to postharvest quality and shelf-life of fruits.



As for pineapples, following UV-C treatment, nine spots which include Glyceraldehyde-3-phosphate dehydrogenase, Ribulose-1,5-bisphosphate carboxylase / oxygenase large subunit, Methionine aminopeptidase 1, ATP synthase, Gibberellin 2-beta-dioxygenase, Pyruvate dehydrogenase E1 beta subunit isoform, Phosphoglycerate kinase, D-3-phosphoglycerate dehydrogenase and Acid phosphatase 1 were found to be reduced in abundance. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been indicated by several reports to be involved in the reversible conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate via the reduction of NAD<sup>+</sup> to NADH (Kim & Dang, 2005; Hara & Snyder, 2006; Min *et al.*, 2007; Lee *et al.*, 2009b). Besides the glycolytic pathway, this enzyme has also been implicated as being important for the generation of metabolites for other anabolic pathways (Baud *et al.*, 2007). The enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase which is also known as the Rubisco, catalyzes the rate-limiting step of CO<sub>2</sub> fixation in photosynthesis (Spreitzer, 2003). Although the exact role of methionine aminopeptidase remains unclear, it has been implicated to be involved in plant metabolic activities (Ross *et al.*, 2005). ATP synthase, as explained earlier, is reported to be involved in the synthesis of ATPs and is linked to plant cell death regulation (Ko *et al.*, 2007; Chivasa *et al.*, 2011). Deterioration of postharvest fruits is contributed by cell death. The ability of UV-C treatment to reduce the abundance of ATP synthase may therefore lead to reduced cell death regulation. The enzyme gibberellin 2-beta-dioxygenase has been reported to catalyze the catabolism of gibberellins, which is vital for plant development (Schomburg *et al.*, 2003). Pyruvate dehydrogenase has been reported to play a pivotal role in the glycolytic pathway of plants. It catalyzes the irreversible reaction that leads to the breakdown of glucose in the energy generating pathway (Patel & Roche, 1990). As for the enzyme phosphoglycerate kinase, it is reported to catalyze the conversion of ADP and 1,3-Bisphosphoglycerate to ATP and 3-Phosphoglycerate, which is the

seventh reaction of the glycolytic pathway (Vas & Varga, 2010). D-3-phosphoglycerate dehydrogenase has been reported to be involved in the synthesis of serine. This enzyme responsible for the oxidation of 3-phosphoglycerate to phosphohydroxypyruvate (Ho *et al.*, 1999). Lastly, for energy and metabolism, the enzyme acid phosphatase has been reported to be involved in the hydrolysis of phosphate esters which renders them important for physiological processes such as phosphorus efficiency (Asaduzzaman *et al.*, 2011). These proteins and their roles mentioned above validate their importance in plants' energy and metabolism. Several proteins were also found to play roles as rate determining enzymes in their respective pathways. Although the importance of these processes in pre-climacteric fruits cannot be over looked, these processes are also one of the causes of postharvest fruit deterioration. The efficacy of UV-C treatment in reducing the abundance of these proteins may therefore be essential for delaying fruit deterioration which could result in extended shelf-life.

Seven spots differentially expressed by UV-C treatment in pineapples were related to stress and defense namely profilin (Allergen Ana c 1), Allergen Ana c 2, Glutathione transferase, Copper/zinc-superoxide dismutase, Thioredoxin H-type, Putative lactoylglutathione lyase and Mannose-binding lectin. The allergen Ana c 1 is a profilin while the allergen Ana c 2 is also known as bromelain. Profilins has been associated with actin polymerization in plants (Haarer & Brown, 2005). Actual functions of bromelains remains vague although it has been reported to possess anti-inflammatory activities which is vital for plant defence (Bhattacharyya, 2008). These proteins have been identified as major allergens in pineapples and have been reported to cause allergic reactions in those allergic to pineapples (Kabir *et al.*, 1993; Nettis *et al.*, 2001; Reindl *et al.*, 2002). Based on our observation, a reduced abundance of these proteins may be beneficial in reducing allergic reactions in patients. However, further research is

required to confirm the efficiency of this treatment as a potential treatment for reducing allergic reactions. Glutathione transferase has been reported to play major roles in pathogen infected plants. Its mode of action involves the reduction of damage caused by pathogens or by limiting the extent of cell death during the hypersensitive response which is attributed to the antioxidative activity of the enzyme (Lieberherr *et al.*, 2003). Glutathione transferase has been implicated to be involved in both normal metabolism as well as several stress responses in plants, which include pathogen attack, heavy-metal toxicity as well as oxidative stress. This highlights the importance of this protein in the plant stress response (Marrs, 1996). Similar to glutathione transferase, the enzyme thioredoxin H-type is also implicated to be involved in cellular protection against oxidative stresses in higher plants, particularly through redox regulation (Gelhaye *et al.*, 2004). The accumulation of these enzymes in ripe fruits has also been observed and is perceived to be a part of the natural ripening process. Furthermore, these enzymes have also been linked to protection against chilling injuries (Dagar *et al.*, 2010). These enzymes have also been speculated to be involved in other processes related to fruit quality such as pulp softening (Andradea *et al.*, 2012). In this study, our observation of a reduced abundance of these proteins may therefore be beneficial in terms of delaying deterioration of fruits caused by softening which leads to susceptibility of pathogens. Reactive O<sub>2</sub> species (ROS) are common threats to plants and the enzyme superoxide dismutase has been reported to act as the first line of defence against them (Alscher *et al.*, 2002). In plants, ROS are produced in both unstressed and stressed cells which may lead to oxygen radical mediated toxicity. The breakdown of superoxide into oxygen and hydrogen under stress conditions highlights its importance for plant development under stressed conditions (Raychaudhuri & Deng, 2000). Various enzymatic and non-enzymatic activities in plants have been reported to lead to the accumulation of methylglyoxal (MG) which is a highly reactive cytotoxic protein. The enzyme

lactoylglutathione lyase is responsible for the detoxification of MGs through the glyoxalase system, which makes this enzyme another important member of plant stress response (Yaday *et al.*, 2008). The protein mannose-binding lectins have also been implicated to play major roles in plant stress and defence (Barre *et al.*, 2001). In this study, along with the other proteins, several allergenic proteins were found to be reduced in abundance as a result of UV-C treatment. A reduced abundance of these allergens may be beneficial in reducing allergic reactions in patients. Besides conferring quality maintenance and extension of shelf-life in fruits, UV-C treatment may also provide new methods for curbing allergic reactions.

Lastly, 2 spots that were reduced in abundance in pineapples were found to be related to ripening and senescence namely, 1-Aminocyclopropane-1-carboxylate oxidase and Ripening-associated protein. The enzyme 1-aminocyclopropane-1-carboxylate oxidase is a non-heme iron containing enzyme which is linked to the biosynthesis of ethylene as described above (Liu & Zhang, 2004; Bassan *et al.*, 2006). Ripening associated proteins have also been reported to be important in fruit ripening regulation particularly in the regulation of the plant hormone ethylene (Osmark *et al.*, 1998). A reduced abundance of the enzyme 1-aminocyclopropane-1-carboxylate oxidase may relate to a reduced rate of ethylene biosynthesis and the adverse effects of ethylene beyond ripening.

To the best of our knowledge, this work reports the first proteomic study of the effects of postharvest UV-C irradiation on the proteomic changes in Chokanan mango and Josephine pineapple. UV-C treatment affected different individual proteins in Chokanan mango and Josephine pineapple. However, the proteins that were affected in both the fruits were of the same functional groups, which include energy and metabolism, stress and response as well as ripening and senescence. Additionally, four of the same proteins

were affected by UV-C treatment in both mango and pineapple namely ATP synthase, profilin, ACC oxidase and superoxide dismutase. Response patterns of Chokanan mango and Josephine pineapple to UV-C treatment are complex, as the differentially abundant proteins are involved in multiple metabolic pathways. Differential analyses of protein maps from UV-C treated mango and pineapple using 2D-Electrophoresis technology provided new information on the changes in protein levels as a result of UV-C treatment. Proteins that were affected by this treatment were found to play important roles in various biological processes that could lead to fruit deterioration during storage. The effects of UV-C irradiation on these proteins and the role that they play may be partly responsible for the maintenance of postharvest fruit quality and shelf-life extension. In this regard, proteomic analysis of UV-C treated fruits has increased our understanding of the effects of this postharvest treatment on the proteome of fruits such as mangoes and pineapples.

## CHAPTER 4: EFFECTS OF THERMAL TREATMENT (70°C) ON THE PROTEOME OF CHOKANAN MANGO (*Mangifera indica* L. cv. Chokanan) AND JOSEPHINE PINEAPPLE (*Ananas comosus* L. Merr. Josephine)

### 4.1 Introduction

Currently, postharvest thermal treatment is preferred over other harmful chemical treatments which are available to control postharvest losses of crops. Postharvest thermal treatment of commodities like fruits can be administered through various methods which include hot water dips and sprays, vapor heat and hot air from temperatures ranging from as low as 35 °C to as high as 70 °C (Lurie & Pedreschi, 2014). The use of thermal treatment in postharvest crops has been reported to confer regulation of fruit ripening, enhancement of treatments such as Ca treatment as well as protection against physiological disorders and pathogens (Klein & Lurie, 1992). Thermal treatment is a plant stressor which affects various aspects of a plant leading to metabolic imbalances (McClung & Davis, 2010; Ruelland & Zachowski, 2010; Suzuki *et al.*, 2011). An understanding of the effects of postharvest thermal treatment on a molecular level is therefore of significant interest but remains unclear to date.

In this chapter, the physiological changes that take place in mangoes and pineapples as a response to thermal treatment on a proteomic level were studied. Proteomic studies were carried out via two-dimensional electrophoresis and protein identification through mass spectrometry as detailed in the previous chapter. An expansion of knowledge regarding the proteomics changes will lead to better quality control of postharvest mangoes and pineapples in the future.

## **4.2. Materials and Methods**

### **4.2.1. Sample Preparation**

Sample preparation was carried out as described in section 3.2.1

### **4.2.2. Thermal Treatment**

Twelve fruit disc samples were placed on sterilized petri dishes (15 x 100 mm; 10 mm depth) and thermally treated at 70°C in an oven (Memmert) for 5, 10 and 20 minutes. Sample discs left at room temperature were treated as controls. Nine biological replicates (n=9) were used for each of the different durations of thermal treatment.

### **4.2.3 Extraction of Total Protein**

Extraction of total protein from treated and non treated samples were carried out as described in section 3.2.3.

### **4.2.4 Total Protein Estimation**

Total protein estimation for treated and non treated samples was carried out as described in section 3.2.4.

### **4.2.5 Protein Extraction for Two Dimensional Gel Electrophoresis (2-DE)**

Protein extraction from treated and non treated samples for 2-DE was carried out as described in section 3.2.5.

### **4.2.6 2-Dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (2D SDS-PAGE) Analysis**

2-D SDS PAGE analysis for samples was carried out as described in section 3.2.6.

#### **4.2.7 Staining Procedure**

Staining of SDS gels was carried out as described in section 3.2.7.

#### **4.2.8 Image Acquisition and Data Analysis**

Image acquisition and data analysis of the gels of samples were carried out as described in section 3.2.8.

#### **4.2.9 Protein In-Gel Digestion**

Spots that showed statistically significant changes (at  $P < 0.05$ ) and above a 2-fold threshold were excised and subjected to protein in-gel digestion as described in section 3.2.9.

#### **4.2.10 Protein Identification by MS and Database Search**

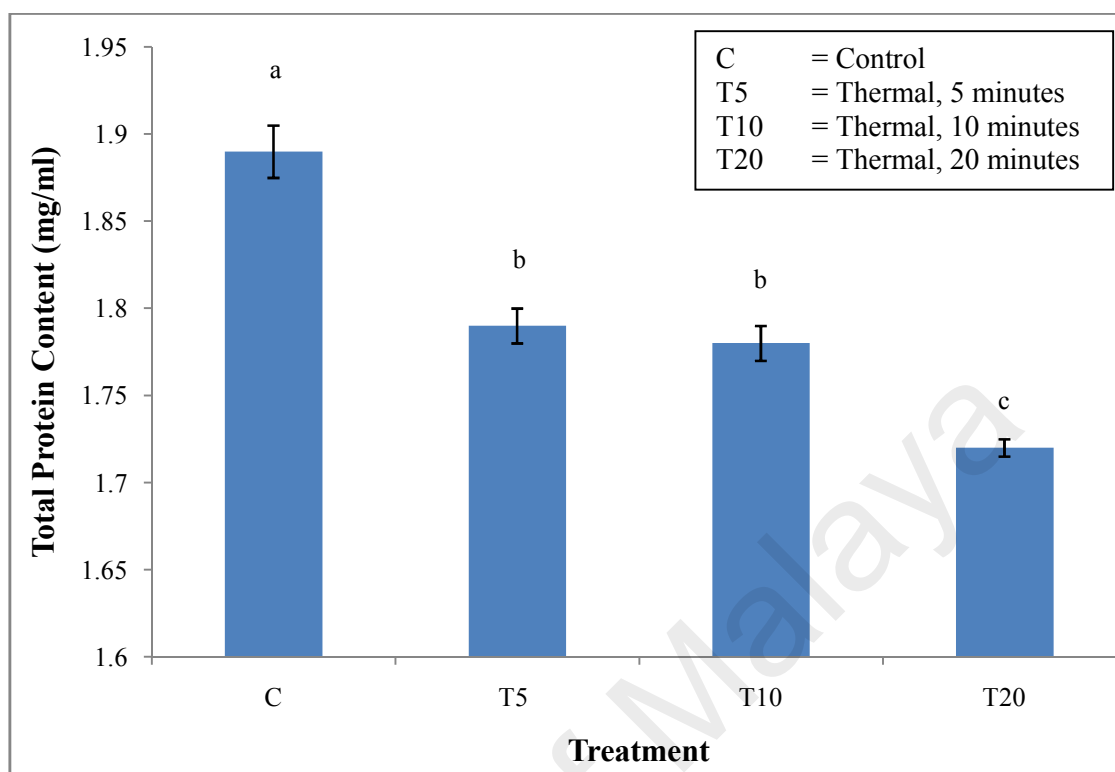
Protein identification of protein in-gel digested spots was carried as described in section 3.2.10.

### **4.3 Results**

#### **4.3.1 Total Protein Content**

Figure 4.1 represents the changes of total protein in mangoes following thermal treatment of 70 °C. Thermal treatment of 70 °C resulted in decrease of total protein content in mangoes. Total protein content in mangoes decreased from 1.89 mg/ml to 1.79 mg/ml following 5 minutes of thermal treatment. Thermal treatment of 10 and 20 minutes resulted in further decreases of total protein content in mangoes. Total protein content decreased from 1.89 mg/ml to 1.78 mg/ml and 1.72 mg/ml following 10 and 20 minutes of treatment respectively.



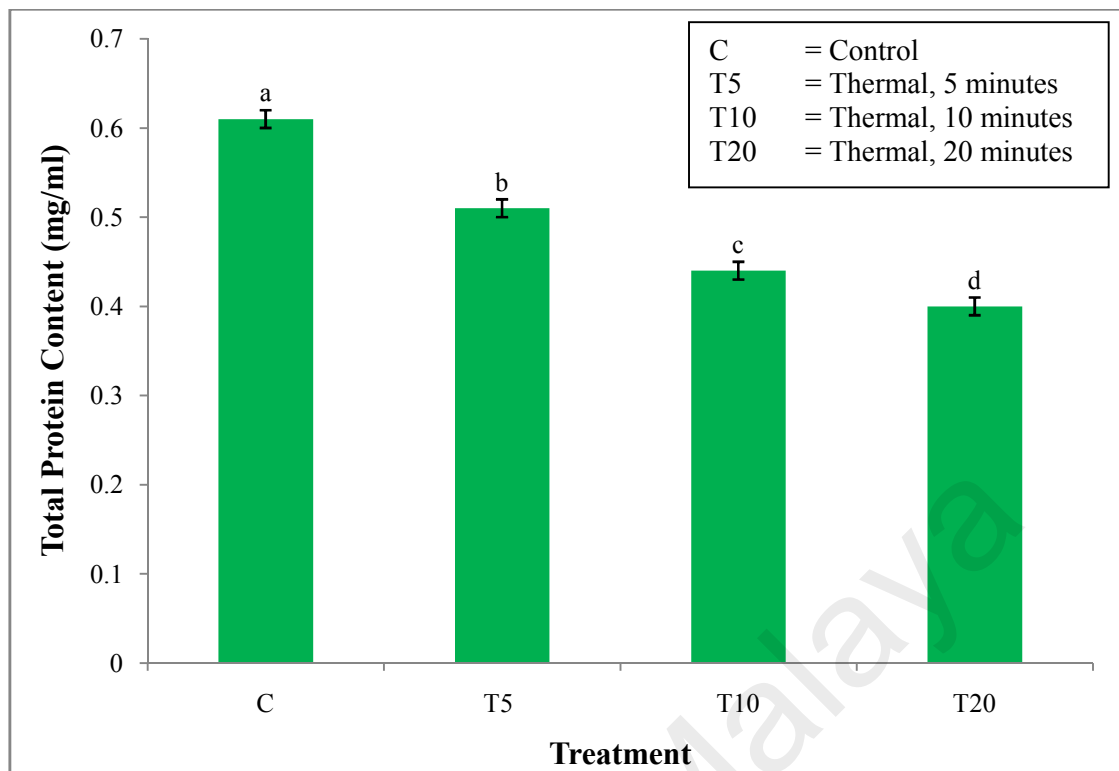


**Figure 4.1: Effects of thermal treatment on the total protein content of Chokanan mango.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different ( $p > 0.05$ ).

Figure 4.2 represents the changes of total protein in pineapple following thermal treatment of 70 °C. As illustrated by Figure 4.3, thermal treatment resulted in decreases of total protein content in pineapples. Total protein content in pineapples decreased from 0.61 mg/ml to 0.51 mg/ml following 5 minutes of thermal treatment. Thermal treatment of 10 and 20 minutes resulted in greater decreases of total protein content in pineapples. Total protein content decreased from 0.61 mg/ml to 0.44 mg/ml and 0.40 mg/ml following 10 and 20 minutes of treatment respectively.



**Figure 4.2: Effects of thermal treatment on the total protein content of Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

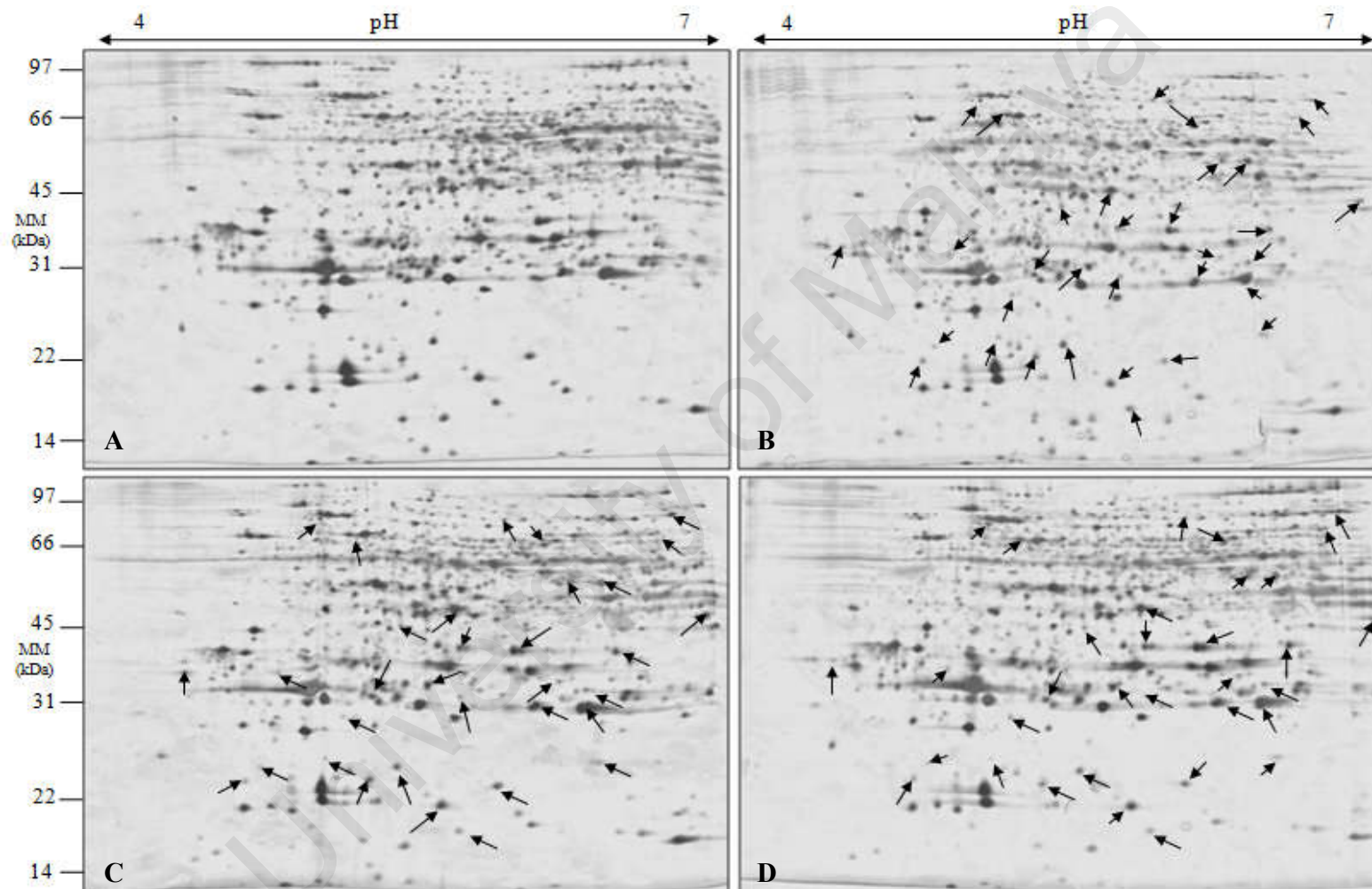
<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

#### 4.3.2 Effects of Thermal Treatment on the Proteome of Chokanan Mango

Results from the software guided comparative visual analysis of the representative 2-DE proteome profiles of Chokanan mango following medium heat (70 °C) treatment of 5, 10 and 20 minutes are represented by Figure 4.3. An average of 450 spots were detected in the gels while only 380 spots out of the 450 detected could be reproducibly detected. Spots detected were in the pH range of 4 to 7 and mainly in the molar mass range of 14 to 97 kDa. Gel analysis of the treated samples against controls via the ImageMaster 2-D Platinum software Version 7.0 (GE Healthcare) revealed 35 spots that were differentially abundant above the 2-fold threshold following heat treatment. Spots that were differentially abundant were excised, in gel digested via trypsin, and finally

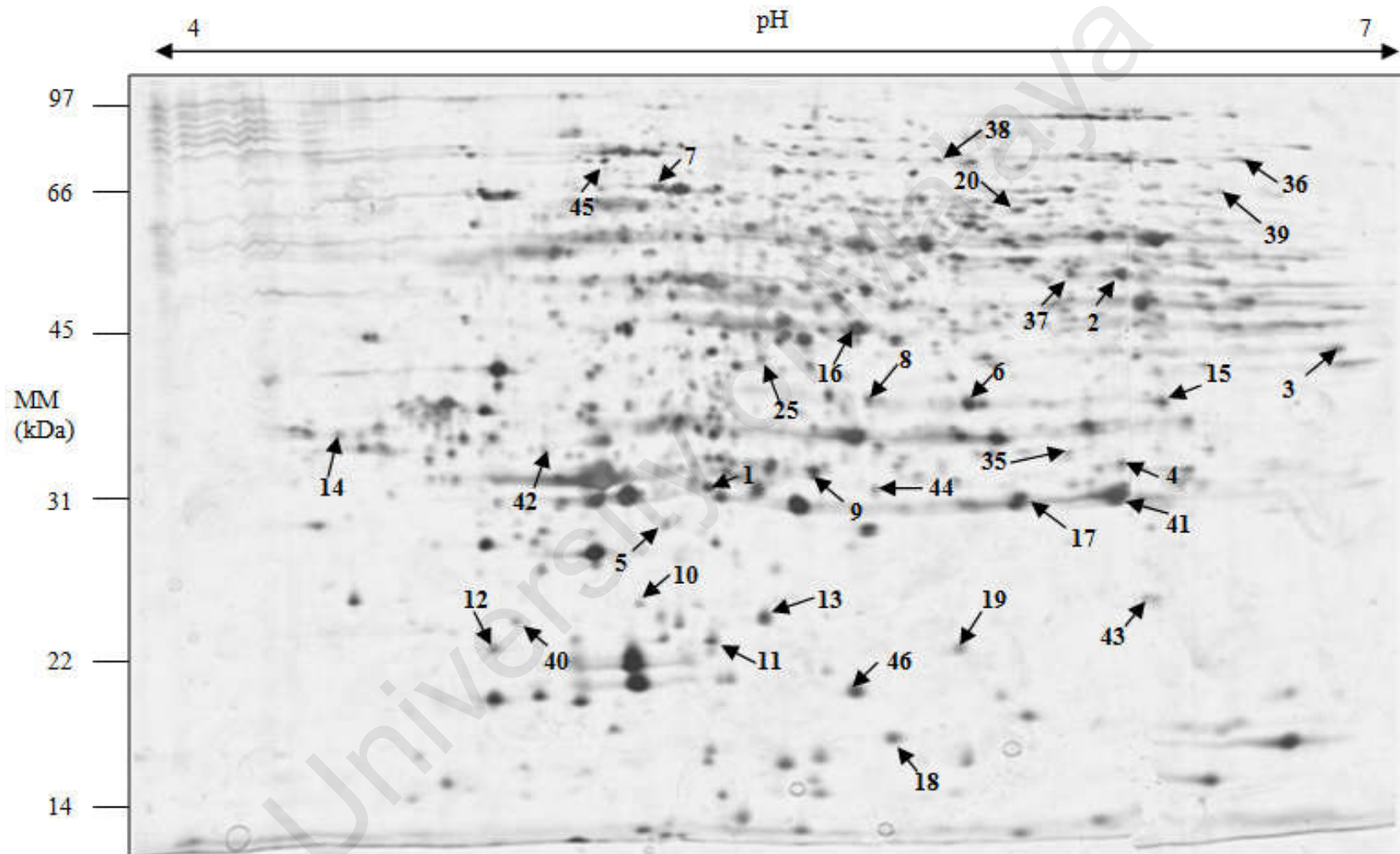
subjected to mass spectrometry. Out of the 35 spots that were differentially abundant, 33 spots were successfully identified using the Ludwig NR database (Figure 4.4).

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**Figure 4.3: Representative spot maps of Chokanan mango A: control; B: Thermal treated for 5 minutes; C: Thermal treated for 10 minutes and D: Thermal treated for 20 minutes.**

<sup>1</sup>Arrows indicate spots that were significantly changed above the 2-fold threshold.



**Figure 4.4: Representative gel of Chokanan mango total proteome after 2-D electrophoresis and silver staining.**

<sup>1</sup>The numbers on top indicate the pH interval of isoelectric focusing (pH 4–7), and the numbers on the left show the position of the molecular mass markers (97 to 14.4 kDa).

<sup>2</sup>Spots differentially abundant above the 2-fold threshold are labelled.

The 33 differentially abundant spots that were successfully identified were divided into respective functional groups according to the homologies of the proteins and literatures (Table 4.1). Ten identified spots were found to correspond to sequences from *Mangifera indica* while the remaining 25 identified spots were found to be similar to sequences of other plants. Fifteen spots were found to be related to energy and metabolism based on their biological functions, 12 spots were found to be related to stress and defence, 4 spots were found to be related to ripening and senescence and 2 spots were found to be related to cell structure. In addition, 5 spots that were allergenic in nature were also identified (Putative Allergen Pru P, Allergen Pyr c 3, Profilin 1, Birch Pollen Allergen Bet V 1 Mutant and Celery Allergen Api G 1).

**Table 4.1: Identity of spots affected by postharvest thermal treatment in Chokanan mango.**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						Thermal 5 mins	Thermal 10 mins	Thermal 20 mins
<b>Energy and Metabolism</b>								
1	6-phosphogluconolactonase [ <i>Mangifera indica</i> ] (Sanyal <i>et al.</i> , 2006b)	ABG23393	3	29886	5.16	2.45	4.17	5.14
2	Methionine gamma-lyase-like [ <i>Cicer arietinum</i> ] (NCBI citation)	XP_004502061	4	47989	6.28	2.72	3.86	4.73
3	Alcohol dehydrogenase 3 [ <i>Vitis vinifera</i> ] (Tesniere & Verries, 2000)	NP_001268071	2	41241	6.76	2.09	3.57	5.13
4	Sucrose synthase [ <i>Mangifera indica</i> ] (Li <i>et al.</i> , 2011c)	AEQ30069	4	30019	6.27	3.01	4.91	5.36
5	Neutral invertase [ <i>Mangifera indica</i> ] (Li <i>et al.</i> , 2011b)	AEQ30068	4	27812	5.13	4.03	5.16	5.54
6	Isoflavone reductase homolog [ <i>Solanum tuberosum</i> ] (NCBI citation)	XP_006353700	3	35258	5.82	2.80	3.44	5.11
7	ATP synthase beta subunit [ <i>Mangifera indica</i> ] (Muellner <i>et al.</i> , 2010)	ADY90124	3	52594	5.04	2.68	3.25	4.16
8	Putative malate dehydrogenase [ <i>Trypanosoma grayi</i> ] (NCBI citation)	XP_009312008	5	37408	5.49	2.22	3.05	3.55
9	Citrate synthase [ <i>Mangifera indica</i> ] (Li <i>et al.</i> , 2011a)	AEQ30066	3	30536	5.57	3.03	3.87	4.32
35	Triose-phosphate isomerase [ <i>Triose-phosphate</i> ] (Marchionni & Gilbert, 1986)	ISZMT	4	34199	5.93	2.37	4.13	5.56
36	Aldehyde dehydrogenase [ <i>Nicotiana tabacum</i> ] (op den Camp & Kuhlemeier, 1997)	T02301	5	64042	6.47	2.87	4.22	6.08
25	Pyruvate dehydrogenase [ <i>Zea Mays</i> ] (Thelen <i>et al.</i> , 1999)	Q9ZQY2	6	41984	5.37	1.98	3.03	4.88
37	Alpha-galactosidase [ <i>Carica papaya</i> ] (Soh <i>et al.</i> , 2006)	Q84QC9	3	51541	5.96	2.63	3.92	5.29
38	ADP-glucose pyrophosphorylase small subunit [ <i>Solanum tuberosum</i> ] (Chakravarty <i>et al.</i> , 2005)	Q2PX19	3	60282	5.75	2.23	3.45	5.88
39	Glutamine synthetase [ <i>Prunus persica</i> ] (Tuskan <i>et al.</i> , 2006)	A9PEY1	4	57300	6.30	2.34	3.61	5.01

**Table 4.1: Identity of spots affected by postharvest thermal treatment in Chokanan mango (Continued).**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						Thermal 5 mins	Thermal 10 mins	Thermal 20 mins
<b>Stress and Defence</b>								
10	Putative allergen Pru P [ <i>Prunus dulcis</i> x <i>Prunus Persica</i> ] (Chen <i>et al.</i> , 2008)	ACE80950	4	17437	5.10	3.15	4.22	5.88
11	Allergen Pyr c 3 [ <i>Pyrus communis</i> ] (Scheurer <i>et al.</i> , 2001)	Q9XF38	3	14064	5.19	2.57	3.89	4.99
12	Profilin 1 – Mango allergen [ <i>Mangifera indica</i> ] (Song <i>et al.</i> , 2006)	ABD62998.1	3	14070	4.89	3.33	5.23	6.73
13	Birch Pollen Allergen Bet V 1 Mutant [ <i>Betula pendula</i> ] (Gajhede <i>et al.</i> , 1996)	1QMR_A	2	17310	5.37	2.56	4.13	5.92
14	Thaumatococin-like protein 1 [ <i>Solanum lycopersicum</i> ] (NCBI citation)	XP_004231024	2	36363	4.31	2.18	3.33	5.47
15	Polygalacturonase inhibiting protein [ <i>Prunus persica</i> ] (Wang <i>et al.</i> , 2010)	AEQ93253	3	36198	6.38	2.55	3.67	5.01
16	Superoxide dismutase [ <i>Zea mays</i> ] (Schnable <i>et al.</i> , 2009)	AFW86132	2	42866	5.63	3.01	4.57	5.90
17	RuBisCO complex protein [ <i>Glycine max</i> ] (Staswick <i>et al.</i> , 1994)	2019481A	7	31258	6.0	2.24	3.55	4.98
41	Abscisic acid stress ripening protein homolog [ <i>Malus domestica</i> ] (NCBI citation)	MDP0000253074	2	30640	6.05	2.56	3.87	5.14
42	Farnesyl pyrophosphate synthase [ <i>Mangifera indica</i> ] (Kulkarni <i>et al.</i> , 2013)	AFJ53077.1	5	39451	5.43	3.01	4.85	5.44
43	Dehydroascorbate reductase [ <i>Solanum lycopersicum</i> ] (Aoki <i>et al.</i> , 2010)	NP_001234822	4	23538	6.32	2.45	5.13	6.33
40	Celery Allergen Api G 1 [ <i>Apium graveolens</i> ] (Schirmer <i>et al.</i> , 2005)	2BK0_B	2	16320	4.63	2.87	4.13	7.05



**Table 4.1: Identity of spots affected by postharvest thermal treatment in Chokanan mango (Continued).**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						Thermal 5 mins	Thermal 10 mins	Thermal 20 mins
<b>Ripening and Senescence</b>								
18	1-aminocyclopropane-1-carboxylate oxidase [ <i>Mangifera indica</i> ] (Gupta <i>et al.</i> , 2004)	AAU06261	5	10573	5.60	2.08	3.56	4.93
19	Beta-1,3-glucanase [ <i>Mangifera indica</i> ] (Sanyal <i>et al.</i> , 2006a)	ABD16200	4	19507	5.77	2.59	3.92	5.07
20	Aminocyclopropane carboxylate synthase [ <i>Glycine max</i> ] (Liu <i>et al.</i> , 1993a)	2019442A	3	54730	5.84	2.89	3.77	5.13
44	Putative aminocyclopropane carboxylic acid synthase [ <i>Mangifera Indica</i> ] (Saiprasad & Lalitha, 2002)	CAD44266	5	31323	5.64	2.44	4.10	6.09
<b>Cell Structure</b>								
45	Actin [ <i>Prunus persica</i> ] (Hannum <i>et al.</i> , 2009)	D5MQE0	2	63000	4.86	2.14	3.12	3.89
46	Actin-depolymerizing factor 2 [ <i>Zea mays</i> ] (Lopez <i>et al.</i> , 1996)	NP_001105590	6	16083	5.57	2.02	2.98	3.16

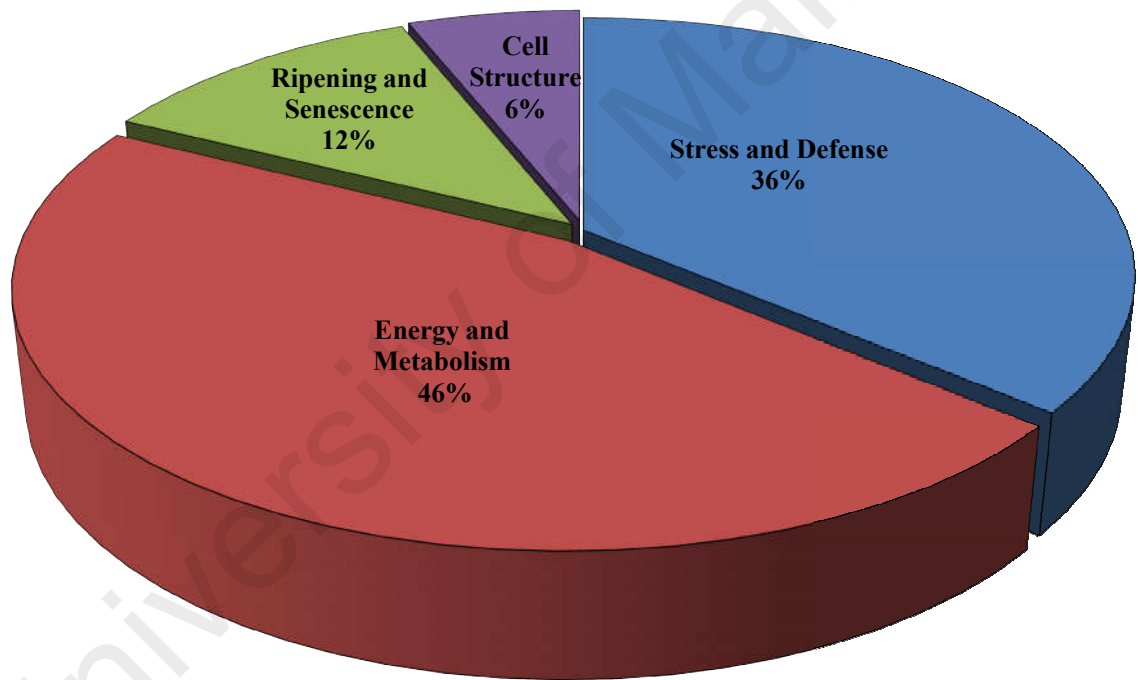
<sup>a</sup>Spot numbers corresponds to the 2-DE in Figure 4.8

<sup>b</sup>Experimental molecular weight

<sup>c</sup>Experimental pI value

<sup>d</sup>Fold change of protein abundance (decrease in abundance) as compared to controls following different durations of thermal treatment

Proteins that were differentially abundant following thermal treatments were found to be related to 4 different functional groups based on their homologies and literatures. The proteins were related to energy and metabolism (46%), stress and defence (36%), ripening and senescence (12%) and cell structure (6%) (Figure 4.5). The relation of the differentially abundant proteins following heat treatment to the quality of Chokanan mango is discussed in the following segments.

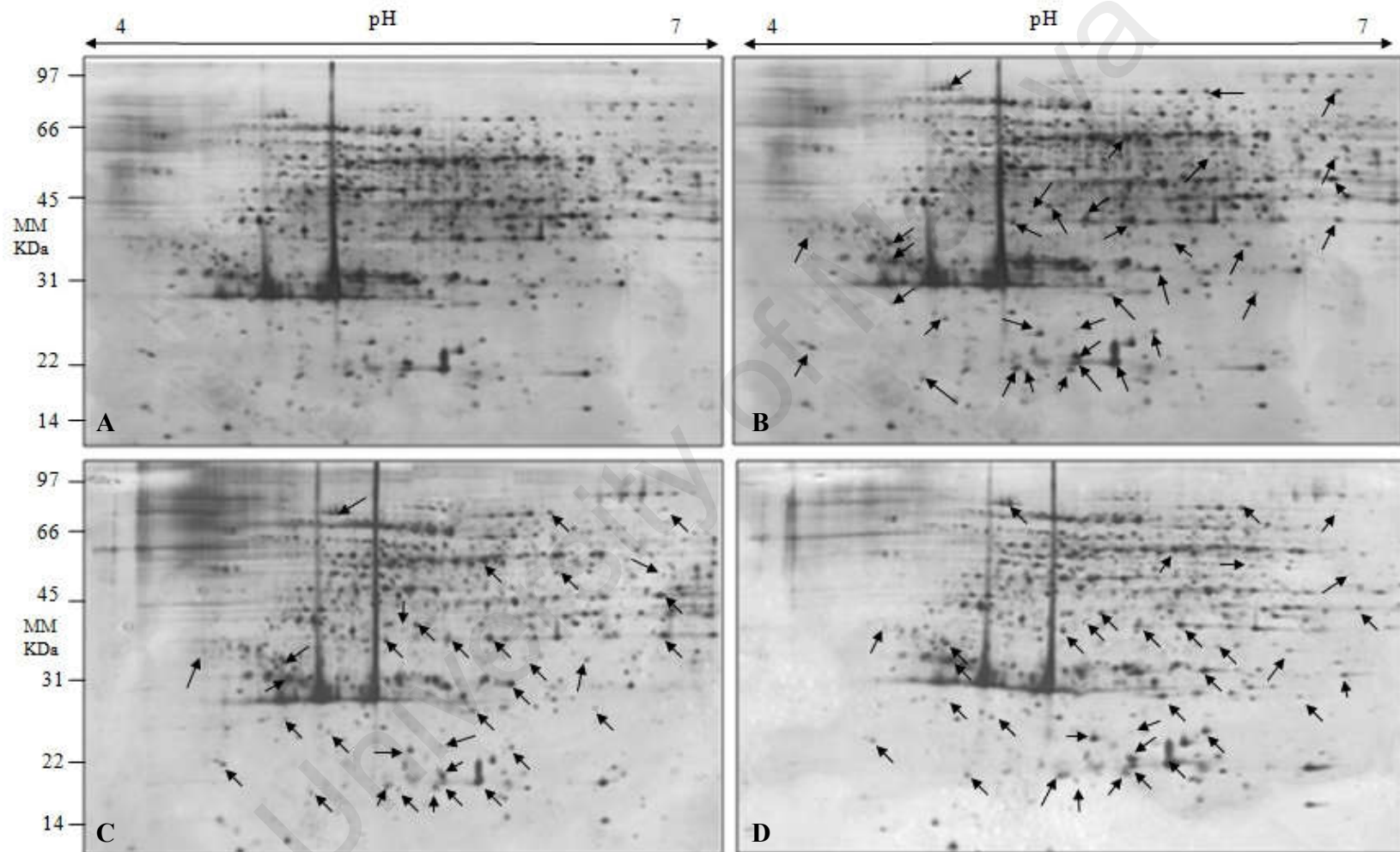


**Figure 4.5: Classification of proteins affected by thermal treatment in Chokanan mango (identified by mass spectrometry analysis and grouped according to its functions).**

### 4.3.3 Effects of Thermal Treatment on the Proteome of Josephine Pineapple

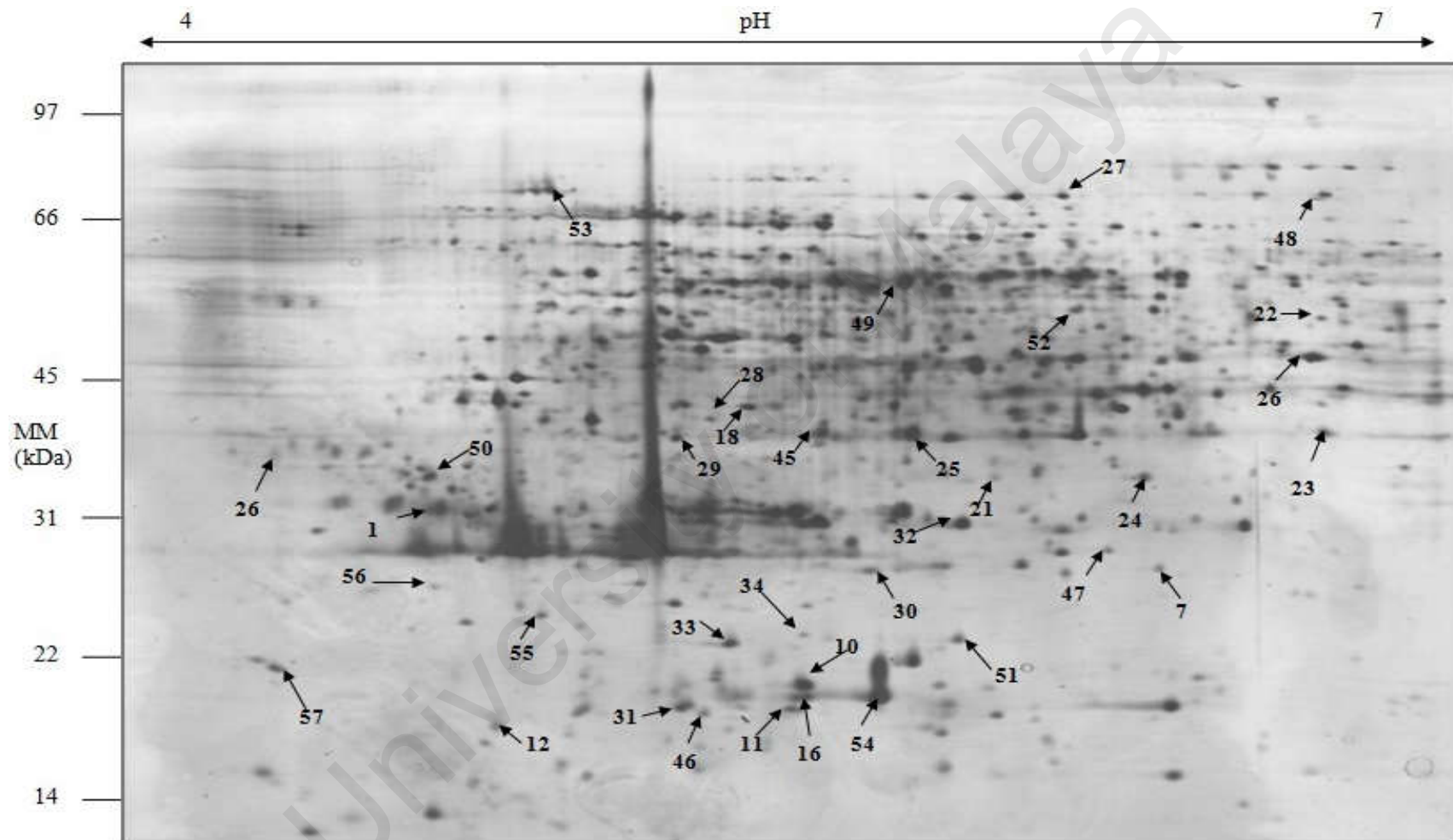
Figure 4.6 represents the 2-DE proteome profiles of untreated and heat treated (5, 10 and 20 minutes) Josephine pineapple following software guided comparative analysis. An average of 400 reproducibly detectable spots was detected in the spots in the pH range of 4 to 7 and molar mass of 14 to 97 kDa. Through gel analysis of the thermal treated samples against controls via the ImageMaster 2-D Platinum software Version 7.0 (GE Healthcare), 40 spots were found to be differentially abundant above the 2-fold threshold following thermal treatment. These 40 spots were excised, in gel digested via trypsin and subjected to mass spectrometry and out of the 40 spots, 35 were successfully identified using the Ludwig NR database (Figure 4.7).

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**Figure 4.6: Representative spot maps of Josephine pineapple A: control; B: thermal treated for 5 minutes; C: thermal treated for 10 minutes and D: thermal treated for 20 minutes.**

<sup>1</sup>Arrows indicate spots that were significantly changed above the 2-fold threshold.



**Figure 4.7: Representative gel of 'Josephine' pineapple total proteome after 2-D electrophoresis and silver staining.**

<sup>1</sup>The numbers on top indicate the pH interval of isoelectric focusing (pH 4–7), and the numbers on the left show the position of the molecular mass markers (97 to 14 kDa).

<sup>2</sup>Spots differentially abundant above the 2-fold threshold are labelled accordingly.

The 35 differentially abundant spots that were successfully identified were divided into respective functional groups according to the homologies of the proteins and literatures (Table 4.2). Eleven identified spots were found to correspond to sequences from *Ananas comosus* while the remaining 24 identified spots were found to be similar to sequences of other plants. Seventeen spots were found to be related to energy and metabolism based on their biological functions, 11 were related to stress and defence, 4 spots were related to cell structure while 3 spots were found to be related to ripening and senescence. Additionally, 4 spots that were allergenic in nature were also identified (Putative Allergen Pru P, Allergen Pyr c 3, Profilin – Allergen Ana c 1 and Allergen Ana c 2).

**Table 4.2: Identity of spots affected by postharvest thermal treatment in Josephine pineapple.**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						Thermal 5 mins	Thermal 10 mins	Thermal 20 mins
<b>Energy and Metabolism</b>								
1	6-phosphogluconolactonase [ <i>Zea mays</i> ] (Alexandrov <i>et al.</i> , 2009)	ACG35132	7	31752	4.52	2.44	3.76	5.12
7	ATP synthase [ <i>Eucalyptus globules</i> ] (Steane, 2005)	AAX21014	2	27524	6.03	2.98	4.01	6.23
21	Glyceraldehyde-3-phosphate dehydrogenase [ <i>Trypanosoma grayi</i> ] (Kelly <i>et al.</i> , 2014)	XP_009308756	6	37521	5.61	2.15	3.27	4.34
22	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [ <i>Ananas comosus</i> ] (Nashima <i>et al.</i> , 2014)	YP_009116350	4	53192	6.33	3.02	4.35	5.87
23	Methionine aminopeptidase 1 [ <i>Ananas comosus</i> ] (Fairbairn <i>et al.</i> , 2005)	ABB91774	6	43757	6.33	3.34	4.97	6.35
24	Gibberellin 2-beta-dioxygenase [ <i>Ananas comosus</i> ] (Zhang <i>et al.</i> , 2009)	ACN30002	5	36223	5.95	2.16	3.93	4.59
25	Pyruvate dehydrogenase E1 beta subunit isoform [ <i>Zea mays</i> ] (Thelen <i>et al.</i> , 1999)	Q9ZQY2	4	41984	5.37	2.01	2.82	3.15
26	Phosphoglycerate kinase [ <i>Capsella rubella</i> ] (NCBI citation)	R0G4P3	5	50147	6.30	2.32	3.87	4.98
26	Phosphoglycerate kinase [ <i>Gossypium hirsutum</i> ] (Pang <i>et al.</i> , 2008)	ACJ11718.1	3	39900	4.14	3.02	4.03	5.44
27	D-3-phosphoglycerate dehydrogenase (GAPDH) [ <i>Ricinus communis</i> ] (Chan <i>et al.</i> , 2008)	XP_002518687.1	2	65100	5.74	2.34	3.56	4.87
28	Acid phosphatase 1 [ <i>Prunus dulcis</i> ] (Tuskan <i>et al.</i> , 2006)	B9H8Y0	7	43100	5.06	2.89	4.14	5.43
47	6-phosphogluconolactonase 2 [ <i>Arabidopsis thaliana</i> ] (Salanoubat <i>et al.</i> , 2000)	AEE78532	3	28863	5.89	2.30	3.59	4.12
48	Sucrose transporter protein [ <i>Ananas comosus</i> ] (Antony <i>et al.</i> , 2008)	ABO21770	3	66455	6.30	2.87	3.99	5.27
49	NADH-plastoquinone oxidoreductase subunit 2 [ <i>Ananas comosus</i> ] (Nashima <i>et al.</i> , 2014)	YP_009116402	5	56806	5.43	2.32	3.15	4.66

**Table 4.2: Identity of spots affected by postharvest thermal treatment in Josephine pineapple.**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						Thermal 5 mins	Thermal 10 mins	Thermal 20 mins
<b>Energy and Metabolism</b>								
50	Fibrillin homolog CDSP34 precursor protein [ <i>Solanum tuberosum</i> ] (Gillet <i>et al.</i> , 1998)	T07825	5	37213	4.50	2.87	3.42	4.69
51	Soluble inorganic pyrophosphatase [ <i>Solanum tuberosum</i> ] (du Jardin <i>et al.</i> , 1995)	Q43187	4	24262	5.59	2.57	3.99	6.01
52	Sugar transporter protein [ <i>Ananas comosus</i> ] (Anthony <i>et al.</i> , 2008)	ABO21769	5	54398	5.70	2.76	4.09	5.15
<b>Stress and Defense</b>								
10	Putative allergen Pru P [ <i>Prunus dulcis x Prunus persica</i> ] (Chen <i>et al.</i> , 2008)	ACE80950.1	3	17500	5.27	2.17	3.08	4.55
11	Allergen Pyr c 3 [ <i>Pyrus communis</i> ] (Scheurer <i>et al.</i> , 2001)	Q9XF38.1	2	13200	5.19	2.99	3.84	4.39
12	Profilin - Allergen Ana c 1 [ <i>Ananas comosus</i> ] (Reindl <i>et al.</i> , 2002)	Q94JN2.1	4	14229	4.71	3.24	4.94	6.18
16	Copper/zinc-superoxide dismutase [ <i>Ananas comosus</i> ] (Lin <i>et al.</i> , 2000)	CAB60191	3	15176	5.29	2.38	3.54	4.89
29	Allergen Ana c 2 [ <i>Ananas comosus</i> ] (NCBI citation)	O23791.1	5	39055	5.00	3.01	4.23	5.09
30	Glutathione transferase [ <i>Carica papaya</i> ] (Lam & Bakar, 1999)	T09781	3	28538	5.43	2.34	3.16	4.73
31	Thioredoxin H-type (Trx-H) [ <i>Oryza sativa Japonica Group</i> ] (NCBI citation)	AAT44260	6	13177	4.96	2.83	4.23	5.22
32	Putative lactoylglutathione lyase [ <i>Malus Domestica</i> ] (NCBI citation)	MDP0000319112	2	35320	5.60	3.15	4.87	6.57
33	Mannose-binding lectin [ <i>Ananas comosus</i> ] (Neuteboom <i>et al.</i> , 2002)	AAM28277	3	17422	5.20	3.55	5.21	6.88
53	Chaperonin 60 alpha chain precursor [ <i>Brassica napus</i> ] (NCBI citation)	S38642	4	66402	4.70	2.70	3.95	5.13
54	Class II small heat shock protein [ <i>Arachis hypogaea</i> ] (Bi <i>et al.</i> , 2006)	ACF74271.1	5	15900	5.41	2.84	3.99	5.09



**Table 4.2: Identity of spots affected by postharvest thermal treatment in Josephine pineapple.**

<sup>a</sup> Spot (#)	Protein Name	Accession No.	Matched peptide	<sup>b</sup> Molecular Weight	<sup>c</sup> pI	<sup>d</sup> Fold change (decrease) in abundance		
						Thermal 5 mins	Thermal 10 mins	Thermal 20 mins
<b>Cell structure</b>								
45	Actin [ <i>Ananas comosus</i> ] (Ma & He, 2010)	ADV91594	2	41730	5.23	2.87	3.58	4.78
46	Actin depolymerizing factor 1 (ADF) [ <i>Populus trichocarpa</i> ] (NCBI citation)	XP_002299887.1	2	16500	5.10	2.55	3.43	4.13
55	Translationally-controlled tumor protein homolog (TCTP) [ <i>Fragaria x ananassa</i> ] (NCBI citation)	O03992.1	3	20000	4.76	2.07	2.43	3.09
56	Calcium ion binding protein (CaBP) [ <i>Ricinus communis</i> ] (NCBI citation)	FC862563	5	28200	4.52	2.05	2.98	3.15
<b>Ripening and Senescence</b>								
18	1-Aminocyclopropane-1-carboxylate oxidase (ACC oxidase) [ <i>Musa acuminata</i> ] (NCBI citation)	Q6LC46 MUSAC	4	42836	5.18	2.11	2.95	3.76
34	Ripening-associated protein [ <i>Musa acuminata</i> ] (Clendennen and May, 1997)	O22322 MUSAC	4	21296	5.24	2.32	3.33	3.98
57	Ripening regulated protein DDTFR8 [ <i>Solanum lycopersicum</i> ] (NCBI citation)	AAG49030	3	20260	4.29	2.22	3.15	4.13

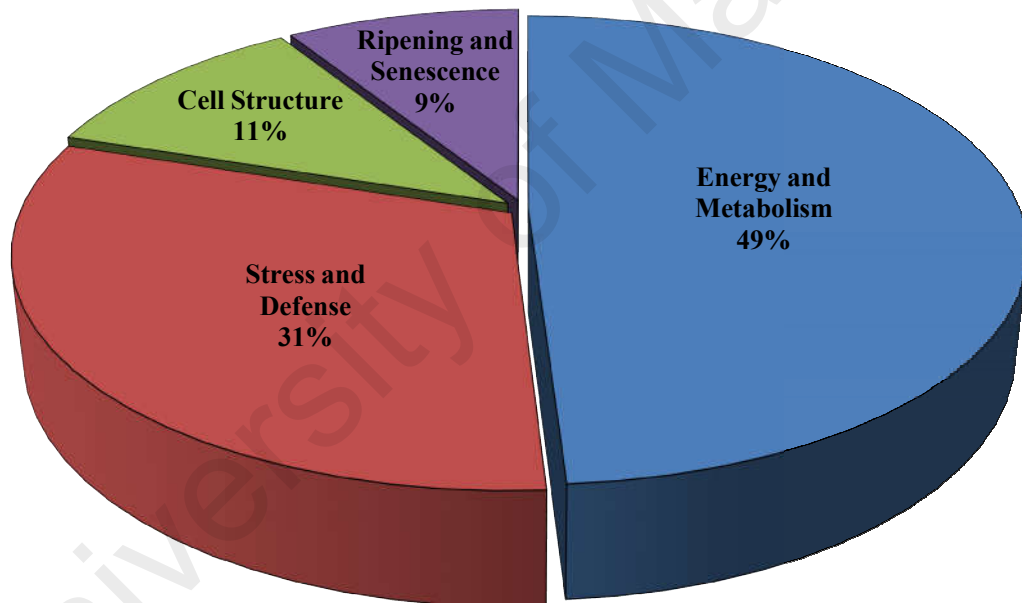
<sup>a</sup>Spot numbers corresponds to the 2-DE in Figure 4.8

<sup>b</sup>Experimental molecular weight

<sup>c</sup>Experimental pI value

<sup>d</sup>Fold change of protein abundance (decrease in abundance) as compared to controls following different durations of thermal treatment

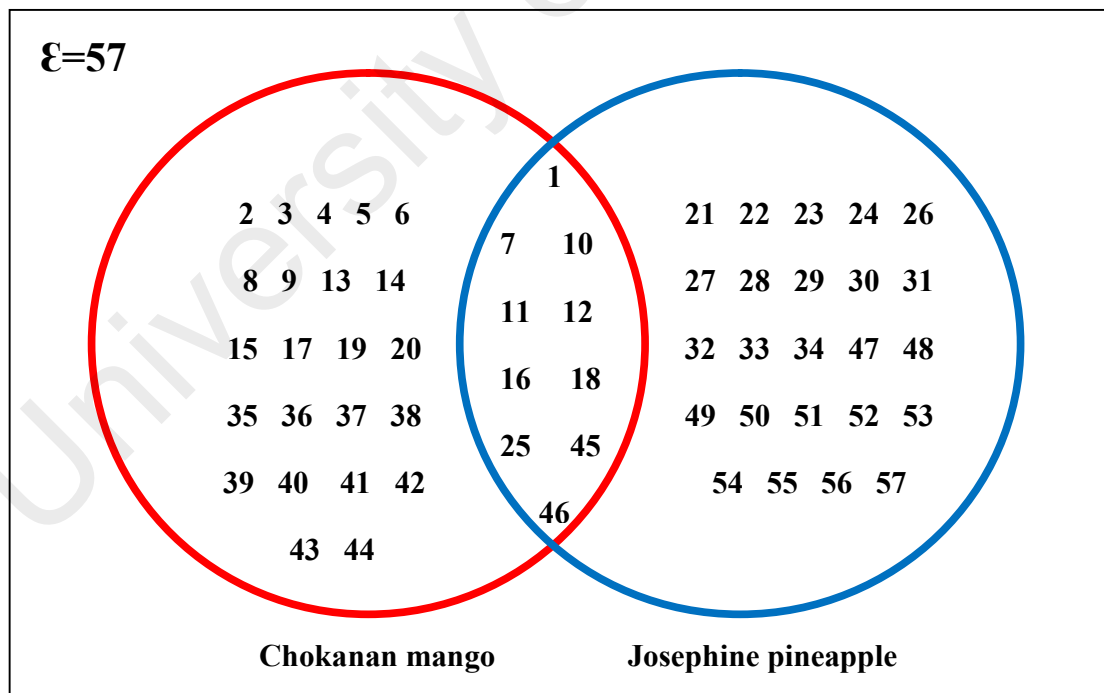
Proteins that were differentially abundant following the different durations of thermal treatment (70°C) were found to be related to energy and metabolism (49%), stress and defence (31%), cell structure (11%) as well as ripening and senescence (9%) (Figure 4.8). Postharvest treatments such as heat treatment have been associated with either the maintenance or deterioration of the quality of crops. This study was designed to identify differentially abundant proteins following postharvest heat treatment and how they could be related to the quality of Josephine pineapple and is discussed in the following segments.



**Figure 4.8: Classification of proteins affected by thermal treatment in Josephine pineapple (identified by mass spectrometry analysis and grouped according to its functions).**

#### 4.3.4 Effects of Thermal Treatment on Proteome of Chokanan Mango and Josephine Pineapple

A total of 57 spots were found to be differentially abundant across both Chokanan mango and Josephine pineapple following thermal treatment. Out of the 57 spots, 21 were differentially abundant only in mangoes, 24 were differentially abundant only in pineapples while 10 were found to be differentially abundant in both thermal treated mango and pineapples namely phosphoglycerate kinase (spot#1), ATP synthase (spot#7), putative allergen Pru 1.06B (spot#10), allergen Pyr c 3 (spot#11), profilin (spot#12), superoxide dismutase (spot#16), 1-Aminocyclopropane-1-carboxylate oxidase (spot#18), pyruvate dehydrogenase (spot#25), actin (spot#45) and actin depolymerizing factor (spot#46) (Figure 4.9). Proteins affected by thermal treatment in both mango and pineapple were those that were from the energy and metabolism, stress and defence, ripening and senescence as well as cell structure functional groups.



**Figure 4.9: Venn diagram of the number of identified proteins that were differentially abundant in Chokanan mango and Josephine pineapple following thermal treatment.**  
<sup>1</sup>Numbers correspond to spot numbers in Table 4.1 and 4.2.

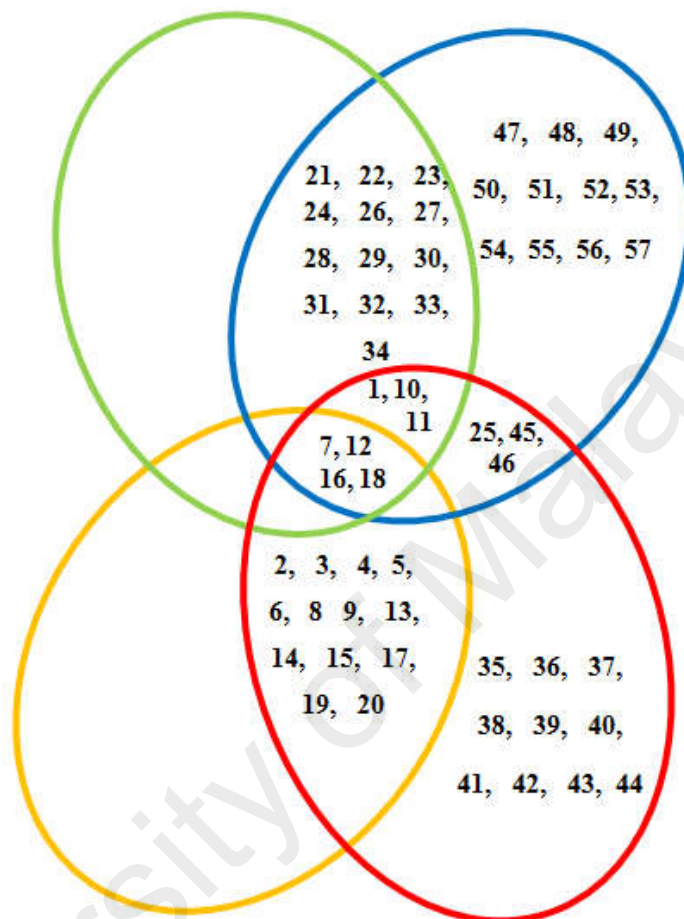
#### **4.3.5 Effects of Postharvest UV-C and Thermal Treatment on the Proteome of Chokanan Mango and Josephine Pineapple**

Following postharvest UV-C and thermal treatment of Chokanan mango and Josephine pineapple, a total of 57 protein spots that were differentially abundant were successfully identified (Figure 4.11). In both the fruits, spots that were affected by UV-C treatment were also affected by thermal treatment. There were no spots that were exclusively affected by UV-C treatment. Thermal treatment in Josephine pineapple significantly reduced 11 additional spots along with those reduced by UV-C treatment. On the other hand, 10 additional spots were significantly reduced in thermal treated Chokanan mango along with those that were reduced by UV-C treatment. Thermal treatment affected 10 spots which were identified in both mango and pineapple. Out of the 57 identified spots, 4 were found to be affected by UV-C and thermal treatment in both mango and pineapples which were ATP synthase (spot#7), profilin (spot#12), superoxide dismutase (spot#16) and ACC oxidase (spot#18) (Figure 4.10).

$\Sigma=57$

UV-C treated Josephine pineapple

Thermal treated Josephine pineapple

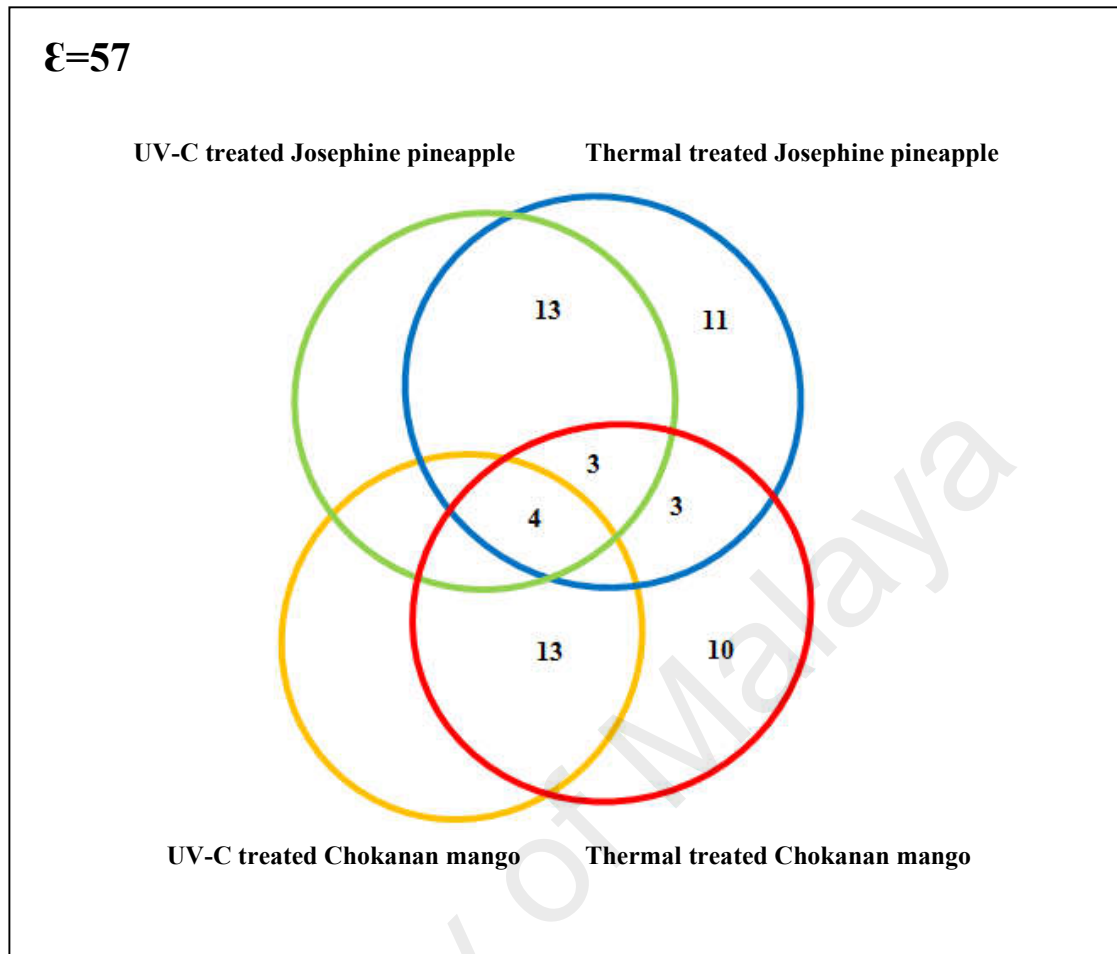


UV-C treated Chokanan mango

Thermal treated Chokanan mango

**Figure 4.10: Venn diagram of the number of identified proteins that were differentially abundant in Chokanan mango and Josephine pineapple following thermal treatment and UV-C treatment.**

<sup>1</sup>Numbers correspond to spot numbers in Table 3.5, 3.6, 4.1 and 4.2.



**Figure 4.11: Venn diagram of the number of identified proteins that were affected in Chokanan mango and Josephine pineapple following thermal treatment and UV-C treatment.**

<sup>1</sup>Numbers indicate total number of spots that were affected by the different treatments.

#### 4.4 Discussion

Changes in postharvest treated fruits are attributed to the disruption of various processes. Although it is evident that biological processes are catalyzed by proteins, information regarding the proteome associated to these biological processes remains scarce. This study therefore, serves as a means of understanding the molecular mechanisms that underline the changes that take place in postharvest thermal (70°C) treated mangoes and pineapples. The comprehensive analysis of this study revealed that the proteins affected by thermal treatment were those that regulate biological processes that contribute to changes in fruits

leading to deterioration of postharvest crops. This present study discusses the effects of this treatment on a proteomic level. Observations of this study are highly relevant to the postharvest industry as well as consumers. Our most substantial observations are discussed in the following segments.

Following thermal treatment of Chokanan mango, 15 spots that were differentially abundant were found to be related to energy and metabolism, namely 6-phosphogluconolactonase, methionine gamma-lyase-like, alcohol dehydrogenase 3, sucrose synthase, neutral invertase, isoflavone reductase homolog, ATP synthase beta subunit, putative malate dehydrogenase, citrate synthase, triose-phosphate isomerase, aldehyde dehydrogenase, pyruvate dehydrogenase, alpha-galactosidase, ADP-glucose pyrophosphorylase small subunit and glutamine synthetase. The roles and functions of the proteins 6-phosphogluconolactonase, methionine gamma-lyase-like, alcohol dehydrogenase 3, sucrose synthase, neutral invertase, isoflavone reductase homolog, ATP synthase beta subunit, putative malate dehydrogenase and citrate synthase in energy and metabolism have been discussed in section 3.4. The protein triose-phosphate isomerase is one of the key enzymes of the glycolytic pathway which catalyzes the reversible interconversion of the triose phosphate isomers dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate (Mathur *et al.*, 2006). Aldehyde dehydrogenase is another enzyme that plays a role in plant energy and metabolism. The protein is reported to catalyze the oxidation of both endogenous and exogenous aldehydes to corresponding carboxylic acids (Gao & Han, 2009). Both pyruvate dehydrogenase and alpha galactosidase are also enzymes that are related to the glycolytic pathway in plants. Pyruvate dehydrogenase catalyzes the irreversible reaction that leads to the breakdown of glucose in the energy generating pathway (Patel & Roche, 1990). ADP-glucose pyrophosphorylase and glutamine synthetase

are also enzymes which are pivotal for plant energy and metabolism. It has been reported to be involved in the rate-limiting step in the synthesis of starch in plants (Georgelis *et al.*, 2009). As stated above, a total of 15 proteins which play pivotal roles in energy and metabolism were significantly reduced in abundance following thermal treatments. The effect of thermal treatment on these proteins may be beneficial in the extension of shelf-life of fruits.

Apart from energy and metabolism, 12 spots that were significantly affected by thermal treatment were found to be related to stress and defence, which were putative allergen Pru P, allergen Pyr c 3, allergen profilin 1, birch Pollen Allergen Bet V 1 Mutant, thaumatin-like protein 1, polygalacturonase inhibiting protein, Celery Allergen Api G 1, superoxide dismutase, RuBisCO complex protein, Abscisic acid stress ripening protein homolog, Farnesyl pyrophosphate synthase and dehydroascorbate reductase. The roles of the proteins putative allergen Pru P, allergen Pyr c 3, profilin - Mango allergen, birch Pollen Allergen Bet V 1 Mutant, thaumatin-like protein 1, polygalacturonase inhibiting protein, superoxide dismutase and RuBisCO complex protein has been discussed in section 3.4. The protein Celery Allergen Api G 1 is known to be involved in actin polymerization according to a previous report (Haarer & Brown, 2005). Along with Celery Allergen Api G 1, thermal treatment was successful in reducing the abundance four other allergens which are putative allergen Pru P, allergen Pyr c 3, profilin - Mango allergen and birch Pollen Allergen Bet V 1 Mutant. The efficacy of thermal treatment in reducing the abundance of allergenic proteins in mangoes may prove to be invaluable in addressing allergic reactions in patients. Similar to UV-C treatment, a reduced abundance of allergenic proteins following thermal treatment may translate to reduced allergic reactions in patients which may pave the way for the advancement of new food processing technologies. Thermal treatment was also



successful in reducing the abundance of these allergenic proteins to lower levels as compared to UV-C. The abscisic acid stress ripening protein is known to be involved in fruit ripening as well as in the response to abiotic stress such as drought and salinity (Golan *et al.*, 2014). Farnesyl pyrophosphate synthase has been reported as being the precursor to many isoprenoids which are the most diverse and abundant group of natural products involved in varying physiological processes in plants (Cervantes-Cervantes *et al.*, 2006). Finally, dehydroascorbate reductase is known to play an important role in the ascorbate-glutathione recycling reaction in higher plants (Yang *et al.*, 2009). A reduced abundance of these proteins may in turn hamper the biological processes that they catalyze and therefore, result in delayed deterioration and eventual shelf-life extension.

Four spots that were reduced in abundance following thermal treatment were found to be related to ripening and senescence namely 1-aminocyclopropane-1-carboxylate oxidase, beta-1,3-glucanase, aminocyclopropane carboxylate synthase, and putative aminocyclopropane carboxylic acid synthase. The proteins 1-aminocyclopropane-1-carboxylate oxidase, aminocyclopropane carboxylate synthase, and putative aminocyclopropane carboxylic acid synthase are related to the biosynthesis of ethylene while beta-1,3-glucanase has been reported to be involved in diverse physiological and developmental processes in plants or fruits as discussed in section 3.4. Thermal treatment, with its efficacy in reducing the abundance of protein related to ethylene biosynthesis, is therefore a valuable processing technology as it could be exploited to reduce the negative effects that may be brought about by exposure of fruits to ethylene during postharvest storage of fruits and vegetables (Martinez *et al.*, 2007).

Finally, in thermally treated mangoes, 2 protein spots that were reduced in abundance were found to be related to cell structure, namely actin and actin-depolymerizing factor. Actin is known to play distinct roles in plant structures and is an integral component of the cytoskeleton. It contributes to the maintenance of the internal structure of the cell, facilitates the movement of components within the cytosol as well as aids cell division (Deeks & Hussey, 2009). The actin depolymerizing factor is also known as small actin-binding proteins. Their roles in higher plants according to reports are in plant growth, development and pathogen defence (Huang *et al.*, 2012). The effects of thermal treatment on these proteins suggest that this treatment may have adverse effects on the integrity and cell structure of the fruit.

As for thermally treated pineapples, 17 spots that were significantly affected were related to energy and metabolism, namely Phosphoglycerate kinase, 6-phosphogluconolactonase, 6-phosphogluconolactonase 2, Glyceraldehyde-3-phosphate dehydrogenase, Sucrose transporter protein, NADH-plastoquinone oxidoreductase subunit 2, Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, Methionine aminopeptidase 1, ATP synthase, Gibberellin 2-beta-dioxygenase, Pyruvate dehydrogenase E1 beta subunit isoform, Fibrillin homolog CDSP34 precursor protein, D-3-phosphoglycerate dehydrogenase (GAPDH), Soluble inorganic pyrophosphatase, Acid phosphatase 1 and Sugar transporter protein. The roles of the proteins Phosphoglycerate kinase, Glyceraldehyde-3-phosphate dehydrogenase, Ribulose-1,5-bisphosphate carboxylase / oxygenase large subunit, Methionine aminopeptidase 1, ATP synthase, Gibberellin 2-beta-dioxygenase, Pyruvate dehydrogenase E1 beta subunit isoform, Phosphoglycerate kinase, D-3-phosphoglycerate dehydrogenase (GAPDH) and Acid phosphatase 1 have been discussed in section 3.4. The enzyme 6-phosphogluconolactonase catalyzes the hydrolysis

of phosphogluconolactone to 6-phosphogluconate which is the second step of pentose phosphate pathway (Miclet *et al.*, 2001). Both the glycolytic and pentose phosphate pathway have been reported to provide ATP for respiration burst in ripe fruits with increased energy demand (Piechulla, 1988). Our observation suggests that the reduced abundance of these proteins involved in these pathways may reduce the rate of respiration in pineapples following thermal treatment. A reduced rate of respiration may translate to a reduced rate of cell deterioration. Sucrose transporter proteins and sugar transporter proteins play similar roles in higher plants. The sucrose transporter protein and sugar transporter proteins have been reported to be involved in allocation of sucrose and sugar intracellularly and at the whole plant level respectively (Lemoine, 2000; Williams *et al.*, 2000). Both sugar and sucrose are important for growth and development of higher plants and these transporters are therefore important for the optimum growth of plants as a whole. NADH-plastoquinone oxidoreductase is an enzyme of the respiration chains and is perceived to play a role in plant respiration (Nakamaru-Ogiso *et al.*, 2010). The activity of soluble inorganic pyrophosphatase has been reported to recycle the pyrophosphate produced by many anabolic reactions. It is also perceived to play additional roles in plants which include the regulations of primary metabolism, sulphur metabolism and growth (Rosales-Leon *et al.*, 2012).

Eleven spots that were differentially abundant in thermal treated pineapples were found to be related to stress and defense which include Putative allergen Pru 1.06B, Allergen Pyr c 3, Glutathione transferase, Chaperonin 60 alpha chain precursor, Class II small heat shock protein, Copper/zinc-superoxide dismutase, Thioredoxin H-type, Putative lactoylglutathione lyase, Mannose-binding lectin, Allergen Ana c 1 and Allergen Ana c 2. The functions and roles of the proteins Glutathione transferase, Copper/zinc-superoxide

dismutase, Thioredoxin H-type, Putative lactoylglutathione lyase, Mannose-binding lectin , Allergen Ana c 1 and Allergen Ana c 2 were discussed in section 3.4. Chaperonins and heat shock proteins play similar roles in plants which are related to stress and response with the difference being that heat shock proteins are induced by heat (Kabir *et al.*, 2011). The allergens Pru P and Pyr c 3 are non-lipid proteins which are implicated to play a role in key processes of plant physiology (Carvalho & Gomes, 2007). A total of 4 allergenic proteins were observed to be reduced in abundance following thermal treatment of pineapples. Thermal treatments was effective in reducing the abundance of 2 additional allergens, which were Putative allergen Pru 1.06B and Allergen Pyr c 3, along with the 2 allergens that were reduced in UV-C treated pineapples. Allergens that were identified in mangoes were also found to be present in pineapples namely Putative allergen Pru 1.06B, Allergen Pyr c 3 and profilin. The presence of the same allergens in different fruits has been reported and is perceived as the cause for cross-reactivity whereby an individual is allergic to several fruits due to presence of the same allergen (Diaz-Perales *et al.*, 1999; Turjanmaa & Mäkinen-Kiljunenb, 2002; Vieths *et al.*, 2006). The potential of thermal treatment in reducing the abundance of these allergenic proteins in pineapples may be an additional benefit of this treatment along with quality maintenance and shelf-life extension. A reduced abundance of allergenic protein may be beneficial in addressing the issue of fruit allergy in susceptible individuals. However, further research is required to confirm the efficacy of this treatment in addressing this issue.

Following thermal treatment, 3 spots that were reduced in abundance were found to be related to ripening and senescence which were 1-Aminocyclopropane-1-carboxylate oxidase, Ripening regulated protein DDTFR8 and Ripening-associated protein. All three proteins are known to be involved in the regulation of ripening as well as senescence via

the plant hormone ethylene (Liu & Zhang, 2004; Bassan *et al.*, 2006; Osmark *et al.*, 1998). Based on our observation, a reduced abundance of these proteins may halt the detrimental effects of ethylene which leads to senescence and eventually, spoilage of fruits.

Finally in thermal treated pineapples, 4 protein spots that were reduced in abundance were found to be related to cell structure namely Actin, Actin depolymerizing factor 1, Translationally-controlled tumor protein homolog and Calcium ion binding protein. Actin is an integral component of the cytoskeleton and has been reported to play distinct roles in plant structures by contributing to the maintenance of the cell's internal structures, facilitating the movement of components within the cytosol and aiding cell division (Deeks & Hussey, 2009). Actin depolymerizing factors have been reported to have major roles in higher plants which include plant growth and development as well as defence against pathogen attacks (Huang *et al.*, 2012). The translationally-controlled tumor protein homolog (TCTP) is another key player in plants' cell structure, whereby it has been reported to be involved in processes such as cell growth and reprogramming, cell shape, malignant transformation, cell cycle progression, stress protection and inhibition of apoptosis (Nagano-Ito & Ichikawa, 2012; Bommer & Thiele, 2004). Calcium ion binding proteins are integral parts of the cell wall and it functions as a transport medium for calcium ions into the cytosol of plant cells. It has been established that calcium ions are crucial regulators of growth and development in plants (Hepler, 2005). The role of calcium ion binding proteins in the transport of calcium ion renders it as an important member for plant growth and development. Thermal treatment of 70 °C was observed to have adverse effects on these proteins suggesting adverse effects on the integrity and cell structure of pineapples. Collectively, the effects of thermal treatment on the proteome changes of

mangoes and pineapples may result in both desirable and undesirable characteristics in the fruits.

It is of interest to focus on the 4 proteins that were reduced in abundance following UV-C and thermal treatment in both Chokanan mango and Josephine pineapple as these proteins may be of significant importance with regards to quality maintenance and shelf-life of fruits. Proteins affected by UV-C and thermal treatment in the fruits were ATP synthase (spot#7), profilin (spot#12), superoxide dismutase (spot#16) and ACC oxidase (spot#18). ATP synthase, with its known role in the synthesis of ATP's, is an important protein for respiration in fruits. Reduced abundance of this protein is therefore related to a reduced respiration rate in fruits during storage delaying deterioration of fruits. ACC oxidase is a key enzyme in the regulation of ethylene biosynthesis. Ethylene is known for its detrimental effect on postharvest life of fruits and previous research has indicated that the removal or reducing of ethylene from the surrounding atmosphere leads to delayed or reduced physiological disorders in fruits (Zauberman & Fuchs, 1973; Lee & Young, 1984). Both UV-C and thermal treatment with the ability to reduce the abundance of ACC oxidase could therefore reduce biosynthesis of ethylene in treated fruits and eventually lead to delayed deterioration leading to a longer shelf-life. These treatments could be exploited for the advancement of better preservation techniques of postharvest crops. Profilin has recently been classified as a multifunctional protein that has both positive and negative regulatory effects on actin polymerization. It is also known to be involved in signal transduction in higher plants. A reduced abundance of profilin in fruits may be detrimental during growth and development; however, it may be insignificant in postharvest crops with regards to quality. On the other hand, a reduced abundance of this protein may be beneficial in addressing the issue of fruit allergy as profilin is a major allergen in several fruits (van

Ree *et al.*, 1992). Finally, the enzyme superoxide dismutase plays an important role in plant stress and defence. A reduce in abundance of this protein is therefore undesirable as it may hamper the scavenging of reactive oxygen species in fruits during senescence (Foyer, 1997).

In conclusion, the proteins that were affected by thermal and UV-C treatment in Chokanan mango and Josephine pineapple could result in delayed fruit deterioration leading to the extension of shelf-life. Reduced metabolic activities of fruits during storage could potentially reduce deterioration in fruits. Furthermore, the effects of ethylene in storage could also be minimized by thermal and UV-C treatment via reduction of ethylene biosynthesis. However, the effects of thermal treatment on proteins that were related to cell structure is a concern as it could cause adverse effects to the quality of the fruits. UV-C treatment, on the other hand, was observed to not affect proteins related to cell structure.

## **CHAPTER 5: EFFECTS OF UV-C AND THERMAL (70°C) TREATMENT ON THE QUALITY OF CHOKANAN MANGO (*Mangifera indica* L. cv. Chokanan) AND JOSEPHINE PINEAPPLE (*Ananas comosus* L. Merr. Josephine)**

### **5.1 Introduction**

Food quality can be defined as the characteristics of food which include external and internal factors that are acceptable to consumers. External factors of food quality comprise of sensory attributes such as appearance, texture, aroma and flavour. Internal factors, on the other hand, involve physicochemical, nutritional and food safety (microbial) properties. One of the most important requirements for food manufacturing and processing is food safety due to the susceptibility of consumers to contaminants in food products (Grunert, 2005). Besides that, food processing is also known to alter the organoleptic, textural and nutritional qualities of food products (Vicknesha *et al.*, 2014). Evaluation of food quality should therefore comprise of analysis to assess both external and internal quality of a particular food commodity. The effects of these treatments on the proteomic changes of the fruits suggest that these treatments could confer quality maintenance and shelf-life extension in treated fruits. Accordingly, in this chapter, the effects of these treatments on the quality (physicochemical, nutritional, microbial, consumers' acceptance and shelf-life) of mango and pineapples were evaluated.

Physicochemical analysis in this chapter comprised of pH, total soluble solids (TSS) and titratable acidity (TA). These tests were selected for quality evaluation due to several reasons. The pH can be used as a reliable indicator to evaluate the overall quality of the fruits as it can prolong the shelf life of the product (Bhat *et al.*, 2011). Another important factor for grading the quality of fruits is TSS as it can be used to indicate the percentage of soluble solids in fruits (McAllister, 1980). The TA value, on



the other hand, is used to indicate the percentage of total acidity in a fruit which is yet another reliable indicator of overall fruit quality (Bhat *et al.*, 2011).

As for nutritional properties, ascorbic acid content and antioxidant levels were evaluated. The awareness of the importance of vitamin C for human nutrition is on the rise and hence, vitamin C has been considered as a quality index for fruits (Groff *et al.*, 1995). Similarly, the role of antioxidants in disease prevention is well established and therefore, antioxidant levels are considered as another reliable quality index in food commodities like fruits and vegetables (Allothman *et al.*, 2009a). In this chapter, three antioxidant assays were carried out which include total polyphenol content, DPPH radical scavenging assay and total antioxidant capacity as these are common methods for evaluating antioxidant levels (Bae & Suh, 2007).

Food safety and sensorial attributes of foods are of significant interest to consumers' and hence, quality assessment was inclusive of the consumers' acceptance test, microbial analysis and shelf-life extension. Consumers' acceptance test was carried out to evaluate the acceptability of treated samples with regards to appearance, aroma, texture and taste to determine the feasibility of the treatments. Microbial and shelf-life analysis was carried out according to the AOAC International Official Methods of Analysis (3M Food Safety, 2010). The different analysis that was carried out in this chapter provides a conclusive effect of treatments such as UV-C and thermal on the quality of Chokanan mango and Josephine pineapple.

## **5.2 Materials and Methods**

### **5.2.1 Plant Material**

Mature mango (*Mangifera indica* L. cv. Chokanan) and pineapple (*Ananas comosus* L. Merr. Josephine) fruits free from external defects were harvested from a mango plantation in Kangar, Perlis, and a pineapple plantation in Batu Pahat, Johor. The fruits were rinsed under running water, dipped in a 5% benomyl solution for 1 minute, rinsed again with water, air dried and left to ripen at room temperature ( $25 \pm 2$  °C) in a respiration chamber aerated humidified air (95% relative humidity) at 0.5 L per hour 9 l/h). Fruits were peeled, cleaned and thinly sliced (5 mm) with the aid of a dial thickness gauge (Mitutoyo MI 7305, Kawasaki, Japan). The fruits were cut into uniform discs with a borer (diameter of 2 cm). The uniform sample discs were used for subsequent food processing.

### **5.2.2. UV-C Treatment**

UV-C treatment was carried out as described in section 3.2.2.

### **5.2.3. Thermal Treatment (70 °C)**

Thermal treatment was carried out as described in section 4.2.2.

### **5.2.4 Physicochemical Analysis**

#### **5.2.4.1 pH**

The pH of sample pastes was determined using a pH meter (Hanna Microprocessor pH 211, Italy) at  $25 \pm 1$  °C. The pH meter was first calibrated using buffer standards of pH

7 and pH 4. The probe was placed in the sample paste and the pH reading was allowed to stabilize before sample readings were recorded.

#### **5.2.4.2 Total soluble solids**

Total soluble solids (TSS) were determined using a digital refractometer (Atago PR-1 digital refractometer, Tokyo, Japan) at  $25 \pm 1$  °C. The refractometer was first calibrated with sterile distilled water (SDW) (0 °Brix). Sample paste (1 gram) was placed on the prism of the refractometer and analysed at  $25 \pm 1$  °C. Results were expressed in standard °Brix unit.

#### **5.2.4.3 Titratable acidity (TA)**

##### **(a) Preparation of reagents**

##### 0.1 N Sodium hydroxide (NaOH)

*4 g of sodium hydroxide (Merck) was dissolved in 1000 ml of SDW. The solution was standardized before use.*

##### 50% ethanol

*50 ml of ethanol (merck) was added into 50 ml of SDW.*

##### Phenolphthalein Solution.

*0.5 g of phenolphthalein powder (East Anglia Chemicals) was dissolved in 100 ml of 50 % ethanol.*

##### **(b) Determination of (TA)**

Five grams of sample paste was diluted with ddH<sub>2</sub>O to a final volume of 250 ml. Diluted paste was then titrated with standardized 0.1 N sodium hydroxide to a definite faint pink end point (colour should persist for at least 15 s) using phenolphthalein as an indicator. The volume of sodium hydroxide used for titration was converted to grams of

citric acid per 100 grams of fruit and the titratable acidity (%TA) was calculated according to the method of Sadler and Murphy:

$$\text{Titratable acidity (\%TA)} = (V_1 \times 0.1 \text{ N NaOH} \times \text{Eq. Wt.} \times 100) / (V_2 \times 1000)$$

where

$V_1$  = volume of titrant (ml)

Eq. wt. = equivalent weight of anhydrous citric acid (64.04 mg/mEq)

$V_2$  = volume of sample (gram)

## 5.2.5 Ascorbic Acid Content

### 5.2.5.1 Preparation of reagents

#### 3% metaphosphoric acid

30 g of metaphosphoric acid sticks ( $HPO_3$ ) (R & M Chemicals) was dissolved in 1000 ml of SDW.

#### 2,6-dichlorophenol-indophenol (DCPIP)

50 mg of sodium salt of 2,6-dichloroindophenol-indophenol (Sigma) and 42 mg of sodium bicarbonate ( $NaHCO_3$ ) (BDH) were dissolved in 150 ml of hot SDW. After cooling, the volume of solution was made up to 200 ml with SDW and filtered through Whatman No. 1 filter paper. The solution was standardized before use.

#### Ascorbic acid standard solution

10 mg of L-ascorbic acid (Baker analysed) was dissolved in 10 ml of  $HPO_3$  solution. Volume of the solution was made up to 100 ml with 3%  $HPO_3$  solution. This stock solution (5 ml) was further diluted with 5 ml of 3%  $HPO_3$  solution.

### 5.2.5.2 Determination of ascorbic acid content

The ascorbic acid content in the samples was determined based on the 2,6-dichlorophenol-indophenol (DCPIP) visual titration method (Ranganna, 1977).

Ascorbic acid was extracted from 5 grams of sample paste using 3% metaphosphoric

acid. The extract was then filtered with Whatman No. 1 filter paper and 5 ml of the resulting filtrate was titrated with a standardized dye solution (2,6-dichloroindophenol-indophenol and sodium bicarbonate) to a pink end point (color should persist for at least 15 s). The results obtained were expressed as milligrams of ascorbic acid per 100 grams sample:

$$\text{Ascorbic acid content (mg/100 g)} = (V_1 \times \text{dye factor} \times V_2 \times 100) / (S_1 \times S_2)$$

Where,

V1 = titre (ml)

V2 = volume made up (ml)

S1 = aliquot of extract taken for estimation (ml)

S2 = volume of sample taken for estimation (ml)

Dye factor = 0.5 / titre

## **5.2.6 Antioxidant Activity**

### **5.2.6.1 Sample preparation**

Sample pastes were extracted according to Xu *et al.*, (2008) with minor modifications. Equal parts of sample paste and 90% acetone was added into a 50 ml Falcon tube to purify the sample. The tubes were placed in a shaking incubator (Shel lab Orbital Shaking Incubator S14, OR, USA) at 250 rpm for 30 minutes at 25 °C, and then centrifuged at 6500 rpm for 15 minutes at 5 °C using a refrigerated centrifuge (Beckman J2-MI Centrifuge, California). The resulting supernatant was saved as crude sample for subsequent antioxidant analysis.

### 5.2.6.2 Total polyphenol content (TPC)

#### (a) Preparation of reagent

##### 20% Sodium carbonate

20 g of sodium carbonate anhydrous (BDH) was dissolved in 100 ml of SDW.

##### Gallic acid standard curve

200 mg of gallic acid (Sigma) was dissolved in 100 ml of SDW. The different concentrations of gallic acid standards were prepared according to Table 5.1 and the final volume was made up to 2 ml.

**Table 5.1: Gallic acid standard preparation.**

Concentration (mg/100ml)	0	20	40	60	80	100	120	140
Volume of stock solution (ml)	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Volume of SDW (ml)	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6

#### (b) Determination of TPC

Total polyphenol content of samples were determined using Folin–Ciocalteu assay modified to a microscale (Bae & Suh, 2007). Sample extract or gallic acid standard solution (10  $\mu$ l) was added to 790  $\mu$ l SDW and 50  $\mu$ l Folin–Ciocalteu reagent (Sigma-Aldrich) in a 1.5 ml microcentrifuge tube, and mixed. After exactly 1 minute, 0.15 ml of sodium carbonate was added to the solution, mixed well and allowed to stand at room temperature ( $25 \pm 1$  °C) for 2 hours. The absorbance of the samples and Gallic acid standards were measured at a wavelength of 750 nm against a prepared blank (replace sample extract with SDW) using a Shimadzu MRC UV-200-RS spectrophotometer. A standard curve of Gallic acid ( $y = 0.0056x$ ,  $R^2 = 0.9955$ ) was prepared and results were reported as milligrams of Gallic acid equivalent (GAE) per 100 grams of fruit extract.

### 5.2.6.3 1,1-di-phenyl- 2-picrylhydrazyl (DPPH) radical scavenging assay

#### (a) Preparation of reagents

##### 80% methanol

80 ml of methanol (System) was added into 20 ml SDW.

##### 80% methanolic 0.1 mM DPPH solution

3.94 mg DPPH (Sigma) was dissolved in 100 ml of 80% methanol.

##### Ascorbic acid standard curve

Stock solution was prepared by dissolving 100mg of L-ascorbic acid (Baker analysed) in 10 ml of SDW. A new stock solution (100 µg/ml) was prepared by diluting 50 µl of this existing stock solution with SDW to a final volume of 5 ml. The different concentrations of ascorbic standards were prepared according to Table 3.8 and the final volume was 2 ml.

**Table 5.2: Ascorbic acid standard preparation for DPPH assay.**

Concentration (µg/ml)	0	0.5	1.0	2.0	4.0	6.0	8.0	10.0
Volume of new stock solution (ml)	0	0.01	0.02	0.04	0.08	0.12	0.16	0.20
Volume of SDW (ml)	2.00	1.99	1.98	1.96	1.92	1.88	1.84	1.80

#### (b) Determination of DPPH assay

This assay is based on the measurement of scavenging ability of antioxidants towards the stable radical DPPH, as described by Bae and Suh (2007). Sample extracts or ascorbic acid standard solution (500 µl) was added to 1 ml of 80% methanolic 0.1 mM DPPH solution in a 2 ml amber microcentrifuge tube. The mixture was vortexed and incubated in the water bath (Memmert, Germany) at 37 °C for 30 minutes (in dark). Absorbance was measured at 517 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (80% methanol) and a control (replace sample extract with 90% acetone). A standard curve of ascorbic acid ( $y=10.145x$ ,  $r^2=0.9907$ ) was prepared, and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml sample extract. The radical scavenging activity was calculated accordingly:

$$\% \text{ DPPH inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

Where,

$A_{\text{control}}$  is absorbance reading of control

$A_{\text{sample}}$  is absorbance reading of the sample

#### 5.2.6.4 Total antioxidant capacity (TAC)

##### (a) Preparation of reagents

###### 0.6 M sulfuric acid

33.33 ml of concentrated (18 N) sulphuric acid (System) was made up to 1L with SDW.

###### 28 mM sodium phosphate

0.336 g of sodium phosphate (Sigma) was dissolved in 100 ml of SDW.

###### 4 mM ammonium molybdate

0.494 g ammonium molybdate (BDH) was dissolved in 100 ml of SDW.

###### Ascorbic acid standard curve

Stock solution was prepared by dissolving 100 mg of L-ascorbic acid (Baker analysed) in 10 ml of SDW. The different concentrations of ascorbic acid standards were prepared according to Table 5.3 and the final volume was 2 ml.

**Table 5.3: Ascorbic acid standard preparation for TAC**

Concentration ( $\mu\text{g/ml}$ )	0	5	25	50	100	200	400	800
Volume of new stock solution (ml)	0	0.001	0.005	0.010	0.020	0.040	0.080	0.160
Volume of SDW (ml)	2.000	1.999	1.995	1.990	1.980	1.960	1.920	1.840

##### (b) Determination of TAC

The antioxidant capacity of sample extracts was determined using the phosphomolybdenum method described by Prieto *et al.*, (1999). Sample extracts or



ascorbic acid standard solution (100 µl) was added to 1 ml of reagent solution in a 1.5 ml microcentrifuge tube. The reagent solution consists of equal volume of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The tubes were incubated in a water bath (Mettler, Germany) at 95°C for 90 minutes (in dark). After cooling to room temperature ( $25 \pm 1$  °C), absorbance was measured at 695 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (replace sample extract with SDW). A standard curve of ascorbic acid ( $y=0.0018x$ ,  $r^2=0.9923$ ) was prepared according to Table 5.3. Results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

## **5.2.7 Microbial Inactivation Analysis**

### **5.2.7.1 Preparation of reagents**

#### 0.1 % Peptone water

*0.1 g of peptone water powder (R & M Chemicals) was dissolved in 100 ml of SDW*

### **5.2.7.2 Sample preparation**

The microbial counts of samples were determined using 3M™ Petrifilm™ plates (3M Center, MN, USA). Petrifilm plate methods are recognized as AOAC International Official Methods of Analysis (3M Food Safety, 2010). Serial dilution bottles were filled with 9 ml of 0.1% peptone water and then autoclaved at 121°C for 20 min. 1 gram of sample paste was added to the stock solution and mixed thoroughly, then serially diluted ( $10^{-1}$  to  $10^{-3}$ ). The final pH of the diluted samples was adjusted to pH 6.6 - 7.2 using 0.1 N sodium hydroxide solution for optimum growth.

### **5.2.7.3 Aerobic plate count (APC)**

Petrifilm aerobic count plates containing an indicator dye (tetrazolium), dehydrated nutrients, and water-soluble gelling agent were used for the enumeration of aerobic bacteria. Plates have two films; top and bottom films. The top film was lifted and 1 ml of diluted sample aliquot was placed on the centre of the bottom film. The top film was released. A plastic spreader (3 M Centre, MN, USA) was used to distribute the inoculums evenly on the circular area. Plates were set aside for 1 minute to allow the gel to solidify, and then incubated at  $35 \pm 1$  °C for 24 and 48 hours. The indicator dye colours the colonies red. All red colonies regardless of their size or colour intensity were counted.

### **5.2.7.4 Yeast and mould count (YMC)**

Petrifilm yeast and mould count plate containing an indicator dye, nutrients supplemented with antibiotics, and water-soluble gelling agent was used for the enumeration of yeast and mould. Diluted sample aliquot (1 ml) was inoculated as described in section 5.2.7.3. Then, plates were incubated at room temperature ( $25 \pm 1$  °C) for 3 to 5 days. The indicator dye colours yeast colonies blue-green with defined edges (small), whereas other variably coloured colonies with diffuse edges (large) are mould colonies.

### **5.2.7.5 Calculation**

The aerobic bacteria and total yeast and mould count colonies were counted using a hand tally counter (Green Show's, Taiwan) under light source. Colony-forming units (CFU) per milliliter of juice were calculated according to the equation:

**CFU per ml = (number of colonies x dilution factor of plate) / aliquot plated**

Results were expressed as log (CFU/mL).

### **5.2.8 Shelf-Life Studies**

Treated samples were aseptically placed into a sterile glass petridish in a laminar flow cabinet (Gelman Science Biological Safety Cabinet Class II, NSW, Australia) and tightly closed with minimum amount of headspace volume. The petridishes were stored at  $4 \pm 1$  °C in a refrigerator (under dark condition) for 15 days and analysed at daily intervals. All analysis was carried out in triplicates.

#### **5.2.8.1 APC**

The APC of samples was determined as described in Section 5.2.7.3.

#### **5.2.8.2 YMC**

The YMC of samples was determined as described in Section 5.2.7.3.

### **5.2.9 Consumers' Acceptance Test**

Consumers' acceptance test was conducted at the Postharvest Biotechnology Laboratory (University of Malaya) with different booths for each sample. A panel of 90 untrained panellists was used to evaluate consumer's acceptance of the UV-C and thermal treated fruit samples. Prior to evaluation, panellists were provided with a questionnaire to record their evaluation. The sample questionnaire is attached in Appendix 1.

Panellists were asked to evaluate 4 sets of samples with a 10 minute break between sets to prevent carryover tastes. The samples were evaluated individually in partitioned booths under fluorescent light at room temperature. Samples were served in randomized order in lunch boxes labelled with a three-digit code number. Acceptance test were performed using a 1 to 9 hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) to evaluate acceptability of the treated fruits' attributes (appearance, texture, aroma and taste/flavour). The panellists were required to cleanse their palate with lime juice between samples (Bayarri *et al.*, 2006).

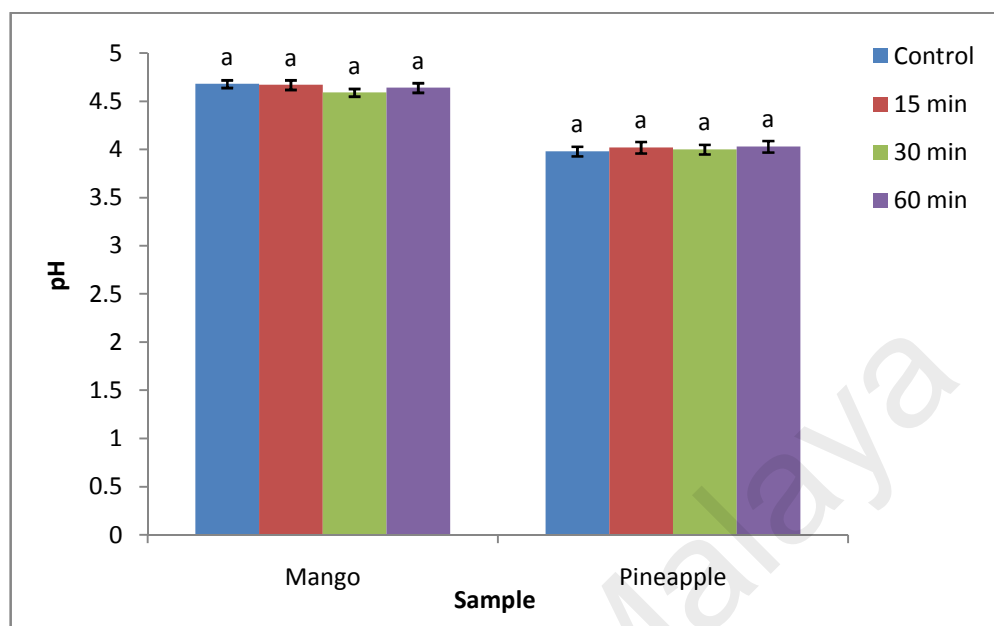
### **5.2.10 Statistical Analysis**

The data obtained were subjected to statistical analysis using SPSS 19.0 software (SPSS Inc., IBM). In this study, data were represented as mean values  $\pm$  standard error (SE) for three batches of triplicates ( $n = 9$ ). The significant differences between mean values of samples were determined by analysis of variance (one way-ANOVA) using Tukey's HSD (Honestly Significant Difference) test at a significance level of  $p < 0.05$ . Comparison was done between the different treatment durations of each fruit.

## **5.3 Results**

### **5.3.1 pH**

The change of pH in mangoes and pineapples post UV-C treatment is illustrated by Figure 5.1. There were no significant changes in pH observed in any of the fruit samples. Treatment of varying time of 15, 30 and 60 minutes resulted in the same outcome without significant changes in pH as compared to respective controls ( $p < 0.05$ ). The pH of control mangoes ( $4.68 \pm 0.109$ ) and control pineapples ( $3.98 \pm 0.106$ ) did not vary significantly following UV-C and thermal treatment.

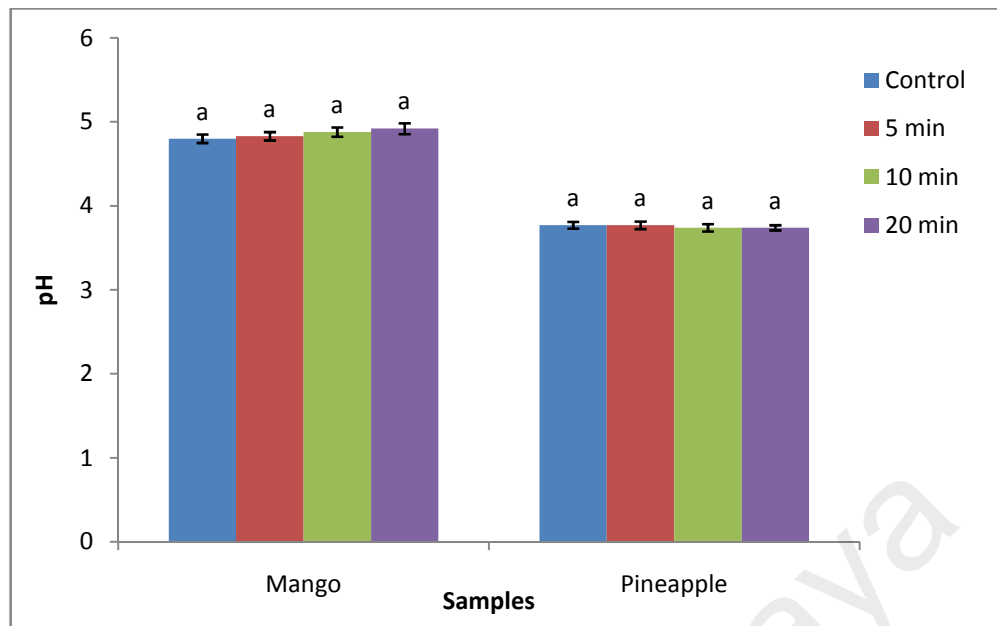


**Figure 5.1: Effects of UV-C treatment on the pH of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

Figure 5.2 represents the changes of pH in mangoes and pineapples after the fruits were thermally treated at 70° C for 5, 10 and 20 minutes. As illustrated in Figure 5.2 no significant changes were observed in any of the fruits in all treatment durations of 5, 10 and 20 minutes as compared to respective controls (p>0.05). The pH of treated pineapples remained within the range of  $3.74 \pm 0.03$  to  $3.77 \pm 0.07$  while the pH of treated mangoes were well within  $4.80 \pm 0.048$  to  $4.92 \pm 0.024$  as compared to the respected controls.



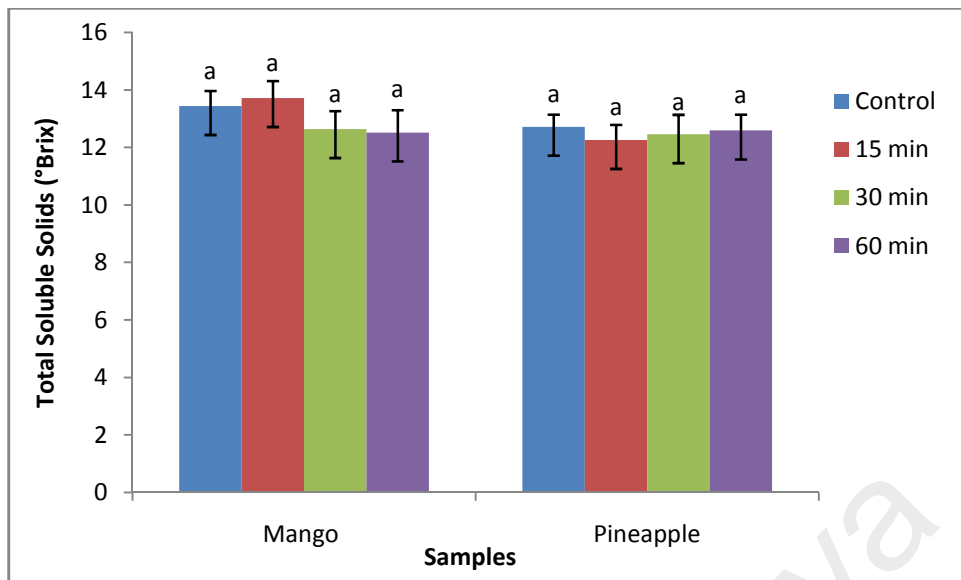
**Figure 5.2: Effects of thermal treatment (70°C) on the pH of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 3.3.2 Total Soluble Solids

The total soluble solids values of mangoes and pineapples following UV-C treatment are presented in Figure 5.3. The TSS of mangoes and pineapples showed no significant changes after UV-C treatment. Treatments at different durations of 15, 30 and 60 minutes did not result in any significant changes in the TSS of both fruits. TSS of mangoes remained within the range of (13.44 ± 0.53 °Brix) to (12.52 ± 0.78 °Brix) while the TSS of pineapples remained within the range of (12.72 ± 0.43 °Brix) to (12.59 ± 0.56 °Brix). Changes in total soluble solids in mangoes and pineapples following treatments were statistically insignificant.

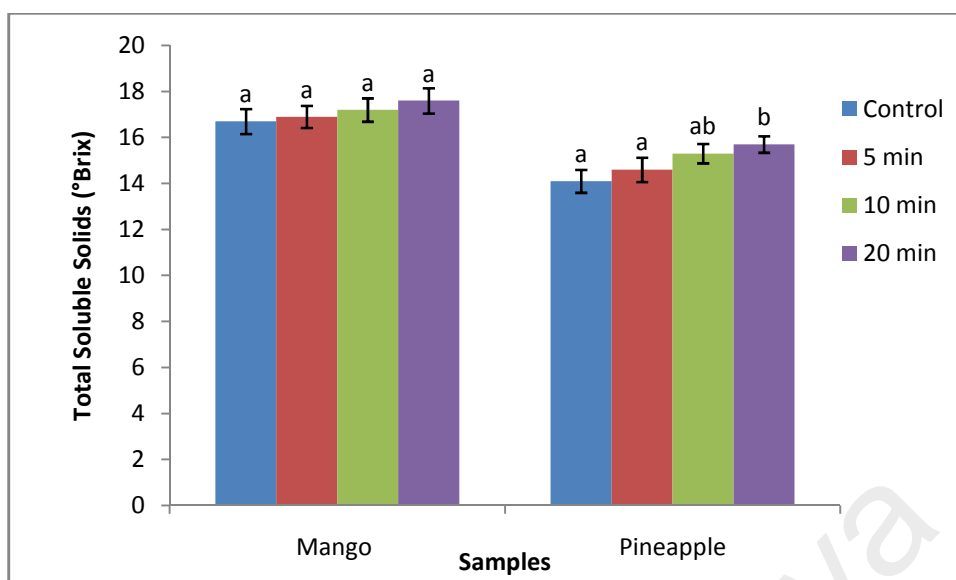


**Figure 5.3: Effects of UV-C treatment on the total soluble solids of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

The changes of TSS in mangoes and pineapples following thermal treatment of 70°C are represented by Figure 5.4. Mangoes showed no significant changes in its TSS through treatments of 5, 10 and 20 minutes. The TSS of mangoes remained within the range of (4.80 ± 0.04 °Brix) to (4.92 ± 0.02 °Brix). Pineapples however, were affected by treatments of 10 and 20 minutes. The TSS content of pineapples increased significantly as treatment persisted for 10 and 20 minutes. An increase of up to 13% from 13.6 ± 0.50 °Brix (control) to 15.7 ± 0.36 °Brix (20 minutes) was observed in pineapples as thermal treatment was carried out.



**Figure 5.4: Effects of thermal treatment (70°C) on the total soluble solids of Chokanan mango and Josephine pineapple.**

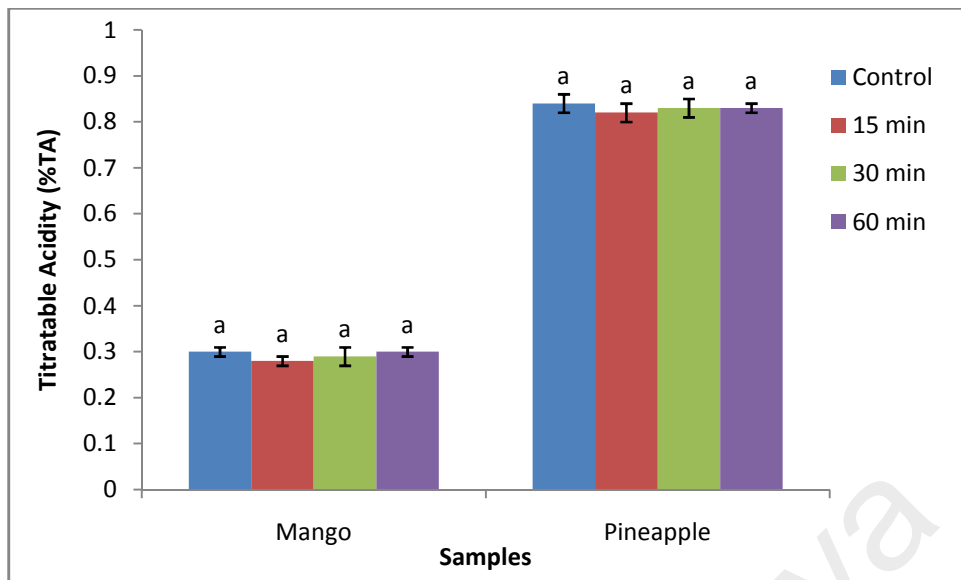
<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 3.3.3 Titratable Acidity

The titratable acidity values of mangoes and pineapples are presented in Figure 5.5. The TA values, similar to pH values, showed no significant differences between the control and UV-C treated samples. TA values were unaffected by UV-C treatment of varying durations of 15, 30 and 60 minutes. The % TA of both fruits remained close to their respective controls with mangoes recording % TA of  $0.30 \pm 0.01$  and pineapples recording % TA of  $0.84 \pm 0.02$ .



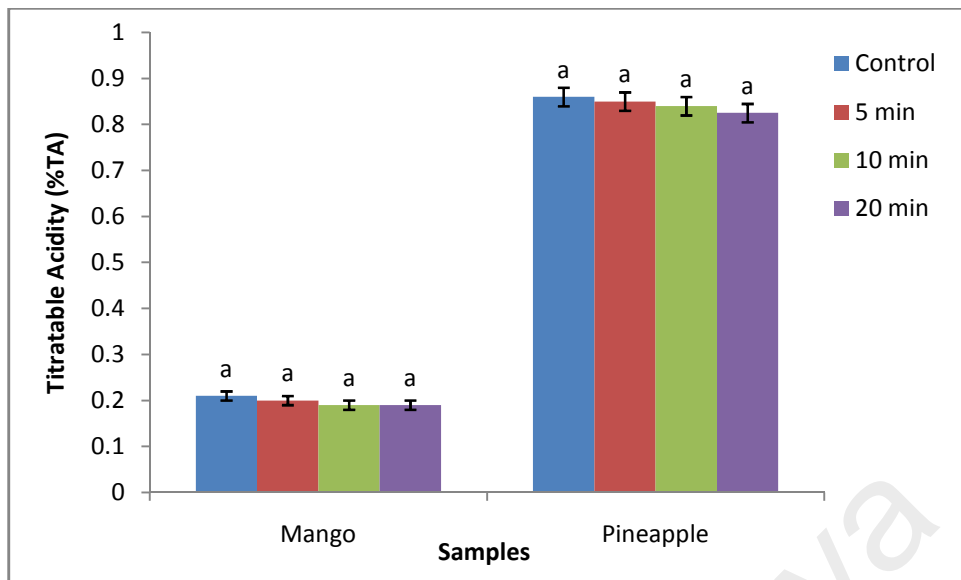


**Figure 5.5: Effects of UV-C treatment on the titratable acidity of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

Figure 5.6 illustrates the changes of TA in mangoes and pineapples following thermal treatment of 70 °C. An overall decreasing trend was observed in both fruits as treatment persisted for longer periods; however changes were negligible as there were no statistically significant changes observed. TA value of both fruits were unaffected by thermal treatment of 70 °C. TA values of mangoes ( $0.21 \pm 0.01$  %TA) and pineapples ( $0.86 \pm 0.02$  %TA) remained constant and did not deviate significantly from respective controls.



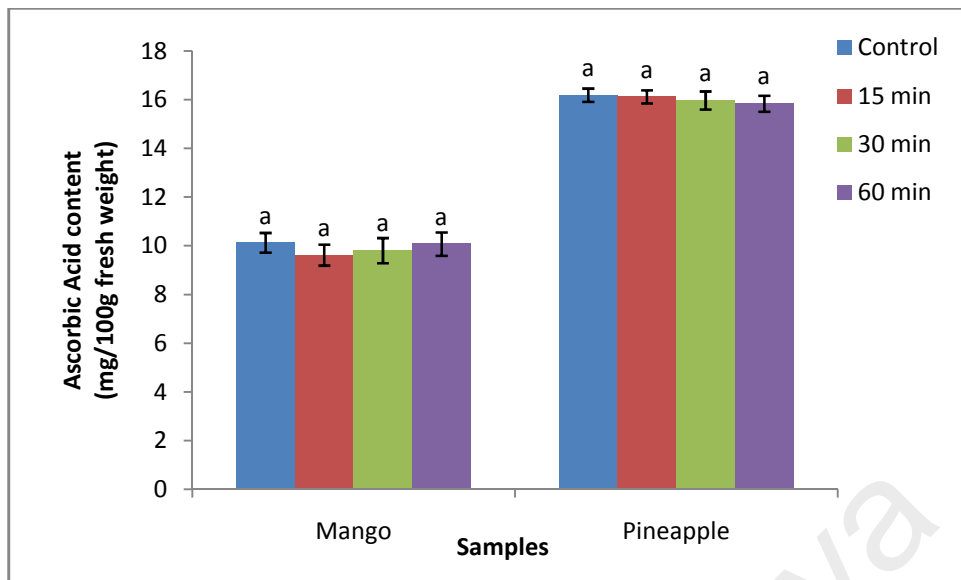
**Figure 5.6: Effects of thermal treatment (70°C) on the titratable acidity of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 3.3.4 Ascorbic Acid Content

The effects of UV-C treatment on the ascorbic acid content in mangoes and pineapples are illustrated by Figure 5.7. As illustrated by Figure 5.7, no significant changes were observed in the ascorbic acid content post UV-C treatment of 15, 30 and 60 minutes. In mangoes, ascorbic acid content of treated samples remained close to that of control ( $10.13 \pm 0.41$  mg/100g fresh weight). In pineapples, ascorbic acid content of treated samples remained close to that of control ( $16.20 \pm 0.27$  mg/100g fresh weight). Ascorbic acid content were unaffected by the UV-C treatment.

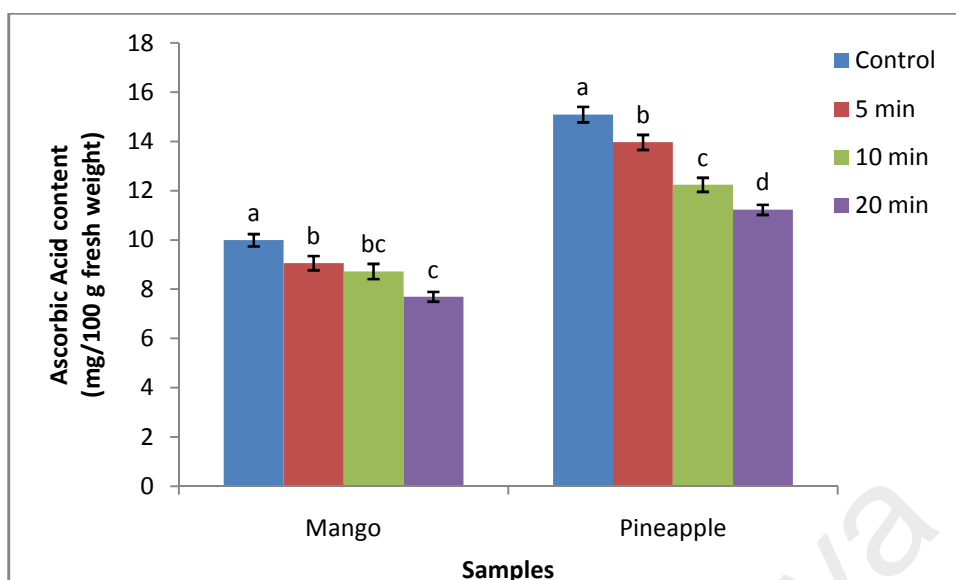


**Figure 5.7: Effects of UV-C treatment on the ascorbic acid content of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

The changes in ascorbic acid content in mangoes and pineapples following thermal treatment (70 °C) of varying durations are represented by Figure 5.8. Mangoes subjected to thermal treatment resulted in a significant decrease in ascorbic acid content. Prominent decreases of the ascorbic acid content were observed as treatment was carried out for 10 and 20 minutes at 70 °C. A total decrease of the ascorbic acid content of up to 23% from  $9.99 \pm 0.25$  mg/100g fresh weight (control) to  $7.70 \pm 0.20$  mg/100g fresh weight (20 minutes) was observed in mangoes. Changes in ascorbic acid content were more significant in pineapples. A decrease was recorded through all the varying duration of treatments. Thermal treatment at 70°C for 20 minutes resulted in the most significant decrease of up to 25% from  $15.09 \pm 0.32$  mg/100g fresh weight (control) to  $11.23 \pm 0.21$  mg/100g fresh weight (20 minutes) in pineapples.



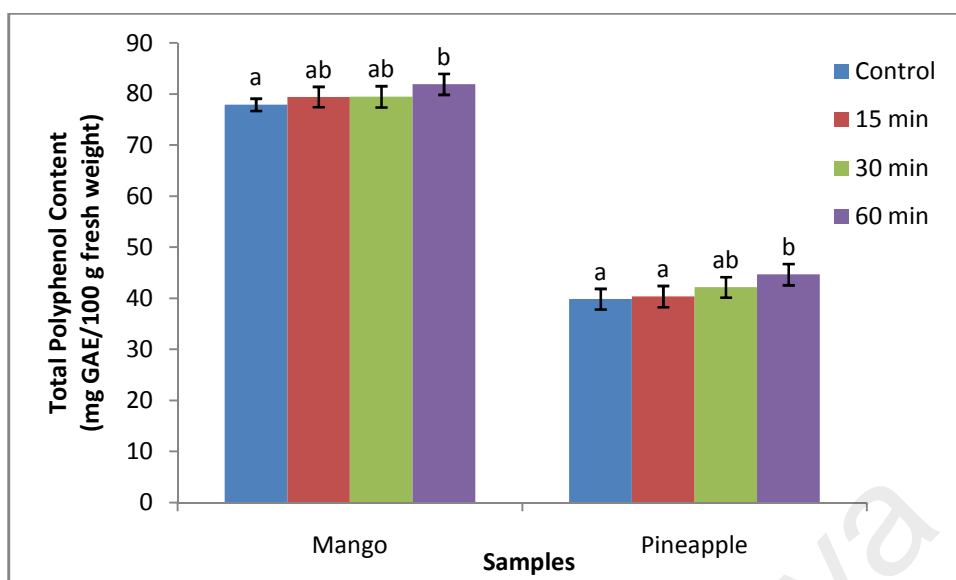
**Figure 5.8: Effects of thermal treatment (70°C) on the ascorbic acid content of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 3.3.5 Total Polyphenol Content

The changes in total polyphenol content of mangoes and pineapples following UV-C treatment is illustrated by Figure 5.9. Total polyphenol content of mangoes increased significantly with UV-C treatment with the highest increase recorded at 60 minutes of treatment. Total polyphenol content in mangoes increased from  $77.89 \pm 2.04$  mg GAE/100 ml to  $80.91 \pm 2.05$  mg GAE/100 ml, recording an increase of up to 4%. A similar outcome was observed in UV-C treated pineapples where a significant increase in the total polyphenol content of the fruit was recorded. In pineapples, total polyphenol content increased from  $39.87 \pm 2.02$  (mg GAE/100 ml) to  $42.67 \pm 2.08$  (mg GAE/100 ml) recording an increase of up to 7%.

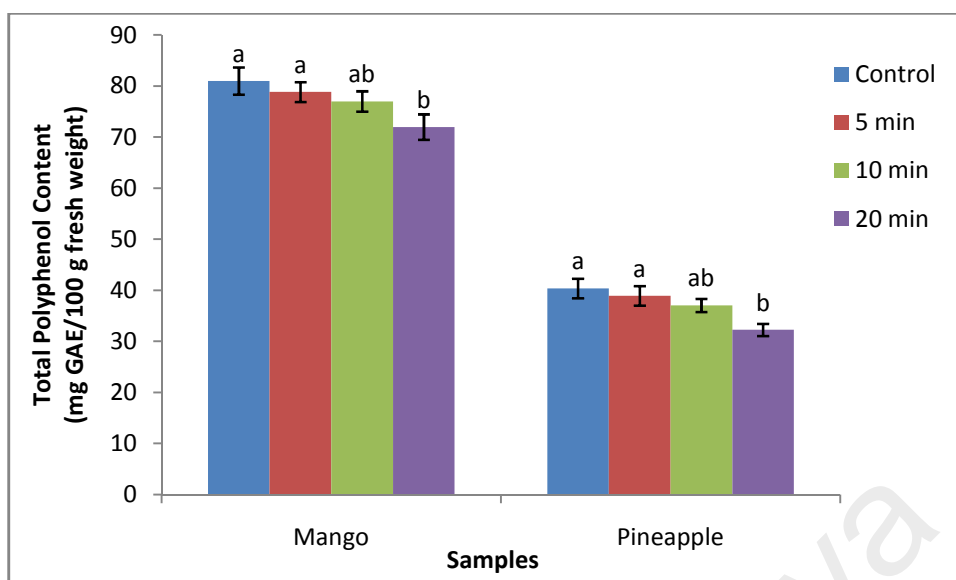


**Figure 5.9: Effects of UV-C treatment on the total polyphenol content of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

A significant decrease in the total polyphenol content was observed in thermal treated (70°C) mangoes and pineapples as illustrated in Figure 5.10. Significant changes were observed largely in prolonged periods of treatment of 10 and 20 minutes. The total polyphenol content in mangoes decreased from  $80.97 \pm 2.67$  mg GAE/100 g fresh weight (control) to  $71.97 \pm 2.49$  mg GAE/100 g fresh weight (20 minutes) recording a decrease of up to 11%. The total polyphenol content in pineapples on the other hand, decreased up to approximately 20% from  $40.39 \pm 1.93$  mg GAE/100 g fresh weight (control) to  $32.27 \pm 1.18$  mg GAE/100 g fresh weight (20 minutes).



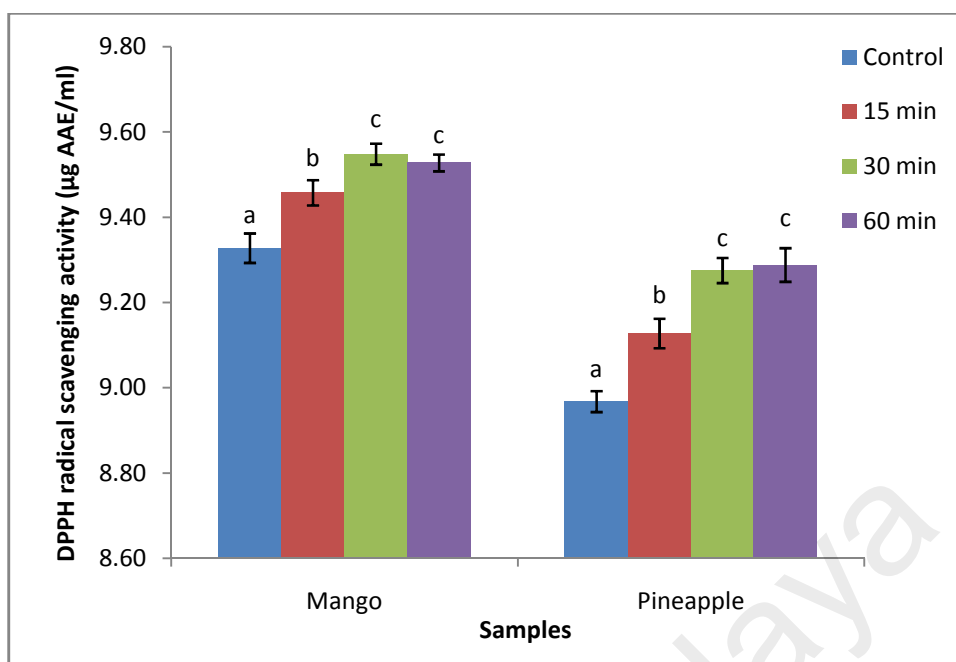
**Figure 5.10: Effects of thermal treatment (70°C) on the total polyphenol content of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 5.3.6 DPPH Scavenging Activity

Figure 5.11 illustrates the changes of the DPPH scavenging activity in mangoes and pineapples following UV-C treatment for 15, 30 and 60 minutes. An increasing trend in the DPPH scavenging activity was observed. In mangoes, the DPPH scavenging activity increase significantly from  $9.33 \pm 0.03 \mu\text{g AAE/ml}$  to  $9.46 \pm 0.03 \mu\text{g AAE/ml}$  following 15 minutes of UV-C irradiation and further increased to  $9.55 \pm 0.02 \mu\text{g AAE/ml}$  following 30 minutes of treatment. A total increase of up to 2% in the DPPH scavenging activity was observed from  $9.33 \pm 0.03 \mu\text{g AAE/ml}$  to  $9.53 \pm 0.02 \mu\text{g AAE/ml}$  following 60 minutes of UV-C treatment. An increase in the DPPH scavenging activity was also observed in pineapples. The DPPH scavenging activity in pineapples increased from  $8.97 \pm 0.02 \mu\text{g AAE/ml}$  to  $9.13 \pm 0.03 \mu\text{g AAE/ml}$  after 15 minutes of treatment and further increased up to  $9.29 \pm 0.04 \mu\text{g AAE/ml}$  following 60 minutes of treatment recording a total increase of up to 4%.

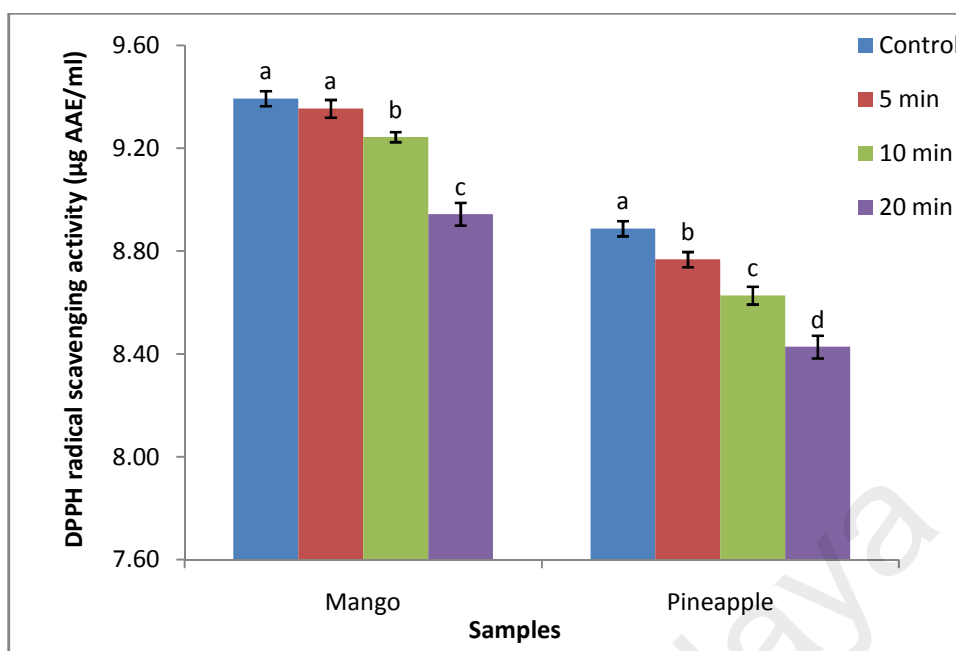


**Figure 5.11: Effects of UV-C treatment on the DPPH radical scavenging activity of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

The changes in DPPH scavenging activity of mangoes and pineapples following thermal treatment (70°C) is illustrated by Figure 5.12. Significant decreases were observed in both crops as treatment was carried out with a more significant decrease observed as treatment prolonged. A total decrease of up to 5% from  $9.39 \pm 0.03 \mu\text{g AAE/ml}$  to  $8.94 \pm 0.14 \mu\text{g AAE/ml}$  was observed in mangoes following thermal treatment of 70°C. In pineapples on the other hand, DPPH scavenging activity decreased from  $8.89 \pm 0.03 \mu\text{g AAE/ml}$  to  $8.77 \pm 0.03 \mu\text{g AAE/ml}$  following 5 minutes of treatment and further decreased to  $8.63 \pm 0.03 \mu\text{g AAE/ml}$ . A total decrease in DPPH scavenging activity of up to 5% was observed in pineapples to  $8.43 \pm 0.04 \mu\text{g AAE/ml}$  was recorded following 20 minutes of thermal treatment.



**Figure 5.12: Effects of thermal treatment (70°C) on the DPPH radical scavenging activity of Chokanan mango and Josephine pineapple.**

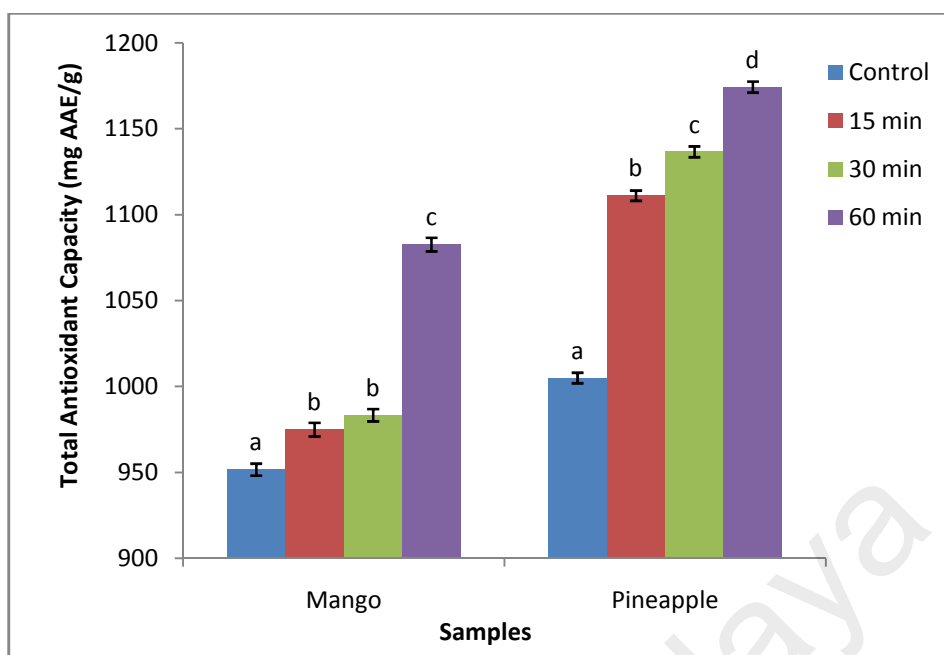
<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 5.3.7 Total Antioxidant Capacity

The effects of UV-C treatment on the total antioxidant capacity in mangoes and pineapples are illustrated by Figure 5.13. As illustrated by Figure 5.13, significant increases were observed in the total antioxidant capacity of samples UV-C treated for 15, 30 and 60 minutes in mangoes and pineapples. In mangoes, total antioxidant capacity increased from  $951.67 \pm 3.49$  mg AAE/g to  $975 \pm 3.18$  mg AAE/g following 15 minutes of treatment and further increased to  $983.33 \pm 2.99$  AAE/g following 30 minutes of treatment. A total increase of up to 14% was recorded in mangoes treated with UV-C for 60 minutes ( $1082.67 \pm 3.98$  AAE/g). The total antioxidant capacity in pineapples increased from  $1005 \pm 3.09$  AAE/g to  $1111.11 \pm 2.98$  AAE/g following 15 minutes of treatment and further increased to  $1174.34 \pm 3.23$  AAE/g following 60 minutes of UV-C irradiation recording an increase of up to 17%.



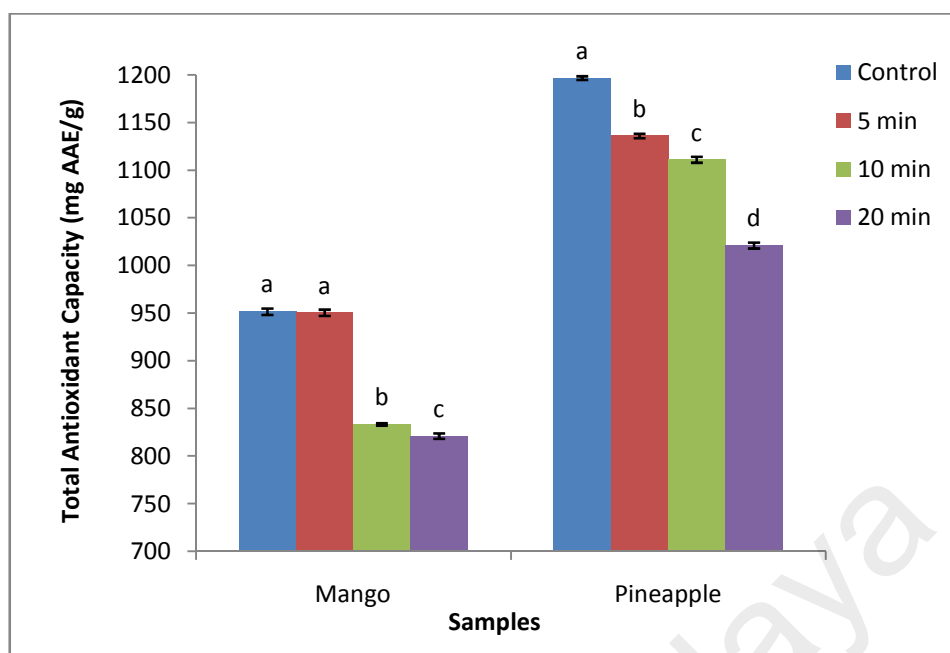


**Figure 5.13: Effects of UV-C treatment on the Total Antioxidant activity of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different ( $p > 0.05$ ).

Figure 5.14 illustrates the changes of the total antioxidant activity in mangoes and pineapples following thermal treatment (70 °C) for 5, 10 and 20 minutes. Significant decreases in the total antioxidant activity were observed in both fruits. In mangoes, the total antioxidant activity decreased from  $951.67 \pm 3.45$  mg AAE/g to  $833.34 \pm 1.23$  mg AAE/g after 10 minutes of treatment and further decreased to  $821.12 \pm 2.87$  mg AAE/g following 20 minutes of treatment. A total decrease in total antioxidant capacity of up to 14% was observed in mangoes following thermal treatment (70 °C). The total antioxidant capacity in pineapples decreased from  $1196.67 \pm 1.98$  mg AAE/g to  $1136.12 \pm 2.34$  mg AAE/g after 5 minutes of treatment and further decreased to  $1021.12 \pm 3.15$  mg AAE/g as treatment was carried out for 20 minutes. A total decrease of up to 15% was observed in pineapples following thermal treatment of 70 °C.



**Figure 5.14: Effects of thermal treatment (70°C) on the Total Antioxidant activity of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different ( $p>0.05$ ).

### 5.3.8 Correlation between Antioxidant Assays

The correlation between total polyphenols and antioxidant assays (DPPH and TAC) are shown in Table 5.4. Total polyphenols exhibited a strong positive correlation ( $p<0.01$ ) with the antioxidant assays studied. Polyphenol content strongly correlated with TAC ( $R^2=0.952$ ) and DPPH ( $R^2=0.912$ ). Besides that, there was a strong correlation between antioxidant assays, DPPH with TAC ( $R^2=0.823$ ).

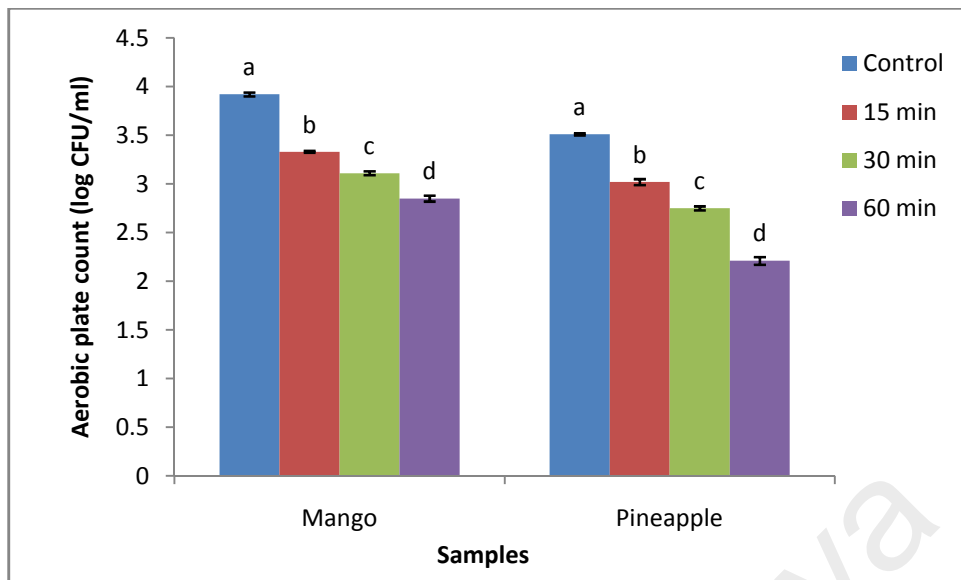
**Table 5.4: Pearson's correlation coefficients between TPC and antioxidant activity measured by different assays (DPPH and TAC).**

	TAC	DPPH	TPC
TAC	1		
DPPH	0.823**	1	
TPC	0.952**	0.912**	1

\*\*Correlation is significant at the 0.01 level.

### 5.3.8 Aerobic Plate Count (APC)

Figure 5.15 illustrates the effects of UV-C treatment on the aerobic plate count in mangoes and pineapples following UV-C treatment for 15, 30 and 60 minutes. Significant decreases in aerobic plate count were observed in both fruits. In mangoes, the APC decreased from  $3.92 \pm 0.02$  log CFU/ml to  $3.33 \pm 0.01$  log CFU/ml after 15 minutes of treatment and further decreased to  $2.85 \pm 0.03$  log CFU/ml following 60 minutes of treatment. In pineapples on the other hand, APC decreased from  $3.51 \pm 0.01$  log CFU/ml to  $3.02 \pm 0.03$  log CFU/ml after 15 minutes of treatment and further decreased to  $2.21 \pm 0.04$  log CFU/ml following 60 minutes of UV-C treatment.

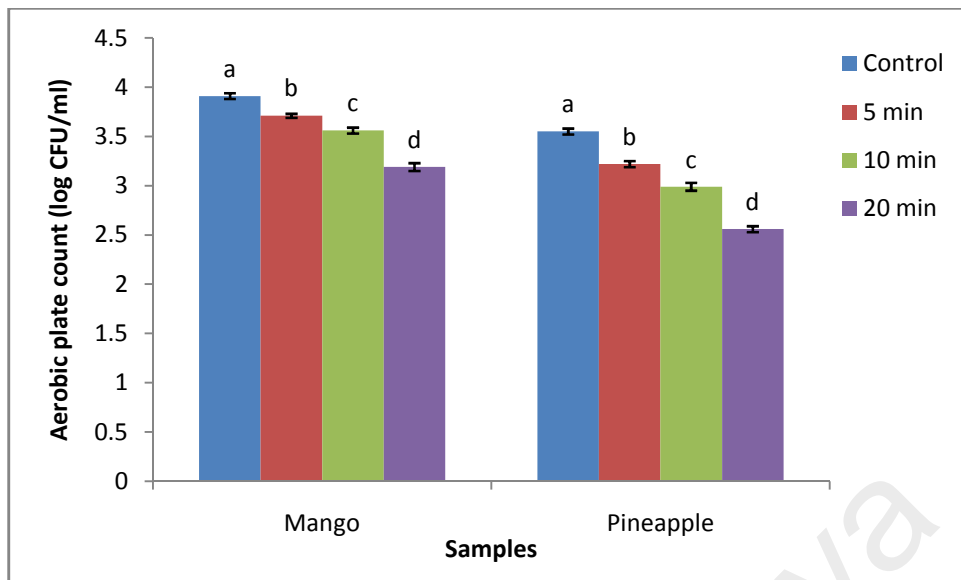


**Figure 5.15: Effects of UV-C treatment on the aerobic plate count of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

The effects of thermal treatment (70°C) on the APC in mangoes and pineapples are illustrated by Figure 5.16. Significant decreases in APC were observed in both fruits. In mangoes, APC decreased from  $3.91 \pm 0.01$  log CFU/ml to  $3.71 \pm 0.02$  log CFU/ml after 5 minutes of treatment and further decreased to  $3.19 \pm 0.02$  log CFU/ml following 20 minutes of treatment. Similarly, in pineapples, APC decreased from  $3.55 \pm 0.01$  log CFU/ml to  $3.22 \pm 0.03$  log CFU/ml after 5 minutes of treatment. APC in pineapples further decreased to  $2.99 \pm 0.04$  log CFU/ml after 10 minutes of treatment and finally to  $2.56 \pm 0.03$  log CFU/ml following 20 minutes of treatment.



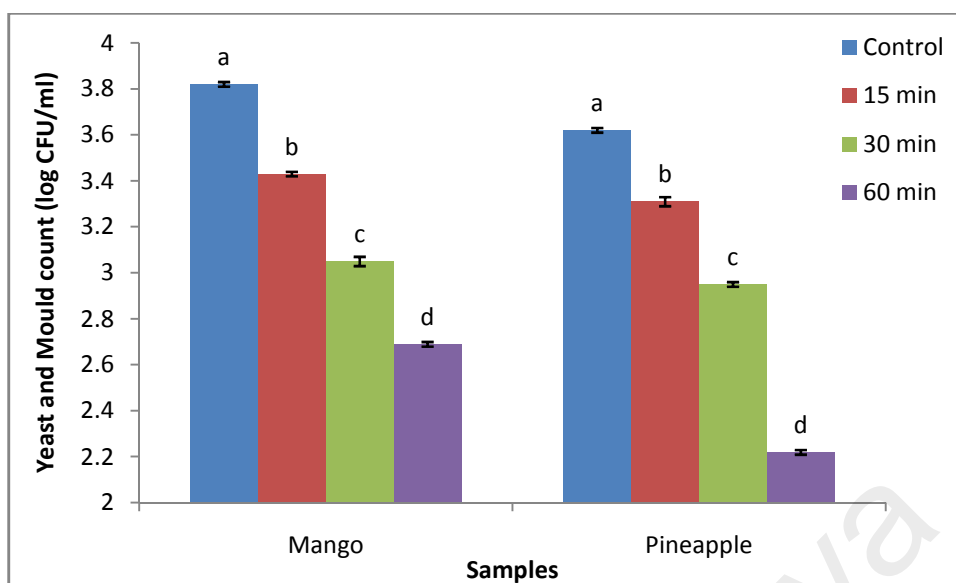
**Figure 5.16: Effects of thermal treatment (70°C) on the aerobic plate count of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 5.3.9 Yeast and Mould Count (YMC)

The effects of UV-C treatment of 15, 30 and 60 minutes on the YMC in mangoes and pineapples are illustrated by Figure 5.17. Significant decreases in YMC were observed in both mangoes and pineapples. In mangoes, YMC decreased from  $3.82 \pm 0.01$  log CFU/ml to  $3.43 \pm 0.01$  log CFU/ml after 15 minutes of treatment and further decreased to  $3.05 \pm 0.02$  log CFU/ml following 30 minutes of treatment. The YMC in mangoes were reduced to a minimum of  $2.69 \pm 0.01$  following 60 minutes of treatment. In pineapples, YMC decreased from  $3.62 \pm 0.01$  log CFU/ml to  $3.31 \pm 0.02$  log CFU/ml after 15 minutes of treatment. YMC in pineapples further decreased to  $2.95 \pm 0.01$  log CFU/ml after 30 minutes of treatment and finally to  $2.22 \pm 0.01$  log CFU/ml following 60 minutes of UV-C treatment.

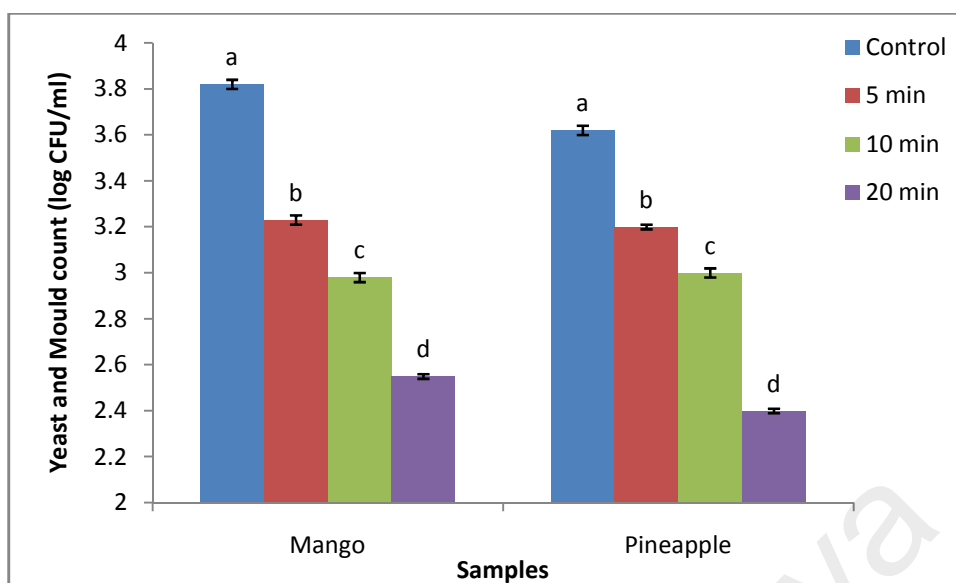


**Figure 5.17: Effects of UV-C treatment on the yeast and mould count of Chokanan mango and Josephine pineapple.**

<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

Figure 5.18 illustrates the effects of thermal treatment (70 °C) on the YMC in mangoes and pineapples. Significant decreases in YMC were observed in both fruits. In mangoes, the YMC decreased from  $3.82 \pm 0.02$  log CFU/ml to  $3.23 \pm 0.02$  log CFU/ml after 5 minutes of treatment and further decreased to  $2.98 \pm 0.02$  log CFU/ml following 10 minutes of treatment. The YMC in mangoes were reduced to a minimum of  $2.55 \pm 0.01$  log CFU/ml following 20 minutes of thermal treatment. In pineapples on the other hand, YMC decreased from  $3.62 \pm 0.02$  log CFU/ml to  $3.20 \pm 0.01$  log CFU/ml after 5 minutes of treatment and further decreased to  $3.00 \pm 0.02$  log CFU/ml following 10 minutes of treatment. The YMC in pineapples were reduced to a minimum of  $2.40 \pm 0.01$  log CFU/ml following 20 minutes of thermal treatment.



**Figure 5.18: Effects of thermal treatment on the yeast and mould count of Chokanan mango and Josephine pineapple.**

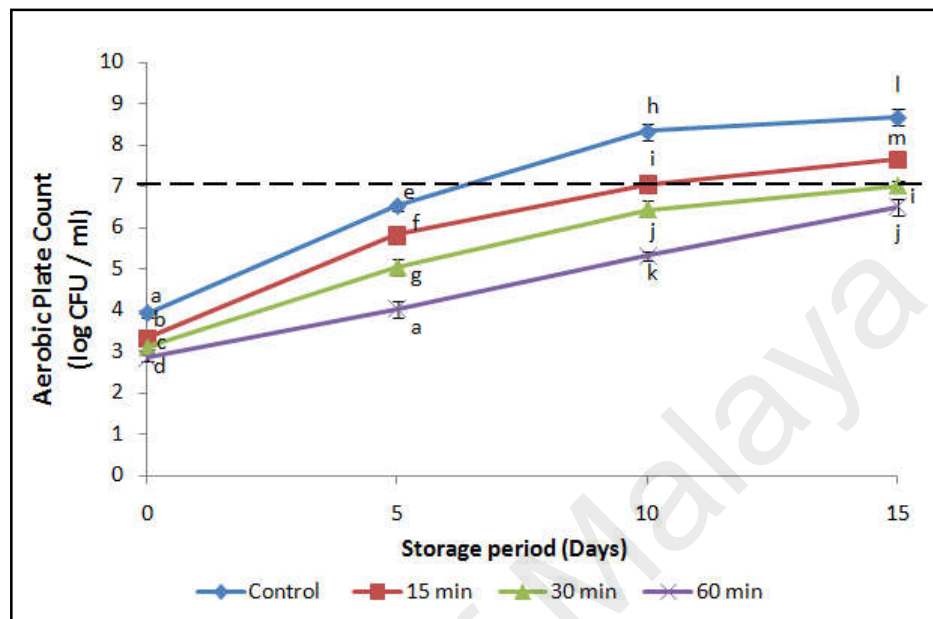
<sup>1</sup>Vertical bars represent SE of the mean (n=9).

<sup>2</sup>Means followed by the same letter are not significantly different (p>0.05).

### 5.3.10 Shelf-Life Studies

Figure 5.19 illustrates the changes in APC of UV-C treated mangoes during storage at 4 °C. APC in control mangoes increased from  $3.92 \pm 0.02$  log CFU/ml to  $6.52 \pm 0.01$  log CFU/ml after 5 days and further increased to  $8.32 \pm 0.02$  log CFU/ml after 10 days of storage at 4 °C. APC in control mangoes increased to a maximum of  $8.66 \pm 0.02$  log CFU/ml after 15 days of storage. APC in mango samples UV-C treated for 15 minutes increased from  $3.33 \pm 0.01$  log CFU/ml to  $5.8 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $7.1 \pm 0.01$  log CFU/ml after 10 days of storage. APC in mangoes UV-C treated for 15 minutes increased to a maximum of  $7.7 \pm 0.02$  log CFU/ml after 15 days of storage. APC in mango sample UV-C treated for 30 minutes increased from  $3.11 \pm 0.01$  log CFU/ml to  $5.1 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $6.45 \pm 0.02$  log CFU/ml after 10 days of storage and finally increased to a maximum of  $7.02 \pm 0.01$  log CFU/ml after 15 days of storage. APC in mangoes UV-C treated for 60 minutes on the other hand increased from  $2.85 \pm 0.01$  log CFU/ml to  $4.0 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $5.32 \pm$

0.01 log CFU/ml after 10 days of storage. APC in mangoes UV-C treated for 60 minutes increased to a maximum of  $6.5 \pm 0.02$  log CFU/ml after 15 days of storage.



**Figure 5.19: Changes in aerobic plate count of UV-C treated Chokanan mango during storage at 4 °C.**

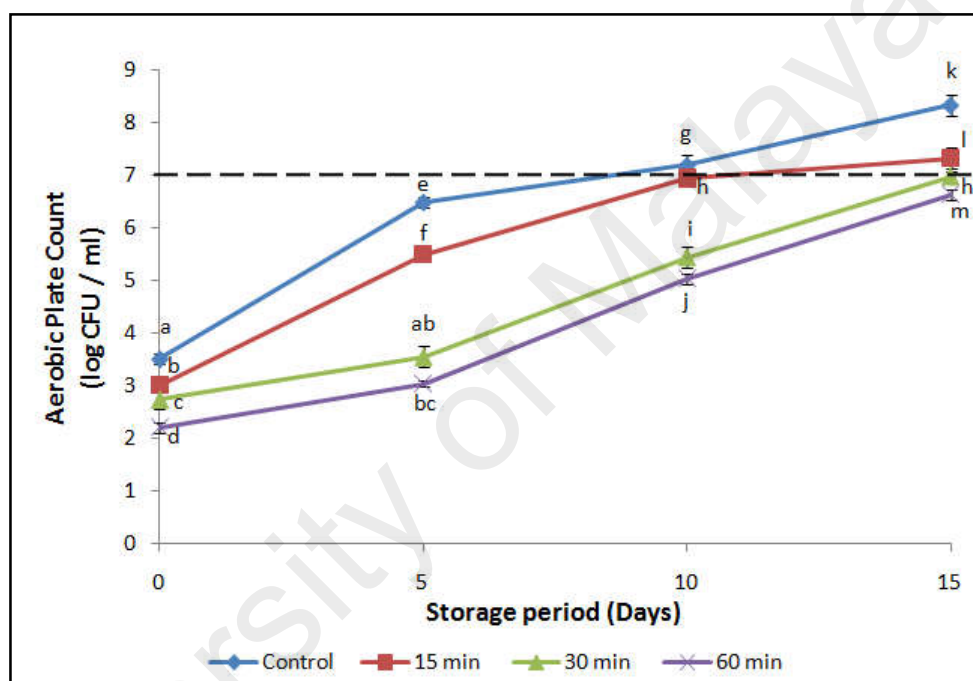
<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

Figure 5.20 illustrates the changes in APC of UV-C treated pineapples during storage at 4 °C. APC in control pineapples increased from  $3.51 \pm 0.01$  log CFU/ml to  $6.5 \pm 0.02$  log CFU/ml after 5 days and further increased to  $7.2 \pm 0.02$  log CFU/ml after 10 days of storage at 4 °C. APC in control pineapple increased to a maximum of  $8.35 \pm 0.02$  log CFU/ml after 15 days of storage. APC in pineapple samples UV-C treated for 15 minutes increased from  $3.02 \pm 0.01$  log CFU/ml to  $5.5 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $6.95 \pm 0.01$  log CFU/ml after 10 days of storage. APC in pineapples UV-C treated for 15 minutes increased to a maximum of  $7.33 \pm 0.02$  log CFU/ml after 15 days of storage. APC in pineapple samples UV-C treated for 30 minutes increased from  $2.75 \pm 0.02$  log CFU/ml to  $3.6 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $5.45 \pm 0.02$  log CFU/ml after 10 days of storage and



finally increased to a maximum of  $6.98 \pm 0.01$  log CFU/ml after 15 days of storage. APC in pineapples UV-C treated for 60 minutes on the other hand increased from  $2.21 \pm 0.01$  log CFU/ml to  $3.1 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $5.05 \pm 0.01$  log CFU/ml after 10 days of storage. APC in pineapples UV-C treated for 60 minutes increased to a maximum of  $6.65 \pm 0.01$  log CFU/ml after 15 days of storage.



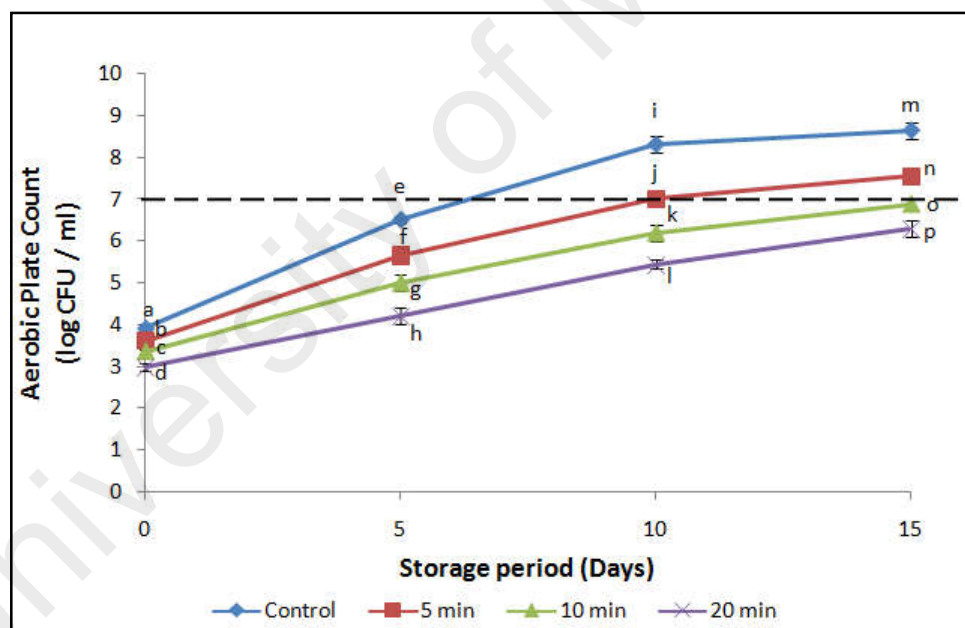
**Figure 5.20: Changes in aerobic plate count of UV-C treated Josephine pineapple during storage at 4 °C.**

<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

Figure 5.21 illustrates the changes in APC of thermal treated mangoes during storage at 4 °C. APC in control mangoes increased from  $3.92 \pm 0.01$  log CFU/ml to  $6.52 \pm 0.01$  log CFU/ml after 5 days and further increased to  $8.32 \pm 0.02$  log CFU/ml after 10 days of storage at 4 °C. APC in control mangoes increased to a maximum of  $8.66 \pm 0.02$  log CFU/ml after 15 days of storage. APC in mango samples thermal treated for 5 minutes increased from  $3.61 \pm 0.01$  log CFU/ml to  $5.7 \pm 0.01$  log CFU/ml after 5 days of storage

and further increased to  $7.0 \pm 0.01$  log CFU/ml after 10 days of storage. APC in mangoes thermal treated for 5 minutes increased to a maximum of  $7.6 \pm 0.02$  log CFU/ml after 15 days of storage. APC in mango sample thermal treated for 10 minutes increased from  $3.36 \pm 0.01$  log CFU/ml to  $5.0 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $6.2 \pm 0.02$  log CFU/ml after 10 days of storage and finally increased to a maximum of  $6.9 \pm 0.01$  log CFU/ml after 15 days of storage. APC in mangoes thermal treated for 20 minutes on the other hand increased from  $2.99 \pm 0.01$  log CFU/ml to  $4.2 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $5.5 \pm 0.01$  log CFU/ml after 10 days of storage. APC in mangoes thermal treated for 20 minutes increased to a maximum of  $6.3 \pm 0.02$  log CFU/ml after 15 days of storage.



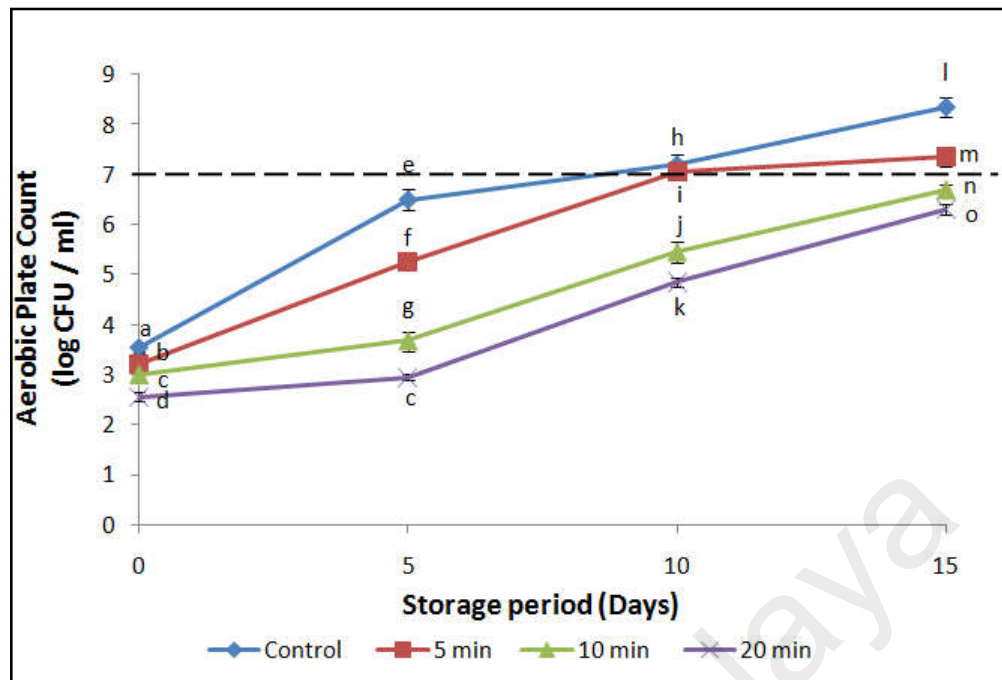
**Figure 5.21: Changes in aerobic plate count of thermal treated (70 °C) Chokanan mango during storage at 4 °C.**

<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

Figure 5.22 illustrates the changes in APC of thermal treated pineapples during storage at 4 °C. APC in control pineapples increased from  $3.55 \pm 0.01$  log CFU/ml to  $6.5 \pm 0.02$  log CFU/ml after 5 days and further increased to  $7.2 \pm 0.02$  log CFU/ml after 10 days of

storage at 4 °C. APC in control pineapple increased to a maximum of  $8.35 \pm 0.02$  log CFU/ml after 15 days of storage. APC in pineapple samples thermal treated for 5 minutes increased from  $3.22 \pm 0.02$  log CFU/ml to  $5.3 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $7.1 \pm 0.01$  log CFU/ml after 10 days of storage. APC in pineapples thermal treated for 5 minutes increased to a maximum of  $7.4 \pm 0.02$  log CFU/ml after 15 days of storage. APC in pineapple samples thermal treated for 10 minutes increased from  $2.99 \pm 0.01$  log CFU/ml to  $3.7 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $5.5 \pm 0.02$  log CFU/ml after 10 days of storage and finally increased to a maximum of  $6.7 \pm 0.01$  log CFU/ml after 15 days of storage. APC in pineapples thermal treated for 20 minutes on the other hand increased from  $2.56 \pm 0.01$  log CFU/ml to  $3.0 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $4.85 \pm 0.01$  log CFU/ml after 10 days of storage. APC in pineapples thermal treated for 60 minutes increased to a maximum of  $6.3 \pm 0.01$  log CFU/ml after 15 days of storage.



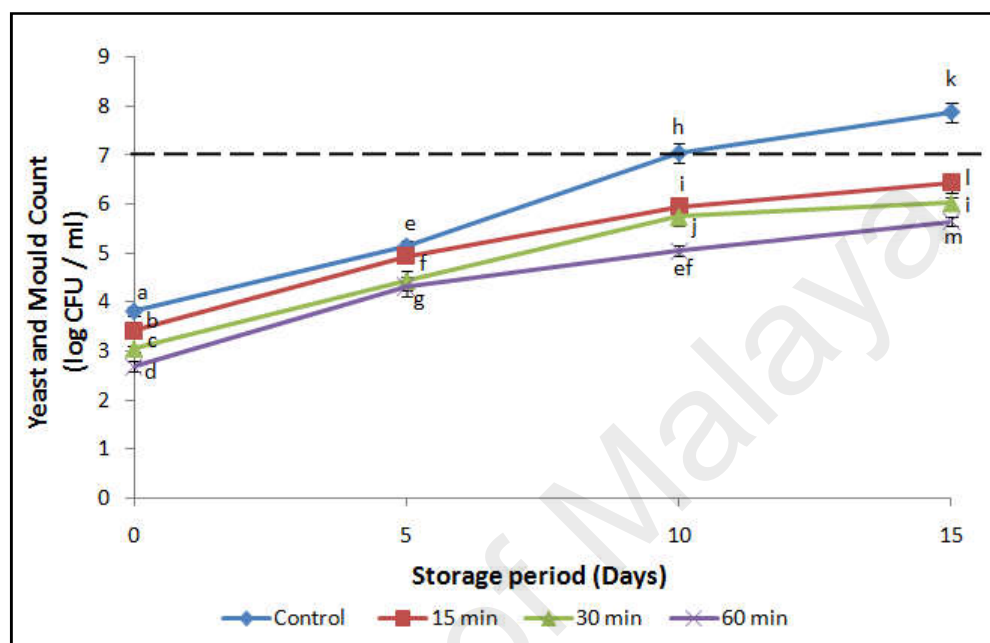
**Figure 5.22: Changes in aerobic plate count of thermal treated (70 °C) Josephine pineapple during storage at 4 °C.**

<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

The changes in YMC of UV-C treated mango samples during storage at 4 °C are illustrated by Figure 5.23. YMC in control mangoes increased from  $3.82 \pm 0.01$  log CFU/ml to  $5.2 \pm 0.01$  log CFU/ml after 5 days of storage. YMC in control mangoes further increased to  $7.05 \pm 0.02$  log CFU/ml after 10 days of storage at 4 °C and finally increased to a  $7.89 \pm 0.02$  log CFU/ml after 15 days of storage. YMC in mango samples UV-C irradiated for 15 minutes increased from  $3.23 \pm 0.01$  log CFU/ml to  $4.8 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $6.0 \pm 0.02$  log CFU/ml after 10 days of storage and finally increased to  $6.2 \pm 0.01$  log CFU/ml after 15 days of storage. YMC in mango samples UV-C treated for 30 minutes increased from  $2.98 \pm 0.01$  log CFU/ml to  $4.3 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $5.7 \pm 0.01$  log CFU/ml after 10 days of storage and finally increased to a maximum of  $6.2 \pm 0.01$  log CFU/ml after 15 days of storage. YMC in mangoes UV-C treated for 60 minutes on the other hand increased from  $2.55 \pm 0.01$  log CFU/ml to  $4.0 \pm 0.02$  log

CFU/ml after 5 days of storage and further increased to  $5.3 \pm 0.01$  log CFU/ml after 10 days of storage. YMC in mangoes UV-C treated for 60 minutes increased to a maximum of  $5.8 \pm 0.01$  log CFU/ml after 15 days of storage.



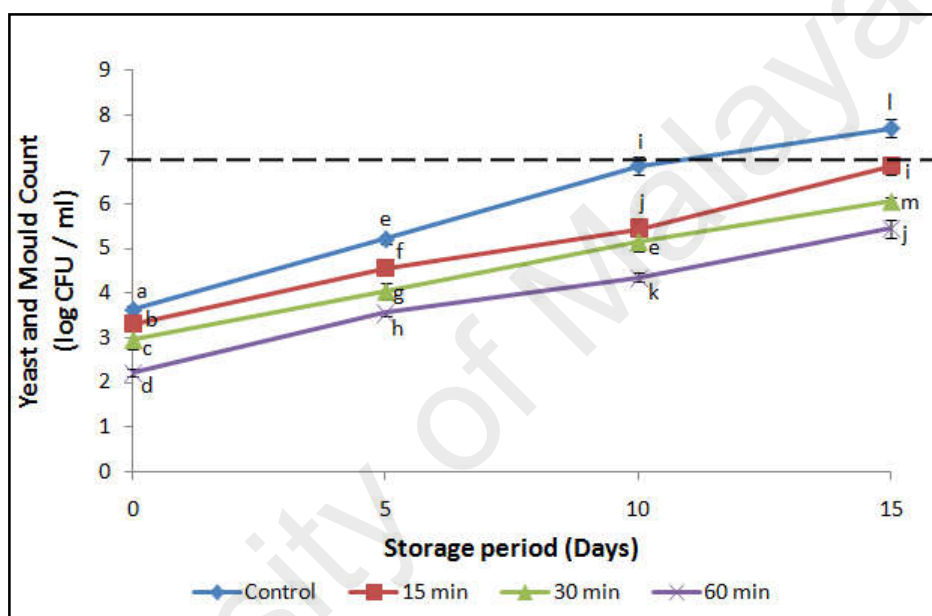
**Figure 5.23: Changes in yeast and mould count of UV-C treated Chokanan mango during storage at 4 °C.**

<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

The changes in YMC of UV-C treated pineapple samples during storage at 4 °C are illustrated by Figure 5.24. YMC in control pineapples increased from  $3.62 \pm 0.01$  log CFU/ml to  $5.2 \pm 0.01$  log CFU/ml after 5 days of storage. YMC in control pineapples further increased to  $6.85 \pm 0.02$  log CFU/ml after 10 days of storage at 4 °C and finally increased to a  $7.69 \pm 0.02$  log CFU/ml after 15 days of storage. YMC in pineapple samples UV-C irradiated for 15 minutes increased from  $3.31 \pm 0.01$  log CFU/ml to  $4.6 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $5.5 \pm 0.01$  log CFU/ml after 10 days of storage and finally increased to  $6.9 \pm 0.02$  log CFU/ml after 15 days of storage. YMC in pineapple samples UV-C treated for 30 minutes increased from  $2.95 \pm 0.02$  log CFU/ml to  $4.0 \pm 0.02$  log CFU/ml after 5 days of storage and further

increased to  $5.2 \pm 0.02$  log CFU/ml after 10 days of storage and finally increased to a maximum of  $6.05 \pm 0.01$  log CFU/ml after 15 days of storage. YMC in pineapples UV-C treated for 60 minutes on the other hand increased from  $2.22 \pm 0.01$  log CFU/ml to  $3.6 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $4.4 \pm 0.01$  log CFU/ml after 10 days of storage. YMC in mangoes UV-C treated for 60 minutes increased to a maximum of  $5.45 \pm 0.02$  log CFU/ml after 15 days of storage.



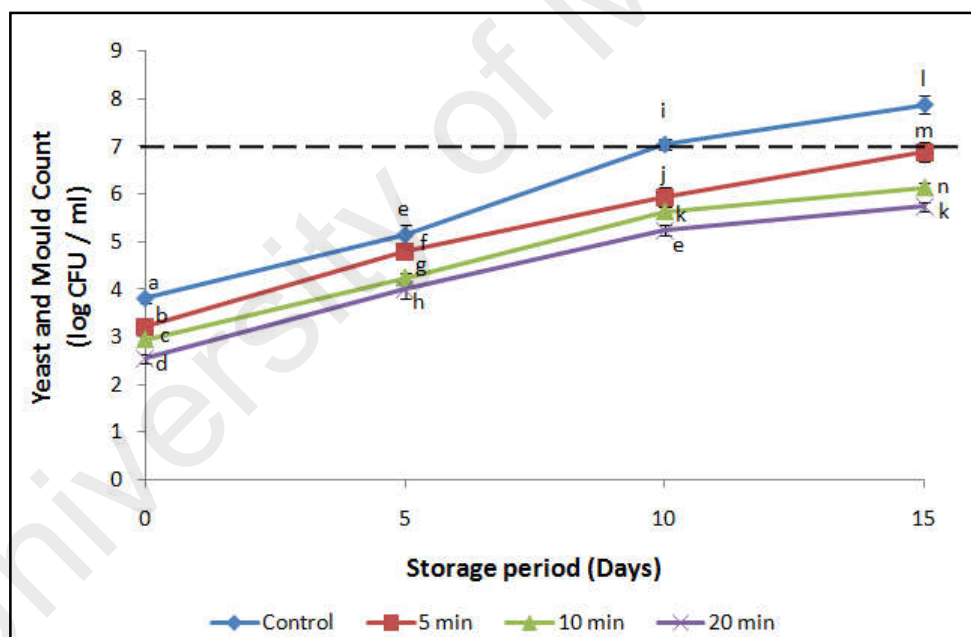
**Figure 5.24: Changes in yeast and mould count of UV-C treated Josephine pineapple during storage at 4 °C.**

<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

Figure 5.25 illustrates the changes in YMC of thermal treated mangoes during storage at 4 °C. YMC in control mangoes increased from  $3.82 \pm 0.01$  log CFU/ml to  $5.2 \pm 0.02$  log CFU/ml after 5 days and further increased to  $7.05 \pm 0.01$  log CFU/ml after 10 days of storage at 4 °C. YMC in control mangoes increased to a maximum of  $7.89 \pm 0.02$  log CFU/ml after 15 days of storage. YMC in mango samples thermal treated for 5 minutes increased from  $3.23 \pm 0.02$  log CFU/ml to  $4.8 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $6.0 \pm 0.02$  log CFU/ml after 10 days of storage. YMC in

mangoes thermal treated for 5 minutes increased to a maximum of  $6.9 \pm 0.02$  log CFU/ml after 15 days of storage. YMC in mango samples thermal treated for 10 minutes increased from  $2.98 \pm 0.01$  log CFU/ml to  $4.3 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $5.7 \pm 0.01$  log CFU/ml after 10 days of storage and finally increased to a maximum of  $6.2 \pm 0.01$  log CFU/ml after 15 days of storage. YMC in mangoes thermal treated for 20 minutes on the other hand increased from  $2.55 \pm 0.01$  log CFU/ml to  $4.0 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $5.30 \pm 0.01$  log CFU/ml after 10 days of storage. YMC in mangoes thermal treated for 60 minutes increased to a maximum of  $5.8 \pm 0.01$  log CFU/ml after 15 days of storage.



**Figure 5.25: Changes in yeast and mould count of thermal treated Chokanan mango during storage at 4 °C.**

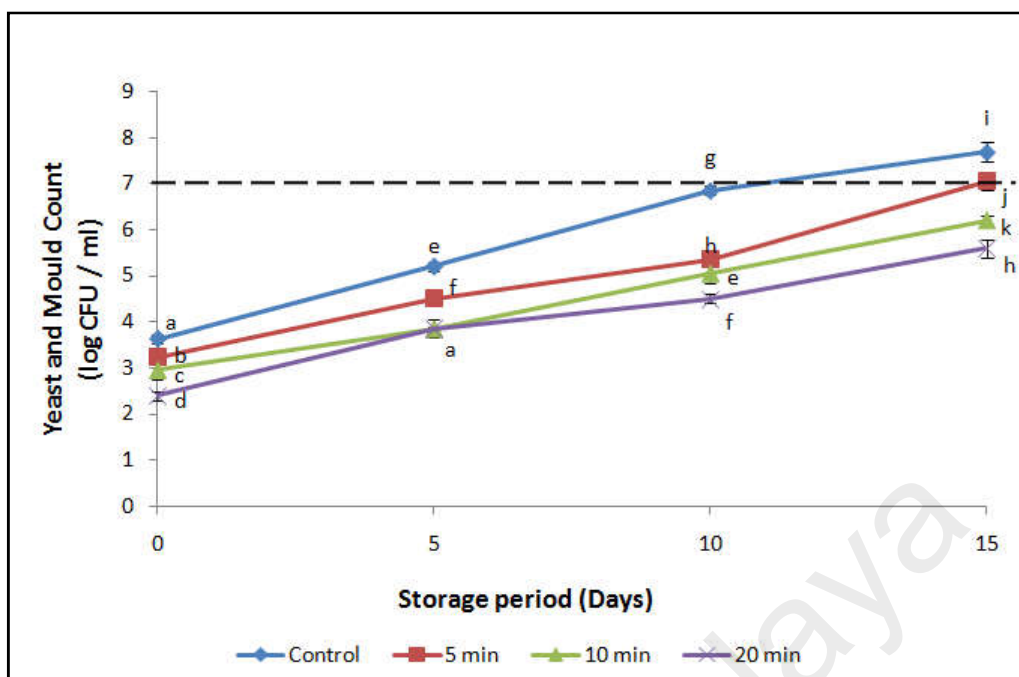
<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

The changes in YMC of thermal treated pineapple samples during storage at 4 °C are illustrated by Figure 5.26. YMC in control pineapples increased from  $3.62 \pm 0.01$  log CFU/ml to  $5.2 \pm 0.01$  log CFU/ml after 5 days and further increased to  $6.85 \pm 0.01$  log

CFU/ml after 10 days of storage at 4 °C. YMC in control pineapple increased to a maximum of  $7.69 \pm 0.02$  log CFU/ml after 15 days of storage. YMC in pineapple samples thermal treated for 5 minutes increased from  $3.2 \pm 0.01$  log CFU/ml to  $4.5 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $5.4 \pm 0.01$  log CFU/ml after 10 days of storage. YMC in pineapples thermal treated for 5 minutes increased to a maximum of  $7.1 \pm 0.02$  log CFU/ml after 15 days of storage. YMC in pineapple samples thermal treated for 10 minutes increased from  $3.00 \pm 0.02$  log CFU/ml to  $3.9 \pm 0.02$  log CFU/ml after 5 days of storage and further increased to  $5.1 \pm 0.02$  log CFU/ml after 10 days of storage and finally increased to a maximum of  $6.2 \pm 0.01$  log CFU/ml after 15 days of storage. YMC in pineapples thermal treated for 20 minutes on the other hand increased from  $2.4 \pm 0.01$  log CFU/ml to  $3.9 \pm 0.01$  log CFU/ml after 5 days of storage and further increased to  $4.5 \pm 0.01$  log CFU/ml after 10 days of storage. YMC in pineapples thermal treated for 20 minutes increased to a maximum of  $5.6 \pm 0.02$  log CFU/ml after 15 days of storage.





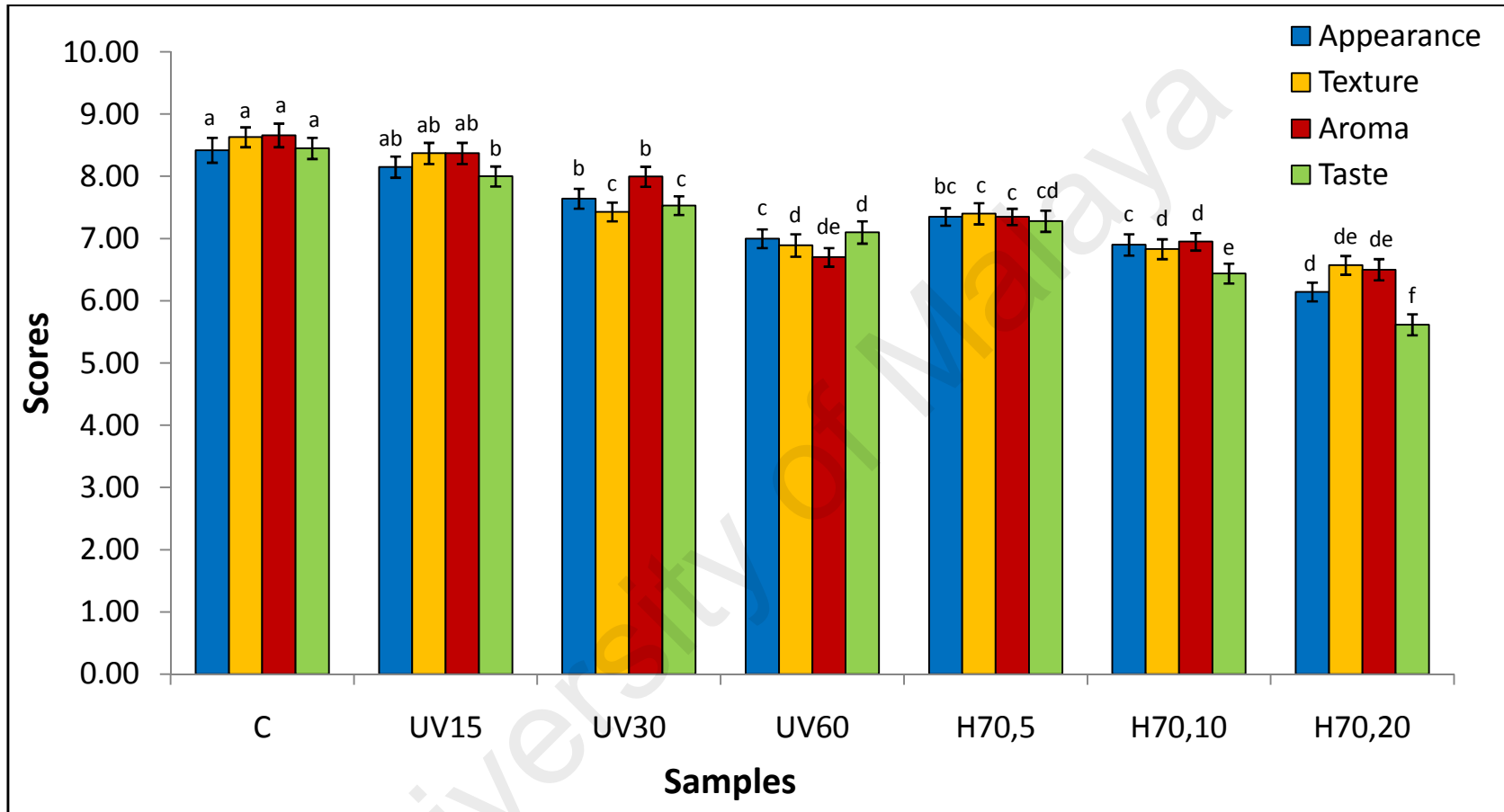
**Figure 5.26: Changes in yeast and mould count of thermal treated Josephine pineapple during storage at 4 °C.**

<sup>1</sup>Values followed by different letters are significantly different ( $p < 0.05$ ) ( $n = 9$ ).

<sup>2</sup>The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit. Samples with values higher than that of the limit are not safe for consumption.

### 5.3.11 Consumers' Acceptance

Figure 5.27 illustrates the effects of UV-C and thermal (70°C) treatments on the consumers' acceptance of Chokanan mango. As illustrated by Figure 5.27, mango samples treated with UV-C for 15 minutes received highest acceptable scores as compared to controls. Scores for mangoes treated with UV-C for 15 minutes deviated the least from the scores of controls. UV-C treatment for 30 minutes resulted in second best scores in all four attributes (Appearance, texture, aroma and taste). Consumers' acceptance of the remaining treatments was in the order: thermal (70 °C) for 5 minutes, UV-C for 60 minutes, thermal (70 °C) for 10 minutes and finally thermal (70 °C) for 20 minutes.

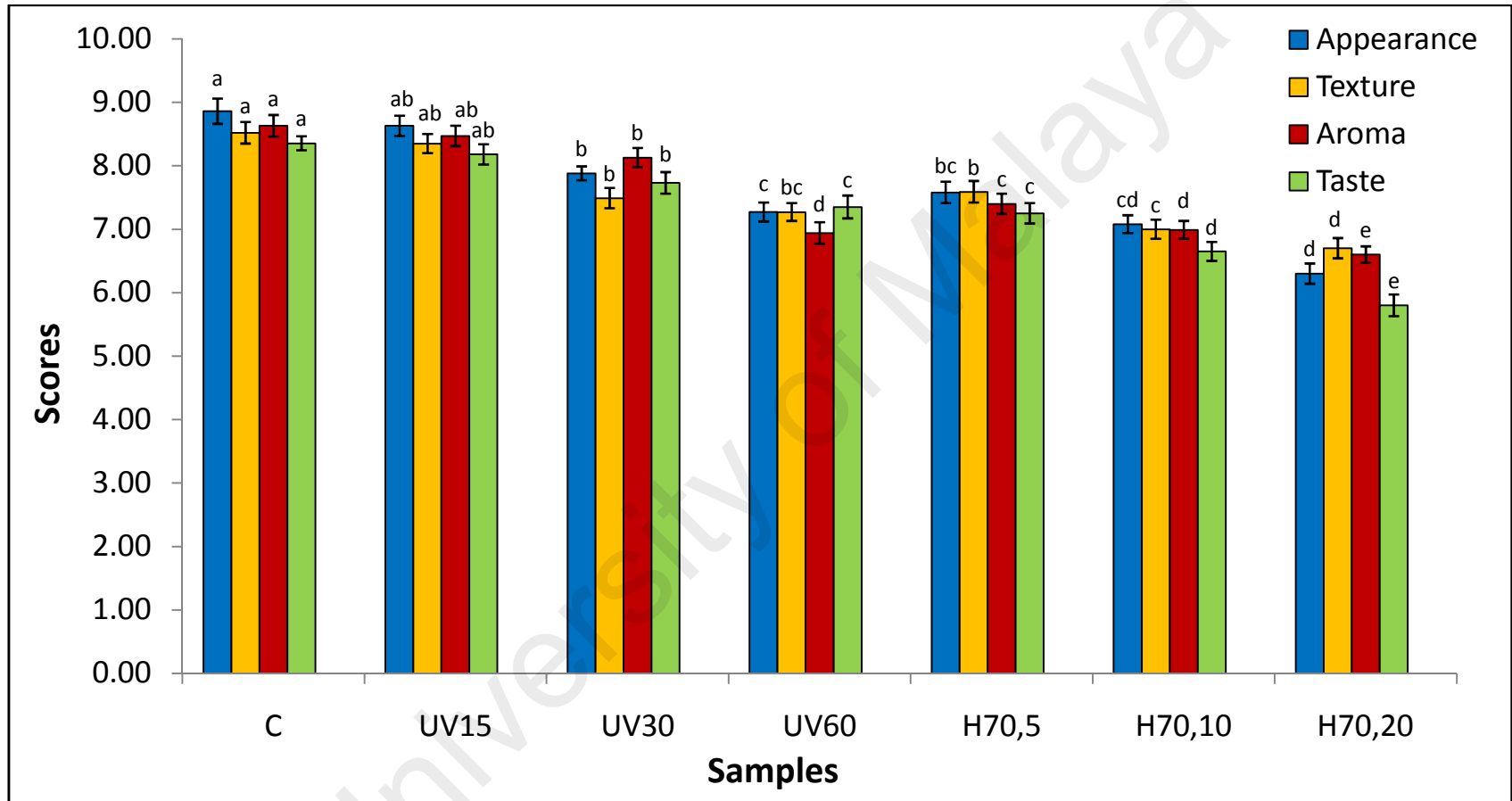


**Figure 5.27: Effects of UV-C and thermal treatment on consumers' acceptance of Chokanan mango.**

<sup>1</sup>Values followed by different letters for vertical bars of the same pattern are significantly different ( $p < 0.05$ ) ( $n = 90$ ).

Figure 5.28 illustrates the effects of UV-C and thermal (70°C) treatment on consumers' acceptance of Josephine pineapple. Pineapple samples treated for 15 minutes with UV-C was the most acceptable taking into account the scores of the different attributes (appearance, texture, aroma and taste) followed by UV-C treatment for 30 minutes . Pineapples that were UV-C treated for 15 minutes received scores that were closest to control followed by pineapple samples that were UV-C treated for 30 minutes. Consumers' acceptance of the remaining treatments taking into account all of the four attributes (appearance, texture, aroma and taste) were in the order: thermal (70 °C) for 5 minutes, thermal (70 °C) for 10 minutes, UV-C for 60 minutes and finally thermal (70 °C) for 20 minutes.

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**Figure 5.28: Effects of UV-C and thermal treatment on consumers' acceptance of Josephine pineapple.**

<sup>1</sup>Values followed by different letters for vertical bars of the same pattern are significantly different ( $p < 0.05$ ) ( $n = 90$ ).

## 5.4 Discussion

The pH of fruits can prolong the shelf life of the product and therefore, it can be used as a reliable indicator to evaluate the overall quality of the fruits (Bhat *et al.*, 2011). In this study, the pH of mangoes and pineapples showed no significant changes after UV-C treatment as compared to their respective controls (Figure 5.1). A similar trend was also observed following thermal treatment of 70 °C where the pH of both fruits remained close to the pH of the respective controls (Figure 5.2). The findings of this research pertaining UV-C treatment on pH were similar to those of previous researches on different fruits. Bal and Kok (2009) observed no significant differences in the mean of pH values of the differently UV-C treated samples. A research conducted on tomatoes also recorded no significant alteration to pH as compared to controls. UV-C treatment did not affect the pH of the fruits regardless of the length of exposure to UV-C irradiation due to the fact that UV has limited penetration into solids (Gardner & Shama, 2000). Maintaining the pH of fruits has been proven to be invaluable in prolonging shelf life (Anthon *et al.*, 2011). The findings of this study pertaining UV-C treatment on pH were similar to those of previous research on kiwifruits (Bal & Kok 2009).

As shown in Figure 5.3 and 5.4, no significant changes in the total soluble solids (TSS) of mangoes and pineapples were observed after UV-C treatment. As for thermal treatment, increases of up to 15% in pineapples were observed while no change was observed in mangoes. TSS is used to indicate the percentage of soluble solids and is one of the important factors for grading the quality of fruits (McAllister, 1980). According to Davies and Hobson (1981), an estimated half of the water soluble portion of the fruit dry matter is in the form of the reducing sugars fructose (25%) and glucose (22%). Furthermore, previous research on kiwi fruit suggests that TSS is often associated with

the eating quality of ripe fruits (Mitchell *et al.*, 1991). In addition, it has also been established that fruits with higher TSS contents are generally more preferred by consumers (Rossiter *et al.*, 2000; Burdon *et al.*, 2004). In the present research, increases were seen in thermal-treated samples. An increase in TSS of fruits post thermal treatment may be attributed to hydrolysis of polysaccharides (Bal, 2012). In addition, heat increases the solubility of certain solids which in turn may have led to the increases of TSS observed in thermal-treated samples. However, previous researches on other fruits have concluded that UV-C irradiation of fruits affected the TSS of fruits during storage. Bal and Kok (2009) illustrated that UV-C irradiation of kiwifruits indirectly reduced the increase of TSS during storage as compared to control. In their research, an increase on TSS was observed in all treated and non-treated samples with controls exhibiting the highest increase, indicating that UV-C irradiation reduces the increase in TSS. Besides maturity of fruits, microbial activity during storage may result in increases of TSS (McAllister, 1980). Therefore, a lower increase in UV-C treated samples as compared to controls in their research may be attributed to microbial inactivation. In contrast, TSS of persimmon fruits increased with storage with all UV-C treated and control showing similar patterns of increase with no significant reduces in UV-C irradiated samples when compared to control (Khademi & Zamani, 2013). A similar pattern was also observed by Bal (2012) in fresh fig where an increase in TSS was eminent in all UV-C irradiated sample as well as control with no variation in increases between treated and non-treated samples.

The TA value is used to indicate the percentage of total acidity in a fruit and is also another reliable indicator to evaluate the overall quality of fruit (Bhat *et al.*, 2011). Generally, as a fruit matures and ripens, the predominant acid in most fruits, citric acid explicitly reduces as it is utilized in the fruit respiratory process (Nagar, 1994). It has

also been established that citric acid is more important in tomatoes but malic acid more important in apples (Davies & Hobson, 1981). Titratable acidity, apart from total soluble solids in fruits such as citrus and pineapple, is responsible for their distinct taste and flavour (Yamaki, 1989). In this present research, there were no significant changes observed in the TA of both the UV-C and thermal-treated fruits. Results observed in the present study for UV-C treated fruits were similar to those of previous researches on blueberry and persimmon (Perkins-Veazie *et al.*, 2008; Khademi & Zamani, 2013).

Consumers' awareness of the importance of vitamin C for human nutrition is constantly on the rise and hence, vitamin C has been considered as a quality index for fruits. Ascorbic acid (vitamin C) is one of the most abundant antioxidants in plants and is a cofactor of many plant dioxygenases. Ascorbic acid is also known to play important regulatory roles indirectly throughout the entire body due to its involvement in the synthesis of hormones, hormone-releasing factors, and neurotransmitters (Groff *et al.*, 1995). In certain fruits such as tomatoes, ascorbic acid content has been reported to increase as the fruit ripens (Cantwell, 2000). Vitamin C levels generally increase with storage which may very likely be credited to the beneficial effect of low temperature during storage (Lingegowdaru, 2007). However, vitamin C content may be altered by the presence of ascorbate oxidase. The activity of ascorbate oxidase brings about the concern of ascorbic acid metabolism whereby in *Oryza sativa* shoot apices it was observed that an increase in the activity of the enzyme resulted in a decreased level of ascorbic acid (Reddy *et al.*, 1986). Current researches have concluded that UV irradiation has the ability to result in the abatement of ascorbate oxidase activity (Barka, 2001; Maccarrone, 1993). Whatever the case may be, in this present research, UV-C irradiation resulted in no significant changes in ascorbic acid content of both the fruits (mango and pineapple; Figure 5.8). As for thermal treatments of 70 °C, significant

changes in the ascorbic acid content were observed. Ascorbic acid content loss of 26% in mangoes and 27% in pineapples was observed in thermal-treated (70 °C) samples. Decreases in the ascorbic acid content of thermal-treated samples are largely due to the fact that ascorbic acid is a heat-sensitive bioactive compound. It is known as the least stable vitamin which is easily destroyed through processing and storage (Ercan & Soysal, 2011). Ascorbic acid is also known to degrade by oxidative processes by enzymes which include ascorbate oxidase and peroxidase (Davey *et al.*, 2000). Results obtained in this study of mangoes and pineapples are similar to results recorded in thermal-treated strawberries and tomatoes (Musto & Satriano, 2010; Ercan & Soysal, 2011). A direct effect on the ascorbic acid content of the fruits may not be apparent, but according to several previous researches, UV-C irradiation coupled with storage resulted in varying effects on the vitamin C contents of the treated fruits. In a research conducted on kiwifruits, UV-C irradiation was found to minimize the losses of vitamin C during storage (Bal & Kok, 2009). UV-C treatment was found to be detrimental in a research on tropical fruits where they concluded that an increase in treatment time resulted in the loss of vitamin C (Allothman *et al.*, 2009b). Similarly, on a separate research on fresh cut pineapples, UV-C irradiation was found to decrease the vitamin C content in the fruit significantly (Yong-Gui & He, 2012). Similar results were also reported in UV-C treated blueberries (Perkins-Veazie *et al.*, 2008). In contrast, ascorbic acid content was found to be increased in UV-C irradiated tomatoes (Lingegowdaru, 2007) while there were no significant changes observed in the vitamin C content of fresh-cut watermelon (Artés-Hernández *et al.*, 2010).

Fruits contain numerous compounds that contribute to their antioxidant activity. Therefore, more than one method was used to measure antioxidant properties according to their ability to scavenge specific radicals, to chelate metal ions and to inhibit lipid



peroxidation (Martinez et al., 2012). Accordingly, antioxidant activities in fruits can be contributed by phenolic compounds. These phenolic compounds due to their biological properties are able to exhibit antioxidant, anti-inflammatory, antiviral, and anticancer actions (Allothman *et al.*, 2009a). The effects of UV-C treatment and thermal (70 °C) treatment on the polyphenol content are shown in Figure 5.9 and 5.10. The Folin–Ciocalteu method was preferred as it encompasses the detection of phenolic compounds by the reduction of the reagent, which contains tungsten and molybdenum oxides (Waterhouse, 2003). According to results, total polyphenols increased as UV-C treatment was carried out for 60 min. An increase of up to 4% in mangoes and up to 7% in pineapples was observed following 60 min of UV-C treatment. Observation for polyphenols following UV-C treatment is similar to those observed in blueberry and pepper (Vicente *et al.*, 2005; Perkins-Veazie *et al.*, 2008). Thermal treatment of 70 °C resulted in decreases of total polyphenol content. Decrease of up to 10% in mangoes and 16% in pineapples were observed in thermal-treated (70 °C) samples. Thermal treatments have been shown to significantly decrease the concentration of polyphenols in apples (Aguilar-Rosas *et al.*, 2007). This reduction in polyphenols of thermal-treated fruits is undesirable as polyphenols give added value to fruits.

The DPPH radical scavenging assay results are shown in Figure 5.11 and 5.12. The assay is used to measure the hydrogen donating capacity of the antioxidant to the stable free radical DPPH, which results in the formation of diphenylpicrylhydrazine (Shon *et al.*, 2003). In this study, UV-C treatments resulted in an increase in DPPH inhibition capacity as compared to controls. An increase of up to 2% was observed in mangoes while an increase of up to 4% was observed in UV-C irradiated pineapples. Conversely, thermal treated samples resulted in decreases of DPPH inhibition capacity. A total

reduction in DPPH inhibition capacity of up to 5% was observed in both thermal-treated mangoes and pineapples.

Total antioxidant capacities of treated samples are shown in Figure 5.13 and 5.14. UV-C treated samples resulted in increases of total antioxidant capacities in both fruits. An increase of up to 14% was observed in mangoes while an increase of up to 17% was observed in pineapples following UV-C irradiation. Thermal treatments, on the other hand, resulted in adverse effects to the total antioxidant capacities of both fruits. A decrease of up to 14% was observed in mangoes while a decrease of up to 15% was observed in pineapples following thermal treatment. The antioxidant activity of UV-C treated samples increased significantly as compared to respective controls as suggested by result of this study. This increase could be due to the formation of free radicals which led to the accumulation of phenolic compounds as a stress response of these fruits against UV-C irradiation. In addition, exposure to UV-C has been linked to increased activity of the enzyme phenylalanine ammonia lyase which contributes to the activation of phenolic biosynthesis pathway, which leads to enhancement of phenolic compounds (Allothman *et al.*, 2009b). Furthermore, UV-C irradiation may inactivate polyphenol oxidase activity which in turn prevents further loss of polyphenols (Oms-Oliu *et al.*, 2012). It has been reported in tomatoes that UV-C treatment induces the production and accumulation of phenolic compounds in epicarp and mesocarp cells as well as biochemical reinforcement of the cell wall (Narayanasamy, 2013).

Total polyphenols exhibited a strong positive correlation with the antioxidant assays studied, thus indicating that phenolic compounds are the major contributor of antioxidant activity, in terms of radical scavenging ability. The correlation between phenolic content and antioxidant activity has been previously reported (Gorinstein *et al.*,

2004; Rama Prabha & Vasantha, 2011). Since TAC results correlated well with those obtained by other assays, therefore it serves as a good estimate of antioxidant capacity in samples.

In this study, a reduced microbial count in both fruits following both treatments (UV-C and thermal) was observed (Figure 5.15, 5.16, 5.17 and 5.18). As for UV-C treatment, a total reduction in microbial count of up to 1.07 log CFU/g in mango and up to 1.3 log CFU/g in pineapple were recorded. Following UV-C treatment, yeast and mold count (YMC) was successfully reduced by 1.13 log CFU/g in mango and 1.40 log CFU/g in pineapples. Thermal treatment (70 °C) was successful in reducing microbial count of up to 0.72 log CFU/g in mango and up to 0.99 log CFU/g in pineapple. YMC was reduced by 1.27 log CFU/g in mango and 1.22 log CFU/g in pineapple following 20 min of thermal treatment. Successful microbial inactivation in thermal-treated fruits may be due to the fact that heat disrupts the integrity of the cellular membrane and damages nucleic acids which eventually lead to cytolytic effects (Santhirasegaram *et al.*, 2013). UV-C treatment on the other hand achieves microbial inactivation in a more complex manner which ultimately leads to cell damage. Absorption of UV-C light by nucleic acids causes pyrimidine bases of cytosine and thymine to form crosslinks which are forms of mutation that are fatal to microbial cells (Bintsis *et al.*, 2000; Shama, 2006).

Changes in microbial counts (aerobic bacteria, yeast, and mold) in Chokanan mango and Josephine pineapple stored at refrigeration temperature ( $4 \pm 1$  °C) for 15 d are shown in Figure 5.19-26. Shelf life of the samples was evaluated according to the Public Health Laboratory Service, PHLS (2000) where the acceptable maximum microbial load including aerobic bacteria, and total yeast and mold in fresh-cut fruits are 7 log CFU/mL. The APC in control (untreated mangoes) increased from 3.92 to 8.66 log

CFU/mL after 15 d of storage. While, YMC increased from 3.82 to 7.89 log CFU/mL after 15 d of storage. The shelf life of UV-C treated mangoes, 15 min (10 d), 30 min (15 d), 60 min (15 d) were longer than control (6 d), in terms of microbial load (APC and YMC) limit. The shelf life of thermal-treated (70 °C) mangoes on the other hand was 9 d (5 min of treatment), 15 d (10 min of treatment) and 15 d (20 min of treatment). As for pineapples, APC, YMC in control (untreated pineapples) increased from 3.51 to 8.35 log CFU/mL and from 3.62 to 7.69 log CFU/mL, respectively. The shelf life of UV-C treated pineapples, 15 min (10 d), 30 and 60 min (15 d) were longer than control (8 d), in terms of microbial load (APC and YMC) limit. The shelf life of thermal-treated (70 °C) mangoes on the other hand was 9 d (5 min of treatment), 15 d (10 min of treatment) and 15 d (20 min of treatment). Hence, the shelf life of UV-C treated and thermal-treated Chokanan mango and Josephine pineapples stored at  $4 \pm 1$  °C was extended to a maximum of 15 d. Results obtained are in agreement with previous studies that reported prolonged shelf life in UV-C treated carrots and fresh-cut melon (Manzocco *et al.*, 2011; Alegria *et al.*, 2012).

The sensory analysis of mango and pineapple following the different treatments are shown in Figure 5.27 and 5.28, respectively. In this study, controls resulted in the most acceptable score, followed by UV-C treatment in all attributes (appearance, texture, aroma, and taste). UV-C treatments of 15 and 30 min were more acceptable as compared to thermal treatment. Scores for Thermal treatment were below acceptable levels. A decline in acceptance of appearance may be due to changes in color which might have been caused by enzymatic browning or Maillard reactions. According to a report, drying at high temperature may degrade the color of fruits (Nowak & Lewicki, 2005). Darker undesirable color in fruits may also be due to significant Maillard reaction which involves hydrolysis of hexose and sucrose (Rhim *et al.*, 1989). A

reduced acceptability of texture was also observed in thermal-treated samples as compared to UV-C treated samples. UV-C treated samples received higher scores for acceptability of texture among the 3 different treatments. The texture can be considered as one of the most important quality characteristics of edible fruits and vegetables (Waldron *et al.*, 2003). Thermal treatment has been reported to affect the degradation of proteins related to cell structure which are related to texture (Roeck *et al.*, 2009). The texture of fruits is perceived based on several attributes of which some of the most important attributes include crispness, hardness, and juiciness or moisture release (Meilgaard *et al.*, 1999). Treatments at elevated temperatures may result in non enzymatic conversion of protein related to cell structure which may affect the hardness and crispness of the fruits which are both attributes of fruit texture (Sila *et al.*, 2006). Elevated temperatures also result in dehydration of fruits which affects the juiciness and moisture release attributes of the fruit's texture. As for the aroma and taste of the differently treated fruits, UV-C treated samples resulted in scores that were closest to controls suggesting better retention of aroma and taste post treatment. Both 10 and 20 min of thermal (70 °C) resulted in low scores, suggesting that the effects of these treatments on the fruits' aroma and taste were unacceptable by consumers. As both aroma and taste are interrelated, the changes or deterioration of these attributes may be due to the changes in the TSS and TA as reported in earlier sections. Heat generated water loss may also be a key factor in the deterioration of aroma and taste. As a whole, the overall acceptability of the treated fruits, taking into consideration the 4 attributes (appearance, texture, aroma, and taste) suggest that UV-C treated samples are the most acceptable as compared to their thermal-treated counterpart.

To conclude, UV-C treatment exhibited better retention in most quality parameters as compared to thermal (70 °C) treatment. UV-C irradiated samples showed significant

improvement of antioxidant activities (total polyphenols, DPPH inhibition and total antioxidant capacity) as compared to controls while thermal treatment posed adverse effects to antioxidant activities and vitamin C content. Thermal-treated samples also received lower scores for consumers' acceptance test as compared to UV-C treated samples. Both UV-C and thermal treated samples showed a significant reduction in microbial load. Both treatments were successful in extending the shelf life of fruits stored at 4 °C to a maximum of 15 days which may prove invaluable to the fresh-cut industry. Both UV-C and thermal treatment with appropriate optimization of processing variables are feasible treatments for shelf-life extension of fresh-cut fruits. This study provides evidence for the implementation of UV-C and thermal treatment on an industrial scale.

## CHAPTER 6: GENERAL DISCUSSION

The increasing health consciousness as well as the growing interest in the role of food in maintaining and improving human well-being and health has led to the increase in fruit consumption. According to epidemiologic studies, there is an inverse relationship between the intake of fruits and vegetables and the risk of developing diseases such as cancers, hypertension as well as cardiovascular diseases (Liu *et al.*, 2000; Hung *et al.*, 2004; He *et al.*, 2007; Bhupathiraju *et al.*, 2013). Furthermore, current consumers' profile of increased consumption of readily available food as compared to cooked food has led to the demand for fresh cut as well as processed fruit products. However, a major problem that is faced by the industry is the limited shelf life which is largely a result of rapid deterioration caused by microbial growth as well as physiological disorders (Das & Kim, 2010).

Various processing technologies have been employed by the fresh-cut industry in the recent years to curb with the problem of limited shelf-life. Preservation techniques currently in use, which include antioxidants, chlorine and modified atmosphere packaging, are inadequate and therefore, the development of new techniques for preservation of fresh-cut commodities is highly recommended. Inappropriate processing technologies may result in adverse effects on the organoleptic, textural and nutritional qualities and hence, the need for technologies which are less degradative. An ideal processing technology should be effective in the inactivation of enzymes and vegetative micro-organisms while having minimal deleterious effects on food quality attributes (for example color, flavor, and nutritional value). Minimal thermal treatment or non-thermal treatment could prove to be better alternatives for preservation of fresh-cut fruits whilst minimizing degradation of its quality.

In this study, fresh-cut Chokanan mango and Josephine pineapple was subjected to UV-C and thermal treatment (70 °C). The effects of these treatments on the proteome of fruits was studied via proteomic analysis which includes 2D electrophoresis followed by mass spectrometry while the effects on the quality of the fruits was determined through analysis of different aspects of quality which include physicochemical, microbial, nutritional as well as sensorial. Proteomics analysis was carried out to better understand the effects of these treatments on the proteome of the different fruits with relation to shelf-life extension while quality analysis was performed to determine the efficacy of these treatments for implementation by the fresh-cut industry. The results obtained were published and have been submitted as research papers to peer-reviewed journals (See publications).

Through proteomic analysis, a plethora of proteins were observed to be reduced in abundance in both the fruits with thermal treatment affecting more proteins as compared to UV-C treatment. Proteins affected by the treatments were identified and were found to be involved in various biochemical processes that could lead to fruit deterioration during storage. Although different individual proteins were affected in mangoes and pineapples following the treatments, these proteins were of the same groups which include energy and metabolism, stress and response, ripening and senescence as well as cell structure. The effects of UV-C and thermal treatment on mangoes and pineapples are complex, as the differentially abundant proteins are involved in multiple metabolic pathways. It can be inferred that the effects of UV-C irradiation and thermal treatment on these proteins and the role that they play may be partly responsible for the shelf-life extension in fruits. In this regard, the differential proteomics of UV-C and thermal treatment has enlarged our understanding of the effects of postharvest treatments on mangoes and pineapples. To the best of our knowledge, this study reports the first



proteomic study of the effects of postharvest UV-C irradiation and thermal treatment of 70 °C on the proteome of Chokanan mango and Josephine pineapple.

Another interesting finding of this study is that proteomic analysis of UV-C and thermal treated Chokanan mangoes and Josephine pineapples revealed that these treatments were effective in reducing the abundance of allergenic proteins in both the fruits. Several allergenic proteins were found to be reduced in abundance in both the fruits following treatments. The efficacy of these treatments in reducing the abundance of allergenic proteins may be beneficial in addressing the problem of fruit allergy in susceptible individuals. However, further research is required to determine the correlation of a reduced abundance of allergenic proteins with a reduced potential to cause allergic reactions in susceptible individuals.

Regarding the quality attributes of mangoes and pineapples following treatments, UV-C treatment of 30 minutes and thermal treatment of 10 minutes were observed to be effective in retention of quality as compared to the other treatments. No significant changes were observed in the fruits' qualities which include physicochemical and nutritional properties. Successful retention of quality in treated fruits validates these treatments as a potential treatment for fresh-cut fruits. Furthermore, UV-C treatment was also successful in increasing the quality of fresh-cut fruits with regards to antioxidant activities. This study provides a holistic quality profile of both thermal treatment and UV-C treatment of fresh-cut Chokanan mango and Josephine pineapple.

Both UV-C and thermal treatment were successful in significantly reducing the microbial loads in mangoes and pineapples. Significant reduction in aerobic bacteria as well as yeast and mould were observed in both fruits following UV-C and thermal

treatment. UV-C treatment of 30 minutes and thermal treatment of 10 minutes with its efficacy to retain quality as well as reduce microbial loads could be exploited as a novel processing procedure for safer and high quality fresh-cut Chokanan mango and Josephine pineapple. Furthermore, the shelf-life of fresh-cut fruits during storage at 4 °C was significantly extended to a maximum of 15 days in mangoes and pineapples following UV-C and thermal treatment with microbial counts not exceeding the microbial shelf-life limit of log 7 CFU. UV-C and thermal treatment are therefore useful technologies for preservation of fresh-cut produce and could be exploited for preservation of fruits and vegetables.

Another highlight of this study was that UV-C treatment was found to increase phenolic compounds and antioxidants in treated Chokanan mango and Josephine pineapple. All three of the tested antioxidant parameters which include total polyphenol content, DPPH radical scavenging activity and total antioxidant capacity exhibited significant increases following UV-C irradiation with higher values observed in increased duration of UV-C irradiation. Increased antioxidants in UV-C irradiated fruits are added value to the food product as it is linked to many health promoting processes which fulfils the demand of health conscious consumers.

Consumers' acceptability test which included the attributes appearance, aroma, taste and texture were evaluated by 90 panelists via a hedonic scale of 1 to 9. Untreated fruit samples (control) for both mango and pineapple received the most acceptable score. UV-C treated mangoes and pineapple received scores that were close to control with minimal deviation for all tested attributes (appearance, texture, aroma, and taste). Scores for thermal treated mangoes and pineapples exhibited a decreasing trend with increasing treatment time. Thermal treatment of 20 minutes received scores that were below

acceptable levels. As for consumers' acceptance, UV-C treatment of 15 and 30 minutes were preferred among the other treatments.

In conclusion, the extensive study of both proteomic and quality analysis of UV-C and thermal treated mangoes and pineapples revealed that shelf-life of fruits could be related to proteins that were affected by the treatments and the disruption of the corresponding biological processes that they catalyze. Both UV-C and thermal treatment were found to be successful in producing a safer food product with an extended shelf-life which is highly beneficial for consumers as well as the fresh-cut industry. Through findings of this study, UV-C treatment of 30 minutes and thermal treatment of 10 minutes are the most effective treatments among UV-C and thermal treatments with regards to quality retention, safer food product and exponential shelf-life extension. These treatments were also observed to be successful in reducing the abundance of several allergenic proteins which could cause allergic reactions in susceptible individuals. These treatments therefore have the potential to curb the issue of fruit allergy. Both UV-C treatment of 30 minutes and thermal treatment (70°C) of 10 minutes are therefore feasible treatments for fresh-cut fruits and its positive effects on various aspects as presented by the results of this thesis will further encourage the development of these technologies on a pilot scale in the processing of fresh-cut produce in the fresh-cut industry. Significantly, this thesis is the first reported study of the holistic effects of UV-C and thermal treatment on Chokanan mango and Josephine pineapple taking into consideration proteomic, safety and quality aspects.

All the proposed objectives of this current study have been achieved. Knowledge and information garnered from this study will pave the way for further research work for the advancement of the fresh-cut processing industry. A profile of proteins affected by UV-

C and thermal treatment as well as the role that they play in biological processes could be exploited in further studies for optimization of processing methods. Proteomic profiles and their link to physiological aspects of fruit deterioration in this study provide new insights into possible regulation mechanisms. Apart from providing proteomic information on fruit regulation, findings from this study also pave the way for further quantitative studies at the transcriptomic and metabolomic levels.

Further studies on a combination of treatments on the shelf-life extension and quality maintenance of fresh-cut fruits will also be beneficial. This study also highlights the potential of UV-C and thermal treatment as a means of addressing the problem of allergy by its ability to reduce the abundance of several allergenic proteins in both fruits. However, further studies which include clinical trials are necessary to evaluate the efficacy of this treatment in reducing allergenicity of these allergens.

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## PUBLICATIONS

- Publication 1** **Dominic Soloman George**, Zuliana Razali, Vicknesha Santhirasegaram, & Chandran Somasundram (2015). Effect of postharvest ultraviolet-C treatment on the proteome changes in fresh cut mango (*Mangifera indica* L. cv. Chokanan). *Journal of the Science of Food and Agriculture*, In press. DOI: 10.1002/jsfa.7454.
- Publication 2** **Dominic Soloman George**, Zuliana Razali, Vicknesha Santhirasegaram, & Chandran Somasundram (2015). Effects of Ultraviolet Light (UV-C) and Heat Treatment on the Quality of Fresh-Cut Chokanan Mango and Josephine Pineapple. *Journal of Food Science*, 80(2): S426–S434. DOI: 10.1111/1750-3841.12762.
- Publication 3** Vicknesha Santhirasegaram, Zuliana Razali, **Dominic Soloman George**, & Chandran Somasundram (2015). Comparison of UV-C treatment and thermal pasteurization on quality of Chokanan mango (*Mangifera indica* L.) juice. *Food and bioprocess processing* 94: 313-321. DOI: 10.1016/j.fbp.2014.03.011.
- Publication 4** Vicknesha Santhirasegaram, Zuliana Razali, **Dominic Soloman George**, & Chandran Somasundram (2015). Effects of thermal and non-thermal processing on phenolic compounds, antioxidant activity and sensory attributes of Chokanan mango (*Mangifera indica* L.) juice. *Food and Bioprocess Technology: An International Journal*. In press. DOI: 10.1007/s11947-015-1576-y.