A COMPARATIVE METABOLOME AND PROTEOME ANALYSIS TO ELUCIDATE DISEASE RESISTANCE IN HOT WATER TREATED *Carica papaya* L. cv. Eksotika II

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A COMPARATIVE METABOLOME AND PROTEOME ANALYSIS TO ELUCIDATE DISEASE RESISTANCE IN HOT WATER TREATED *Carica papaya* L. cv. Eksotika II

ABSTRACT

The metabolome and proteome analyses are powerful research techniques utilized to understand the molecular alterations in metabolic and protein profiles of organisms in response to environmental factors. This study uses these methods to investigate the differences in disease resistance between hot water-treated and untreated papaya fruit. The primary objectives are to uncover the molecular pathways involved in disease resistance and identify potential biomarkers of resistance in papaya. Proteome analysis aims to study the impact of environmental factors on protein expression patterns in organisms. In the context of papaya, this analysis helps determine changes in the expression of disease-fighting proteins in hot water-treated fruit. Examples of such proteins include chitinases and pathogenesis-related (PR) proteins. Metabolome analysis focuses on investigating alterations in the metabolic profiles of organisms. In this study, changes in metabolite levels, including sugars, organic acids, and phytohormones, in hot water-treated papaya are assessed. Metabolites are known to play a role in disease resistance. Combining the findings from proteome and metabolome analyses provides a better understanding of the molecular changes associated with disease resistance in hot water-treated papaya. This information can be applied to enhance disease resistance through strategies such as selecting appropriate cultivars or optimizing hot water treatment protocols. In conclusion, comparing metabolome and proteome analyses is an effective approach for investigating the molecular basis of disease resistance in hot water-treated papaya. This knowledge can provide crucial insights into the molecular pathways underlying disease resistance and be utilized to develop strategies for enhancing papaya's resistance to diseases.

Keywords: Hot water treatment, Eksotika II, metobolome, proteome, anthracnose

A COMPARATIVE METABOLOME AND PROTEOME ANALYSIS TO ELUCIDATE DISEASE RESISTANCE IN HOT WATER TREATED *Carica papaya* L. cv. Eksotika II

ABSTRAK

Analisis metabolom dan proteom adalah teknik penyelidikan yang berkuasa yang digunakan untuk memahami perubahan molekul dalam profil metabolik dan protein organisma sebagai tindak balas terhadap faktor-faktor persekitaran. Dalam kajian ini, kami menyiasat perbezaan dalam daya tahan penyakit antara buah betik yang dirawat dengan air panas dan yang tidak dirawat menggunakan kaedah ini. Objektif utama adalah untuk mendedahkan laluan molekul yang terlibat dalam daya tahan penyakit dan mengenal pasti biomarker berpotensi bagi daya tahan dalam buah betik. Analisis proteom bertujuan untuk mengkaji impak faktor-faktor persekitaran terhadap corak ekspresi protein dalam organisma. Dalam konteks buah betik, analisis ini membantu menentukan perubahan dalam ekspresi protein yang melawan penyakit dalam buah yang dirawat dengan air panas. Contoh protein seperti itu termasuk kitinase dan protein yang berkaitan dengan patogenesis (PR). Analisis metabolom memberi tumpuan kepada penyelidikan perubahan dalam profil metabolik organisma. Dalam kajian ini, kami menilai perubahan dalam tahap metabolit, termasuk gula, asid organik, dan fitohormon dalam buah betik yang dirawat dengan air panas. Metabolit diketahui memainkan peranan dalam daya tahan penyakit. Dengan menggabungkan dapatan daripada analisis proteom dan metabolom, kami memperoleh pemahaman yang lebih mendalam tentang perubahan molekul yang berkaitan dengan daya tahan penyakit dalam buah betik yang dirawat dengan air panas. Maklumat ini boleh digunakan untuk meningkatkan daya tahan penyakit melalui strategi seperti memilih varieti yang sesuai atau mengoptimumkan protokol rawatan air panas. Sebagai kesimpulan, membandingkan analisis metabolom dan proteom adalah pendekatan yang berkesan untuk menyiasat asas molekul daya tahan penyakit dalam buah betik yang dirawat dengan air panas. Pengetahuan ini dapat memberikan wawasan penting tentang laluan molekul yang mendasari daya tahan penyakit dan boleh digunakan untuk membangunkan strategi meningkatkan daya tahan buah betik terhadap penyakit.

Kata kunci: Rawatan air panas, Eksotika II, metabolom, proteom, antraknosa.

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"What comes, when it comes, will be what it is."

Alberto Caeiro

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LIST OF SYMBOLS AND ABBREVIATION

μg : Microgram
μl : Microlitre
$\sum$: Sum
°*Brix* : Degree brix
℃ : Degree celcius
AAE : Ascorbic acid equivalent
ACN : Acetonitrile
BC : Biocontrol
BCA : Biological control agent
CE : Catechin equivalent
$CHCl_3$: Trichloromethane
CI : Chilling injury
CO_2 : Carbon dioxide
COG : Clusters of Orthologous Groups
Da : Dalton
DCPIP : 2,6-dichlorophenol-indophenol
DI : Disease resistance
DPPH : 1,1-di-phenyl-2-picrylhydrazyl
DTT : Dithiothreitol
EBDC : Dialkyldithiocarbamates
EI : Electron impact
EMS : Enhanced mass spectra
EPI : Enhanced product ion
ESI : Electrospray Ionisation

Fe^{3+} : Ferric ion

FOASTAT : Statistics Division of the Food and Agriculture Organisation of the United Nations

GAE : Gallic acid equivalent

GC-MS : Gas chromatography–mass spectrometry

GELPs : GDSL esterase/lipases

GO : Gene Ontology

H_2O : Water

HEPES : (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

HSPs : Heat shock proteins

HWT : Hot water treatment

HWT-CG : Hot water treated papaya inoculated with C. gloeosporioides

HWT-DW : Hot water treated papaya inoculated with distilled water

IAA : Indole-3-acetic acid

IDA : Information dependent acquisition (IDA)

IPG : Immobilized pH gradient

IPR : InterPro

KEGG : Kyoto Encyclopedia of Genes and Genomes

LC-MS : Liquid chromatography-mass spectrometry

M : Molarity

MARDI : Malaysian Agricultural Research and Development Institute

MeOH : Methanol

mg : milligram

ml : Millilitre

mM : Milimolar

mm : Millimetre

N : Normality

nm : Nanometre

NaOCl : Sodium hypochlorite

NH_4HCO_3 : Ammonium bicarbonate

O_2 : Oxygen

PA : Polyamine

PDA : Potato dextrose agar

PG : Polygalacturonase

pI : Isoelectric point

PL : Phospholipase

PME : Pectin methylesterase

RNS : Reactive nitrogen species

ROS : Reactive oxygen species

rpm : Resolution per minute

SDS : Sodium dodecyl sulphate

SDW : Sterile distilled water

TA : Titratable acidity

TAC : Total antioxidant capacity

TEAB : Triethylammonium bicarbonate

TFC : Total flavonoid content

TMT : Tandem mass tag

TSS : Total soluble solids

UHPLC-QTRAP MSMS : Ultraperformance liquid chromatography quadrupole time-of-flight mass spectrometry

UV-C : Ultraviolet-C

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CHAPTER 1: INTRODUCTION

Analyses of the metabolome and proteome are important research techniques to investigate how organisms' metabolic and protein profiles alter in response to various environmental factors. In the context of papaya fruit, these methods can be utilised to examine the differences in disease resistance between papaya that has been treated with hot water and papaya that has not been treated with hot water. This study was conducted to understand the molecular pathways involved in disease resistance and locate possible biomarkers of disease resistance in papaya.

Studying how different environmental factors influence protein expression patterns in a given organism is the primary objective of proteome analysis. This analysis can determine whether there have been changes in the expression of disease-fighting proteins in the case of papaya treated with hot water. These proteins include chitinases and pathogenesis-related (PR) proteins, among other (Chouhan *et al.*, 2023)

On the other hand, metabolome analysis is centred on investigating alterations that occur within the metabolic profiles of an organism. This study can determine whether there have been changes in metabolites such as sugars, organic acids, and phytohormones in the case of papaya treated with hot water. Metabolites are known to have a role in disease resistance.

When the findings from the analyses of the proteome and the metabolome are combined, a more in-depth comprehension of the molecular alterations involved in the disease resistance of hot water-treated papaya can be obtained. This information can then be utilised to design methods for improving disease resistance in papaya, such as selecting appropriate cultivars or optimising hot water treatment protocols (Belay *et al.,* 2022)

In conclusion, comparing metabolome and proteome analysis is an effective method for researching the molecular basis of disease resistance in papaya treated with hot water. This knowledge can provide vital insights into the molecular pathways involved in disease resistance and can be exploited to develop strategies for increasing papaya disease resistance.

1.1 Research Questions

The primary focus of this study is to explore the effects of hot water treatment (HWT) on fruits, specifically papaya. Correspondingly, the research questions are:

1. What metabolites are influenced by hot water treatment?
2. Are there metabolites that are found exclusively in HWT-treated fruits?
3. What changes occur in the global protein expression of fruits treated with HWT?
4. Are there specific proteins that are unique to HWT-treated fruits?

1.2 Objectives

In alignment with these questions, the objectives of this study are:

1. To investigate the effects of physicochemical properties of papaya influenced by hot water treatment
2. To investigate the metabolites affected by hot water treatment and identify any changes or influences on their behaviour.
3. To determine if specific metabolites are exclusive to HWT-treated fruits by comparing treated and untreated samples.
4. To analyze the changes in global protein expression of HWT-treated fruits, understanding the overall impact on protein levels.
5. Identifying any specific proteins unique to HWT-treated fruits provides insights into the exclusive effects of the treatment.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to Papaya (*Carica papaya* L.)

The papaya tree (*Carica papaya* L.) is an iconic tropical American fruit tree renowned for its succulent and nutritious produce. Thriving in tropical and sub-tropical climates worldwide, this fruit-bearing tree has captured the attention of both producers and consumers across the globe.

The taxonomical journey of *Carica papaya* has witnessed its classification under various plant families over time, eventually finding its place within the Caricaceae family, which encompasses 35 latex-producing species distributed across four genera: Carica, Jarillaand, Jacaratia, and Cyclicomorpha. Some previously ascribed Carica species have undergone reclassification and now fall under the genus Vasconcella.

The origins of *Carica papaya* L. can be traced back to southern Mexico, the Philippines, and Central America. These regions, particularly the Caribbean coast of Central America, Argentina, Chile, and southern Mexico, have been identified as the cradle of papaya, with its existence attributed to natural hybridization involving *Carica peltata* and other wild species. This tropical treasure has found its way into domestication in various parts of the world, particularly in Asia, Africa, Oceania, and North America.

In addition to its delectable fruits, papaya offers an abundance of gifts to humanity in the form of vitamins, macro and micro minerals, bioactive compounds, and secondary metabolites. Beyond the luscious fruit, papaya's leaves stems, seeds, and other plant components are rich sources of alkaloids and flavonoids, endowing them with remarkable antimicrobial and medicinal properties. Over the years, the manifold therapeutic benefits of papaya have been unveiled, establishing it as a plant of significant therapeutic relevance (Veersain *et al.*, 2023).

2.1.1 Botanical Description

The Caricaceae are a small family of flowering plants comprising six genera of around 35 species. The first taxonomic treatment, which worked with the family under the name Papayaceae, was done by Alphonse De Candolle (1885), who documented three genera: Papaya with three species; *Vasconcellea* with 15 species divided into two parts (*Hemipapaya* and *Euvasconcellea*); and *Jacaratia* with four species.

The nomenclature of *C.papaya* is as follows (National Plant Data Centre, 2000)

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridiplantae – green plants
Infrakingdom	Streptophyta – land plants
Superdivision	Embryophyta
Division	Tracheophyta – vascular plants
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Rosanae
Order	Brassicales
Family	Caricaceae
Genus	Carica
Species	Carica papaya

C. papaya is a perennial herbaceous plant that endlessly bears fruit on the axis of the leaves, spirally arranged around the single vertical trunk. In addition, it is a small, soft-wooded, short-lived and rapidly growing pantropical, lacticiferous tree (Wiart, 2002). The generic names used to identify *C. papaya* are betik (Indonesia and Malaysia), kepaya, papayer (French), lechosa (Spanish), melonenbum (German), mugua (Chinese), papali (Tamil) and pawpaw (Australia and New Zealand) (Tietze, 2003; Chan and Paull, 2008).

In nature, the papaya tree grows upright up to 3 – 8m and even up to 10m in special conditions, ending with a crown of large leaves. It has a hollow, semi-woody stem and a smooth bark marked with protruding leaf scars that are half-moon shaped. The tree normally grows without branching except in cases where the growing point is damaged (Chan and Paull, 2008). The leaves are arranged in a spiral manner at the top of the trunk. Usually, the length of the leaf stems is about 30 cm to 1 m. Most leaves have 5-9 key fingers with a diameter of 30 cm to 70 cm (Tietze, 2001).

The papaya fruit weighs 200 g to over 10 kg and is a fleshy berry. The fruit hangs below the younger fruit from the stalks connected to the upper trunk where the mature fruit is. The shape of the fruit is a sex-linked character that produces round to ovoid fruit for female flowers and long cylindrical or pyriform shapes for hermaphrodite flowers. When young, the fruit's skin is shrill, smooth and green, turning yellow to bright orange when ripening and maturing. The fruit typically comprises five united carpels to form a central ovarian cavity reinforced with a placenta carrying abundant black seeds. The fruit seeds are removed easily (Chan and Paull, 2008). The flesh is succulent and thick but susceptible to bruising injury. The colour of the flesh when the fruit is immature is white. It then turns yellow to orange and, lastly, red when it ripens. The fruit cultivar also decides the flesh's colour when it ripens (Singh and Rao, 2011).

A ripe papaya fruit has a mild aroma and sweet taste. The flavour varies across varieties; for instance, ‘Eksotika’ and ‘Solo’ fruit have a pleasingly aromatic scent, while the ‘Maradol’ variety is known for its unpleasantly musky odour.

2.1.2 *Carica papaya* L. cv. Eksotika II

Eksotika II is a successor to Eksotika I. It is a hybrid of the Sunrise Solo with the local Subang 6 variety. It was introduced on 15 October 1991 by the Malaysian Agricultural Research and Development Institute (MARDI). After 7 years of breeding and development Eksotika II was cultivated. It is a hybridization product between two purelines, Eksotika and Line 19 (Chan *et al.,* 1993).

The advantages of the trees of Eksotika II over Eksotika I are that they exhibit heterosis, and grow taller and more vigorously than Eksotika I. They have the essence of precocity or early fruiting where the fruit is borne around 42-44 weeks after seed sowing, 60-90 cm from the ground level. The seeds of Eksotika II are derived from the hybridization of female flowers with hermaphrodite pollen compared to the Eksotika I whose seeds have been developed from self-pollination (hermaphrodite × hermaphrodite). This will cause the ratio of female Eksotika II trees to be higher than Eksotika I. As a result of the straining task to extract the stamen, the seeds produced from hermaphrodite x hermaphrodite will cost more. However, there is some evidence that using hermaphrodite flowers for seed production is still cost-effective because of the higher proportion of hermaphrodite fruit that contributes to higher rates (Chan *et al.,* 2000).

The fruits of Eksotika I and Eksotika II are comparable; however, certain characteristics differentiate them. Fruits of Eksotika II are larger and weigh between 600g and 800g. Moreover, the smoother skin with fewer freckles contributes to a more attractive appearance of the Eksotika II fruit. The skin of Eksotika II is thicker than

Eksotika I, with firmer flesh and a richer orange-red hue. The fruit of Eksotika II has a higher total soluble solid content (12-14%) and a prolonged postharvest shelf-life, making it a preferable candidate for export than its predecessor. In multiple attempts over various locations, Eksotika II yield is far greater than its inbred parents (Chan and Kwok, 1995). During a two-year crop cycle, the mean yield of Eksotika II ranged from 40-100 tons per hectare, which accounts for 25% more yield than Eksotika I. Eksotika II is a hybrid of F1, which means that the growers could not replicate its pure seeds. Only by crossing the two inbred parents, Line 19 and Eksotika, can the seeds of Eksotika II be achieved. Malaysian Agriculture Research and Development Institute (MARDI) and the Department of Agriculture, Malaysia, are responsible for seed production for this hybrid.

2.2. Commercial Cultivation and Production

2.2.1 Economic and Commercial Value of Eksotika II Papaya

Before MARDI introduced the Eksotika and Eksotika II cultivars, Eksotika II fruits were productively exported to the foreign market. Since Eksotika II produces healthy, standardized quality fruits, it is readily accepted in many markets and has spurred the fruit market's expansion in recent years. Eksotika II is exported to Singapore, Brunei, Czech Republic and Maldives, where the prices are very profitable (Table 2.1). In addition, Malaysian papayas are also approved in the United Kingdom (UK) market. Nevertheless, in the UK, papayas come predominantly from Pakistan, Brazil and India.

The benefit of papaya farming is the high yield and the swift return on investment. Papaya, a non-seasonal plant, could continuously flower and bear fruit throughout the year. Furthermore, the fruits could be harvested 8 to 9 months after seed sowing in the tropics. Therefore, papaya harvest occurs all year round. Papaya cultivation in Malaysia is primarily carried out in Johor and the central parts of the Malaysian Peninsula. The amount of papaya production in Malaysia, as shown in Figure 2.1, increased from 43,640 tonnes in 2011 to 60,844 tonnes in 2021.The increase in the area planted and the production volume of papaya shows that papaya is one of the most prominent tropical fruit crops in Malaysia, with a high commercial value that can contribute to the income of the country.

Table 2.1: Top importers of Malaysian papaya in 2021 (Source: Worldbank, 2023)

No	Country	Quantity (tonnes)	Value (1000USD)
1.	Singapore	12,954.200	4993.74
2.	Brunei	1,615,540	624.03
3.	Czech Republic	300	5.05
4.	Maldives	38	0.23

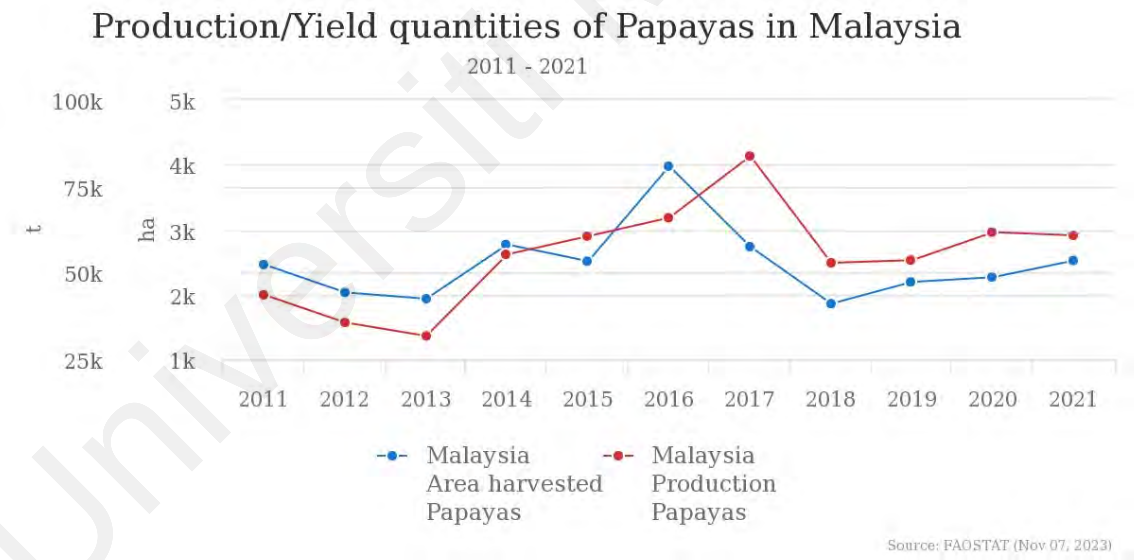

Figure 2.1: Area harvested and production of papaya in Malaysia (2011 – 2021) (Source: FAO, 2023)

The majority of papaya produced is eaten fresh. Smaller parts are processed into pickles, cakes, chilli sauce fillers, and fruit cocktails. Government agencies, individual entrepreneurs, and the private sector have been commercially planting papaya ranging from 10 to 30 hectares. The government also introduced incentives to encourage farmers to plant papayas commercially. Farmers are granted Pioneer Status and the Investment Tax allowance to assist in the fruit industry's development.

There are, however, several barriers to the rapid growth of the papaya industry in Malaysia. One of the biggest issues includes the lack of appropriate land, particularly in Peninsular Malaysia. The current crops are situated on the fringe with a small and steep area, making it unsuitable for commercial papaya cultivation. In addition, other commercial crops threaten the papaya market, such as rubber, oil palm and cocoa. In addition, another problem is the papaya market structure, which is centred on mixed smallholder orchards that are disorganized and scattered throughout the region. This results in the absence of quality uniformity and the size of the fruit entering the market. Nowadays, papaya fruit diseases and pests are still the biggest challenges to the Malaysian papaya industry. To export papaya internationally, a general disinfestation procedure is required. This impedes exports to foreign markets where the quarantine requirements are strict. The fresh papayas from Malaysia could not be exported to countries such as New Zealand, Australia, Japan and the United States of America due to the strict postharvest import quarantine regulations demanded by the countries.

2.3 Postharvest Disease Affecting Papaya

To minimize papaya yield and sales quality, orchard and post-harvest diseases are critical and largely responsible for the losses that occur during crop shipping. Almost one-third of the food produced (about 1.3 billion tonnes), worth roughly US $1 trillion, is wasted annually worldwide during post-harvest operations (Mpagalile *et al.,* 2024). Depending on post-harvest handling and packaging methods, disease losses ranged from 33% (FAO, 2015).

2.3.1 Wet Rot

Lesions of fruit caused by *Phomopsis sp*. They happen infrequently, but they do extensive damage. The entire infected region is soft and translucent, and black pycnidia can form in the central portion of the lesion. A wet rot progresses easily into the fruit cavity from the surface and the infected tissue can be lifted away from the remainder of the fruit. This fungus is often commonly related to papaya stem-end rots.

2.3.2 Alternaria Fruit Spot

Circular to oval black lesions surrounded by black spore masses of *Alternaria alternata* (Fr.) Keissler is distinguished by this disorder. Lesions are generally confined to the fruit surface and do not allow the parenchyma tissues to rot extensively. Refrigeration during surface shipping increases the development of infections, and on unrefrigerated fruit, symptoms rarely arise. Previously, the *Alternaria* fruit spot was a significant disease in fruit grown in papaya orchards in moderately dry areas of Maui. *Alternaria* was found to colonize senescent petioles, and at the time of picking, significant numbers (13,700-36,900 spores per fruit) were found on fruit surfaces; hence, the primary source of inoculum tended to be petioles. Infection was minimised by biweekly orchard sprays and post-harvest hot-water treatment (Neils *et al.*, 2021).

2.3.3 Stem-End Rot

When fungi enter the detached peduncle after harvest, stem-end rots of papaya occur. Via cracks between the peduncle and the papaya tissue, spores can also infiltrate or infiltrate via tiny wounds during harvest. Caused by *Mycosphaerella* sp. stem-end rot. Initially, a transparent region across the peduncle characterizes it. Just a mild browning of the peduncle is visible at the early stages when the fungal hyphae penetrate the vascular tissue. As the infection spreads, although the remaining infected tissue becomes black, wrinkled, and sterile, the lesion margin appears translucent. At an advanced infection stage, white mycelium develops at the stem end (Oliver, 2024).

2.3.4 Anthracnose

Colletotrichum is a large genus of important species in several tropical and subtropical fruits that are among the most prevalent fungal pathogens causing diseases. Virtually all crops planted worldwide are susceptible to one or more species of *Colletrotrichum*. The genus is correlated with economically significant diseases of cereals, legumes, fruits and vegetables (Christopher *et al*., 2022).

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc triggers anthracnose disease which is a rising threat to global agriculture. *Colletotrichum* species are accountable for more than 50% loss of fresh vegetables and fruit (Bordoh *et al*., 2020). The late onset of disease symptoms caused by the fungus is destructive and leads to substantial postharvest losses. *C. gloeosporioides* causes severe losses of important fruits such as mango, papaya and avocado found in tropical regions. These fruits are highly perishable in nature, requiring an urgent need to develop technologies that not only lengthen their shelf-life but also reduce the rate of deterioration of the fruits.

Colletotrichum gloeosporioides was first identified in 1884 and its perfect state (telomorph) was given the generic name *Glomerella cingulata* (Stonem) in 1903. The fungus forms Hyaline, one-celled, ovoid to oblong, strongly curved or dumbbell-shaped conidia with obtuse ends. They are usually borne on distinct, well-developed hyaline conidiophores, measuring 12.5–14.8µm × 4.1–4.7µm (Xie *et al.,* 2010). The waxy acervuli of *C. gloeosporioides* that develop freely on parts of the plants that are diseased (fruits, petioles, and leaves) are subepidermal. The acervuli have been reported to measure 80–250µm (Sattar and Malik, 1939) and 115–467 × 95–22µm (Bose *et al.,* 1973). The acervuli, when mature, exude pink- or salmon-coloured masses of conidia under moist conditions.

Largely, it is commonly regarded as anthracnose if *Colletotrichum* species cause disease symptoms. The anthracnose pathogen targets preharvest in various fruit trees' inflorescences, fruits, leaves, and stems. The fungus secretes extracellular cutinolytic enzymes (pectate lyase and polygalacturonase) that degrade plant cell walls and cause significant economic losses (Peralta-Ruiz *et al*., 2023). The disease symptoms caused by *C. gloeosporioides* are normally depicted by tiny, sunken, water-soaked, subcircular or angular lesions with translucent light brown margins (Talhinhas *et al.*, 2023). The lesions are occasionally referred to as chocolate spots. As infection strides in these circular sunken lesions, erumpent orange to pink conidial masses develop and often appear in concentric rings. With greyish-white discolouration, the internal tissue is solid and turns brown later (Alvarez and Nishijima, 1987; Sivakumar *et al*., 2002; Masyahit *et al*., 2009). Verhoeff (1974) and Swinburne *et al.* (1983) have described these infections as ‘quiescent or dormant parasitic relationship which, after a specified duration, becomes active’. During penetration, germination initiation, germ tube elongation, appressorium formation or eventual establishment are the well-known growth stages where the fungus could become inactive.

2.4 Alternatives Used to Control Anthracnose

A sequence of fungicides belonging to the benzimidazole (Thiabendazole and Benomyl), Imidazole (Phrochloraz) and ethylene, bisdiothiocarbamate (EBDC) classes rely on the chemical regulation of post-harvest papaya diseases. The chemical group strobulins (Azoxystrobin) and the Benzonitrile Compound (Chlorothalonil) are other registered fungicides. Apart from Benomyl, the U.S. registers most fungicides. Committee for Environmental Conservation (EPA). Among other things, the efficacy of these fungicides is associated with the dosage administered, the ripening stage, the targeted fungi's susceptibility response and the application timing. Thiabendazole and benomyl are the most effective fungicides used post-harvest, demonstrating up to 50% disease control (Ons *et al*., 2020). During the post-harvest handling of papaya, the application of fungicides depends on the destination market. Post-harvest rot control has relied on synthetic fungicides for the national market. In this respect, there are little to no regulations for the use of chemicals in certain countries, while the use of fungicides is supervised for the export market by the various food security agencies of the importing nation. In this context, combining fungicides with other non-chemical substitutes such as waxes and hot water/vapour at lower concentrations is typical during fruit handling, resulting in effective post-harvest disease control (Wan *et al*., 2021).

2.4.1 Controlled Atmospheric Storage

As a supplement to proper temperature and relative humidity regulation, controlled or modified atmosphere storage can be used (Yahia *et al.,* 2019). Controlled atmosphere storage refers to a gas atmosphere that is constantly regulated, whereas modified atmosphere storage refers to an originally modified gas composition. Due to the respiration rate of food products and the permeability of the packaging (if any) containing the food products, the gas composition inside a modified atmosphere storage can alter with time (Qu *et al*., 2022). The key advantage of decreasing O_2 and increasing CO_2 across the commodity is its impact on decreasing the commodity's respiration / metabolic rate, contributing to slowing the natural senescence process. Table 2.2 summarizes the other effects of modified O_2 and CO_2 levels (Thompson *et al.,* 2018). Careful consideration should be paid to keeping reasonable control of the precise levels of modified gases from Table 2.2, since too high levels of CO_2 or O_2 are likely to harm fruits and vegetables. Table 2.3 lists examples of optimized modified atmosphere conditions (Kader, 1994). The decrease in food degradation from pests and diseases is another significant advantage of controlled atmosphere storage since higher CO_2 levels usually harm the production and growth of microorganisms. Preserving fruits and vegetables in a controlled environment will also substantially decrease the post-harvest chemicals that shield them from insects and microorganisms (Mostafidi *et al*., 2020).

Table 2.2: Some effects of modified O_2 and CO_2 levels on crops (Thompson, 1998).

Modified O_2 levels	**Modified CO_2 levels**
Reduced respiration rate	Decreased discoloration levels
Reduced substrate oxidation	Decreased synthetic reaction in climacteric fruits
Delayed ripening of climacteric fruits	Inhibition of some enzymatic reactions
Prolonged storage life	Decreased production of some organic volatiles
Reduced rate of production of ethylene	Modified metabolism of some organic acids
Reduced degradation rate of soluble pectin	Inhibition of the effects of ethylene
Formation of undesirable odours and flavours	Retarded fungal growth
Altered texture	Production of off-flavours
Development of physiological disorders	Development of physiological disorders

Table 2.3: Examples of Modified Atmosphere Conditions and Benefits for Selected Tropical Fruits (Kader, 1994)

	Avocadas		Papayas		Bananas	
	Reduced O_2	Increased CO_2	Reduced O_2	Increased CO_2	Reduced O_2	Increased CO_2
Beneficial levels	2–5%	3–10%	2–5%	5–8%	2–5%	2–5%
Benefits	Delay in ripening, reduced rate of CO2 and C2H4 production	Delay in softening, reduced chilling injury symptoms	Delay in ripening	Firmness retention	Delay in ripening	Delay in ripening
Potential for benefits	Good	Good	Slight to moderate	Slight to moderate	Very good	Very good
Injury symptoms	Off-flavor, internal flesh browning	Skin browning, off-flavor	Failure to ripen, off-flavors	Off-flavors, may aggravate chilling injury at <12 °C	Discoloration, failure to ripen, off-flavors	Green fruit softening, undesirable texture and flavor
Potential for injury	Moderate	Moderate	Moderate	Moderate	High	Moderate to high
Optimum storage temperature	10 °C		12 °C		14 °C	

2.4.2 Bio-Control Agents

According to Rao *et al.* (2020), biocontrol (BC) is concerned with the purposeful use of introduced or resident living species, aside from the disease-resistant host plant, to inhibit the behaviours and populations of one or more plant pathogens or the reproduction of one organism using another organism. Similarly, biological regulation was regarded by Mrid *et al.* (2021) as the use of beneficial species, their genes, or products, such as metabolites, to minimize the harmful effects of plant pathogens and to stimulate favourable plant responses. This suggests that the biological control agent (BCA) is referred to as the organism that contributes to the suppression of the pathogen. Typically, using natural products derived from different sources has also been connected to BC. BCA formulations can range from a simple combination of natural active ingredients to complex combinations of multiple effects on the host and the target pest or pathogen. The natural mixtures may mimic the behaviours of living organisms, while, based on the primary advantage they offer to the host plant, the non-living inputs may be more properly treated as biopesticides or biofertilizers (Tariq *et al.*, 2020).

By direct or indirect manipulation of microorganisms (Francis *et al.*, 2020) or the host ecosystem (Prusky *et al.*, 2014), the basic biocontrol model is to minimize disease incidence or its intensity. Any unique characteristics of microbial species make them very competent in the latest approach to managing post-harvest diseases. These microbial organism characteristics include their ability to compete for space and nutrients that allow rapid establishment in the host plant within the stable microbial communities, control through various mechanisms of pathogenic and competitive/deleterious microflora, boost plant health, and stimulate root growth (Liu *et al.*, 2022)

It has also been documented that during pre- and post-harvest yeasts are tolerant of harsh environmental conditions, have unique adaptations to the fruit microenvironment, can grow quickly on a less expensive medium and are easy to produce in large quantities

(Zhang *et al.,* 2023). Compared to filamentous fungi, other promising characteristics of yeasts are that they do not develop allergenic spores or mycotoxins and have clear dietary requirements that enable them to colonize dry surfaces for a prolonged period. As an essential mechanism, their competitiveness for nutrients such as amino acids, sugars and Fe^{3+} helps leaves to compete against other antagonists for nutrients (Bencheqroun *et al*., 2007; Saravanakumar *et al*., 2008; Sipiczki, 2006). In addition, modes of action such as mycoparasitism, mediated resistance and development of yeast-reported lytic enzymes viz., β-1, 3-glucanase and chitinase have been reported (Ippolito and Nigro, 2000, Saligkarias *et al.*, 2002, Yu *et al.*, 2009, Mukherjee *et al*., 2022).

Microorganisms used to biocontrol pathogens have many modes of action, classified into two broad fields (direct and indirect effects). The direct microbial effect is when the microbial antagonists inhabiting the same ecological niche communicate directly with the target pathogen (Weiland-Bräuer, 2021). Here, the interaction mechanisms are parasitism, competition for space, nutrients or 'chemical warfare' using antibiotics or other secondary metabolites that damage the target pathogen. Second, an indirect effect is when a resistance reaction in the host is caused by the biocontrol agent that gives it protection against plant pathogens (Lahlali *et al*., 2022). A new strain of the plant pathogen with low virulence, a particular microorganism species or a natural product may be used by the 'inducer' for this type of control (Timilsina *et al*., 2020).

In practice, however, the extensive use and acceptance of postharvest biocontrol products remains limited. This can be due to many shortcomings linked to decreased and erratic efficiency as biocontrol agents (BCAs) are used under commercial conditions and to the growth and commercialization of limited- and small-size businesses (Khulbe *et al*., 2024). Numerous analyses of host, pathogen and environmental factors influenced the decreased effectiveness of post-harvest BCAs and techniques for their enhancement (Sare *et al*., 2021).

2.4.3 Irradiation

2.4.3.1 Ultraviolet Irradiation (UV)

UV-C irradiation has also been effective for decontaminating food surfaces, with a dose range between 190 and 280 nm as a feasible option. The value of this approach is that UV-C specifically prevents the activity of pathogens and activates the host's defence mechanism. (Ledermann *et al.*, 2021). It has been implemented in a postharvest treatment to delay the spoilage fungi growth and to decrease the lesion diameter of *P. expansum* or *B. cinerea* on kiwi (Beirao-da-Costa *et al.*, 2014), peach (Gonzalez-Aguilar *et al.*, 2004), pear (Syamaladevi *et al.*, 2014), apple, cherry, strawberry and raspberry (Syamaladevi *et al.*, 2015) fruit after pathogens are artificial inoculated. The inactivation of fungi responsible for fresh fruit post-harvest diseases depends on the dosage and timing of UV-C exposure and the morphology of the fruit surface (e.g., roughness), but there were no substantial variations in weight loss, content of soluble solids and texture for treated and untreated UV-C fruits. (Abdipour *et al.*, 2019).

2.4.3.2 Blue Light

Blue light (400-500 nm) is part of the visible spectrum and regulates certain vegetative tissue biochemical pathways (El-Esawi *et al.*, 2017; Lafuente & Alférez, 2015). Several experiments have shown that blue light may regulate fungal production (Ballester & Lafuente, 2017; Liao *et al.*, 2013; Yamaga, Takahashi, Ishii, Kato, & Kobayashi, 2015). It may be speculated that the resistance caused after treatment with blue light could be due to a direct effect of light on fungal growth an indirect effect of light on fruit elicit resistance, or both (Ballester & Lafuente, 2017; Lafuente & Alférez, 2015; Liao *et al.*, 2013). *In vitro* studies have shown that fungal morphology and sporulation are influenced by blue light, while effectiveness improves with the length of the application and the quantum flux of light (Lafuente & Alférez, 2015). The consequence of blue light in circadian rhythms and the development of ROS in fungal cells may be attributed to these effects (El-Esawi *et al*., 2017; Tisch & Schmoll, 2010). The inhibitory effect of blue light on the fungal mould allows the affected fruit surface to be directly exposed to light (Liao *et al*., 2013). In addition to the changes in the fungal cells caused by blue light, it also controls metabolic pathways into the plant tissues, which may be involved in fungal resistance. Blue light, for example, induces phospholipase A_2 (PLA_2) gene expression on treated fruits, and is a key component in the pathway of lipid signalling and is involved in plant immune responses (Mehta *et al*., 2021). In addition, high blue light quantum flux stimulates the metabolism of phenylpropanoids, and this contributes to an improvement in phytoalexin scoparone, which has been related to antifungal activities (Bhatta *et al*., 2021).

2.4.3.3 Gamma-Irradiation

Gamma-irradiation is a sustainable process that could prolong the life of fruits and vegetables after harvest (Guerreiro *et al.,* 2016). Low-dose gamma-irradiation treatment can delay fruit ripening by inhibiting ethylene development and respiration rate and controlling the activity of enzymes involved in scavenging free radicals (Wang *et al.,* 2017). Gamma irradiators have used either cobalt-60 or cesium-137 as radioactive sources. Due to its adverse effects on fungal morphology and its penetration potential, gamma-irradiation is a promising therapy for reducing the post-harvest decay of citrus products (Kahramanoğlu *et al.,* 2020). The utility of this technique is proportional to each pathogen's radiosensitivity (Jeong *et al.,* 2015). However, the mistrust of customers over irradiated foods should be resolved before this approach is commercially implemented (Castell-Perez *et al*., 2021). The mechanism by which fungal growth is hindered by gamma-irradiation is related to its ability to destroy the fungal cell membrane, resulting in a loss of intracellular content. (Jeong *et al*., 2016).

2.4.3.4 X-Ray

X-ray is an electric irradiation with 1016-1019 Hz frequencies (Turini *et al.*, 2023). Previous experiments on various food items have shown that X-ray irradiation is a revolutionary technique for decontamination that may supplement traditional sanitisers because it has antimicrobial activities against different pathogenic bacteria (Chadha *et al.*, 2022). The major X-ray disadvantage, however, is connected to customers' perception of irradiated goods. Water is the primary focus of the energy (photons) produced by the sources of X-rays. Free hydroxyl and hydrogen radicals can be produced after a photon reaction with water, activating physiological functions in living organisms (Wu *et al.*, 2022).

Irradiation can induce the synthesis of antifungal activity by secondary metabolites. Under particular conditions, the aggregation of these compounds is preferred and requires a certain time. Rojas-Argudo *et al*., (2012) found that X-ray irradiation of 510 Gy caused the synthesis of scoparone, scopoletin and phytoalexins after storage at 20 °C for 14 days, while the accumulation of these compounds was delayed for 60 days when the fruit was stored at 5 °C.

2.4.4 Natural Products

2.4.4.1 Plant Extracts And Active Compounds

Plants have long been known to create antimicrobial compounds in various organs, such as seeds, leaves, bulbs, etc. Different plant species from different botanical families and their derivatives have been shown to have fungicidal or fungistatic activity against fungal diseases to some degree (Deresa *et al.*, 2023). In this sense, plant extracts from the botanical families Sapotaceae (*Achras sapota*, *Chrysophyllum cainito* and *Pouteria sapota*), Caricaceae (*C. papaya*), Fabaceae (*Pachyrrizus erosus*), Leguminosae (*Phythecellobium dulce*), Solanaceae (*Cestrum nocturnum*) and Verbenaceae (*Lantana* *camara*) have been used to provide exceptional regulation of different fungal diseases, such as *Mucor* spp. (Bautista-Bañoset *et al.*, 2002; Barrera-Necha *et al.*, 2003; Tasiwal, 2008; Nwinyi *et al.,* 2010). Additional analyses of 40 separate plant species for the regulation of *C. gloeosporioides*. Optical microscopy and spectrophotometric methods contributed to the classification of two notable plant species, *Cestrum nocturnum* and *Annona cherimola*. Aqueous and acetonic extracts of *C. nocturnum* were further evaluated to determine the control they exert on postharvest rot incidence of papaya (Hernández-Albíter *et al.,* 2005). Results found that leaf extracts were only as effective at 1.5% concentration of this plant species as or more than the fungicide Mirage® in reducing disease occurrence (Cruz-Cerino *et al.*, 2023). A remarkable regulation of numerous post-harvest fungi was shown by the constituents and secondary metabolites of *P. dulce* and *P. erosus* (Barrera-Necha *et al*., 2003). Several triterpene saponins named Pithedulside A, B, E, F and I retarded and inhibited the mycelial growth of *R. stolonifer* and *C. gloeosporioides*, while for *P. erosus*, the active compounds identified as rotenone, paquirrizone and dehidroneotenone (250 μg ml^{-1}) has been the best fungicidal action on *R. stolonifera, Fusarium oxysporum* and *C. gloeosporioides*, respectively.

2.4.4.2 Essential Oils

It has long been recognized that essential oils produced by plants, a mixture of volatile compounds resulting from secondary plant metabolism, effectively control fungal phytopathogens. Barrera-Necha *et al.* (2008) tested the effectiveness of nine essential oils in regulating *C. gloeosporioides* and their effect on postharvest rots. In those trials, the best control was provided by the essential oils *Cinnamomum zeylanicum* and *Syzygium aromaticum* at a concentration of 50 μg ml^{-1}, as both effectively controlled anthracnose disease. In this situation, the occurrence of the disease reached no more than 13% compared to 35% on the untreated fruit, while, Espitia *et al.* (2012) recorded that the presence of different forms of pathogenic microorganisms like moulds during papaya storage was delayed by 12 days by polymeric resin sachets containing 20% of cinnamon essential oil. In other research, the inclusion of thyme essential oil at 0.10% and 0.15% with mesquite gum-based and candelilla wax significantly reduced *R. stolonifer* and *C. gloeosporioides* by as much as 40% and 100% (Bosquez-Molina *et al*., 2010), while the formulation of gum Arabic with cinnamon essential oil at 0.4% was reported to decrease anthracnose disease by 80% during storage (Maqbool *et al*., 2011).

2.4.4.3 Chitosan

Chitosan, a natural compound obtained from chitin in the outer shell of crustaceans such as shrimps, crabs and krills, is well-documented for its antifungal effects on various plant pathogenic bacteria and fungi. In this respect, chitosan has also been tested to evaluate its efficacy in controlling fungi *in vitro* and *in situ* studies.

In *in vitro* evaluations, the development of various isolates such as *C. gloeosporioides*, *R. stolonifer*, *P. digitatum* and *F. oxysporum* was completely inhibited in a range of chitosan concentrations from 1.0% to 3.0% (Bautista-Baños *et al*., 2006; Hewajulige *et al*., 2009; Ali *et al*., 2011), while in *in situ* evaluations, Luna *et al*. (2011) reported infection levels of chitosan-treated fruits were halved at 3% concentration, Ali *et al*. (2013) reported that infection levels were from 7.5 to 7.0% in comparison with the 100% infection level observed in the control fruit, and Ramos-García *et al*. (2012) reports that irrespective of molecular weight, postharvest control of rots was accomplished at concentrations of 0.15% and 0.25%. In other research, the effectiveness of chitosan in managing post-harvest diseases was tested during three harvest seasons when applied from the flowering stage to harvest. In 2005, among all therapies, including those with synthetic fungicides, infection levels were high at 14 °C at the end of the storage time (Rojas *et al.,* 2009). However, during 2009 and 2011, infection levels of approximately 50% were registered, coinciding with the application of higher chitosan concentrations (1.0% and 1.5%). Compared with the untreated and fungicide-treated fruit, the shelf life of the chitosan-treated fruit was increased by up to 3 days over all sampling times. Chitosan may be used as a carrier of antimicrobial agents. Different mechanisms of action have been suggested, but the most supported are related to the cationic nature of chitosan, closely correlated with the fungi's cell membrane composition, affecting their integrity and permeability (Ke *et al.*, 2021).

2.4.5 Salts and Minerals

It is documented that after harvest, carbonate salts effectively influence various fruit and vegetable fungi (Lyousfi *et al.*, 2023). Three per cent ammonium carbonate followed by 2% sodium carbonate, measured alone or in combination with wax, among the various salts measured for anthracnose disease prevention, had a beneficial effect on the reduction of *C. gloeosporioides* in normal and chemically inoculated fruit by up to 50% (Martínez-Blay *et al.*, 2022). One of the most important factors that can induce post-harvest disease is calcium, which is an agent that improves fruit firmness and decreases the decay of some fruits and vegetables. Studies by Saborio *et al.* (1996) showed that fruits immersed in calcium chloride had a disease prevalence of 10% compared to the 23% observed in untreated fruits. In more studies, the effectiveness of calcium (2.5%) and chitosan (0.7%) penetration in regulating *C. gloeosporioides* (Al Eryani-Raqeeb *et al.*, 2009). Since no conidial germination took place with the above combination and the incidence of anthracnose was 38% to 88% seen in the untreated fruits, the development of this fungus was badly hampered. Additional benefits of this medication were an improved shelf life of almost 15 days and reduced weight and firmness loss.

2.5 Heat Treatment

Post-harvest heat treatments, including herbs, fruits, and vegetables, have been used to disinfect a growing range of crops. Applying heat levels (hot water, vapour heat and hot air) to fruit temperature treatments has been successful (Usall *et al.,* 2016). Heat treatments cause mild and reversible stress that inhibits the product's natural metabolism (senescence or fruit maturation) (Corpas *et al.*, 2023). In situations where heat is used for curing produce to facilitate wound healing or kill organisms (nematodes, insects or viruses), heating times are normally longer than for regulating post-harvest decay. Owing to the position of the target pathogens on the surface or inside the few exterior cell layers of the product, post-harvest heat treatments for decay frequently take a few minutes. For this purpose, only the outer surfaces require heat to reach a significant degree of pathogen control. Such restrictions can exist, including low persistence, the possibility of adverse effects on manufacturing efficiency or technical difficulties for industrial use.

Heat application before storage can alter gene expression and fruit ripening (delayed or disrupted). The degree of alternation during fruit ripening is a function of the sensitivity to temperature and length and how easily the product is cooled after heat application. The most assessed components of fruit ripening impacted by heat treatments are fruit softening, membrane and taste shifts, respiration rate, ethylene production, and volatile production. In addition, decaying enzymes from the cell wall and the development of ethylene are also the most disrupted and are occasionally not produced or their presence is postponed after heating. Other ripening-related mechanisms are often not altered to the same degree or rebound rapidly. Preharvest weather conditions, cultivar, heating rate, and subsequent storage conditions have been found to alter fruit susceptibility to heat treatment. A commodity's heat stress exposure or resistance is linked to the degree of heat protein at harvest and the production of heat shock proteins after harvest. Two types of heat responses are detected. First is a natural cellular response (< 42 °C) that can lead to diminished sensitivity to chilling, delayed or slowed maturation, and quality improvement. The second form of heat response occurs close to the damage threshold of > 45 °C; thus, the environmental conditions of pre-stress modify the cellular response to stress and cellular recovery. The lack of integrity of the membrane is a consequence and not a source of injury. However, when an injury threshold has been exceeded, the location of the injury lesion is still uncertain and could contribute to transcription, translation and cellular recovery capacity.

Heat pre-treatment impairs endogenous ethylene production; however, fruit tissue does not respond to exogenous ethylene. An earlier study revealed that this situation could be attributed to the degradation or inactivation of ethylene receptors or the failure to relay the signal to a subsequent sequence of ripening events (Lurie, 1998), usually correlated with lower tolerance to fungal pathogens. Elevated temperatures in tomato fruits impair the expression of ripening genes (Picton & Grierson, 1988). Relevant mRNAs associated with ripening cycles, for example, vanished during high temperature (38 °C) hot air treatment of tomatoes but reappeared during recovery from heat (Chaurasia *et al.*, 2024). Therefore, the suppression of ethylene formation is reversed as the fruits are separated from the sun. The level of ethylene will also rise to higher levels than in unheated fruits; such treatment involves protein synthesis, as seen in the analysis by Lurie *et al.* (1996), where both mRNA and ACC oxidase protein accumulate during hot air treatment recovery (38 °C). More recently, Lin *et al.* (2022) reported that heat treatment delays senescence and preservation of the overall product quality during storage. Chaurasia *et al.* (2024) stated that elevated temperatures activate certain developmental and physiological responses; hence, certain ripening characteristics are more advanced in heated fruit than in unheated fruit thus retaining their consistency.

2.5.1 Hot Water Treatment

In 1922, hot water treatments were first reported to control citrus fruit decay (Fawcett, 1922), but their use was applied for insect disinfestation (Lurie, 1998). Pre-storage heat treatments to control oxidation are often performed for a comparatively brief amount of time (minutes) since the target pathogens are located under the skin of the fruit or vegetable on the surface or in the first few cell layers. Heat can be applied to fruit and vegetables in several ways: by hot water dips, vapour heat, or hot, dry air (Hao *et al.*, 2020), or by hot water rinsing and brushing (Fallik *et al*., 2021). Vapour heat treatment was developed mainly for insect control, while hot dry air was used for fungal and insect control (Lurie, 1998). Water is the chosen medium for most situations because it is a more effective medium for heat transfer than air. Heat treatments may also be used to prevent processes of ripening or to cause resistance to chilling injury (CI) and external skin damage during storage, thereby increasing storability and marketing (Rai *et al.*, 2022).

Numerous temperate, sub-tropical and tropical fruits, vegetables and flowers have demonstrated the beneficial effect of pre-storage hot water immersion treatment (HWT) to prevent rot development (Heyes *et al.*, 2023). This procedure has various benefits, including relative ease of use, short treatment time, effective fruit and water temperature control, and killing of skin-borne decay-causing agents (Sharp, 1994). Another major economic benefit of hot water immersion technology is that it only costs 10% of the total cost of a commercial device (Pambudi *et al*., 2022). However, by season and growing area, the physiological responses of cultivars of various fruit or flower species to heat treatments can differ (Chaudhary *et al.*, 2020). Differences in temperature, soil type, season, production methods, and harvest fruit maturity can cause variations in response between production regions (Medda *et al*., 2022).

The quality of fresh produce handled at optimum hot water temperatures and exposure periods was typically considerably higher than that of untreated fruit, as measured by a sharp decrease in the rate of decay and several quality characteristics (Botondi *et al.,* 2021).

For control of *Penicillium digitatum* and *Penicillium italicum,* immersion (HWT) of citrus fruit in 50–53 °C water for 2–3 min is recommended which causes rots in oranges, lemons, or grapefruit (Bhatta *et al*., 2022). Hot water treatments of 56 °C for 10 seconds reduced *Colletotrichum* body rots in avocado fruit (Majola *et al*., 2020). Such treatments also help preserve the condition of the external and internal avocado fruit after cold disinfestation (Hofman *et al*., 2002). A short-term HWT of mature-green tomato at 39–45 °C is a cost-effective, efficient and environmentally safe method for reducing decay, but a 48 °C dip was harmful to overall fruit condition and quality (Rai *et al*., 2022). Immersion of guavas (*Psidium guajava* L.) for 35 min in hot water at 46.1 °C increased fruit susceptibility to decay but delayed fruit ripening by 2 days (McGuire, 1997). Immersing bell pepper at 53 °C for 4 min before storage effectively reduced decay after 14- and 28-days storage at 8 °C (Gonzalez-Aguilar *et al*., 2000).

Heat treatments can also inhibit the development of chilling injury and external skin damage during storage and marketing (Rodrigues *et al.*, 2022). Hot water treatment for 1 h at 38 °C reduced external skin damage of ‘Hass’ avocado caused by subsequent heat and cold treatments (Setagane *et al*., 2021). The effect of postharvest HWT treatments on chilling tolerance and polyamine (PA) induction in flavedo tissue of mandarin (*Citrus reticulata*, Blanco, cv. ‘Fortune’) was investigated by Morales *et al*. (2024). After 45 days at 2 °C, the major reduction in CI was found in fruit dipped for 6 min at 47 °C or 3 min at 53 °C. The researchers concluded that the pattern of change in PA content was not related to HWT-induced cold tolerance.

2.6 Proteomics

Proteomics is a complex field focused on the study of proteins, their modifications, interactions, localization, and roles within biological systems. Unlike the relatively static nature of DNA, proteins are dynamic molecules that undergo extensive biochemical modifications during and after their synthesis, known as post-translational modifications (PTMs) (Ramazi *et al.*, 2021). These modifications can significantly influence protein function and are crucial for understanding cellular processes.

The proteome is inherently more complex than the genome due to several factors. One of the primary contributors is alternative splicing, where a single gene can produce multiple protein isoforms through the splicing of messenger RNA (mRNA) (Reixachs-Solé *et al.*, 2022). This process leads to diverse protein functions from a single genetic code and is regulated by various factors, including cell type, developmental stage, and environmental conditions. Additionally, different genes can encode for the same protein, a phenomenon known as gene duplication, which allows for the evolution of new protein functions and the maintenance of essential cellular processes (Kuzmin *et al.*, 2022). Furthermore, more than 300 types of PTMs have been identified, including phosphorylation, acetylation, and ubiquitination. These modifications can alter protein activity, stability, and interactions, adding another layer of complexity to the proteome (Lee *et al.*, 2023)

A primary method for comparative proteomics is Two-Dimensional Gel Electrophoresis (2-DE). This technique separates proteins based on their isoelectric point (pI) in the first dimension and their molecular weight in the second dimension. 2-DE can resolve thousands of proteins in a single gel, making it a powerful tool for protein analysis. The high resolution of this technique allows for the detection of subtle differences in protein expression and modification patterns between samples. Various stains, such as Coomassie Brilliant Blue, silver stain, SYPRO Ruby, and Deep Purple, are used to visualize proteins on the gel. These stains differ in their sensitivity, compatibility with downstream analyses, and ability to detect specific types of proteins or modifications.

Despite its advantages, 2-DE is labor-intensive and time-consuming. It primarily detects denatured proteins within a specific pH range (3.5-11.5) and size range (10-200 kDa) (Yuan *et al.*, 2018) Additionally, it struggles with low-abundance proteins and small molecular weight proteins (<10 kDa) and often under-represents membrane proteins due to solubility issues. Vertical and horizontal protein streaking can also complicate analysis, making it challenging to accurately quantify and identify specific proteins of interest.

Recent developments have aimed to enhance the capabilities of 2-DE and other proteomic techniques. Innovations include changing buffer systems to improve resolution for small proteins and employing different isoelectric focusing methods, such as immobilized pH gradients (IPG) and carrier ampholytes, to better separate proteins based on their pI (Lomeli *et al.,* 2024) These modifications have led to the detection of extreme-size and pI proteins, expanding the range of proteins that can be analyzed using 2-DE.

Coupling 2-DE with mass spectrometry (MS) has revolutionized proteomics, allowing for detailed identification and quantification of proteins and their modifications. Techniques such as Data-Independent Acquisition (DIA) and label-free quantification have emerged, enabling high-throughput analysis of PTMs (Wang *et al.,* 2024). These advancements have significantly improved the sensitivity and accuracy of protein identification and quantification compared to traditional gel-based methods. Additionally, immunoaffinity enrichment enhances the detection of specific PTMs by using antibodies to isolate modified peptides before mass spectrometric analysis, facilitating the study of dynamic changes in protein modifications under various conditions.

Targeted proteomics is another emerging approach that involves the selective monitoring of pre-defined proteins or peptides using mass spectrometry. Techniques like Selected Reaction Monitoring (SRM) and Parallel Reaction Monitoring (PRM) enable the quantification of specific proteins of interest with high sensitivity and reproducibility (Schmidt *et al.,* 2022). This targeted approach is particularly useful for validating findings from discovery-based proteomics and for monitoring changes in specific proteins across different conditions or disease states.

The study of proteomics is essential for understanding the functional complexities of proteins in biological systems. While traditional methods like 2-DE provide valuable insights, ongoing advancements in mass spectrometry and enrichment techniques are paving the way for more efficient and comprehensive proteomic analyses. These developments are crucial for elucidating the roles of proteins and their modifications in health and disease. By combining the power of high-throughput protein identification and quantification with targeted approaches, researchers can gain a deeper understanding of the dynamic interplay between proteins and their modifications in complex biological systems (Cui *et al*., 2022).

2.6.1 Role of Proteomics in Plant Defence Mechanism

Plants display several responses to biotic stresses that allow them to avoid or survive in adverse circumstances. In response to the upcoming pathogenic attack, the plant defence system requires vision, pathogen identification and many signalling activities. To survive the damage caused by stress, these reaction mechanisms must be fast and precise. (Jain *et al*., 2022).

Plant defence strategy requires the synthesis of phytohormones such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) that play important roles in the signalling pathways (Gilroy *et al*., 2022). In addition, auxin, abscisic acid, cytokinin, and brassinosteroids function to fine-tune the defensive response and are also documented to be manipulated by beneficial rhizospheric microbes (Pieterse *et al*., 2012). Within a few minutes of pathogen perception, reactive oxygen species (ROS) and reactive nitrogen species (RNS), along with different kinases like MAP kinase (MAPK) are produced (Giulietti *et al.,* 2023). For necrotrophic and hemibiotrophic pathogens, MAPK cascade activation by heterotrimeric, Guanine nucleotide-binding proteins or 'G-proteins' has also been investigated (Patel *et al.,* 2016). ROS provides strength by cross-linking and antimicrobial action in cell wall polymers (Jain *et al*., 2013).

The existence of such interaction depends not only on the life cycle of the invading pathogen (Doehlemann and Hemetsberger, 2013), and the host plant's genetic structure (Gimenez-Ibanez and Solano, 2013), but also on the beneficial microbes interacting. (Jain *et al*., 2013). Appropriate mRNA does not generally correlate with the corresponding protein in the plant cells to be involved or abundant during protection (Jones *et al.*, 2012). In the living cell, proteins are the true workhorses, continuously reacting to their external environment, denoting the cell's active state. Vast numbers of studies have been conducted to unravel plant-microbe relationships. To deal with the pathogenic challenge, Proteomic explains intensive knowledge of many facets of plant protection (Sarethy *et al*., 2021). This method has already been analysed regarding differential protein abundance, post-transcriptional, post-translational alterations, protein stimulation/repression, and turnover (Jain *et al.,* 2021). The proteomic method will assist in evaluating the large range of protein profiles that may, in turn, offer insights to consider the plant's defensive responses as the product of the relationship between host and pathogen (Jain *et al.,* 2022).

The rapid response of plants to pathogen attacks involves not only the production of phytohormones and reactive species but also the activation of various signaling molecules and transcription factors. These molecules play crucial roles in regulating the expression of defense-related genes. For instance, WRKY transcription factors are known to modulate the expression of genes involved in both SA and JA signaling pathways, thereby enhancing the plant's defense mechanisms (Wani *et al.,* 2021). Similarly, the NAC and MYB families of transcription factors have been identified as key regulators of stress responses, helping to orchestrate a coordinated defense response (Javed *et al.,* 2020).

An important aspect of plant defense is the crosstalk between different signaling pathways, which allows plants to fine-tune their responses to specific pathogens. For example, the antagonistic interaction between SA and JA signaling pathways enables plants to effectively allocate resources towards defense against biotrophic pathogens (which require living host tissue) or necrotrophic pathogens (which kill host tissue) as needed (Biere *et al.*, 2016). This crosstalk ensures that the defense response is appropriate for the type of pathogen encountered, optimizing the plant's chances of survival.

Epigenetic modifications, such as DNA methylation and histone modifications, also play a significant role in regulating plant defense responses. These modifications can lead to changes in gene expression without altering the underlying DNA sequence, allowing plants to quickly adapt to biotic stresses. For example, histone acetylation has been shown to activate the expression of defense genes in response to pathogen attack (Kang *et al.,* 2022). Such epigenetic changes can be heritable, providing a form of "memory" that enhances the plant's ability to respond to future attacks by the same pathogen.

Beneficial microbes, particularly those in the rhizosphere, can induce systemic resistance in plants, a phenomenon known as induced systemic resistance (ISR). ISR is characterized by the enhanced defensive capacity of the entire plant, not just the site of initial microbial interaction. This is often mediated by JA and ET signaling pathways and involves the priming of plant defenses, which enables a faster and stronger response to subsequent pathogen attacks (Honig *et al.,* 2023). The manipulation of plant-associated microbiomes to enhance ISR represents a promising strategy for sustainable agriculture.

Despite significant advances in understanding plant defense mechanisms, many aspects remain to be explored. Future research could focus on the integration of omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics, to gain a comprehensive understanding of plant-pathogen interactions. Additionally, the development of advanced genetic and biotechnological tools could facilitate the engineering of crops with enhanced resistance to a wide range of pathogens. Understanding the molecular basis of plant defense and leveraging this knowledge to improve crop resilience will be crucial for ensuring food security in the face of increasing biotic stresses.

2.7 Metabolomics

Metabolomics is devoted to studying small molecules present throughout living organisms. These small molecules determine many of the significant features of crop plants and their related food items. Nutritional value, taste, smell, colour, disease resistance, immunity to abiotic stress, appearance, spoilage of off-flavours and many more are included here. The most important nutritional food ingredients such as sugars, amino acids, fatty acids, and so on are, but also a multitude of 'minor' components like phenolics, terpenoids, alkaloids, and so on, which determine the quality of food and influence the perception and preference of consumers (Jacobs *et al*., 2021).

Compared to genomics and proteomics, the value of metabolomics is its strong relation to the characteristics of the phenotype or food. However, the wide variety of chemical structures and the great variations in quantity are major obstacles, and no single technology can cover the entire metabolome. Untargeted or selective profiling methods focused on nuclear magnetic resonance spectroscopy, liquid chromatography-mass spectrometry or gas-chromatography-mass spectrometry are usually implemented depending on the question(s) to be answered and coupled to multivariate statistics and pathway analysis to obtain the appropriate data (Jacobs *et al*., 2021).

The application of metabolomics in crop improvement has immense potential for enhancing the quality, yield, and resilience of agricultural products. By analyzing the metabolic profiles of plants, researchers can identify biomarkers associated with desirable traits, such as higher nutritional content, better taste, and increased resistance to diseases and environmental stresses. For instance, metabolic profiling has been used to identify compounds that confer drought tolerance in crops, enabling the development of more resilient varieties through breeding or genetic engineering (Yadav *et al.*, 2021).

Despite its potential, metabolomics faces several challenges, including the complexity of plant metabolomes and the lack of standardized protocols for sample preparation, data acquisition, and analysis. Advances in analytical techniques, such as high-resolution mass spectrometry and improved bioinformatics tools, are needed to overcome these challenges and enhance the reliability and reproducibility of metabolomic studies. Moreover, expanding the metabolite databases and improving the annotation of unknown metabolites will be critical for advancing the field (Chaleckis *et al.,* 2019).

2.7.1 Role of Metabolomics in Plant Defence Mechanism

Plants, also referred to as natural chemists, may generate thousands of specific metabolites that attract pollinators, repel herbivores, battle microbial pathogens, and defend against environmental stresses (Kessler *et al*., 2018). Primary metabolism includes compounds essential for plant development, reproduction, and growth, while specialized metabolism includes compounds important for the plant to deal successfully with abiotic and biotic stresses (Castro-Moretti *et al*., 2020). Plants rely heavily on chemical protection from biotic and abiotic stresses because of their sessile existence. Plant metabolism is, therefore, a dynamic process which responds to external stimuli. Environmental changes, such as light quality, water stress, or temperature, have been shown to influence metabolism (Garzon *et al*., 2020). Metabolomics may provide a snapshot of plant metabolism during growth and in reaction to various biotic and abiotic stimuli, including environmental or nutritional stresses (Wu *et al*., 2018). Due to the universality of primary metabolite structures and the closely retained structures of specialized metabolites, Metabolomics is a highly powerful way to analyse plant pathogen associations of plant varieties and races (Castro-Moretti *et al*., 2020). In plant-pathogen interactions, metabolites perform various functions, including monitoring against pathogen attack, signal transduction, enzyme control, cell-to-cell signalling, and anti-microbial activity (Vinayavekhin *et al*., 2010). Metabolomics can identify a few metabolites associated with infection, such as compounds secreted during invasion by pathogens or amino acids and sugars caused or mislocated in the host to boost the growth of pathogens (Mehrotra *et al*., 2022).

Metabolites are the intermediaries and components of gene-coordinated metabolic pathways and related products, complementing and corroborating transcriptomic or proteomic evidence on plant-pathogen interactions by tracking their levels (Peyraud *et al.*, 2017), thereby revealing mechanisms of pathogen assault. Transcriptomic and proteomic analyses are examined through messenger RNA and protein shifts. Metabolites may also control the products of these gene-centred mechanisms (Sandling *et al.*, 2021). The incorporation of metabolomics data with other omics data thus adds additional layers of information to plant-pathogen interaction studies, including the detection of antimicrobial action metabolites (Seraj *et al.*, 2023), variations in metabolomic profile between infected and non-infected plants, and pathogenic infection and colonization requirements (Castro-Moretti *et al*., 2020).

The detailed metabolic profiles obtained through metabolomics can reveal specific changes in the levels of these defense-related metabolites, offering insights into how plants respond to different stress conditions. For instance, during a pathogen attack, plants may increase the production of salicylic acid (SA) and jasmonic acid (JA), which are crucial for activating systemic acquired resistance (SAR) and induced systemic resistance

(ISR), respectively (Kamle *et al.*, 2020). Metabolomics allows for the precise measurement of these hormone levels and their downstream metabolites, providing a clearer picture of the defense signaling network.

Furthermore, metabolomics can aid in the discovery of novel defense-related metabolites that were previously unidentified. By comparing the metabolic profiles of resistant and susceptible plant varieties, researchers can pinpoint specific compounds that contribute to resistance. This knowledge can be used to develop new strategies for crop protection, such as breeding programs that select for higher levels of these beneficial metabolites or the application of biostimulants that enhance their production.

Another significant application of metabolomics in plant defense is the study of induced resistance by beneficial microbes. Certain soil microbes, such as mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR), can induce systemic resistance in plants, enhancing their ability to withstand pathogen attacks and environmental stresses (Meena *et al.*, 2020). Metabolomic studies can elucidate the metabolic changes induced by these beneficial interactions, identifying key metabolites involved in the priming of plant defenses (Bertrand *et al.*, 2021). This information can be leveraged to develop microbial inoculants that boost plant immunity in agricultural settings.

CHAPTER 3: MATERIALS AND METHOD

3.1 Plant Material

Mature-green papaya fruits (*Carica. papaya* L., cv. Eksotika II) of colour index 2 (green with traces of yellow) (Figure 3.1) were obtained from a local exporter, Exotic Star (M) Sdn Bhd, Sg. Chua, Kajang, Selangor, Malaysia, on the same day of harvesting. The cultivar ‘Eksotika II’ fruits of uniform size (400–500 g), shape and maturity, free from any indication of mechanical injury, insect or pathogenic infection, were selected for the experiment.

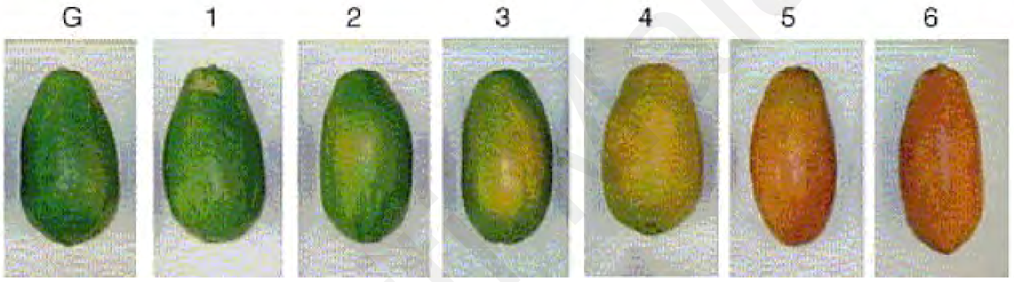

Figure 3.1: Color index of *Carica papaya* L. (Source: Santamaría Basulto *et al.*, 2009)

3.2 Inoculum Preparation

Papaya fruits (‘Eksotika II’) were placed in wet chambers (plastic trays with wet paper towels inside, sealed with a plastic sheet) and kept at room temperature (25 ± °C) for 24 h. Papaya fruits showing typical anthracnose symptoms or lesions of rot were examined. Small pieces of tissue were excised (5 mm in diameter) from the border of an actively growing lesion and surface sterilized in 1.0% sodium hypochlorite (NaOCl) for 2–3 min, followed by washing in two changes of sterile distilled water. The plant tissues were dried on a sterilized filter paper, plated on Potato Dextrose Agar (PDA) (90mm, Difco Brand, USA) and incubated at 25 ± 2 °C for 8 days. Once mycelial growth was observed, the colonies were re-isolated on fresh PDA plates to obtain pure cultures (Figure 3.2). *C. gloeosporioides* was isolated and morphologically identified under an optical microscope

(Weir *et al.*, 2012). Cultures identified as *C. gloeosporioides* were maintained on PDA agar and sub-cultured weekly. Figures 3.3 (a) and 3.3 (b) show the top and bottom view growth of a single *C. gleosporioides* on day 10. Ten millilitres (10 ml) of sterilised water were added to each petri dish. The conidia suspension was filtered through a double layer of gauze, and the conidia concentration was estimated using a Neubauer plate and adjusted to 10^6 spores/ml (Bautista-Banõs *et al.*, 2003).

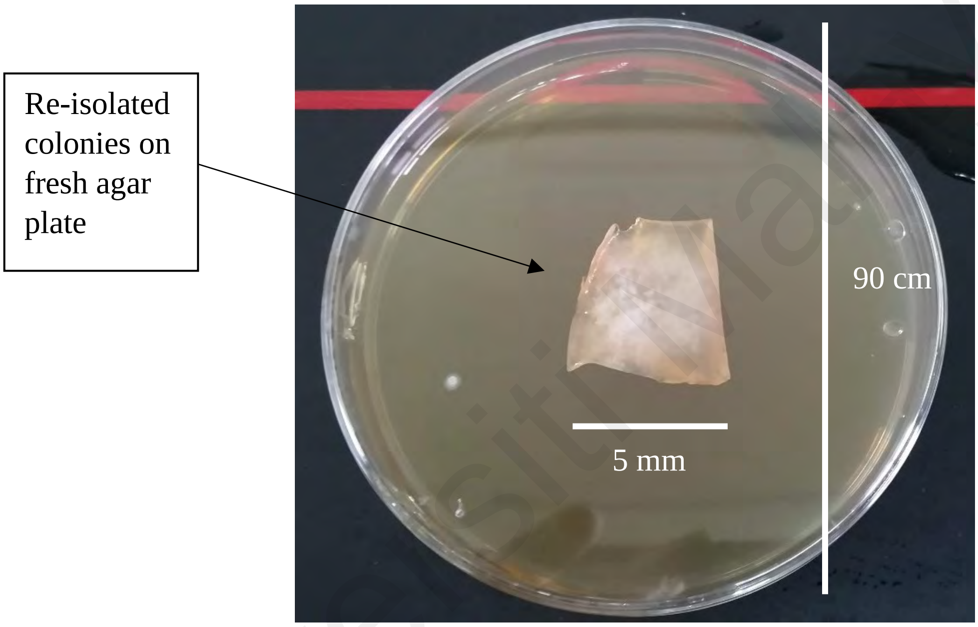

Figure 3.2: Re-isolated *C. gloeosporioides* colonies on fresh PDA plates to obtain pure cultures.

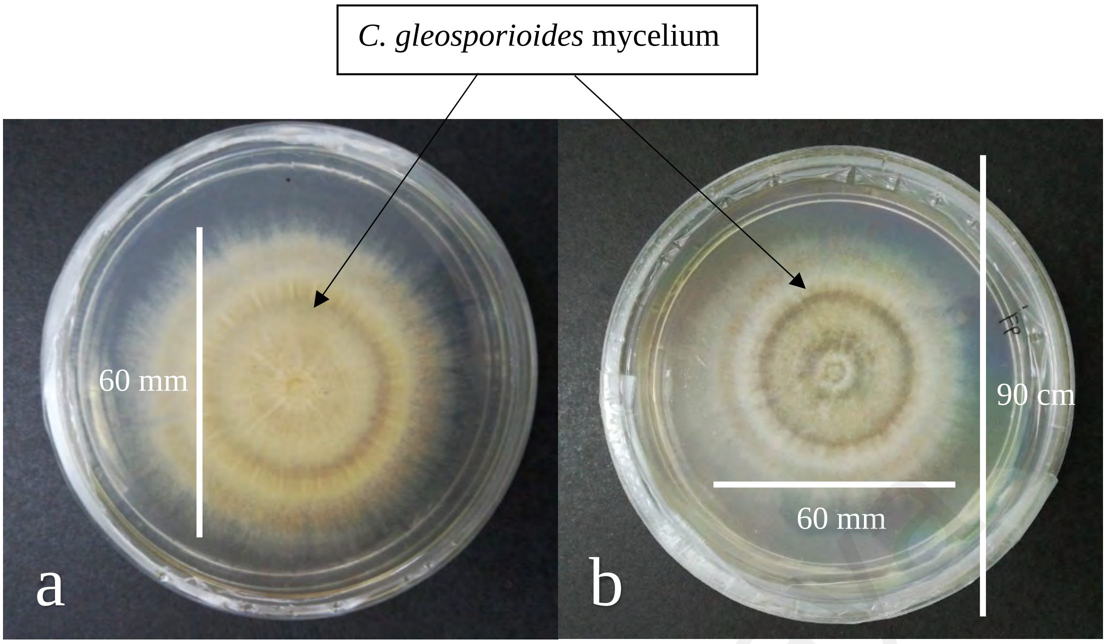

Figure 3.3: *Colletotrichum gloeosporioides*. Cultures on PDA, 10 d growth from single conidia, viewed from top (a) and bottom (b).

3.3 HOT WATER TREATMENT

Fruits were randomly selected, cleaned and dipped in a 0.3% hypochlorite solution for 10 min. The fruits were rinsed under running tap water and left to air dry at 25 °C. Preliminary investigations revealed that treatment with 54 ºC for 5 min was the most effective in controlling diseases and delaying the ripening of papaya fruit. The selected whole papaya fruit was dipped into hot water (54 °C) for 5 min (Memmert, L4930720, 160(W) x 90(H) x 210(L) mm, Germany) (Figure 3.4), taken out, and then air-dried at 25 °C. The control group was dipped into water at room temperature (25 °C) for 5 min. After air-drying, the control group and a portion of the HWT group were inoculated with the conidial suspension (HWT-CG). The other portion of the HWT group was inoculated with distilled water (HWT-DW). Fruits that were not treated with hot water were treated as controls. Nine biological replicates (n=9) were used for each HWT treatment. After treatment, papaya samples were placed onto rectangular polypropylene basins, sealed with stainless steel wire (0-10 squares per inch) and stored at 25 °C for 8 days. All analyses were carried out in triplicates.

The following terms are used to describe papaya samples in this study, as shown in Table 3.1.

Table 3.1: Papaya samples subjected to different hot water treatments (HWT).

Treatment	**Papaya samples**	
Control	Control	Dipped in distilled water at 25°C for 5 minutes
HWT	HWT-CG (Inoculated with *C. gloeosporioides)*	Dipped in hot water at 54°C for 5 minutes
	HWT-DW (Inoculated with distilled water)	Dipped in hot water at 54°C for 5 minutes

Figure 3.4: Hot water treatment of papaya in a water bath at 54 °C for 5 min.

3.4 Physicochemical Analysis

3.4.1 Disease Incidence and Lesion Diameter

Disease incidence (DI) was recorded based on the anthracnose symptoms on fruit surfaces. The symptoms are small, well-defined dried pink spots on the surface of ripening fruit, rounded, sunken (3–5mm deep) and brown to black in colour (Torres-Calzada *et al.,* 2013). The effect of hot water treatment on disease incidence for fruits from each treatment was evaluated at 0,1,2,4,6 and 8 days during storage at room temperature. Disease incidence was expressed as the number of fruits showing anthracnose out of the total number of fruits in each treatment (Sivakumar *et al.,* 2002).

The lesion diameter (mm) of fruits was measured after 0,1,2,4,6 and 8 days. Lesion diameter (mm) per fruit was expressed as the average of the three values from each fruit.

3.4.2 Fruit Peel Colour

Fruit peel colour was rated on a scale of 1 to 6 as described by Miller and McDonald (1999), where 1 = entirely green, 2 = < 25% yellow, 3 = 25–50% yellow, 4 = 50–75% yellow, 5 = ≥75% yellow, and 6 = orange blush/yellow. The fruit colouring index was calculated daily as colouring index=∑ (colouring grade × number of fruit)/ total number of fruits.

3.4.3 Extraction of Papaya Juice

Each fruit was peeled, and the seeds were discarded. Papaya pulp was macerated using a domestic juice extractor (Philips Juice Extractor HR 2820, Holland) and then centrifuged (Beckman J2-MI Centrifuge, California) at 12000 rpm for 10 min at 4 °C. The supernatant was removed and stored in sterile glass bottles before deployment into the experiment.

3.4.4 pH

The pH of the juice sample was determined using a pH meter by inserting the probe into the juice sample (Hanna Microprocessor pH 211, Italy) at 25 ± 1 °C. The pH meter was calibrated before analysis using pH 7 and pH 4 buffer standards.

3.4.5 Total Soluble Solids (TSS)

TSS of the juice sample was determined using a digital refractometer (Atago, Japan) at 25 ± 1 °C. Before measuring the samples, the refractometer was calibrated with sterile distilled water (SDW) (0 °Brix). Results were expressed in standard °Brix unit.

3.4.6 Weight Loss

For weight loss determination, fruits were weighed using a digital balance at the beginning of the experiment and the end of each storage period (0, 1, 2, 4, 6, and 8). The results were expressed as percentage loss of initial weight.

3.4.7 Titratable Acidity (TA)

3.4.7.1 Preparation of reagent

0.1 N Sodium hydroxide

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.

3.4.7.2 Determination of TA

Five ml (5 ml) of juice sample was diluted with distilled water to a final volume of 100 ml. The diluted sample was titrated against 0.1 N sodium hydroxide to a faint pink endpoint (colour should persist for at least 15s) using phenolphthalein as an indicator. The volume of sodium hydroxide used to titrate the solution was recorded and then converted to grams of citric acid per 100 grams of fruit according to the method by Sadler and Murphy (2010). Titratable acidity (TA) was then calculated using the following formula:

Titratable acidity (%TA) = (V_1 x 0.1 N NaOH x Eq. Wt. x 100)/(V_2 x 1000)

where

V_1 = volume of titrant (ml)

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

V_2 = weight of sample (gram)

3.4.8 Ascorbic Acid Content

Preparation of reagents

3% metaphosphoric acid

30 g of metaphosphoric acid sticks (HPO3) (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 (NaHCO3)(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 HPO3 solution. Volume of the solution was made up to 100 ml with 3% HPO3 solution. This stock solution (5 ml) was further diluted with 5 ml of 3% HPO3 solution.

5.2.5.2 Determination of ascorbic acid content

The ascorbic acid content in samples was determined based on the 2,6-dichlorophenol-indophenol (DCPIP) visual titration method (Ranganna & Ranganna, 1977). Five grams (5g) of pulp sample was ground with liquid nitrogen until it became powdered and then was added to 100 ml of 3% metaphosphoric acid. The extract was filtered with fluted Whatman No.1 filter paper, and then 5 ml of the filtrate was titrated with a dye solution (2,6-dichloroindophenol-indophenol and sodium carbonate) to a pink endpoint (colour should persist for at least 15 s). The results obtained were expressed as milligrams of ascorbic acid per 100 gram sample using the following equation:

Ascorbic acid content (mg/100 g) = (V_1 x dye factor x V_2 x 100)/(S_1 x S_2)

Where

V_1 = titre (ml)

V_2 = volume made up (ml)

S_1 = aliquot of extract taken for estimation (ml)

S_2 = volume of sample taken for estimation (ml)

Dye factor = 0.5 / titre

3.4.9 Total Carotenoid Content

Carotenoid was extracted using the method by Lee *et al.* (2001) with slight modifications. Five grams (5 g) of pulp sample was ground in liquid nitrogen until powdered and homogenized (Omni Mixer Homogenizer, CT, USA) for 30 seconds (speed 4) with 20 ml of extracting solvent. The extracting solvent consists of 10 ml of hexane (Baker Analysed), 5 ml of acetone (Systerm) and 5 ml of ethanol (Merck). The mixture was centrifuged (Beckman J2-MI Centrifuge, California) at 6500 rpm for 10 min at 5 °C. The top layer of hexane containing carotenoids (yellow) was collected and adjusted to 10 ml with hexane.

Total carotenoid content was determined according to Scott (2001). The absorbance of extracted hexane was measured at 450 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (hexane). The total carotenoid content using β-carotene as a reference was calculated according to the following formula:

Total carotenoid content = $(A \times V_1 \times C^{1\%}) / A^{1\%}$

Where

A = absorbance reading of the diluted sample

V_1 = dilution factor

$A^{1\%}$ = absorbance of a 1% solution (the extinction coefficient for β-carotene: 2592 AU)

$C^{1\%}$ = concentration of a 1% solution (10 mg/ml)

3.5 Antioxidant Activity

3.5.1 Sample Preparation

The antioxidant extraction was performed based on Xu *et al.* (2008) with slight modifications. The pulp sample was ground in liquid nitrogen until powdered and was transferred to 80 % methanol in a Falcon tube with a ratio of 1:1 to purify the sample. The tubes containing the mixture were placed on the rotary shaker (Orbital Shaker S01, Stuart) at speed 8 for 30 min at room temperature and then centrifuged at 6500 rpm for 15 min at 4 ℃ using a refrigerated centrifuge (Beckman J2-MI Centrifuge, California). The supernatant obtained was collected and kept in 2.0 ml Eppendorf tubes at -20 °C. The samples were used for subsequent antioxidant analysis.

3.5.2 Total Polyphenol Content (TPC)

3.5.2.1 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 3.2 and the final volume was made up to 2 ml.

Table 3.2: Gallic acid standard preparation for TPC.

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

3.5.2.2 Determination of TPC

The total polyphenol content of samples was determined using the Folin-Ciocalteu assay (Singleton & Rossi, 1965) modified to a microscale (Bae & Suh, 2007). 10 µl of sample extract or gallic acid standard solution was added to 790 µl of distilled water and 50 µl Folin-Ciocalteu reagent (Sigma-Aldrich) in a 2.0 ml microcentrifuge tube, and the solution was mixed. After exactly 1 min, 0.15 ml of 20 % sodium carbonate was added, and the solution was mixed by inverting the tubes. The mixture was allowed to stand at room temperature (25 ± 1 °C) for 120 min (in dark). The absorbance reading of the samples and gallic acid standard were measured at 750 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (replace sample extract with distilled water). A standard curve of gallic acid ($y = 0.0056\,x$, $r^2 = 0.9955$) was prepared ranging from 0 – 140 mg/100 ml, and the results were expressed as milligrams of gallic acid equivalent (GAE) per 100 ml of sample extract.

3.5.3 Total Antioxidant Capacity (TAC)

3.5.3.1 Preparation of reagents

0.6 M sulfuric acid

33.33 ml of concentrated (18 N) sulphuric acid (Systerm) 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 3.3 and the final volume was 2 ml.

Table 3.3: Ascorbic acid standard preparation for TAC.

Concentration (µg/ml)	0	5	25	50	100	200	400	800
Volume of 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

3.5.3.2 Determination of TAC

The antioxidant capacity in pulp sample extracts was determined based on the phosphomolybdenum method described by (Prieto *et al.*, 1999). 100 µl of the sample (5 µl of the extract was diluted with 95 µL 80 % methanol) or ascorbic acid standard solution was added to 1 ml of reagent solution in a 1.5 ml microcentrifuge tube. The reagent solution consists of equal volumes of 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The tubes were incubated in a water bath (Memmert, Germany) at 95 °C for 90 min (in the dark). After incubation, the tubes were cooled to room temperature (25 ± 1 °C), and absorbance was measured at 695 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (distilled water). A standard curve of ascorbic acid ($y = 0.0018x$, $r^2 = 0.9981$) was prepared ranging from 0 to 800 µg/ml, and the results were reported as micrograms of ascorbic acid equivalent (AAE) per gram of sample extract.

3.5.4 Total Flavonoid Content (TFC)

3.5.4.1 Preparation of reagents

5% sodium nitrite

2.5 g of anhydrous sodium carbonate (Systerm) was dissolved in 50 ml of SDW.

10% aluminium chloride

5 g of anhydrous aluminium chloride (Fisher Scientific) was dissolved in 50 ml of SDW.

1N sodium hydroxide

2 g of sodium hydroxide (Merck) was dissolved in 50 ml of SDW.

95% ethanol

95 ml of ethanol (Merck) was added into 5 ml of SDW.

Catechin standard curve

Stock solution was prepared by dissolving 10 mg of (+)-catechin (Sigma-Aldrich) in 10 ml of 95% ethanol. The different concentrations of catechin standards were prepared according to Table 3.4 and the final volume was 1.5 ml.

Table 3.4: Catechin standard preparation for TFC.

Concentration (µg/ml)	0	5	10	20	40	60	80	100
Volume of stock solution (ml)	0	0.08	0.20	0.30	0.60	0.90	1.20	1.50
Volume of SDW (ml)	1.5	1.4	1.3	1.2	0.9	0.6	0.3	0

3.5.4.2 Determination of TFC

Total flavonoid content in sample extracts was determined based on the aluminium chloride colourimetric method described by Sakanaka *et al.* (2005). 0.25 ml of pulp sample extracts was added to 1.25 ml of distilled water and 75 µl of a 5 % sodium nitrite solution in a test tube, and mixed. After incubation for 5 min at room temperature (25 ± 1 °C), 150 µl of 10 % aluminium chloride solution was added to the mixture. The mixture was allowed to stand for another 5 min. Then, 0.5 ml of 1 N sodium hydroxide was added and the mixture was made to 2.5 ml with distilled water. The mixture was vortexed thoroughly at speed 4. The absorbance of the light-yellow colour developed was determined at 510 nm using a spectrophotometer against a blank (the juice sample was replaced with distilled water). A standard curve of catechin ($y = 0.0135x$, $r^2 = 0.9943$) was prepared ranging from 0 to 100 mg/100ml. Results were reported as catechin equivalent (CE) milligrams per 100 ml juice sample.

3.5.5 1,1-di-phenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

3.5.5.1 Preparation of reagents

80% methanol

80 ml of methanol (Systerm) 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 y 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 3.5 and the final volume was 2 ml.

Table 3.5: 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 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

3.5.5.2 Determination of DPPH assay

This assay is based on measuring the scavenging ability of antioxidants towards the stable radical DPPH, as described by Oyaizu (1986) and Bae and Suh (2007). Juice extract 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 ºC for 30 min (in the dark). Absorbance was measured at 517 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (80% methanol) and a control (replace juice extract with 80% methanol). A standard curve of ascorbic acid ($y=10.145x$, $r^2=0.9907$) was prepared ranging from 0 to 10 µg/ml, and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

The radical scavenging activity was calculated accordingly:

% DPPH inhibition = (Acontrol - Asample / Acontrol) x 100

where

Acontrol is the absorbance reading of control

3.6 Metabolomic Analysis

3.6.1 Papaya Sample Extraction

A plant pulp sample was used for this analysis. Homogenization of papaya pulp was performed with mortar and pestle in liquid N_2, after which 150 ± 5 mg of pooled homogenized sample was weighed in an Eppendorf tube. Each extraction procedure was performed in triplicates on the same pool of plant pulp. The extraction solvent combinations, comprising 300 µl of liquid for each extraction, were spiked in advance with ribitol (internal standard, GC–MS) obtaining a 5 µg/ml concentration. Extraction procedures A–C are based on liquid extraction with MeOH/H_2O:

(A)

The sample was extracted with 300 µl cold MeOH/H_2O 80/20 (v/v) in a Thermomixer (Eppendorf AG, Hamburg, Germany) for 15 min (1250 rpm, 4 °C) (R. t'Kindt *et al.*, 2008).

(B)

240 µl of cold MeOH was added to the sample in the Eppendorf tube after which extraction was carried out in a Thermomixer (15′, 1250 rpm, 4 °C). 60 µl of H_2O was added subsequently and samples were remixed (5′, 1250 rpm, 4 °C).

(C)

It is identical to procedure B, except the Thermomixer temperature was set at 70 °C during extraction with MeOH. After 1 min of incubation, the Eppendorf tubes were opened briefly to relieve built-up gas pressure. The vials remained closed for the rest of the incubation. Immediately after the incubation, all samples were cooled down to 4 °C. As such, all gaseous solvent was liquefied again through condensation. Extraction procedures D–F are based on liquid extraction with a one-phase $CHCl_3$/MeOH/H_2O mixture:

(D)

The sample was extracted with 300 µl cold $CHCl_3$/MeOH/H_2O 20/60/20 (v/v) in a Thermomixer (15′, 1250 rpm, 4 °C).

(E)

60 µl of cold $CHCl_3$ was added to the sample in an Eppendorf tube, after which the extraction was carried out in a Thermomixer (5′, 1250 rpm, 4 °C). 240 µl of MeOH/H_2O 60/20 (v/v) was added subsequently and samples were placed in the Thermomixer again (15′, 1250 rpm, 4 °C) (J. Gullberg *et al.*, 2004).

(F)

180 µl of cold MeOH was added to the sample, after which the samples were placed in a Thermomixer for 15 min (1250 rpm, 70 °C, with relief of build-up pressure; see above). 60 µl of $CHCl_3$ was then added and samples were mixed again (5′, 1250 rpm, 4 °C). Finally, 60 µl of H_2O was added to the Eppendorf tubes. This method was adopted from the Golm Metabolome Database (http://csbdb.mpimp golm.mpg.de/csbdb/gmd/analytic/gmd_prot.html).

All extracts were finally sonicated for 5 min (Bransonic Ultrasonic Cleaner 1210, Danbury, CT, USA) and centrifuged (Sigma 3-18K, Sartorius AG, Göttingen, Germany) for 15 min (4 °C, 15,000 rpm). The supernatant (300 µl) was isolated for subsequent LC-MS analysis. For GC–MS analysis, a 50 µl aliquot of the metabolite sample supernatant was further derivatized by methoxyamination, using a 20 mg/ml solution of methoxyamine hydrochloride in pyridine, and subsequent trimethylsilylation with N-methyl-N-(trimethylsilyl)-trifluoroacetamide (O. Fiehn *et al.*, 2000; U. Roessner *et al.*, 2000). A mixture of C12, C15, C19, C22, C28, C32, and C36 n-alkanes was used to determine retention time indices (C. Wagner *et al.*, 2003).

For the quality control (QC) samples, an aliquot (40 µl) of all prepared sample extracts, regardless of extraction procedure, was mixed in an Eppendorf tube in cold conditions. Due to visible contamination, extract D3 was excluded from the QC preparation and the subsequent LC–MS and GC–MS analysis. The quality control pool was subsequently divided over several vials and analysed regularly throughout the whole analysis batch, in both GC–MS and LC–MS. The quality control approach for GC–MS and LC–MS metabolomics has been adopted by Sangster *et al*. (T. Sangster *et al.*, 2006). All extracts and QCs were analysed in parallel on both the LC–MS and GC–MS platforms in a 1-day time window.

3.6.2 Primary Metabolite Analysis

Samples were analysed using an Agilent 7683B Series Injector (Agilent, Santa Clara, CA) coupled to an Agilent HP6890 Series gas chromatograph system and a 5973 Mass Selective Detector (Agilent, Santa Clara, CA), i.e., a quadrupole type GC–MS system. A Varian factor FOUR capillary column VF-5 ms (5% phenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID, df = 0.25 µm) connected to a 10 m EZ-guard column was used. A constant column flow of 1 ml/min helium was applied. The injector was kept at 230 °C. Samples were split less injected (1 µl) during 1.5 min using a total flow of 39 ml/min which was reduced to 24 ml/min after 2 min. The programmed temperature separation started at 70 °C for 5 minutes and then ramped by 5 °C/minute to 325 °C within 51 minutes. After 1 minute at 325 °C, the oven was cooled to the initial temperature of 70 °C within 5.10 minutes. A temperature equilibration phase of 5 min was allowed before the next injection. The transfer line and EI source temperatures were 250 and 200 °C, respectively. EI spectra were acquired between 60 and 600 Da. The electron multiplier voltage was set to 1700 V.

3.6.3 Secondary Metabolite Analysis

An Agilent 1100 series HPLC system coupled to Sciex 3200 hybrid quadrupole trap triple quad tandem mass spectrometer (UHPLC-QTRAP MSMS) (Santa Clara, California, USA) was used for the liquid chromatography part. The LC column was a Phenomenex Synergy RP C18, 100A, 100mm x 3uM x 2.0mm (Torrance, California, USA). The LC mobile phase consisted of (A) water with 0.1% (v/v) formic acid and (B) acetonitrile with 0.1% (v/v) formic acid. Both eluents (A) and (B) were filtered through a 0.45 µm membrane filter before use. Gradient elution chromatography was always performed, starting with 95% solvent A. Within an 8 min interval, % B composition was increased to 70%, followed by a %B increase up to 95% within 2 min. For the last 4 min, the composition of solvent B was reduced to 5% while the composition of solvent A was increased to 95%.

MS experiments were performed using a Q-TRAP Linear Ion Trap Quadrupole mass spectrometer (Framingham, MA, USA). The instrument was operated in negative ion electrospray mode. The detection mode used was a full scan enhanced mass spectra mode (EMS) Information dependent acquisition (IDA) triggered enhanced product ion (EPI). Agilent 1100 Autosampler (Santa Clara, California, USA) with an injection volume 0f 20 µl and Agilent 1100 LC Quaternary Pump (Santa Clara, California, USA) with a maximum flow ramp of 100.0 ml/min^2 was used. Electrospray Ionisation (ESI) capillary voltage was optimized to 3000 V and cone voltage was set to 30 V. Full scan spectra were acquired over an *m*/*z* 100–1500 range at a scan rate of 1000 Da/s and an interscan delay of 0.1 s. Identification via MSMS spectra searched with the internal Sciex natural product database.

3.7 Tandem Mass Tag (Tmt) Labelling and Peptide Fractionation

3.7.1 Total Protein Extraction

3.7.1.1 Preparation of reagents

100 mM ammonium bicarbonate at pH 8.0

0.7906 grams of ammonium bicarbonate (Sigma) was dissolved in 100 ml of SDW. The pH was adjusted to pH 8.0 by adding small amounts of hydrochloric acid (HCl) to lower the pH or sodium hydroxide (NaOH) to raise the pH.

6M Urea

36.036 grams of urea (Sigma) was dissolved in 100 ml of SDW.

0.2 % Sodium dodecyl sulfate

0.2 grams of sodium dodecyl sulphate (Sigma) was dissolved in 100 ml of SDW.

3.7.1.2 Protein extraction

The total protein was extracted from papaya pulp from control and HWT groups at 0 d and 8 d of storage. The papaya pulp was finely ground into powder in liquid nitrogen, then lysed with ice-cold buffer (100 mM NH_4HCO_3 pH 8.0, 6 M Urea, and 0.2 % sodium dodecyl sulfate). After 5 min, the lysate was centrifuged at 12000 *g* and 4 ± 0.5 °C for 15 min. The supernatant was transferred into a clean tube. Then 4 times volume of precooled acetone was added and shaken to allow it to mix well with the samples and incubated at -20 ± 0.5 °C for 2 h. The precipitate was collected once the samples were centrifuged at 12000 g at 4 ± 0.5 °C for 15 min. After washing twice with the cold acetone, the extracted proteins were collected for further analysis.

3.7.2 Sample Digestion

3.7.2.1 Preparation of reagents

5 % Sodium dodecyl sulfate

5.0 grams of sodium dodecyl sulphate (Sigma) was dissolved in 100 ml of SDW.

100 mM triethylammonium bicarbonate at pH 7.55

1.0119 grams of triethylammonium bicarbonate (Sigma) was dissolved in 100 ml of SDW. The pH was adjusted to pH 7.55 by adding small amounts of hydrochloric acid (HCl) to lower the pH or sodium hydroxide (NaOH) to raise the pH.

10 mM of dithiothreitol

0.1543 grams of dithiothreitol (Sigma) was dissolved in 100 ml of SDW.

25 mM of iodoacetamide

0.4624 grams of iodoacetamide (Sigma) was dissolved in 100 ml of SDW.

2% phosphoric acid

1.40 ml of phosphoric acid (Sigma) was dissolved in 100 ml of SDW.

Trypsin solution

10 µg of trypsin was dissolved in 100 µL of the TEAB solution.

0.2 % aqueous formic acid

0.186 ml of formic acid (Sigma) was dissolved in 100 ml of SDW.

50 % aqueous acetonitrile

50 ml of acetonitrile (Sigma) was dissolved in 100 ml of SDW.

200 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 8.8

4.766 grams of HEPES (Sigma) was dissolved in 100 ml of SDW. The pH was adjusted to pH 8.8 by adding small amounts of hydrochloric acid (HCl) to lower the pH or sodium hydroxide (NaOH) to raise the pH.

3.7.2.1 Digestion of sample protein

Half of the sample pellet was suspended in S-Trap lysis buffer (5 % SDS, 100 mM triethylammonium bicarbonate, TEAB, pH 7.55). To enable dissolution of the protein, pellets were sonicated in a water bath followed by probe sonication, finally boiled briefly at 90 °C, cooled to room temperature and stored at 4 °C overnight. The samples' volumes were equalized using S-Trap lysis buffer before further processing. Cysteine disulphide bonds were reduced with 10 mM DTT at 56 °C for 30 min, then alkylated with 25 mM IAA for 30 min in the dark at room temperature. The pH of the samples was adjusted using 12% aqueous phosphoric acid, added at 1:10 for a final concentration of ~1.2% phosphoric acid and diluted using S-Trap binding buffer (90% aqueous methanol containing a final concentration of 100 mM TEAB, pH 7.55). S-Trap binding buffer was added to the acidified lysis buffer and the sample mixture was transferred to a labelled S-Trap column and centrifuged at 4,000 g, after which the flow through was discarded. The column was washed twice using S-Trap binding buffer and proteins retained on the column were digested in the presence of 125 µL trypsin solution (~10 ug total trypsin in 50 mM triethylammonium bicarbonate) for 3 hours at 47 °C. After digestion, peptides were eluted off the column after adding 50 mM triethylammonium bicarbonate and centrifugation. The remaining peptides were eluted from the column using sequential centrifugation with the addition of 0.2 % aqueous formic acid followed by 50 % aqueous ACN containing 0.2 % formic acid. Peptides were dried by vacuum centrifugation and then reconstituted in 100 uL of 200 mM HEPES (pH 8.8). The peptide concentration was determined using the Pierce quantitative colourimetric peptide assay (Thermo Scientific, USA).

3.7.3 Sample Labelling

3.7.3.1 Preparation of reagents

5 % hydroxylamine

5 grams of hydroxylamine (Sigma) was dissolved in 100 ml of SDW.

0.1 % formic acid

0.093 ml of formic acid (Sigma) was dissolved in 100 ml of SDW.

2 % aqueous acetonitrile

2 ml of acetonitrile (Sigma) was dissolved in 100 ml of SDW.

3.7.3.2 Labelling of samples

Equal total peptide quantities from each sample were used for subsequent sample processing. The samples were labelled in a 10-plex TMT label batch. Each sample's TMT reagent (Thermo Scientific, USA) labelling was performed as per APAF’s SOP MS-096. To each TMT label vial, 41 µL of anhydrous acetonitrile was added followed by vortexing and brief centrifugation. Aliquots (100µL) of individual peptide samples were labelled with 41 µL of the TMT Label Reagent. Labelling was performed at room temperature for 1 h with occasional vortexing. To quench the excess TMT label in the sample, 8 µL of 5 % hydroxylamine was added to each sample and vortexed briefly, then incubated at room temperature for 15 min. Before pooling the samples, a ‘label check’ experiment was performed to ensure equal amounts of total peptide were pooled from all samples. The label check was performed by mixing small, equal amounts of each individually labelled TMT sample, and the mixed sample was vacuum dried using a vacuum centrifuge.

Samples were reconstituted in 0.1% formic acid in 2% acetonitrile and analysed by LC-MS/MS (Q-Exactive, Thermo Fisher, USA). A normalization factor was obtained from the label check experiment and the original TMT-labelled peptide samples were then pooled at an equal, 1:1 ratio across all individual samples in the respective set.

3.7.4 TMT Labelled Peptide Fractionation

3.7.4.1 Preparation of reagents

0.1% trifluoroacetic acid

0.067 ml of trifluoroacetic acid (Sigma) was dissolved in 100 ml of SDW.

0.1 % formic acid

0.093 ml of formic acid (Sigma) was dissolved in 100 ml of SDW.

3.7.4.1 Fractionation of peptides

The combined sample mixture was vacuum dried, resuspended in 0.1% trifluoroacetic acid, and then fractionated by Pierce High pH Reversed-Phase Peptide Fractionation Kit (Thermo Scientific, USA) according to APAF's SOP, MS-079. Three fractions (12.5 %, 17.5 % and 50 % ACN with 0.1% triethylamine) were collected. Each fraction was vacuum centrifuged to complete dryness and then reconstituted in 0.1% formic acid for LC-MS/MS analysis.

3.7.5 Data Analysis And Protein Quantification

The raw data files were processed using Proteome Discoverer (Version 2.1.0.81, Thermo Scientific). The data was searched in a single attempt against the following two databases provided by the client, using the search engine SequestHT. The parameters for peptide identification and quantification were as follows: Enzyme: Trypsin; Maximum missed cleavages: 2; Precursor mass tolerance: 20 ppm; Fragment mass tolerance: 0.02

Da; Dynamic modifications: Oxidation (M), Deamidated (N, Q), Glu->PyroGlu, Gln->PyroGlu, Acetyl (Protein N-Terminus), and TMT6plex (K) and TMT6plex (N-term); Static Modification: Carbamidomethyl (C); FDR and result display filters: Protein, Peptide and PSM FDR<1%, and Master proteins only. The raw quantitative data were used for final statistical analysis.

3.4.3 Bioinformatics Analysis

For further functional analyses, Gene Ontology (GO) and InterPro (IPR) analysis were conducted using the InterPro Scan-5 program against the non-redundant protein database (including Pfam, PRINTS, ProDom, SMART, ProSiteProfiles, PANTHER). Clusters of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were applied to analyze the protein family and pathway, respectively. The enrichment pipeline conducted the enrichment analysis of GO, IPR, and KEGG.

3.8 Statistical Analysis

Data obtained was subjected to statistical analysis using SPSS 22.0 software (SPSS Inc., IBM). This study's data was represented as mean values ± standard error (SE). The significant differences between mean values of juice samples were determined by analysis of variance (one-way ANOVA) using Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$. The relationship between variables was determined using Pearson's correlation test at a significance level of $p < 0.01$ and $p < 0.05$.

CHAPTER 4: RESULTS

4.1 Disease Incidence and Severity

Figure 4.1 shows that the incidence of anthracnose in papaya fruit was affected by the HWT and storage time (*P*<0.05). Incidentally, the HWT protected the fruit. On days 0 and 1, all the fruits were free of symptoms; however, on day 2, the control fruits began to show symptoms. On day 4, almost half of the control fruits began showing symptoms, while HWT-CG began to show symptoms. The HWT-DW remained symptom-free. On day 8, there was a significant difference in the disease incidence of the control and treated papayas. All the control fruits showed disease symptoms while half the HWT-CG samples showed signs of disease. The HWT-DW showed significantly less disease incidence with only a third of the papayas showing disease symptoms.

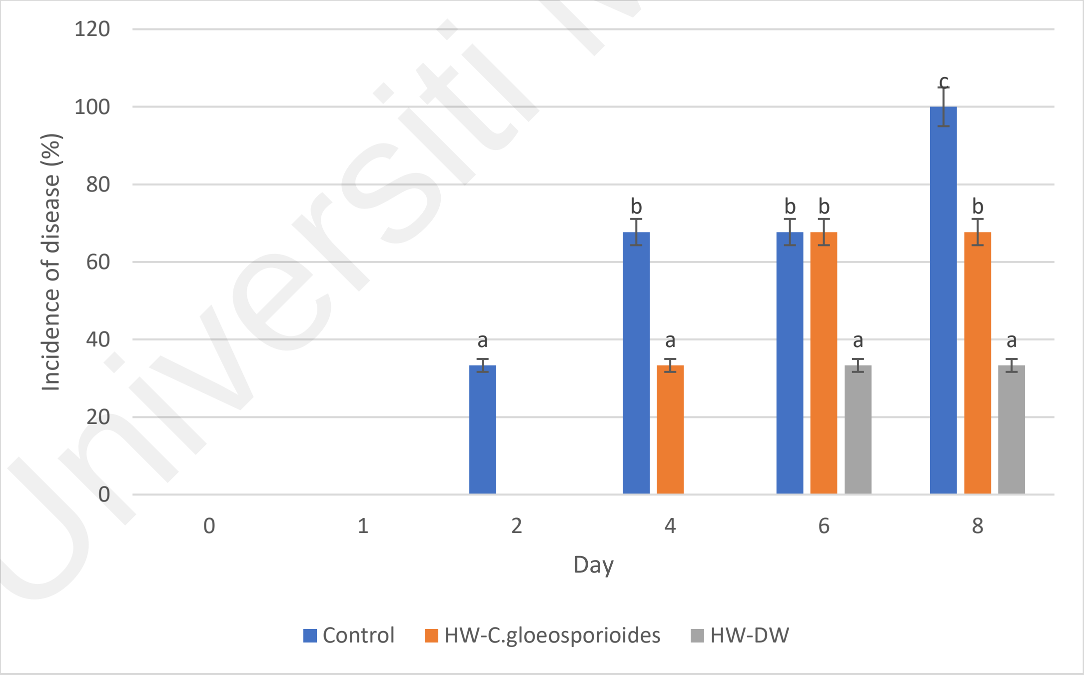

Figure 4.1: Effect of hot water treatment (HWT) on disease incidence.
^{1}Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).
^{2}Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*); HWT-DW (hot water treated inoculated with distilled water)

The severity of anthracnose on the papaya fruit depended on the time of storage and HWT (*P*<0.05). According to Figure 4.2, the disease severity was evaluated by measuring the lesion length (Sivakumar *et al.*, 2002). The disease severity of HWT fruits was significantly reduced compared to control fruits. On the final day of storage, the control fruit (22 mm) showed significantly higher disease severity compared to HWT-CG (9 mm) and HWT-DW (7 mm).

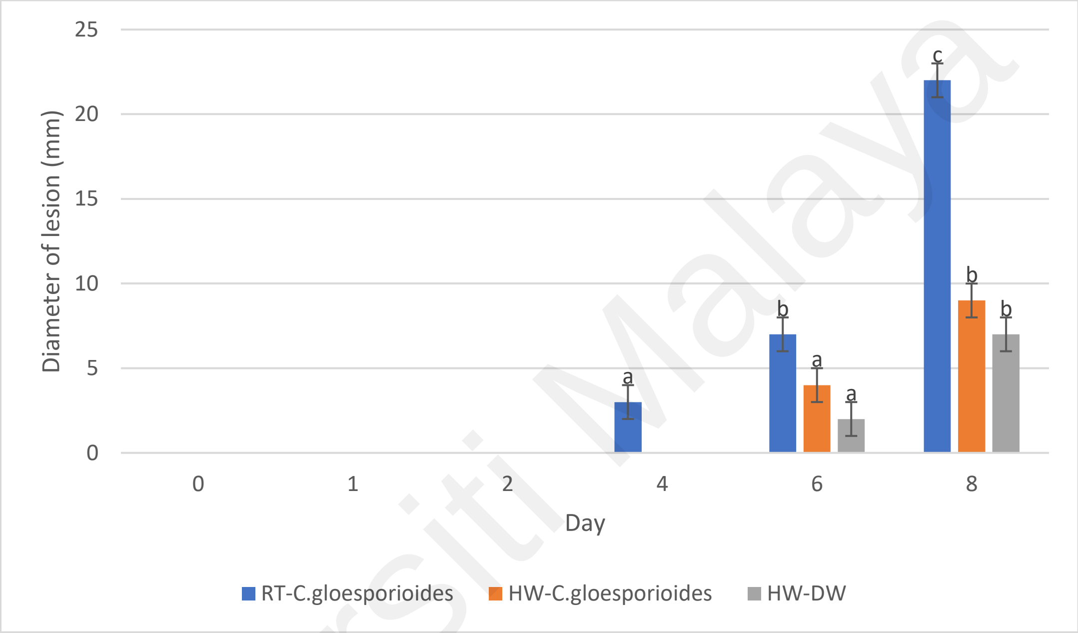

Figure 4.2: Effect of hot water treatment (HWT) on disease severity.

[1] Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

4.2 Fruit Peel Colour

Fruit peel colour was rated on a scale from 1 to 6 as described by Miller and McDonald (1999), where 1 = entirely green, 2 = < 25% yellow, 3 = 25–50% yellow, 4 = 50–75% yellow, 5 = ≥75% yellow, and 6 = orange blush/yellow. Based on Figure 4.3, on the first day all fruits recorded a rating of 1. On day 1, the control samples recorded a colour grade of 2 while the HWT samples scored 2 respectively. From day 4 onwards, all samples gradually ripened with no significant difference. On day 8, all fruits recorded a colour grade of 6.

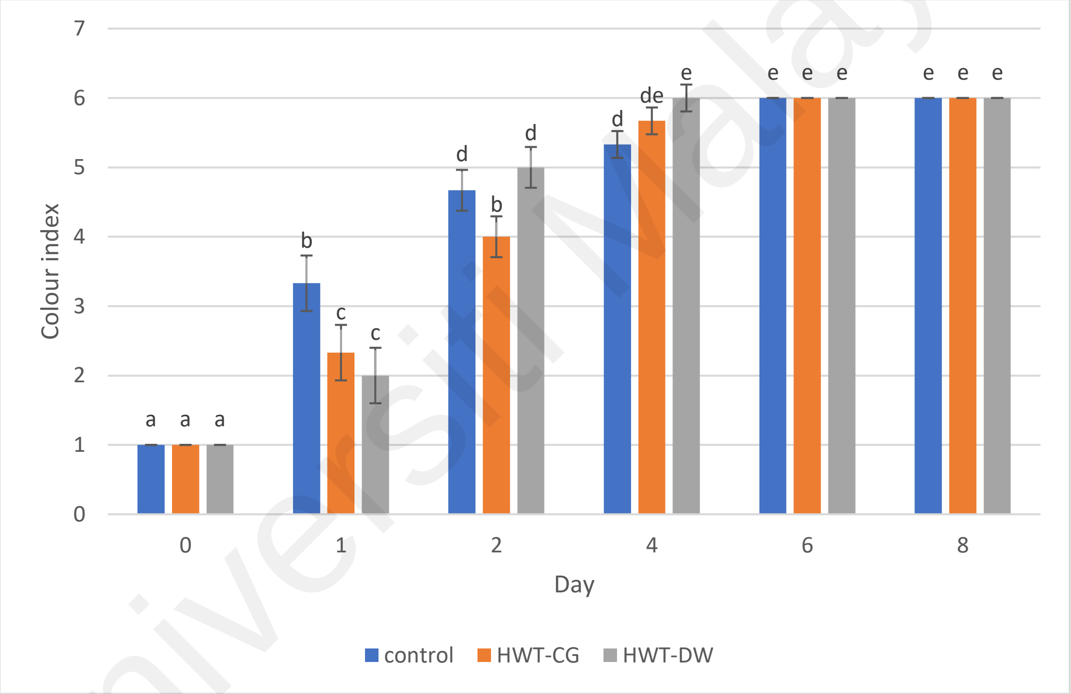

Figure 4.3: Effect of hot water treatment (HWT) on the colour index.

[1] Values followed by different letters within the same column are significantly different (p < 0.05) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

4.3 Physicochemical Analysis

The pH of both HWT samples increased after the second day of storage and remained higher than the control until day 6, as shown in Figure 4.4. On the last day of storage, the pH of HWT-DW decreased slightly below the control samples. The pH value did not significantly change between the control and hot water-treated samples.

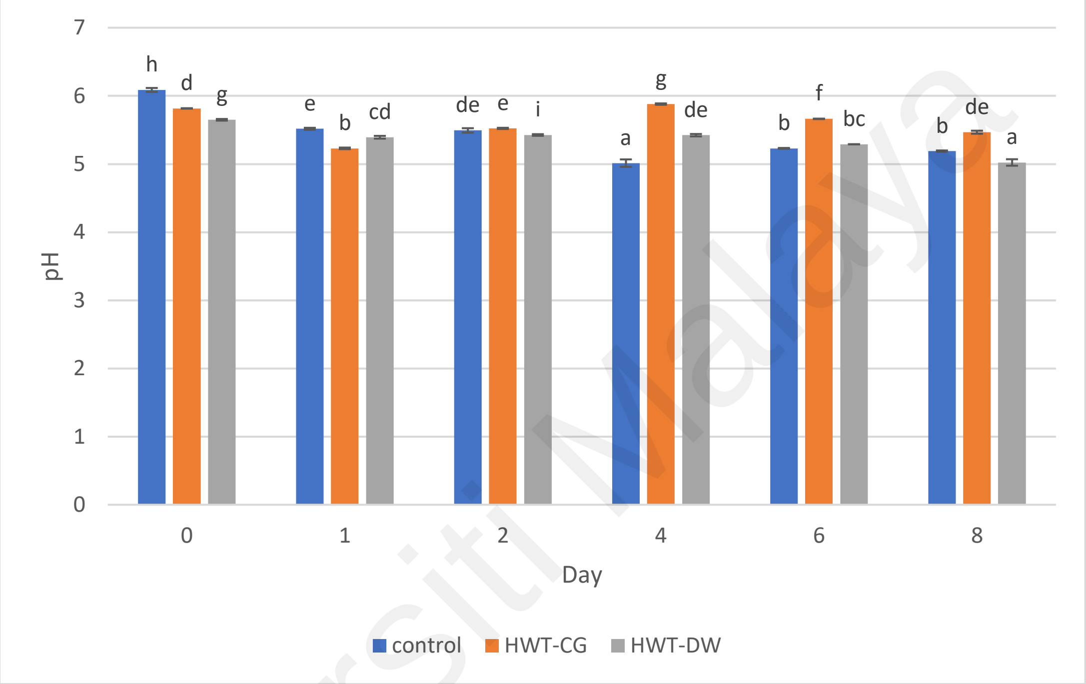

Figure 4.4: Effect of hot water treatment (HWT) on the pH.

[1] Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

Values for TSS (10.2 to 13.2 ºBrix) of samples were within the range of desirable qualities for Eksotika II papaya. According to Figure 4.5, the highest reading was obtained on day 1 for HWT-DW, which was 13.2 ºBrix and the lowest was recorded on day 8 for HWT-DW which was 10.2 ºBrix.

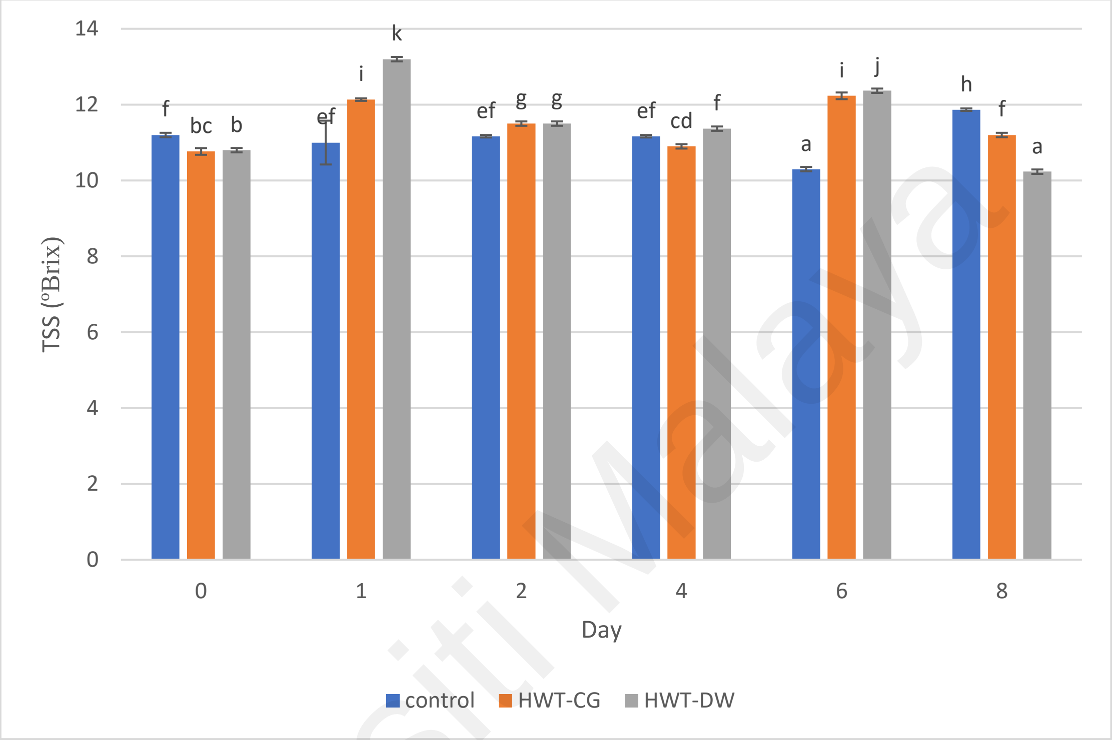

Figure 4.5: Effect of hot water treatment (HWT) on the total soluble solids (TSS) .

[1]Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).

[2]Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

Storage life and quality of papaya fruit could be determined by measuring the weight loss (Figure 4.6). No significant weight loss was measured among the HWT samples compared to control samples.

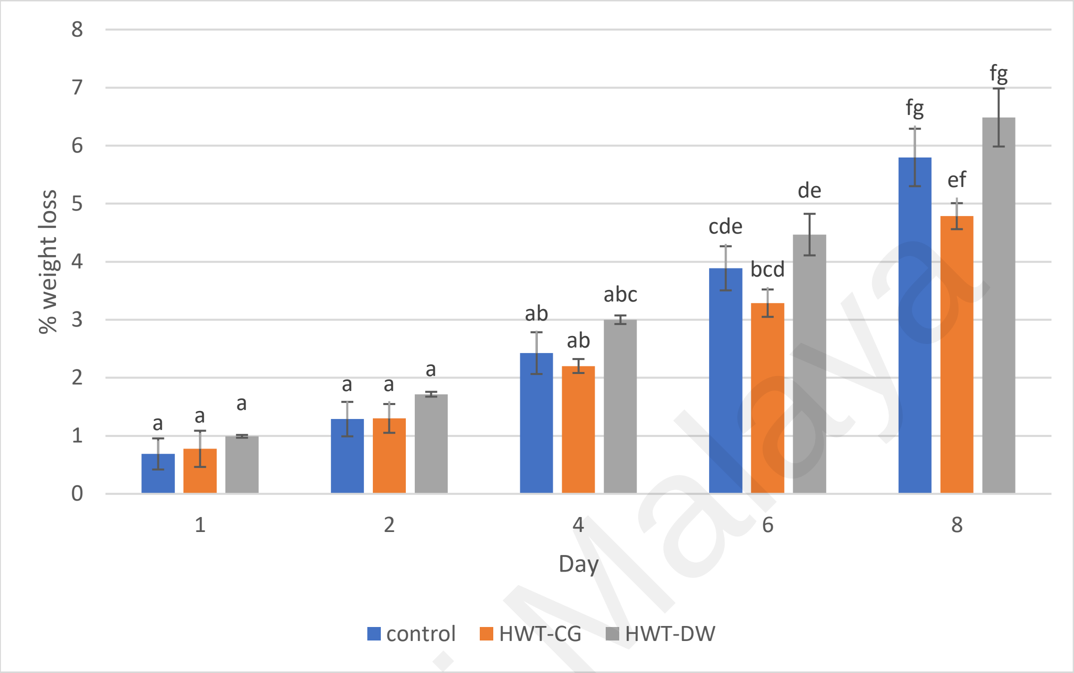

Figure 4.6: Effect of hot water treatment (HWT) on weight loss.
[1]Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).
[2]Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

The TA values (Figure 4.7) showed not significant difference.

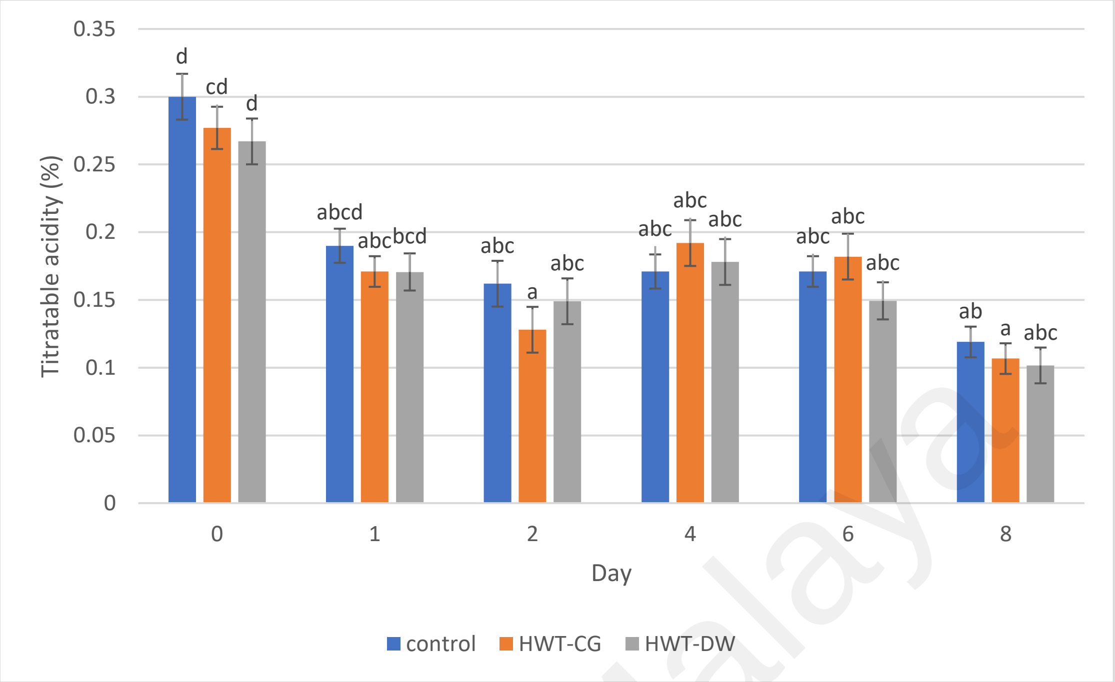

Figure 4.7: Effect of hot water treatment (HWT) on the titratable acidity (TA).

^{1}Values followed by different letters within the same column are significantly different (*p* < 0.05) (n = 9).

^{2}Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

4.4 Total Carotenoid and Ascorbic Acid Content

From Figure 4.8, there was a significant increase in the carotenoid content of HWT-CG after day 1 with a value of 175.74 µg/100 ml. The lowest value of 58.6 µg/100ml was also recorded by HWT-CG on day 1.

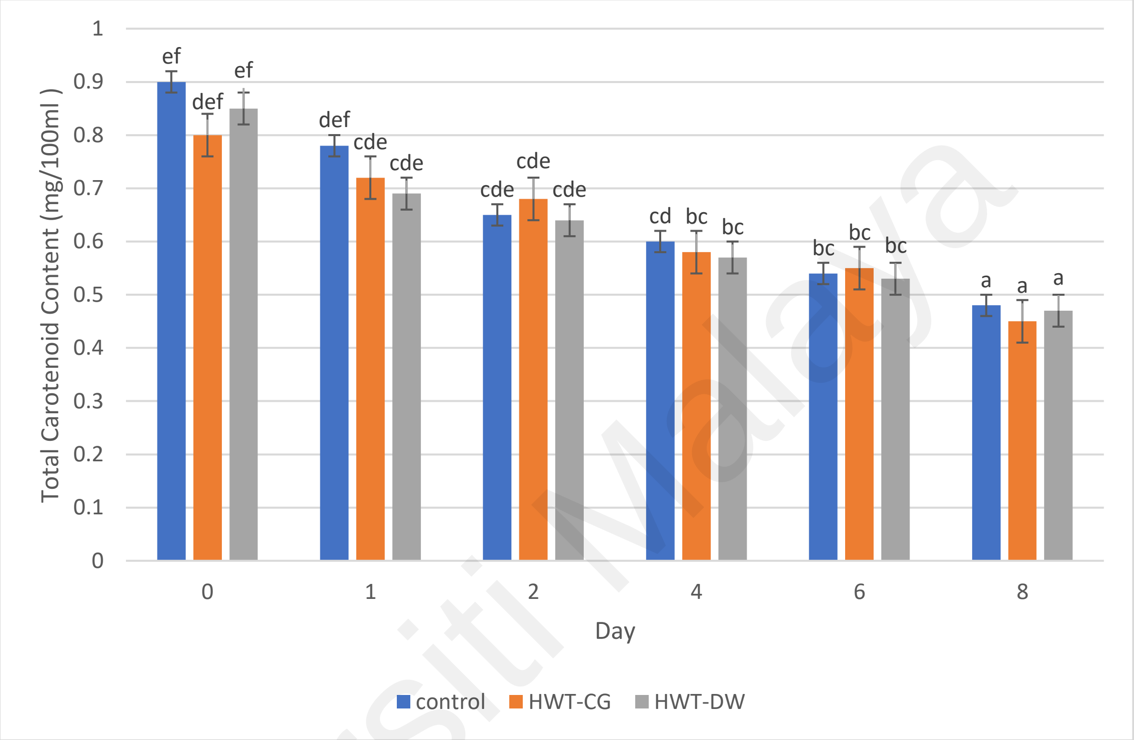

Figure 4.8: Effect of hot water treatment (HWT) on the total carotenoid content. [1]Values followed by different letters within the same column are significantly different (*p* < 0.05) (n = 9).
[2]Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

The ascorbic acid content decreased from day 0 to day 1 but increased gradually throughout the storage period (Table 4.1). Moreover, the ascorbic acid content of HWT-CG samples remained significantly higher than control samples on day 8.

Table 4.1: Effect of hot water treatment (HWT) on the ascorbic acid content.

Ascorbic acid Content (mg/100ml)	0	1	2	4	6	8
Control	42.24 ± 3.50^{c}	40.65 ± 3.53^{b}	40.65 ± 3.46^{b}	42.24 ± 3.79^{c}	43.83 ± 1.33^{d}	42.92 ± 2.31^{cd}
HWT-CG	52.92 ± 4.12^{cd}	60.87 ± 1.33^{e}	62.54 ± 4.21^{e}	64.28 ± 4.00^{d}	62.47 ± 2.67^{bcd}	66.21 ± 1.33^{ef}
HWT-DW	50.43 ± 3.90^{b}	52.92 ± 4.12^{d}	51.56 ± 3.46^{bc}	51.79 ± 3.99^{bc}	52.15 ± 3.53^{bc}	55.09 ± 3.53^{e}

[1] Values followed by different letters within the same column are significantly different (p < 0.05) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

4.5 Antioxidant Activity

The total polyphenol content (Table 4.2) showed a gradual increase until day 4 then decreased significantly on day 6 and increased slightly on day 8. The highest value was recorded by HWT-DW on day 4 with a value of 50.77 mg GAE/100 ml while the lowest value (16.94 mg GAE/100 ml) was on day 1 for HWC-CG.

Table 4.2: Effect of hot water treatment (HWT) on the total polyphenol content.

Concentration (mg GAE/100ml)	0	1	2	4	6	8
Control	26.31 ±0.5^{bc}	24.286 ±3.4^{b}	25.119 ±0.6^{bc}	40.179 ±7.1^{g}	27.619 ±0.3^{bc}	26.191 ±0.2^{bcde}
HWT-CG	24.048 ±0.2^{b}	16.964 ±0.3^{a}	34.643 ±1.3^{defg}	29.464 ±0.4^{bcd}	27.441 ±0.5^{bc}	32.143 ±0.4^{cdef}
HWT-DW	29.643 ±0.4^{bcd}	38.631 ±0.9^{fg}	29.048 ±0.3^{bcd}	50.774 ±0.4^{h}	30.417 ±3.5^{bcde}	36.726 ±0.7^{efg}

[1] Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

The total antioxidant capacity (TAC) showed no significant difference from day 0 to day 2 (Table 4.3). However, the TAC of treated samples increased from day 4 to the final day of storage. The highest value (298.89 µg AAE/ml) was recorded by HWC-CG on day 6 while the lowest value (192.96 µg AAE/ml) was recorded by HWT-DW on day 8.

Table 4.3: Effect of hot water treatment (HWT) on the total antioxidant capacity.

Concentration µg AAE/ml	0	1	2	4	6	8
Control	263.52 ±12.5^{cdefg}	255.28 ±13.1^{bcdefg}	239.26 ±4.1^{bcd}	253.52 ±6.3^{bcdef}	270.19 ±13.7^{efgh}	239.44 ±12.1^{bcd}
HWT-CG	247.59 ±4.4^{bcdef}	242.8 ±3.5^{bcde}	236.48 ±7.7^{bc}	290.0 ±19.3^{hi}	298.89 ±7.5^{i}	244.07 ±7.9^{bcde}
HWT-DW	269.26 ±2.4^{defgh}	230.23 ±4.0^{b}	239.44 ±7.5^{bcd}	282.96 ±2.9^{ghi}	275.93 ±6.1^{fghi}	192.96 ±0.5.4^{a}

[1] Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

As shown in Table 4.4, the total flavonoid content showed no significant increase in the first days of storage. However, the polyphenol levels began to increase on day 6. The flavonoid content of treated samples peaked on day 8 with 1.423 mg CE/100ml and 1.926 mg CE/100ml for HWT-CG and HWT-DW, respectively.

Table 4.4: Effect of hot water treatment (HWT) on the total flavonoid content.

Concentration mg CE/100ml	0	1	2	4	6	8
Control	0.62 $\pm 0.04^{ab}$	0.72 $\pm 0.03^{abc}$	1.14 $\pm 0.15^{defg}$	0.62 $\pm 0.07^{ab}$	1.41 $\pm 0.09^{fg}$	1.16 $\pm 0.09^{def}$
HWT-CG	1.11 $\pm 0.04^{def}$	0.72 $\pm 0.09^{abc}$	0.99 $\pm 0.25^{cde}$	0.57 $\pm 0.09^{a}$	1.28 $\pm 0.09^{efg}$	1.43 $\pm 0.03^{g}$
HWT-DW	0.74 $\pm 0.00^{abc}$	0.89 $\pm 0.04^{bcd}$	0.79 $\pm 0.09^{abc}$	0.62 $\pm 0.02^{ab}$	1.21 $\pm 0.12^{efg}$	1.93 $\pm 0.03^{h}$

[1] Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

Corresponding to Table 4.5, DPPH radical scavenging activity was significantly increased in the treated samples on the first 2 days of storage. The values significantly decreased on day 4 and continued throughout the storage period. The highest DPPH radical scavenging activity was recorded on day 0 which was 97.84 ± 0.42 µg AAE/ml in HWT-CG samples, and the lowest value was recorded on day 8 by HWT-DW with a value of 90.81 ± 0.67 µg AAE/ml.

Table 4.5: Effect of hot water treatment (HWT) on the DPPH radical scavenging activity.

Concentration µg AAE/ml	0	1	2	4	6	8
Control	95.48 ±0.07^{ef}	96.66 ±0.24^{fgh}	96.10 ±0.18^{fg}	94.01 ±0.25^{de}	92.14 ±1.14^{abc}	92.76 ±0.18^{bcd}
HWT-CG	97.84 ±0.42^{h}	97.22 ±0.07^{gh}	96.87 ±0.36^{fgh}	91.51 ±0.18^{ab}	94.16 ±0.48^{de}	93.25 ±0.80^{cd}
HWT-DW	96.38 ±0.28^{fgh}	95.83 ±0.60^{fg}	95.55 ±0.77^{ef}	93.74 ±0.21^{d}	95.41 ±0.24^{ef}	90.81 ±0.67^{a}

[1] Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 9).

[2] Control (no treatment); HWT-CG (hot water treated inoculated with *C.gloeosporioides*) ; HWT-DW (hot water treated inoculated with distilled water)

4.6 Primary and Secondary Metabolite Analysis

Differentially accumulated metabolites were studied to investigate the changes in metabolic composition between HWT and control fruits. GC-MS determined the biochemical changes, and 52 identified metabolites were monitored in the same sample sets using GC-MS. After analyzing significance with ANOVA ($P < 0.05$), 17 detected metabolites, mostly fatty acids were found to be significantly up/downregulated in HWT fruits when compared to control fruits with the same storage period (Table 4.6).

Table 4.6: Differential accumulated primary metabolites in hot water-treated fruits compared to control fruits.

Compounds	D0	D4	D8
Alcohols			
Glycerin	1.04*	1.01*	1.15*
Fatty acids			
Pentadecanoic acid	0.26	0.328	
n-Hexadecanoic acid	1.25*	UP	0.92
Octadecanoic acid	1.61*	DOWN	0.66
11,14,17-Eicosatrienoic acid		DOWN	
Eicosanoic acid		DOWN	
9,12,15-Octadecatrienoic acid	1.32*	UP	0.62
Others			
Hydrazine, 1,2-dimethyl-	1.12*	0.98	0.98
O-Butylisourea	DOWN	UP	
Isopropyl myristate (Ester)	1.11*	1.43*	0.89
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	1.74*		0.71
1,2-Cyclopentanedione		DOWN	DOWN
2-Butanone, 4-hydroxy-3-methyl-		UP	2.48*
1,2-Cyclopentanedione			DOWN
1H-Pyrazole, 1,5-dimethyl-	UP		
2(3H)-Furanone, 5-methyl-			UP
Benzophenone			UP

The table shows the ratio (HWT/control during the same storage period).
*: significant difference (P <0.05). UP: metabolite was detected in the HWT pulp but not in the control pulp.

For secondary metabolite analysis, ultra-high performance liquid chromatography coupled with a hybrid triple quadrupole linear ion trap mass spectrometer (UHPLC-QTRAP MS/MS) was done. Analysis of samples was done for control and HWT samples. The detected and identified secondary metabolites from the papaya sample by UHPLC-QTRAP MS/MS were shown in Table 4.7. For liquid chromatography , an Agilent 1100 series HPLC system coupled to Sciex 3200 hybrid quadrupole trap triple quad tandem mass spectrometer (UHPLC-QTRAP MS/MS) (Santa Clara, California, USA) was used. The LC column was a Phenomenex Synergy RP C18, 100A, 100mm x 3uM x 2.0mm (Torrance, California, USA). The LC mobile phase consisted of (A) water with 0.1 % (v/v) formic acid and (B) acetonitrile with 0.1 % (v/v) formic acid. Both eluents (A) and (B) were filtered through a 0.45 µm membrane filter before use. Gradient elution chromatography was always performed, starting with 95 % solvent A. Within an 8 min interval, % B composition was increased to 70 %, followed by a % B increase up to 95 % within 2 min. For the last 4 min, the composition of solvent B was reduced to 5 % while the composition of solvent A was increased to 95 %. MS experiments were performed using a Q-TRAP Linear Ion Trap Quadrupole mass spectrometer (Framingham, MA, USA). The instrument was operated in negative ion electrospray mode. The detection mode was a full scan enhanced mass spectra mode (EMS). Information-dependent acquisition IDA triggered enhanced product ion (EPI). Agilent 1100 Autosampler (Santa Clara, California, USA) with an injection volume 0f 20 µl and Agilent 1100 LC Quaternary Pump (Santa Clara, California, USA) with a maximum flow ramp of 100.0 ml/min^2 was used. ESI capillary voltage was optimized to 3000 V and cone voltage was set to 30 V. Full scan spectra were acquired over an *m*/*z* 100–1500 range at a scan rate of 1000 Da/s and an interscan delay of 0.1 s.

Table 4.7 lists the metabolic characterization of chemicals which induced resistance in HWT fruits. Contents of decreased 1,2-di-O-palmitoyl-3-O-(60-deoxy-60-sulfo-D-glycopyranosyl)-glycerol, caffeic acid and α-Hederin were downregulated on HWT fruits when compared to the control fruits, especially at D0 and D4. On the other hand, contents of 2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzene propanoic acid, caffeoyl glucose, lambda-carrageenan and sucrose were upregulated on HWT fruits when compared to the control fruits, especially at D0 and D4.

Table 4.7: Differential accumulated secondary metabolites in hot water-treated fruits compared to control fruits.

Compounds	D0	D4	D8
Lipids			
2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzene propanoic acid	UP		
1,2-di-O-palmitoyl-3-O-(60-deoxy-60-sulfo-D-glycopyranosyl)-glycerol	DOWN	DOWN	
15,16-dihydroxy- 9Z,12Z-octadecadienoic acid	2.72*	0.43	1.05
Decanoic acid	1.37*	0.92	3.82
Octadecanoic acid (stearic acid)	0.73	4.75	0.37
Carbohydrate			
2-Decyltetradecyl 4-O-α-d-glucopyranosyl-β-d-glucopyranoside	0.86	0.53	1.54
Monogalloyl Glucose	1.1*		
Caffeoyl glucose	UP	UP	1.63
Lamda-Carrageenan	UP	UP	2.29
Sucrose	UP		
Phenolics			
3,7-O-diferuloyl-4-O-caffeoyl quinic acid	1.47*	DOWN	
Caffeic acid	DOWN	DOWN	
Terpenoids			
α-Hederin	DOWN	DOWN	
β-Ecdysone	1.16*	DOWN	DOWN

The table shows the ratio (HWT/control during the same storage period).
*: significant difference ($P < 0.05$). UP: metabolite was detected in the HWT pulp but not in the control pulp.

4.7 Quantitative Proteomic Analysis of Eksotika II Papaya

Based on the morphology of the papaya, a TMT-based quantitative proteomics analysis was carried out to seek more information and obtain a global perspective of the mechanism of HWT on postharvest papaya at the protein level. Proteins were extracted from the papaya pulp at day 0 and day 8 for control and HWT papaya. A total of 361 proteins were identified from the papaya pulp and 18 of them were differentially accumulated among treatments. To further obtain insight into these proteins' functional and structural features, GO (Gene Ontology) and IPR (InterPro) analyses were conducted, and COG (Clusters of Orthologous Groups) and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases were applied to analyse the protein family and pathway.

The identified proteins involved an extensive array of biological processes, including cellular components and molecular functions, which were classified into 10 categories each (Figure 4.9). These proteins' biological process functions primarily involved protein folding (17 proteins), translation (15 proteins), and carbohydrate metabolic activities (8 proteins). The primary cellular component activities of these proteins were cytoplasm (32 proteins), membrane integral component (13 proteins), and chloroplast (11 proteins) (9 proteins). Within the area of molecular function, the largest group was ATP binding (16 proteins), followed by ATP hydrolase activity (13 proteins) and ribosome structural component (7 proteins).

The structural domain is the basic protein structure, function, and evolution unit. In the present study, the IPR analysis was conducted using Interproscan software based on Pfam, ProDom, and SMART protein domains to obtain a complete knowledge of the identified proteins. Figure 4.10 illustrates the top 18 which illustrates that the NAD(P)-binding domain (29 proteins) was the most abundant domain within identified proteins, followed by ATPase, F1/V1/A1 complex, alpha/beta subunit, N-terminal domain superfamily (14 proteins), and Tetratricopeptide-like helical domain (8 proteins).

The COG proteins database is constructed and maintained by NCBI, based on the classification of the evolutionary relationship of the complete genome-encoded proteins of bacteria, algae, and eukaryotes. It divides the proteins into 26 categories according to their function. As shown in Figure 4.11, the proteins identified in the present study were mainly related to post-translational modification, protein turnover, chaperone functions (104 proteins), energy production and conversion (59 proteins) and carbohydrate metabolism and transport (44 proteins). Most proteins enriched within these GO terms were upregulated in hot water-treated fruits compared to the control fruits.

The KEGG database was also applied to annotate the extracted proteins. Figure 4.12 presented 1450 maps using the identified proteins in response to HWT, which were mainly involved in the 43 pathways, and many proteins of these pathways participated in energy, carbohydrate, and protein metabolism. The predictions suggested that the most differentially expressed proteins were involved in the pathways.

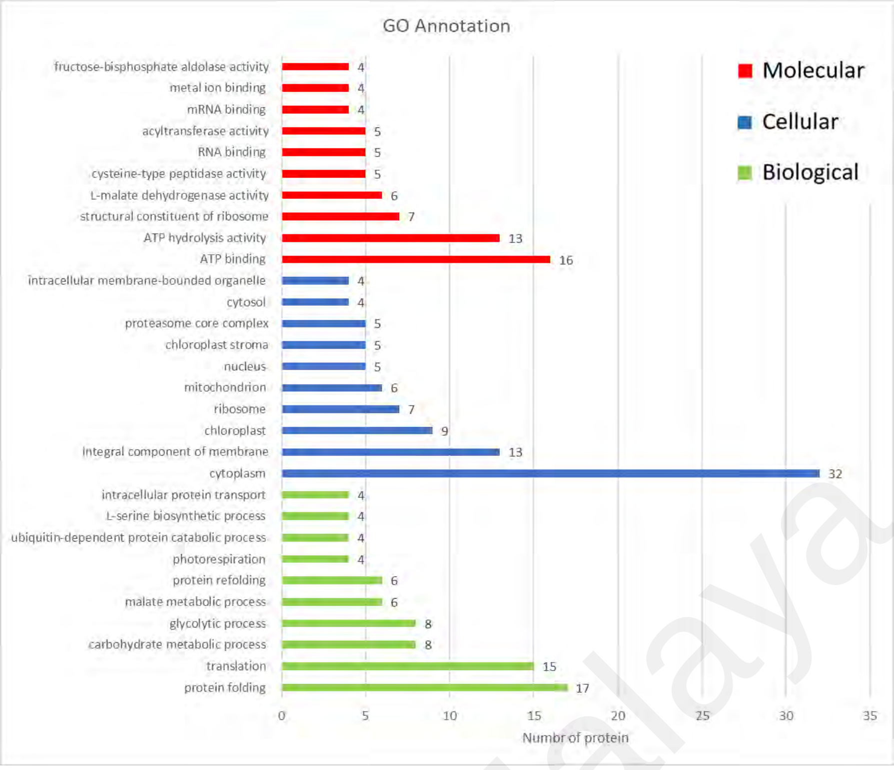

Figure 4.9: The Gene Ontology (GO) annotation of identified proteins from TMT quantitative proteomic analysis.

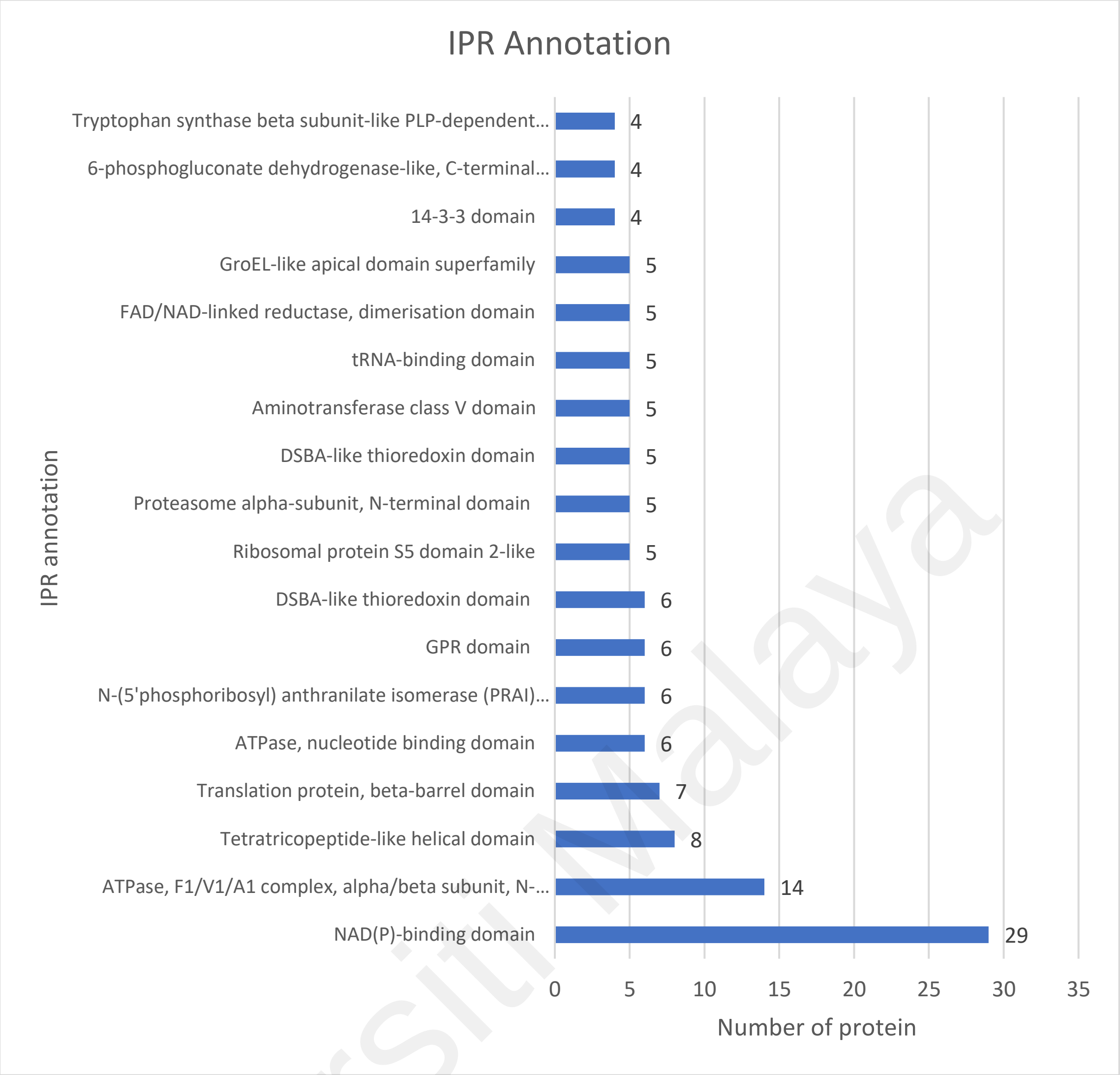

Figure 4.10: The IPR annotation of identified proteins from TMT quantitative proteomic analysis.

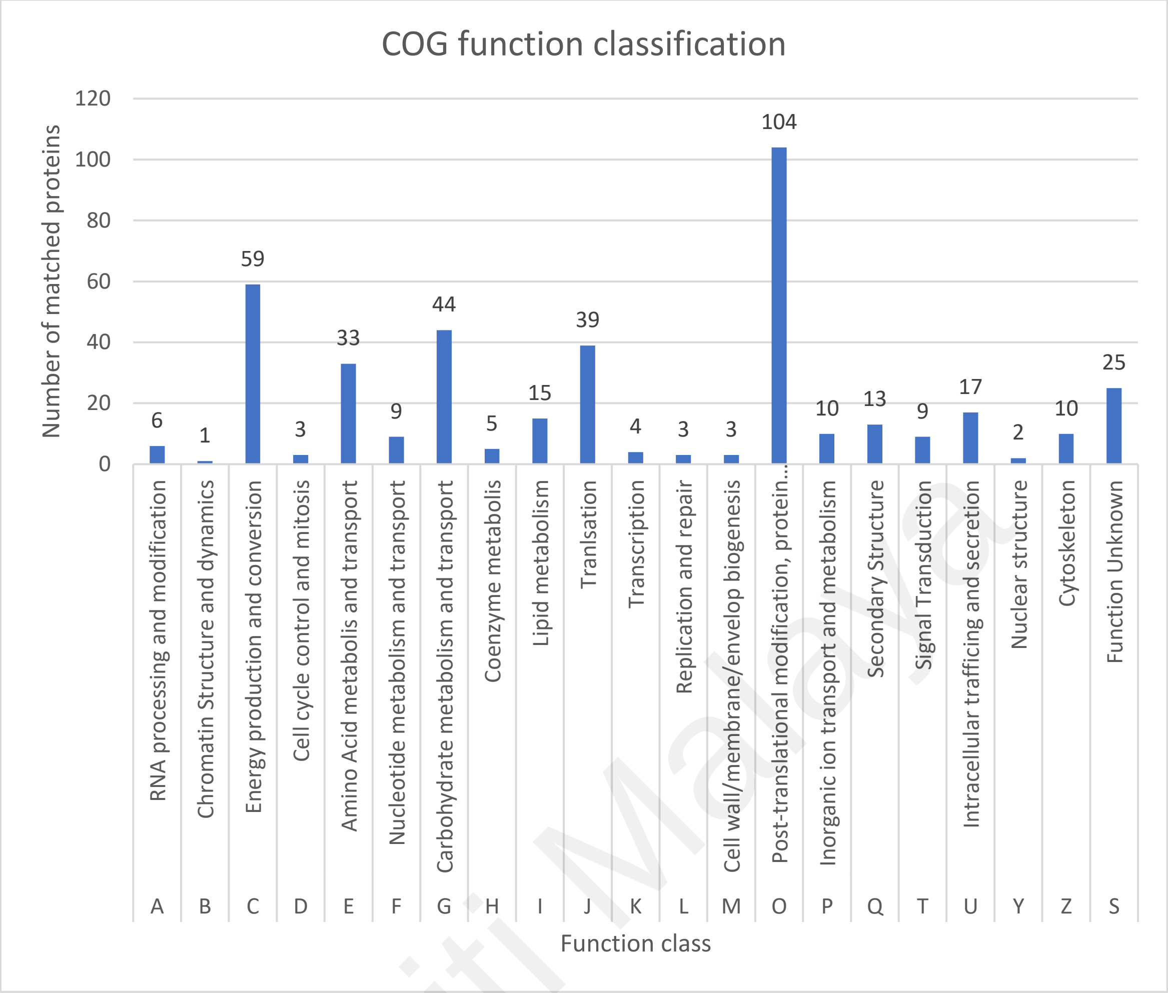

Figure 4.11: The COG function classification of identified proteins from TMT quantitative proteomic analysis.

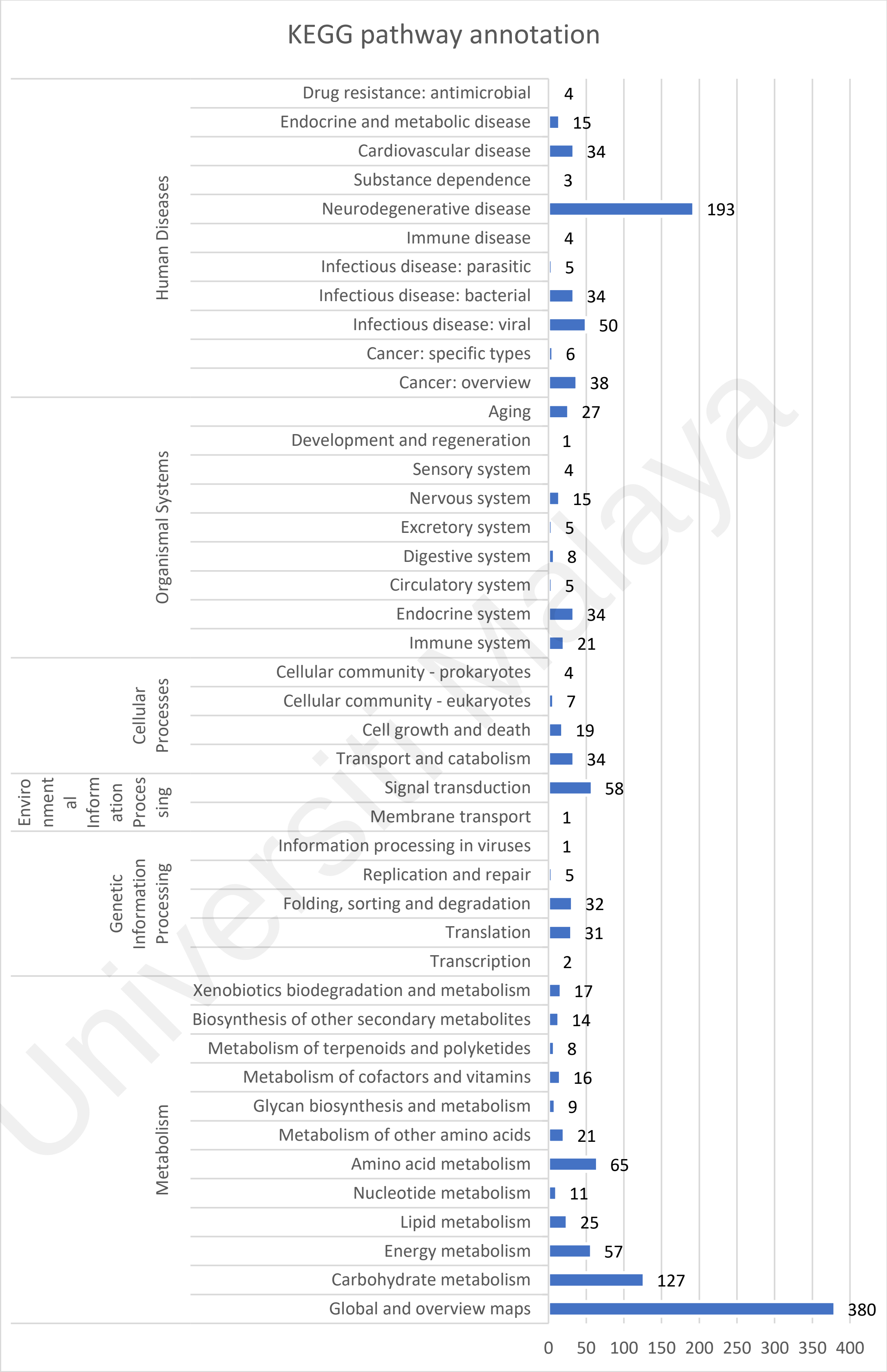

Figure 4.12: The KEGG pathway annotation of identified proteins from TMT quantitative proteomic analysis.

On day 8 of storage, 25 proteins were identified in control samples, and 16 were upregulated (Figure 4.13 (a)). In contrast, 54 proteins were identified in HWT samples, of which 23 were upregulated (Figure 4.13 (b)). In total, 31 proteins in the HWT group were identified compared to the control group on 0 d of storage, including 24 upregulated proteins (Figure 4.13 (c)). These findings indicated that HWT samples undergo a vigorous metabolic process. Apparent differences in protein abundance were also observed between the control and HWT samples on day 0.

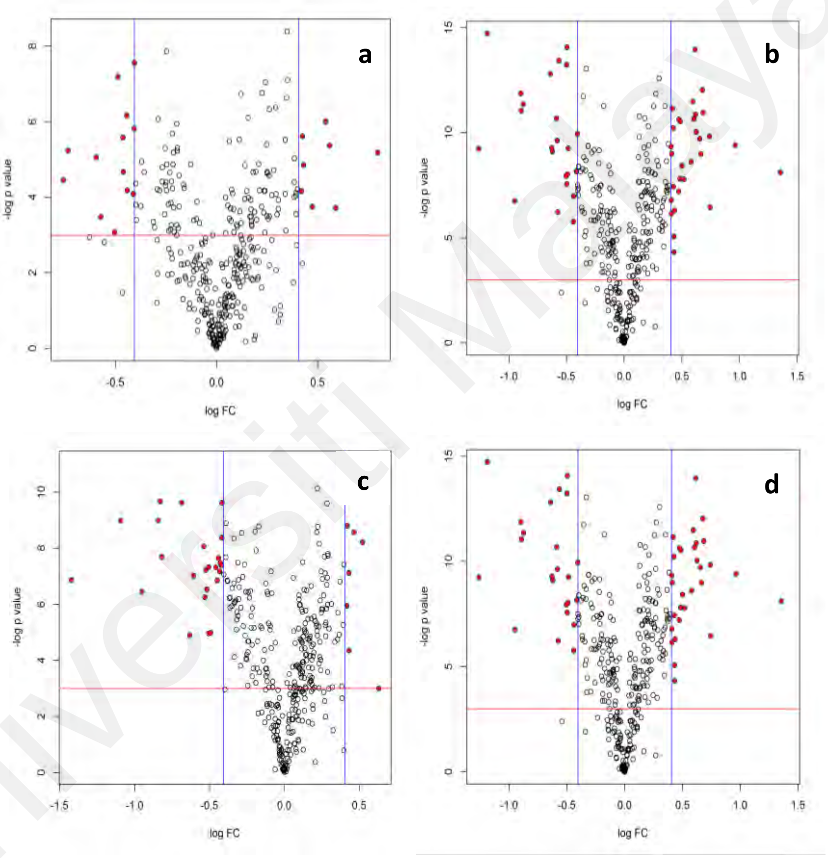

Figure 4.13: Volcano map of differentially expressed protein in papaya fruit; (a) CD0 vs CD8; (b) HWD0 vs HWD8; (c) CD0 vs HWD8 and (d) HWD0 vs HWD8.

To better show the differentially expressed proteins among samples, the protein expression in different groups was analyzed by hierarchical cluster analysis (Figure 4.14). The Z-score method was used to standardize the expression of proteins in different samples, and different colours (red indicates up-regulation and green indicates down-regulation) were displayed in the heat map.

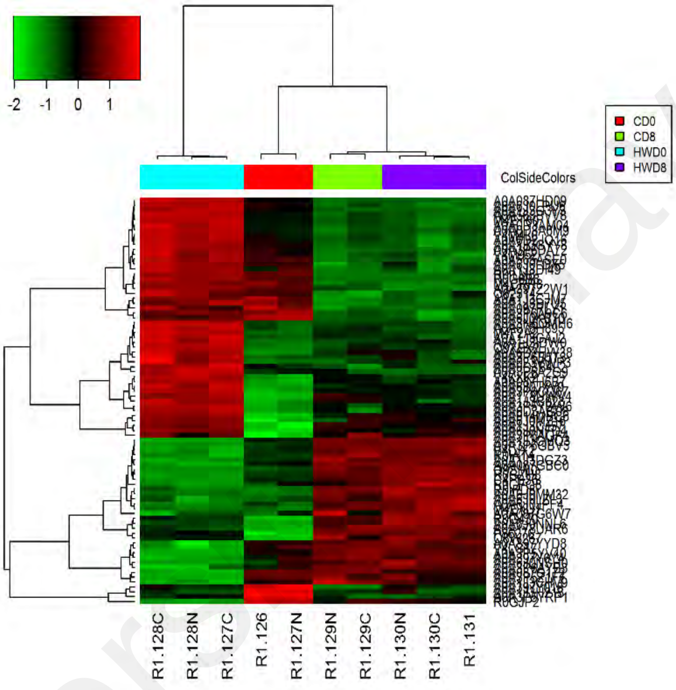

Figure 4.14: Hierarchical cluster analysis of differentially expressed proteins in papaya fruit.

The cluster plot provided shows each cluster's average length of proteins. The X-axis represents the cluster number, and the Y-axis represents the average length of proteins in that cluster (Figure 4.15). The plot shows four main clusters of proteins, with average lengths of 30, 24, 24, and 25, respectively. This suggests significant variation in the length of proteins, even within the same cluster. The cluster plot also shows a slight trend towards longer proteins in the higher-numbered clusters. This suggests that proteins in the higher-numbered clusters may be more complex or involved in more specialized functions.

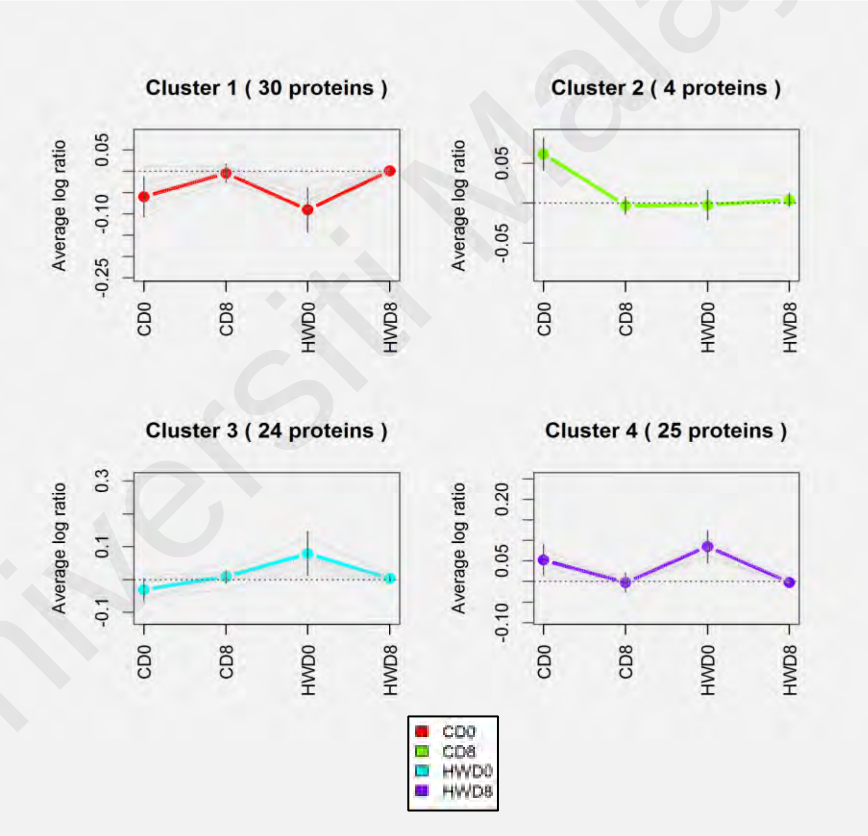

Figure 4.15: Cluster profile of each cluster of differentially expressed proteins in papaya fruit.

Table 4.8: Differentially accumulated proteins in hot water-treated fruits compared to control fruits.

Accession	Description	Abbreviation	Fold change (FC)	Organism Species (OS)	Taxonomy ID	Gene Name
	Heat stress response					
A0A6J0LDX5	Heat shock protein 90-5, chloroplastic	Hsp90C	0.68	OS=Raphanus sativus	OX=3726	GN=LOC108829222
A0A1I9RH42	Heat shock protein 70	Hsp70	0.64	OS=*Carica papaya*	OX=3649	
A0A0H4JN20	Heat shock protein 90 (Fragment)	Hsp90	0.65	OS=Capparis spinosa	OX=65558	
A0A654FTG3	sHSP domain-containing protein	sHSPs	0.69	OS=Arabidopsis thaliana	OX=3702	GN=AN1_LOCUS19542
	Carbohydrate metabolism					
R0G430	Pectinesterase (Fragment)	PEs	1.66	OS=Capsella rubella	OX=81985	GN=CARUB_v10013226mg
A0A6J0NNL6	Triosephosphate isomerase, cytosolic	TPI	0.61	OS=Raphanus sativus	OX=3726	GN=LOC108856736
A0A1J3CJ57	Fructose-bisphosphate aldolase (Fragment)	FBA	0.66	OS=Noccaea caerulescens	OX=107243	GN=GA_TR1990_c0_g1_i1_g.6406
	Cellular detoxification					
R0IPD8	EF1B class glutathione S-transferase	GSTs	0.64	OS=Capsella rubella	OX=81985	GN=EF1BG1
A0A6J0MM32	Glutathione S-transferase U24-like	GST	0.46	OS=Raphanus sativus	OX=3726	GN=LOC108844242
	Protein transport					
A0A1J3H213	Luminal-binding protein 5 (Fragment)	BIP5	1.53	OS=Noccaea caerulescens	OX=107243	GN=LE_TR13727_c1_g1_i1_g.44168
A0A3P6CQ67	Coatomer subunit delta	ARCN1	1.63	OS=Brassica campestris	OX=3711	GN=BRAA10T45158Z
	Protein translation					
A0A1J3CJM7	Protein translation factor SUI1-like protein	SUI1	1.81	OS=Noccaea caerulescens	OX=107243	GN=GA_TR14473_c0_g1_i1_g.44910
	Protein folding					
Q8W267	Maturation-associated SRC1-like protein	SRC1	0.62	OS=*Carica papaya*	OX=3649	GN=SRC
	Amino acid metabolism					
R0GZH8	Adenosylhomocysteinase	AHCY	0.63	OS=Capsella rubella	OX=81985	GN=CARUB_v10004693mg
	Cell signalling					
A0A097PLY1	LOS4 (Fragment)	LOS4	1.61	OS=*Carica papaya*	OX=3649	
	Energy metabolism					
O82514	Adenylate kinase 4	AK4	1.54	OS=Arabidopsis thaliana	OX=3702	GN=ADK1
	Lipid metabolism					
P86276	GDSL esterase/lipase	GELPs	0.55	OS=*Carica papaya*	OX=3649	
	Oxidation-reduction					
A0A1J3DGZ3	Aldehyde dehydrogenase family 2 member B7, mitochondrial	ALDH	0.56	OS=Noccaea caerulescens	OX=107243	GN=GA_TR15826_c3_g1_i1_g.48481

Table 4.8 shows the 18 differential accumulated proteins in hot water-treated fruits compared to control fruits. The identified proteins are involved in heat stress response, carbohydrate metabolism, cellular detoxification, protein transport, protein translation, protein folding, amino acid metabolism, cell signalling, energy metabolism, lipid metabolism, and oxidation-reduction.

The HWT induced qualitative and quantitative changes in the abundance of several proteins in papaya fruit. Compared with the levels of proteins in the control fruit, the HWT fruit showed a higher accumulation of 13 proteins, including 3 heat shock protein (HSP), 1 small heat shock protein (sHSP), 2 enzymes associated with carbohydrate metabolism (triosephosphate isomerase and fructose-bisphosphate aldolase), 2 cellular detoxification proteins (EF1B class glutathione S-transferase and glutathione S-transferase U24-like), 1 protein folding enzyme (Maturation-associated SRC1-like protein), 1 protein related to amino acid metabolism (Adenosylhomocysteinase), 1 enzyme related to lipid metabolism (GDSL esterase/lipase) and as well as 1 enzyme involved in oxidation-reduction (Aldehyde dehydrogenase family 2 member B7, mitochondrial).

On the other hand, 4 proteins were down-regulated by HWT which are 2 transport proteins [Luminal-binding protein 5 (Fragment) and Coatomer subunit delta], 1 enzyme involved in protein translation (Protein translation factor SUI1-like protein), 1 protein associated with cell signalling [LOS4 (Fragment)], 1 enzyme involved in energy metabolism (Adenylate kinase 4) and 1 enzyme involved in carbohydrate metabolism (Pectinesterase, Pes).

CHAPTER 5: DISCUSSION

The hot water treatment method has garnered significant attention from researchers due to its relative ease of use, short treatment time, effective fruit and water temperature control, and the killing of skin-borne decay-causing agents (Fallik *et al.,* 2021). Nevertheless, the impact of hot water treatment (HWT) on the postharvest quality of *Carica. papaya* L., cv. Eksotika II has yet to be thoroughly examined. The current investigation demonstrated that the application of hot water treatment resulted in enhanced disease resistance through the upregulation of specific metabolites and proteins that effectively impeded the advancement of anthracnose while preserving the physicochemical features of the papaya fruit. These findings corroborate those of Li *et al.* (2019), who found that heat-treated pericarp showed up-regulation of resistance-associated proteins and a notable build-up of metabolites linked to disease resistance.

According to Peralta-Ruiz *et al.*, (2023), the mode of action of hot water treatment against anthracnose is by reducing the carrier rate of *C. gloeosporioides* in fruit peel, which significantly inhibits the incidence of anthracnose and stem-end rot, effectively delaying fruit softening, but slightly promoting the rate of fruit colouring. HWT reduced anthracnose index and fruit ripeness to some extent and induced changes in wax arrangements on the treated fruit surface, causing the wax to melt. It is assumed that the molten wax caused by HWT wholly or partially sealed natural openings in the epidermis could physically prevent the invasion of the pathogen, thus reducing disease rates.

A direct effect on the pathogen causing the disease and an indirect effect on the fruit host can be involved (Zakaria, L., 2021). It has been reported that heat treatment, performed at non-lethal conditions, causes moderate stress to fruit, provoking a temporary inhibition of normal metabolism, that is recovered once the fruit is returned to a non-stressing temperature (Haider *et al.,* 2021). The result is delayed ripening and softening of treated fruit.

According to Hasan *et al.* (2018), exposure to temperatures higher than 40 °C for a short period could result in a rapid loss of 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) in papaya, but after fruits are removed from the heat and transferred to ambient condition for three days, the ACC oxidase activity recovered. The inhibition of ripening due to lack of ethylene is reversible if the heat treatment is not too extended and does not cause damage.

The pH of both HWT samples increased after the second day of storage and remained higher than the control until day 6 as shown in Figure 4.4. On the last day of storage, the pH of HWT-DW decreased slightly below the control samples. The pH value did not significantly change between the control and hot water-treated samples. These results are consistent with Vilaplana *et al.* (2020), who reported pH values were between 5.2 and 5.4 for both treated and untreated fruits.

The sweet taste is an essential parameter of fruit quality. Taste is usually associated with sucrose, glucose, and fructose concentrations, which are used as a ripening index to assess the stage of maturity and papaya quality standards. TSS is an indicator of sweetness routinely used for evaluating the quality of fruits. Values for TSS (10.2 to 13.2 ºBrix) of samples were within the range of desirable qualities for Eksotika II papaya (Azhar *et al.*, 2020). According to Figure 4.5, the highest reading was obtained on day 1 for HWT-DW which was 13.2 ºBrix and the lowest was recorded on day 8 for HWT-DW which was 10.2 ºBrix. The results obtained are in tandem with those of Madani *et al.* (2021), who reported that the TSS of HWT and untreated papayas did not significantly change throughout the ripening period.

After harvesting and postharvest, climacteric fresh fruits lose weight primarily due to respiration and transpiration activity (Karagiannis *et al.*, 2024). The quality of papaya fruit could be determined by measuring the weight loss. The weight loss of HWT-DW was highest (6.49 %) compared to HWT-CG (4.79 %) and control (5.80 %). However, no significant weight loss was measured among the HWT samples compared to control samples. Similar trends were observed in hot water-treated papaya (*Carica papaya* cv. ‘Maradol’) where the treated fruit did not show significant weight loss differences (P<0.05) in comparison to the control fruit throughout the storage period (Al-Dairi *et al.,* 2021). The decision to choose only 8 days of storage for the project can be attributed to the preliminary findings indicating that the shelf life of papaya is limited to this duration. Papayas are climacteric fruits, meaning they continue to ripen after being harvested. This ripening process is associated with a high respiration rate, leading to a rapid decline in quality post-harvest (Gundewadi *et al.*, 2018)

The TA values (Figure 4.7) decreased gradually throughout storage. The TA of both hot water-treated samples remained significantly lower than the control samples on the last day of storage with values of 0.107 % for HWT-CG and 0.10 2% for HWT-DW. Rabiei *et al*. (2011) reported a significantly lower TA in heat-treated than unheated ' Red Delicious’ apples, consistent with the results obtained. Comparable results were also reported by Fan *et al.* (2011), who found that the TA in ‘Cortland’ and ‘Jonagold’ apples decreased during storage at 0 °C. The reduction in titratable acidity seen immediately after heat treatment is consistent with the reduction previously reported after short-term increased temperature fluctuations (Dash *et al*., 2022). It may be that hot-water immersion raises fruit temperature, and thus respiration, enough to speed up the metabolic aspects of maturation that deplete organic acids (Silveira Alexandre *et al*., 2022). Based on all the physicochemical parameters investigated, there were no significant differences between the quality of control and treated fruits, except for TA. This further supports the view that HWT does not impair the important qualities of the fruits.

Ascorbic acid has strong antioxidant properties, and the colour of the fruit is determined by the amount of carotenoids present (Zacarías-García *et al*., 2021). The ascorbic acid content decreased from day 0 to day 1 but increased gradually throughout the storage period. The ascorbic acid content of treated samples remained significantly higher than control samples on day 8. Similar results were also observed in apples infected with *Penicillium expansum, Botrytis cinerea,* or *Colletotrichum gloeosporioides*; fruit with the lowest decay area had the highest ascorbic acid content (Sharma, Singh, & Pal, 2013; Zhao & Wang, 2015). Yin *et al*. (2022) suggested that heat treatment stabilized the ascorbic acid by enzyme inactivation, including ascorbate oxidase.

The total carotenoid content showed a decreasing trend throughout the storage period. The highest recorded value was on Day 0 (0.9 µg/100ml) by the control samples and the lowest was recorded on Day 8 (0.45 µg/100ml) by the HWT-CG samples. According to Rey *et al*. (2020), the hot water dip and the control samples did not enhance the carotenoid content of 'Fortune' mandarin fruit and remained constant throughout storage. Moreover, the decrease in total carotenoid content might be due to the oxidative reactions during storage (Meléndez-Martínez *et al.*, 2023).

The antioxidant activity of papaya fruit was measured by Folin-Ciocalteu, phosphomolybdenum and aluminium chloride colourimetric assays. The total polyphenol content gradually increased until day 4 after which it decreased significantly on day 6 and increased slightly on day 8. The highest value was recorded by HWT-DW on day 4 with a value of 50.77 mg GAE/100ml while the lowest value (16.94 mg GAE/100ml) was on day 1 for HWC-CG. Plant tissues, including fruit, produce phenolics as a defence mechanism (Kumar *et al.*, 2020), increasing their levels in response to stress such as damage caused by fungal infection or unfavourable environmental conditions (e.g., high temperatures) (Sonawane *et al.*, 2022). Denoya *et al*. (2021) reported that the heating of fruits induced stress and activated phenolic synthesis. In addition, other studies have shown that hot water treatment increased the expression of enzymes involved in the biosynthesis of phenolic compounds (Yang *et al*., 2020). Phenolics are substrates to oxidative reactions yielding fungitoxic quinones, and plant cells accumulating phenolics are an unfavourable medium for pathogen development (Lattanzio *et al*., 2021). In pepper fruit, infected tissue with *Colletotrichum coccodes* showed higher phenolic content than healthy tissue. In this regard, Ayón-Reyna *et al.* (2017), Madani *et al.* (2016), and Zhao and Wang (2015) reported that papaya and apple fruit with high phenolics content had the lowest infection rate caused by *C. gloeosporioides*.

The total antioxidant capacity (TAC) showed no significant difference from day 0 to day 2. However, the TAC of treated samples increased from day 4 to the final day of storage. The highest value (298.89 µg AAE/ml) was recorded by HWC-CG on day 6 while the lowest value (192.96 µg AAE/ml) was recorded by HWT-DW on day 8. Several authors have also reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products (Djenidi *et al*., 2020). Phenolic compounds act as antioxidants because they donate hydrogen or electrons and generate stable radical intermediates (Parcheta *et al.*, 2021). However, the antioxidant activity varies with the stages of ripening.

The total flavonoid content showed no significant increase in the first four days of storage. However, the polyphenol levels began to increase on day 6. The flavonoid content of treated samples peaked on day 8 with 1.423 mg CE/100ml and 1.926 mg CE/100ml for HWT-CG and HWT-DW respectively. This increase may be due to the stress of heat treatment, as this condition plays an important role in the activation and accumulation of phenol synthesis. (Šamec *et al*., 2021). Generally, plants show a rapid response to increased ROS (reactive oxygen species) produced by abiotic stresses like changes in storage temperature (Devireddy *et al.*, 2021). These changes lead to an enhancement in cellular antioxidants that increase the activity of natural antioxidants (López-Pedrouso *et al*., 2022). Preconditioning treatments of fruit with hot water may delay fruit ripening by modulating antioxidant chemicals such as AA (ascorbic acid), GSH (glutathione), free phenolics and free flavonoids that would work singly and synergistically to prevent the accumulation of ROS (Chen *et al.,* 2022).

The statement describes the DPPH test, a widely used method for evaluating the antioxidant properties of compounds. Antioxidants are compounds that protect cells from the damaging effects of free radicals, which are unstable molecules that can cause oxidative stress and damage to cells. The DPPH test works by measuring the ability of a compound to scavenge free radicals and donate hydrogen atoms to stabilize them (Gulcin *et al*., 2022). The test uses a dark-coloured crystalline substance called DPPH, which is a stable free radical. When an antioxidant compound is added to a solution of DPPH, it reacts with the free radicals and reduces their concentration, causing the solution to become less coloured. The statement further explains that the DPPH radical scavenging activity of a tested substance showed no significant difference on the first two days of storage, but on day 4, the values significantly decreased and continued to decline throughout the storage period. However, the rate of decline in DPPH scavenging activity was considerably lower in the HWT-DW treatment followed by HWT-CG, compared to the control. This suggests that the tested substances, HWT-DW and HWT-CG, have higher antioxidant properties than the control. HWT-DW and HWT-CG could be potential sources of natural antioxidants and can be used to develop functional foods, nutraceuticals, and dietary supplements with antioxidant properties (Das *et al*., 2020).

Differentially accumulated primary metabolites were studied to investigate the changes in metabolic composition between HWT and control fruits. GC-MS determined the biochemical changes. 52 identified metabolites were monitored in the same sample sets using GC-MS. After analyzing significance with ANOVA ($P < 0.05$), 17 detected metabolites, mostly fatty acids, were significantly up/downregulated in HWT fruits compared to control fruits with the same storage period. Primary metabolites are directly involved in normal growth, development, and reproduction. In the present study, the content of ornithine, oleic acid and tetradecanoic acid increased in the treated pericarp compared to the control during storage, and levels of seven types of sugars increased in heat-treated pericarp compared to the control at 2 h after HWT. In plants, ornithine is required to synthesise polyamines and alkaloids, which contribute to oxidative stress tolerance in plants subjected to severe water stress (Yadav *et al*., 2021). The findings suggest that heat treatment can affect the metabolic composition of fruits, resulting in changes in the levels of various metabolites. Specifically, the increased levels of ornithine, oleic acid, and tetradecanoic acid in heat-treated pericarp may contribute to the fruit's ability to tolerate oxidative stress. The increase in the levels of sugars in heat-treated pericarp may also affect the fruit's taste and nutritional value (Malik *et al*., 2021). Overall, this study provides valuable insights into the effects of heat treatment on fruit metabolism and can aid in developing new fruit storage and preservation strategies.

Differentially accumulated secondary metabolites were studied to investigate the changes in metabolic composition between HWT and control fruits. The study found that certain metabolites were downregulated in HWT fruits compared to control fruits, while other metabolites were upregulated. The metabolites that were downregulated in HWT fruits included, 2-di-O-palmitoyl-3-O-(60-deoxy-60-sulfo-D-glycopyranosyl)-glycerol, caffeic acid, and α-Hederin, especially on Day 0 and Day 4 of storage. In contrast, the study found that the levels of certain metabolites were upregulated in HWT fruits compared to control fruits, especially on Day 0 and Day 4. These metabolites included 2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzene propanoic acid, caffeoyl glucose, lamda-carrageenan, and sucrose.

2(3,4-Dihydroxyphenyl)-7-hydroxy-5-benzene propanoic acid, also known as homogentisic acid, is a phenolic compound that has been shown to have antimicrobial activity against a wide range of pathogens, including bacteria and fungi. It can also enhance plant resistance to oxidative stress caused by pathogen attack and may play a role in the synthesis of lignin, a component of plant cell walls that provides mechanical strength and protection against pathogens (Alshwyeh *et al*., 2020).

Caffeoyl glucose is another phenolic compound that has been shown to have antimicrobial properties, particularly against fungal pathogens. It can also enhance plant defence against oxidative stress and UV radiation (Singh *et al*., 2023). Lambda-carrageenan is a type of polysaccharide found in red seaweeds and has been shown to have antimicrobial activity against various plant pathogens, including bacteria and fungi. It can also induce systemic acquired resistance in plants, a long-lasting defence mechanism that protects against various pathogens (Pradhan *et al*., 2023). Sucrose is a common plant metabolite involved in various physiological processes, including energy storage and transport. Although the mechanisms are poorly understood, they have also been shown to play a role in plant defence against pathogens. Some studies suggest that sucrose may act as a signalling molecule to induce plant defence responses, while others suggest that it may enhance the synthesis of antimicrobial compounds (Zehra *et al*., 2021).

The results indicate that subjecting fruits to heat treatment induces alterations in the concentrations of specific metabolites. Notably, certain metabolites associated with stress exhibit downregulation, while others linked to defence mechanisms and metabolism show upregulation.

The application of TMT-based proteomic analysis alongside bioinformatic tools aimed to elucidate the potential mechanisms of Hot Water Treatment (HWT) in postharvest papaya. A total of 361 proteins were identified within the postharvest papaya pulp. Gene Ontology (GO) analysis was utilized to gain functional insights into these proteins, revealing a prevalence of proteins associated with protein folding and translation. This suggests the formation of new proteins, potentially linked to the induction of Heat Shock Proteins (HSPs) due to HWT. The observed elevation in ATP binding and ATP hydrolase activity further supports this notion. This observation was validated by Salazar-Salas *et* *al.* (2022), who documented the emergence of eleven HSPs in 'Keitt' mango fruit following hot water treatment.

KEGG pathway analysis unveiled the significant influence of HWT on global maps, including carbohydrate, energy, and amino acid metabolism. Additionally, it affected processes such as translation, signal transduction, and cellular folding, sorting, and degradation. These findings aligned with previous KEGG analyses of *C. papaya* (Jiang *et al.,* 2019), underscoring the importance of these annotations in investigating the function of *C. papaya*.

The proteomic analysis showcased the induction of multiple HSPs in papaya fruit following HWT, with their accumulation notably higher compared to control fruit (Table 4.8). Heat treatment is a well-recognized postharvest technology effective in controlling pathogens like *Penicillium, Alternaria, and Colletotrichum* spp. in various fruits such as citrus, avocado, and mango (Ravichandra, 2021). This treatment enhances plant stress tolerance, wherein HSP induction plays a pivotal role. For instance, a proteomics study demonstrated that a 48 °C hot water dip for 10 minutes induced the accumulation of low molecular weight HSPs, retarding *Penicillium* growth in peach fruits (Shin *et al.*, 2023). Similarly, a transcriptomic study indicated the upregulation of small HSPs and other defence genes in 'Ataulfo' mango following quarantine hot water treatment (Dautt-Castro *et al*., 2018). In this investigation, a 54 °C hot water treatment led to the upregulation of HSP90C, HSP70, HSP90, and sHSPs (Table 4.8).

Heat Shock Proteins (HSPs) constitute a class of proteins known in cellular biology for their increased concentration under elevated temperatures. Their crucial role in assisting protein folding and preserving functional structure during stressful events, both biotic and abiotic, is well established (Covarrubias *et al.,* 2020). Additionally, small Heat Shock Proteins (sHSPs) possess the ability to modify membrane properties, impacting membrane fluidity under varying temperature conditions (Salazar-Salas *et al.,* 2022).

These proteins also exhibit antioxidant activity and contribute to pectin depolymerization (Escobar *et al.,* 2021; Guo *et al.,* 2020). sHSPs typically collaborate with high molecular weight proteins like HSP70 to prevent protein aggregation and facilitate refolding (Guin *et al.,* 2019).

The pivotal involvement of HSPs in plant immunity against pathogens is well-documented. Induction of HSPs during pathogenic attacks fine-tunes the chaperone system, regulating the production and interactions of proteins crucial for defence responses (Park and Seo, 2015; Wei *et al.,* 2021; Berka *et al.,* 2022). Transcriptomic studies have demonstrated the induction of HSP genes in response to anthracnose infections in tea leaves and lentils, indicating their involvement in defence mechanisms against anthracnose (Cheng *et al*., 2023).

Pectinesterase (PEs) significantly modify pectin, a crucial component of plant cell walls. Alterations in pectin structure can impact plant resistance against certain pathogens. The study observed the down-regulation of PEs in HWT samples, suggesting their potential efficacy in controlling the progression of *Colletotrichum gleosporioides* in HWT fruits (Bambalele *et al*., 2021). Reports suggest the importance of various pectin-related genes in plant cell wall degradation, affecting pathogenic factors in *C. falcatum* (Prasanth *et al*., 2022). Mirshekari *et al.,* (2018) stated that PG (Polygalacturonase), PME (Pectin methylesterase), and (Phospholipase) PL activities were reduced in the treated bananas when compared with the control treatment. Hot water treatment disturbed cell wall degrading enzymes activity and delayed the disassembling of pectin fraction of Berangan banana.

The GDSL esterase/lipase family encompasses several functional genes contributing to growth, development, seed oil synthesis, and defence responses in plants. Upregulation of GDSL esterase/lipase observed in HWT treated samples may have inhibited the growth of *C. gleosporioides*. Although not extensively studied in disease resistance contexts, proteins involved in plant development often indirectly influence plant defence against pathogens. This aligns with research by Yao-Guang *et al.* (2022), demonstrating that the expression of GDSL esterase/lipase genes suppressed the advancement of *S. lycopersici* in tomato fruits.

Aldehyde dehydrogenases (ALDHs) participate in diverse biological processes in plants, including stress responses and detoxification. Certain ALDHs have been associated with plant defence against pathogens by aiding in the synthesis of defensive compounds or regulating signalling pathways involved in defence responses (Cuatlayotl-Olarte *et al*., 2023). The findings suggest that the upregulation of ALDH in the fruit may have contributed to resistance against *C. gleosporiodes*. For instance, the expression of ALDH resulted in enhanced resistance to citrus green mold in *Citrus unshiu Marc*. cv. *Miyagawa Wase* , presumably via the Salicyclic acid (SA)-signalling pathway (Duan *et al.*, 2024).

The overall data collected from proteomic and bioinformatic analyses provide substantial insights into the molecular mechanisms of Heat Water Treatment (HWT) in postharvest papaya. The observed alterations in protein profiles and metabolic pathways, alongside the induction of Heat Shock Proteins (HSPs) and other defence-related enzymes, signify potential avenues for enhancing fruit quality and resilience against pathogens. Further investigations are warranted to elucidate the precise mechanisms underlying these observations.

CHAPTER 6: CONCLUSION

In conclusion, this study comprehensively demonstrated that hot water treatment (HWT) positively impacts the postharvest quality of Carica papaya L., cv. Eksotika II. Specifically, HWT enhanced disease resistance against anthracnose by upregulating specific metabolites and proteins, thereby preserving the fruit's physicochemical properties. The treatment influenced the metabolite composition, leading to an accumulation of stress-response metabolites such as phenolic compounds and antioxidants. Proteomic analysis revealed the formation of new proteins, including heat shock proteins, which are crucial for stress adaptation. Overall, HWT effectively improved the fruit's disease resistance, altered metabolite profiles, and induced the formation of protective proteins, highlighting its potential as a tool for enhancing the shelf life and quality of papaya. Further research should explore the optimization of HWT parameters for different papaya varieties, investigate long-term storage effects, and evaluate the economic feasibility of large-scale HWT implementation. Additionally, exploring the integration of HWT with other postharvest treatments could provide a more comprehensive approach to fruit preservation. The identification of important compounds could also potentially lead to the development of organic pesticides, while specific proteins upregulated by HWT could be targeted for developing transgenic crops with enhanced disease resistance.

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