Antioxidant and antimicrobial activity of *Carica papaya* extracts in minimally processed fresh produce

By

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DEDICATION

This dissertation is dedicated to God and all my loved ones. To my mother, father, siblings, friends and the rest of my family.

I hope I made you all proud.

DECLARATION

I, Lilian Kafuko declare that the dissertation I hereby submit for the degree MSc Food Science at the University of Pretoria is my work and has not been previously submitted at any other institution of higher education. Reference material contained in this dissertation has been duly acknowledged.

Signature: Lilian Kafuko

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ABSTRACT

Antioxidant and antimicrobial activity of *Carica papaya* extracts in minimally processed fresh produce

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Food (especially fruit derived) waste has a detrimental influence on the environment and food security of a country. As the world's population continues to rise, the challenge becomes feeding more people nutrient dense meals and preventing wastage of what we already produce. Fresh produce is important for a healthy diet, however, challenges arise around retaining their freshness and quality during processing and distribution. A lot of produce gets wasted along the supply chain and so technologies that help in retaining the quality are needed. The conversion of by-products obtained during processing to high end food additives is one way of reducing fresh produce waste. Papaya (*Carica papaya* L.) is one such produce whose by-products are underutilised. Based on the nutritional quality of the edible part, there was potential in utilising the papaya peel in the study. There is not much that has been reported on papaya with regards to utilization of its by-products as preservatives within the food industry. Therefore, the antioxidant and antimicrobial properties of *C. papaya* peel crude extracts were studied in this work. The bioactive compounds in the peel crude extracts were also identified. The ability of the crude extracts to inhibit more common foodborne pathogens was investigated. Furthermore, the crude extracts were also tested as anti-browning agents using apples and potatoes as model systems.

Using the agar well diffusion technique, the antibacterial activity of the peel crude extracts against bacteria (*Listeria monocytogenes* and *Escherichia coli*) was examined. The crude extracts were active against both *L. monocytogenes* and *E. coli*. Because of the difference in

cell wall structure between the two bacteria (the former is a gram positive bacterium and the latter is a gram negative bacterium), antimicrobial activity was more pronounced in *L. monocytogenes* than in *E. coli*. The crude extracts were also stored at 4°C and 10°C for 5 days and thereafter their activity tested against the two bacterial species. The results showed that the crude extracts generally retained their antimicrobial properties even after exposure to the low temperatures for the 5 day period. The antimicrobial activity of the crude extracts was found to be due to presence of bioactive compounds.

The bioactive component profiles of the peel crude extracts were investigated using a high-resolution ultra-performance liquid chromatography system with diode array detection, quadrupole time-of-flight and mass spectrometer (UPLC-DAD-QTOF-MS). Metabolites such as citric acid, ascorbic acid, gluconic acid, malic acid, ρ-coumaric acid, ferulic acid, caffeic acid glucoside, vanillic acid, sinapic acid, rutin, quercetin-3-*O*- rhamnosyl rutinoside, Isorhamnetin-3-*O*- dirhamnosyl glucoside and benzyl glucosinolate were identified. Phenolic compounds were found to be the most predominant in the crude extracts. Overall, the bioactive compounds identified within the crude extracts were organic acids, phenolic acids, flavonols and a glucosinolate. The Folin-Ciocalteu (F-C) technique, the aluminium chloride test and the ferric reducing antioxidant power (FRAP) assay were used to measure the total phenolic (TPC), total flavonoid content (TFC), and metal chelating activity of the crude extracts, respectively. The TPC, TFC and FRAP in the crude extracts were 6865 ± 153 mg GAE/g *dw*, 3638 ± 252 mg QE/g *dw* and 7968 ± 38 mM TE/g *dw*, respectively. The crude extracts therefore showed antioxidant activity.

The presence of the enzymes chymopapain and papain in the crude extracts was determined using SDS-PAGE (Sodium dodecyl-sulphate polyacrylamide gel electrophoresis). This was done to test for toxicity of the crude extracts. Additionally, the BCA (Bicinchoninic acid) assay was used to determine the overall protein concentration in the crude extracts. There were low concentrations of proteins observed in the crude extracts. Ultimately, the absence of enzymes chymopapain and papain in the crude extracts gave evidence that the extracts were not toxic.

To determine the crude extracts' ability to inhibit enzymatic browning, potato and apple pieces were immersed in treatments containing the extracts and these were compared with other standard and control treatments. Browning was measured as change in colour using a CR-400 Chromameter where *Lab* values were obtained and used to calculate the overall colour change (ΔE) and the browning index (BI). Browning was also measured through visual assessment where browning scores (BS) for the apples and potatoes were recorded. The crude extracts

inhibited enzymatic browning but were less effective than the standard treatment where the apples and potatoes had been immersed in a solution containing sodium metabisulphite, citric and ascorbic acids. The ability of these crude extracts to inhibit browning is supported by the presence of antioxidants and their ability to slow down the enzymatic browning process. The outcomes and findings of this research open further avenues and opportunities for the increased utilisation of *C. papaya* peels which is are derived from the consumption of papaya.

Keywords: *C. papaya* peel, crude extracts, antioxidant activity, antimicrobial activity, enzymatic browning

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CHAPTER 1: INTRODUCTION

1.1 Background

Food waste happens throughout agricultural production, manufacturing and processing, transportation, distribution and storage (Zhao et al., 2019) due to inefficiencies along the food supply chain. One-third of food generated for human use is lost or wasted (Vilariño et al., 2017). Food waste is estimated to reach over 1.3 billion tons per year globally (FAO, 2019). This has a major impact on both food security and sustainability of food systems because food waste contributes to inaccessibility and unavailability of food for human consumption which later contributes to malnutrition (MacRae et al., 2016). The Food and Agricultural Organisation (FAO) reported that over 220 million people in Sub-Saharan Africa were malnourished (FAO, 2016). Post-harvest food losses are major contributors to food waste within the food industry. Any loss in quantity or quality that occurs between the time of harvest and consumption is referred to as post-harvest food loss (Buzby & Hyman, 2012). Food waste is described as the loss of edible food as a result of human activity or inaction, such discarding the product due to noncompliance with quality criteria, sell by date, rot, decay or insect infestation (Aulakh et al., 2013). Food prepared and not consumed also falls in this category as is more commonly associated with the hospitality industry or at home level. Food waste leads to a reduction in the amount or quality of food as a result of retailer, food service, and customer decisions and behaviours. The issue to do with food losses and waste is therefore of great significance in the efforts to fight hunger and improve food security especially in developing countries (Kumar & Kalita, 2017).

1.2 Problem statement

Fruits and vegetables (fresh produce) are sources of vitamins and minerals known to have health benefits and are part of a healthy diet. They also contain bioactive compounds and extensive studies on these compounds show their health promoting abilities through antioxidant and antimicrobial properties (Singh et al., 2016). The increase in consumer awareness about the health benefits associated with consumption of fresh produce has led to their increase in production and purchase. However, fresh produce are the major contributors (60%) to food waste globally (Campos et al., 2020). A major contributing factor to fresh produce waste is microbial deterioration. In the world of postharvest technology, the focus is on retaining quality and reducing losses due to postharvest pathogens. Innovative technologies

are developed to address the above. The issue around waste as such is more about the circular economy capturing value. Reduction of fresh produce waste is an approach that has been implemented within the unit operations used for their processing (Plazzotta et al., 2017). The fresh produce industry also employs tactics that rely on the recovery of waste after it has been modified in some way (Manzocco et al., 2017). These can be divided into strategies where the whole waste mass is recycled and specific compounds are extracted. Bioactive compounds, oils, fibres and natural dyes are the major targets of this recycle strategy (Ayala-Zavala et al., 2010). Because of their antioxidant and antimicrobial capabilities, more emphasis has been placed on extracting high-value bioactive compounds (Singh et al., 2016).

During processing, storage and transportation, fresh produce is prone to microbiological attack resulting in spoilage and thus the fresh produce becomes unfit for sale and subsequently consumption (Tarabih & El-Metwally, 2014). Microbial spoilage is ultimately a limiting factor in extending the shelf life of fresh produce (Hodges et al., 2011). To prevent microbial contamination and prolong the shelf life of fresh produce; post-harvest technologies and synthetic preservatives have been used, raising concerns amongst consumers. This is because studies have linked the use of synthetic preservatives to life threatening diseases like cancer and obesity (Suez et al., 2014). Therefore, there is need to implement the use of natural compounds as preservatives to replace synthetic preservatives. Plants are deemed as sources of feasible and natural alternatives to synthetic preservatives (Žugić et al., 2014). In this respect, research interest is geared towards the abundant bioactive secondary metabolites in plants which possess antioxidant and antimicrobial properties for their application in the food industry. Waste from fruits such as papaya contain bioactive compounds that act as preservatives due to their antioxidant and antimicrobial properties (Sacho et al., 2011). *Carica papaya* L. is one of Africa's most underutilized fruits but is planted for local fresh consumption due to its nutritional value (the fruit contains a variety of minerals, bioactive compounds like phytochemicals and vitamins) (Martial-Didier et al., 2017). Its peels and seeds which are by-products could therefore be potential sources of bioactive compounds. Bioactive compounds especially phenolic compounds have been found in papaya peel and seeds (Sofi et al., 2016). Currently, there is not enough information on papaya-extracted bioactive compounds and their application in the food industry. Therefore, this study will focus on investigating whether the antioxidant and antimicrobial properties of papaya peel extracts can prolong the shelf life of fresh cut produce.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The United Nations established an ambitious goal of halving global per capita food waste by 2030, a choice that numerous governments have adopted in light of the growing attention on food waste and loss reduction (FAO, 2019). To effectively achieve this, many international initiatives of relevance have been put in place for example the "Zero Hunger Challenge" that was launched by the United Nations in 2012. The goal of this challenge is to eliminate hunger and malnutrition while also developing sustainable food systems that prevent food waste and losses (FAO, 2018). Along the food supply chain; lack of optimum storage facilities, poor infrastructure, inadequate processing facilities and poor market facilities lead to high food losses in developing countries. According to FAO (2013), 54% of global food waste comes from agricultural production, post-harvest handling and storage while 46% of global food waste originates from processing, distribution, retail and consumption. Most of the food losses generated in Sub-Saharan Africa is from production, storage and handling practices (**Figure 1**). This gives an idea of poor and inefficient post-harvest handling practices within Sub-Saharan Africa.

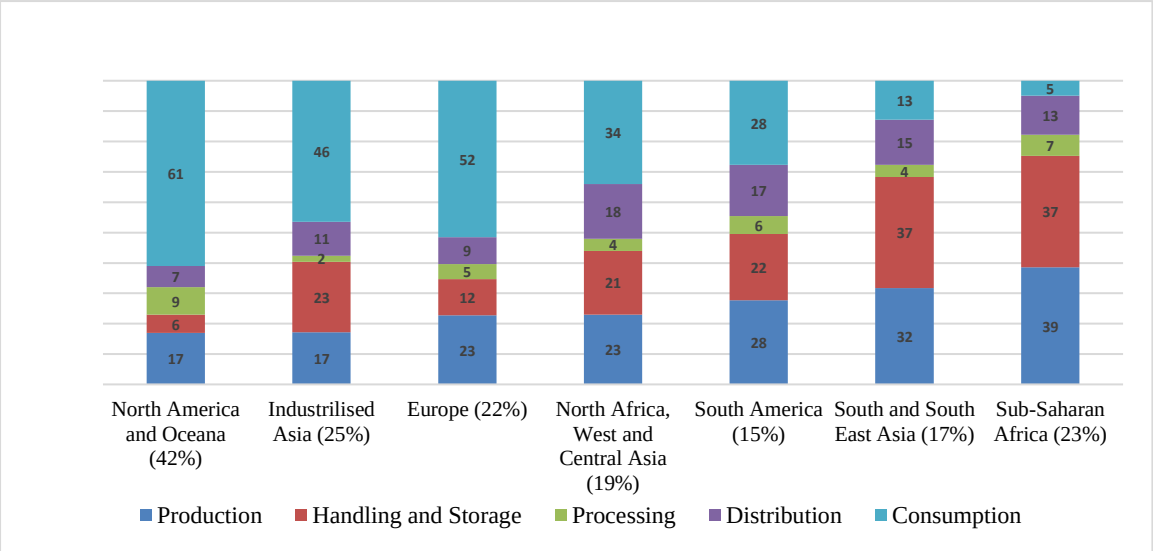


Figure 1: Regional food losses and waste distribution throughout the food supply chain (Adapted from Gustavsson et al., 2011)

Whilst a variety of different foods contribute to global food waste, Campos et al. (2020) highlights fresh produce as a major contributor to waste. Fresh produce waste accounts for 60% of the global food waste (Gustavsson et al., 2011). Garcia-Garcia et al. (2017) has however

reported on how fresh produce by-products can be incorporated back into food systems, highlighting ways in which the management and reduction of this waste can be attained. Fruits contains a variety of bioactive compounds useful in several food and pharmaceutical industrial applications. Focus has been majorly on their antioxidant and antimicrobial characteristics which enable these compounds which range from the least complex phenolic acids to the most complex flavonoids to be applied within the food industry as means of extending the shelf life of different food products. These compounds are distributed throughout the fruit including the non-edible parts (peels, skin, leaves, roots, stems and seeds) of the fruit. Waste from several processed fruits can be recycled to extract active compounds thus making the entire fruit useful.

2.2 Minimally processed fresh cut produce

There is an increase in demand for conveniently prepared fresh produce (peeled and sliced) compared to the processed fresh produce products (canned, dried or frozen) by consumers. According to International Fresh cut Produce Association (IFEA) "*Fresh cut produce are fruits and vegetables which are either trimmed, peeled or cut into 100% usable product that is pre-packaged to offer consumers high nutrition, convenience and flavour while still maintaining its freshness*" (Lockrey et al., 2019). The shift in the demand for fresh produce by consumers is due to the increasing awareness of the health benefits that are associated with fresh produce consumption as well as lifestyle changes (Pimentel, 2018). Fresh produce is high in vitamins and minerals, which have been associated to a lower risk of noncommunicable illnesses including type 2 diabetes, cardiovascular disease, and several malignancies (Aune et al., 2017). These characteristics are all strong selling points for many consumers who are health conscious. Consumers however still want convenience thus minimally processed fresh produce is purchased more than the whole produce (Putnik et al., 2017). Sillani & Nassivera (2015) reported that purchases made on minimally processed vegetables had a stable upward trend despite prices being more than whole fresh products. Research on fresh cut produce pays emphasis on microbiological quality and safety (De Azeredo et al., 2011). The cutting, peeling, grating and shredding processes are some of the unit operations during minimal processing where by-products (mainly peels and seeds) accumulate as waste (**Figure 2**) (Banerjee et al., 2018). Physiological decline, metabolic alterations, and microbiological destruction are all accelerated when fresh food is processed. This causes the commodity's colour, texture, and flavour to alter. While traditional methods like refrigeration and postharvest chemical treatments enhance the shelf life of fresh fruit, the minimum processing that fresh produce

undergoes makes it very perishable, necessitating cold storage to maintain an acceptable shelf life (Pavani et al., 2017).

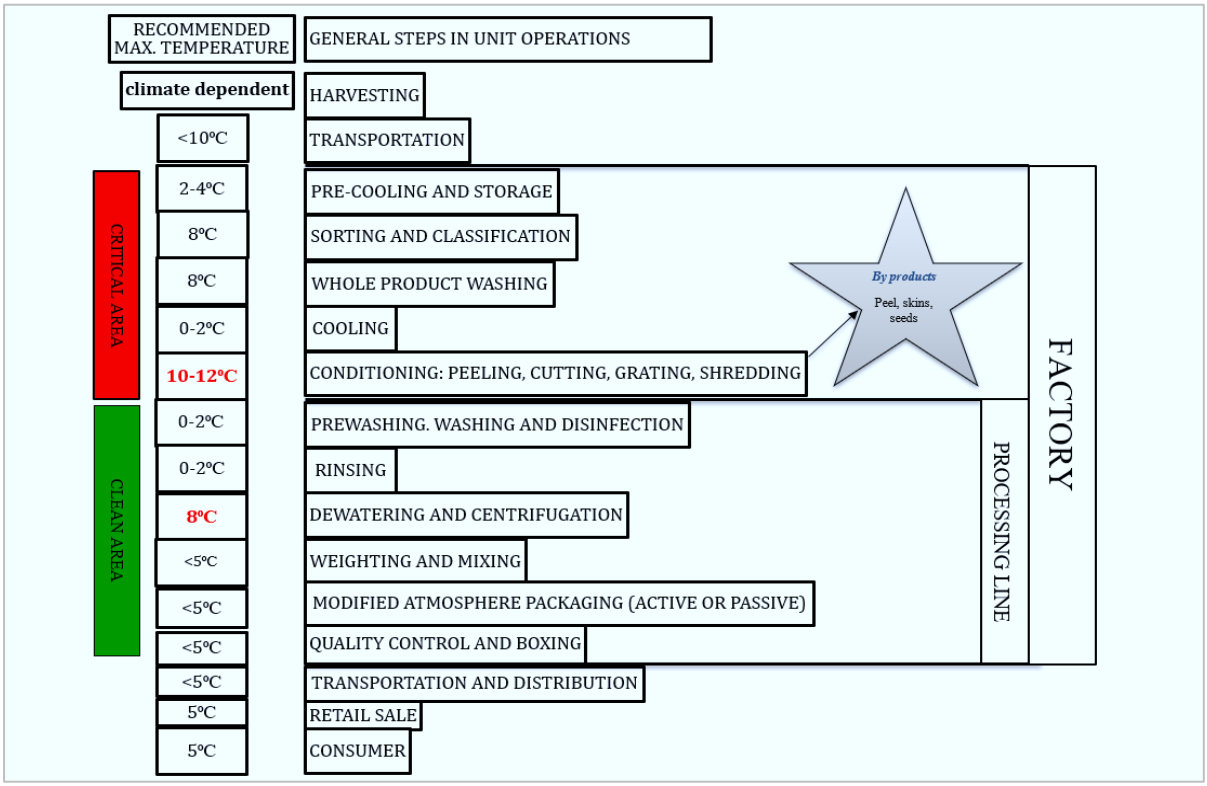


Figure 2: Schematic diagram showing general unit operations in fresh produce treatments (Banerjee et al., 2018)

2.2.1 How pre-harvest and post-harvest conditions affect the quality of fresh produce

Due to production and agronomic practices that result in changes in quality, affecting the suitability for transport, storage stability and shelf life after harvest, conditions and activities during pre-harvest in the field can indirectly lead to losses at later stages within the food chain (Gutwa Oino et al., 2017). A variety of biotic and abiotic factors alter the appearance of fresh produce prior to harvest (Petropoulos et al., 2017). Even with optimal conditions, a fraction of the crop yield is downgraded due to appearance defects. The decisions made during the production stages of crops i.e. the soil type, irrigation and application of fertilisers influence the nutritional quality of the harvested crops (Herencia et al., 2011). Temperature and light intensity, in particular, have a significant impact on the nutritional content of fresh food (Kyriacou & Rouphael, 2018). In this regard, the preharvest phase gives a better understanding

on the quality of the crops prior to harvesting hence farmers have knowledge on how to avoid pre-harvest losses.

Post-harvest factors greatly influence fresh produce quality. Fresh food must be stored at the correct temperature and relative humidity to maintain freshness and reduce post-harvest losses (Elik et al., 2019). Delays in chilling or processing can result in direct losses owing to decay and water loss, as well as indirect losses due to texture, colour, flavour and aroma. The extent to which these losses take place depends entirely on the condition of the commodity at harvest and its temperature which in most cases is higher than the ambient temperature especially when exposed to direct sunlight. The major catalyst in post-harvest losses during harvesting of fresh produce for example fruits like avocados, apples, pears and vegetables like cabbages, spinach, radishes and potatoes is the handling practices (Dodd & Bouwer, 2014). This is where majority of fresh produce waste usually comes from because if damage occurs at this point, there is no unit operation that can reverse this damage.

2.2.2 Microbial deterioration of fresh cut produce

Melons, mangoes, papaya, pineapples, fruit mixes, shredded leafy greens and salad mixes, cooking vegetables such as peeled baby carrots, broccoli and cauliflower florets, sliced sweet potatoes and other fresh cut food are available on the market. Despite the health advantages of eating raw, fresh vegetables, food safety remains a worry because these items are known to transmit non-infectious diseases (Bennet et al., 2018). Rapid microbial development is aided by high water activity and tissue pH that is either neutral (vegetables) or low acidic (many fruits) (Siddiqui & Ali., 2017). These properties are critical for microbe development, resulting in fresh sliced produce deteriorating faster than whole fresh produce. Microorganisms are present on the surface of the fruit and when the epidermis is removed, microbial contamination of the flesh occurs. The natural microflora of fresh cut produce contains bacteria, yeast and moulds. This is dependent on the type of fresh produce, the environment, season and the conditions under which it is grown (Leff & Fierer, 2013).

The key characteristics of fresh cut or minimally processed fresh produce that increase the occurrence of microbial contamination include cut surfaces, inherent high moisture content and the active metabolism of the plant tissue (Berger et al., 2010). There are various unit processes in the fresh cut produce manufacturing chain, and each of these has the potential for microbial contamination (**Figure 2**). Fresh cut fruits and vegetables experience increased deterioration and quality loss as a result of processing and storage. Damage to fresh produce tissues results

in the loss of nutrients and cellular fluids, which serve as microbial growth substrates (Heard, 2002). Due to the passage of bacteria from the surface to the fruit flesh, which functions as a full substrate for microbial growth, minimal processing may enhance the microbial contamination of fresh cut produce.

2.2.2.1 Microbial contamination as a limiting factor in extending the shelf life of minimally processed produce postharvest

Fresh cut produce is contaminated by a range of human pathogens, and the incidence of food-borne outbreaks linked to fresh and fresh cut vegetables has increased in recent years. For example, in 2018, an outbreak due to romaine lettuce-associated *Escherichia coli* O157:H7 in the United States resulted in more than 200 illnesses (Baber, 2018). Salmonellosis caused by *Salmonella* has been linked to the eating of fresh cut fruits in a number of cases (Sim et al., 2013).

The presence of microbial pathogens except moulds cannot be detected by consumers and requires further testing to determine their presence. The occurrence of spoilage microbes can be observed because their presence often produces effects that are seen, smelled or tasted and in most cases indicate the presence of microbial pathogens (Kaczmareck et al., 2019). Controlling pathogens and spoilage bacteria is therefore important. *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* are common pathogens linked to human illnesses upon consumption of contaminated food (Deering et al., 2012). The physical properties (no protective epidermis and high moisture), biochemical characteristics (browning and degreening), physiological characteristics (enhanced ethylene and respiration rates) and handling environment (processed and stored at refrigerated temperatures and packed under modified atmospheres) of fresh cut produce differ significantly from whole produce (Ergun, 2006). These variations have an impact on the microflora that can colonize the skin and flesh. Microbial levels on the outer surface may decline in some cases, and the pace of reduction is determined by the type of product and the unit procedures used (Kaczmarek et al., 2019). Freshly cut produce has a water activity of 0.90-0.99 and nutrients, such as vitamins and sugars, have pH values greater than 4.5. These qualities make them ideal hosts for bacteria, since they provide ideal conditions for survival and development (Francis et al., 2012).

Refrigerated conditions and modified atmosphere conditions used during the packaging of fresh produce enable bacteria to successfully survive in these foods because majority of the bacterial species that are responsible for postharvest spoilage of refrigerated fresh cut produce

are psychrotrophic (Jideani et al., 2017). Within the minimal processing plant, a disinfection step is incorporated as a critical control point especially after the cutting and peeling processes. This is done to avoid any microbial contamination given that during conditioning, the temperatures are slightly higher, and the machinery used might also be possible sources of contamination. Direct handling with hands is a risk due to possible contamination with human-associated pathogens like *Staphylococcus*, *E. coli*, *Salmonella*, *Shigella* etc. The disinfection step, therefore, proves necessary and the most common form of disinfectant used is chlorine water at 0.2-0.4 mg/L due to its efficiency and affordability.

2.2.3 Deterioration of fresh cut produce through enzymatic browning

One of the key attributes that characterizes the freshness of most fresh vegetables is colour. The discolouration of fresh cut produce due to injuries acquired because of physical alteration is a key issue in minimal processing of fresh produce (Wiley, 2017). This may be seen in fruits like avocados, apples, bananas and pears, as well as vegetables like potatoes, eggplants and carrots. Discolouration of the product causes quality deterioration resulting in its rejection by consumers. Polyphenol oxidase (PPO) is a naturally occurring enzyme found within plastids (double membrane organelles) in fresh produce which brings about the brown colour of minimally processed fresh produce (Singh et al., 2018). It contains two copper atoms at the active site. The mechanism of action of PPO involves its ability to oxidize phenolic compounds in plant tissues (**Figure 3**). The rupturing of plastids produces PPO when plant tissue is damaged. This results in the enzyme interacting with the phenolic compounds released by the rupture of the cell vacuole which is the main storage organelle of these compounds (Singh et al., 2018).

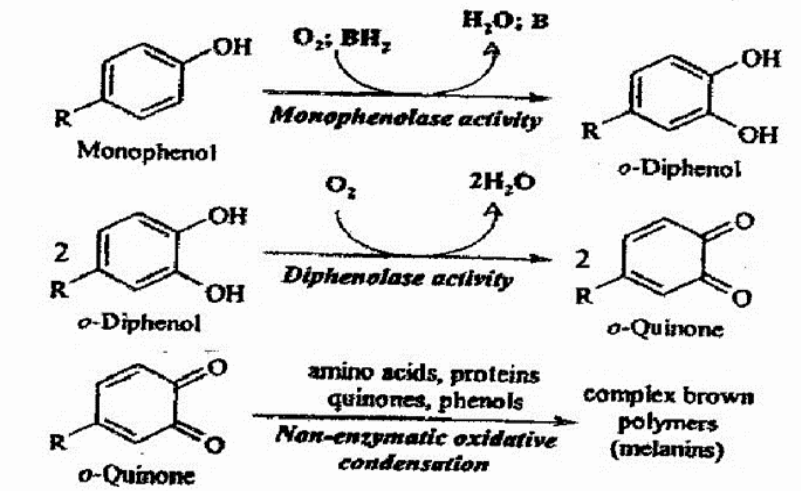


Figure 3: Mechanism of action of PPO during enzymatic browning (Adopted from Singh et al., 2018).

Synthetic antioxidants and chemicals such as Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), sulphites, nitrates and benzoates have been used to prevent the browning process of fresh produce during and after minimal processing. Due to concerns by consumers about the health implications these synthetic additives have, using more natural-based additives from plant sources has been adopted (Saeed et al., 2019).

To prevent the enzymatic browning response caused by PPO in fresh cut produce, phenolic substances form stable phenoxyl radicals by reacting the radical molecule with an antioxidant molecule. This makes initiating a complicated chemical cascade that results in the formation of reactive oxygen species (ortho-quinones) challenging. Phenolic compounds may also operate as hydrogen donors, supplying atoms directly to the radicals needed for browning. Chelating bivalent transition metal ions can efficiently minimize oxidation by preventing the oxidative processes that lead to the generation of hydroxyl radicals and the breakdown of hydroperoxides. The enzyme PPO contains copper ions and the binding effect of these ions by phenolic compounds lowers enzyme activity (Singh et al., 2018).

2.3 Synthetic additives versus natural additives

Chemical additives are used in foods to maintain and/or improve biological, physicochemical, rheological and sensory qualities, as well as to increase shelf life. Different compounds with specific functionalities are used to achieve these objectives to promote quality in processed foods (Martins et al., 2019). Synthetic additives are added to prevent quality degradation.

However, these compounds have life-threatening side effects and are reported to be carcinogenic (den Braver-Sewradi et al., 2020).

According to the Codex Alimentarius (FAO/WHO, 2016), "*a food additive is a compound not typically consumed as a food on its own and is not normally used as an ingredient in the food but is intentionally added in the manufacture, processing, preparation, treatment, packaging, transport and holding of the food, to perform a technological function (including organoleptic)*". The diversity of technology and changes in consumer consumption patterns has fuelled the use of food additives in the food industry. In this way, there has been development and standardisation of new formulations, reduction in losses, extension of shelf life and an increase in the quality of food products (Gokoglu, 2019).

Food additives have been classified into preservatives (antimicrobial agents and/or antioxidants), texture developers, colourants, nutritional value protectors and developers (Carocho et al., 2015). Most of these additives have more than one function. Fruits have a variety of value high-end additive compounds that can be obtained from their by-products. Natural antioxidants and antimicrobials of biological origin have gained considerable attention due to their reported safety, potential nutritional and therapeutic value and can be recovered from fruit waste (Mărgăoan et al., 2021). The increased interest in natural preservatives has led to the antioxidant and antimicrobial assessment of many classes of fruits, vegetables, herbs, spices and cereals. Much attention has been paid to fruits and fruit waste as they are a rich source of phenolic compounds (Swallah et al., 2020; Sir Elkhatim et al., 2018).

2.3.1 Fruit waste derived additives

The consumption and production of fruits has increased significantly both on local and international markets because of their nutritional and therapeutic value and attractive sensory properties (Abstract of Agricultural Statistics, 2020). Processing of fruit juice from fruits like pineapples, mango, papaya and oranges involves separating the flesh from the peels and seeds by-products that are undesirable. The mass of by-products obtained from fruit processing is usually more than the corresponding valuable flesh (da Silva et al., 2014). Previously this problem was dealt with by processing these by-products further to produce high-end products like nutraceuticals (Gorinstein et al., 2011). Apart from these products, fruit by-products have the potential to develop food additives. These food additives are in the form of antioxidants, antimicrobials, colourants, flavourings and thickeners. Vitamin C, a natural compound common in most plant tissues is a good example of a by-product that is used in the food industry

(Ayaza-Zavala et al., 2009). Demand for more natural raw food components has prompted increasing study into recovering natural value-added chemicals from fruit waste (Mahato et al., 2018). By-products from the processing of papaya, pineapple and mango account for 10-60% of their total weight (Ayala-Zavala et al., 2010)

During papaya processing, peel and seeds are the major by-products and these are not exploited for any commercial purpose, thus are disposed of as waste (Cheok et al., 2018). Santana-Méridas et al. (2012) reports that papaya peels and seeds contain phenolic compounds which possess antioxidant and antimicrobial properties.

Table 1: Proposed properties of different fruit by-products

Type of fruit	Scientific name	Common name	Nature of by-product	Proposed properties	References
Citrus	*Citrus sinesis*	orange	peel, seeds	Antioxidant, antimicrobial, flavouring, acidulants	Bustamante et al., 2016; Pathak et al., 2017, Hong et al., 2017; Pathak et al., 2017, Wu et al., 2017
	Citrus limon	lemon			
	Citrus paradisi	grapefruit			
Tropical	*Magnifera indica*	mango	peel, seed kernel, solid residues	Flavouring, colourant, antioxidant, antimicrobial	Araya-Cloutier et al., 2012; Dorta and Sogi, 2017, Rivera-Pastrana et al., 2010; Sancho et al., 2011
	Carica papaya	papaya			
	Ananas comosus	pineapple			
Berries	*Fragaria ananassa*	strawberry	Pomace, seeds, skin	Antioxidant, colourant, antimicrobial	Altunkaya et al., 2013; Avalos-Llano et al., 2018 Bhol et al., 2016; Hwang et al., 2019; Szymanowska & Baraniak, 2019
	Punica granatum	pomegranate			
	Rubus idaeus	raspberry			

Stones	*Prunus armeniaca*	apricot	seeds, pomace	Antioxidant, colourant	Cantin et al., 2009; Dragović-Uzelac et al., 2009; Melgarejo et al., 2014; Sochor et al., 2010
	Prunus persica	peach			
Vines	*Vitis vinifera*	grape	seeds, pomace, skin	Antimicrobial, antioxidant, colourants, acidulants	Hayta et al., 2014; Prudencio et al., 2008; Ramli et al., 2020
	Passiflora ligularis	passion fruit			
Pomme	*Malus domestica*	apple	peel, pomace	Antioxidant, antimicrobial	Bchir et al., 2014

2.3.2 Bioactive compounds in fruit and fruit waste

Bioactive compounds are present in foods though in small quantities, primarily in fruits, vegetables and cereals (Hamzalıoğlu & Gökmen, 2016). Agriculture generates a lot of waste, which contains many useful bioactive compounds, the majority of which are phenolic compounds including phenolic acids, flavonoids, procyanidins and anthocyanins, as well as other compounds like carotenoids and saponins (Nguyen, 2017). These compounds have a variety of biological activities; including antioxidant, antimicrobial, anti-inflammatory and anti-cardiovascular properties (Santana-Méridas et al., 2012).

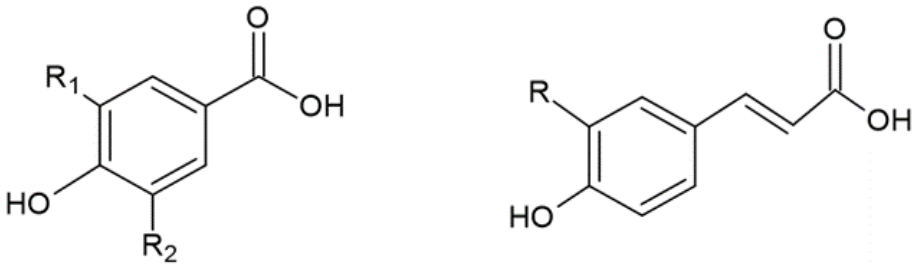
Epidemiological studies demonstrate that eating a lot of foods high in bioactive compounds with antioxidant activity is excellent for your health and lowers your risk of non-communicable diseases (de Melo Ribeiro et al., 2019; Nediani et al., 2019; Siriwardhana et al., 2013). The recovery of bioactive compounds from plant materials offers a solution to the problem of food waste. This is because many of the by-products of fruit and vegetable processing that in most cases would be disposed of can be further processed to obtain these high-end useful compounds.

2.3.2.1 Phenolic compounds

Phenolic compounds in fruit by-products mostly include phenolic acids and flavonoids, which have potential antioxidant and antimicrobial properties. The chemistry and content of phenolic acids and flavonoids in fruit waste is discussed below.

2.3.2.1.1 Chemistry of phenolic acids and their content in fruit by-products

Phenolic acids are "secondary plant metabolites" found in different kinds of fruit by-products (Daud et al., 2017; Roda et al., 2017). They are organic acids that possess one carboxylic acid group (Robbins, 2003). Phenolic acids are divided into hydroxybenzoic acid and hydroxycinnamic acid derivatives (**Figure 4**). Hydroxybenzoic acids have a phenol ring with a carboxylic group attached forming a C6-C1 backbone (**Figure 4**). The most common hydroxybenzoic acids encountered are "ρ-hydroxybenzoic, protocatechuic, vanillic and syringic acids" (Kumar & Goel, 2019). Hydroxycinnamic acids on the other hand have a chemical backbone with a phenylpropanoid C6-C3 structure (Teixeira et al., 2013) (**Figure 4**). "The most abundant hydroxycinnamic acids present in plants are ferulic, caffeic, ρ-coumaric and sinapic acids" (Kumar & Goel, 2019). The number and placements of the hydroxyl groups on the aromatic ring determine the distinctions between hydroxycinnamic acids and hydroxybenzoic acids, despite the fact that their basic skeletons are identical (Robbins, 2003).



Hydroxybenzoic acids Hydroxycinnamic acid

Figure 4: The two different classes of phenolic acids

Pinsirodom et al. (2018) reported that the Khiew Sawoey mango seed variety contained "334.94 mg/100g ρ-coumaric acid, 826.36 mg/100g cinnamic acid and 283.98 mg/100g ferulic acid" while the Kaew Dum mango peel variety contained about "30.09 mg/100g caffeic acid, 158.72 mg/100g cinnapic acid and 73.30 mg/100g ferulic acid". Bitter orange peel was reported to contain about "0.03 mg/g gallic acid, 0.02 mg/g hydroxybenzoic acid, 0.12 mg/g chlorogenic acid, 0.34 mg/g ρ-coumaric acid and 0.33 mg/g ferulic acid" (Marzouk, 2013). Meral & Köse (2019) also reported that adding grape seed to bread increased the content of the phenolic compounds Additionally, bread with pomegranate seed contained "0.04 mg/kg more ferulic acid and 0.41 mg/kg more gallic acid" than bread without pomegranate seed.

2.3.2.1.2 Chemistry of flavonoids and their content in fruit waste

Flavonoids are secondary plant metabolites characterised by a C6-C3-C6 backbone structure labelled A, B and C (Zhang et al., 2018) (**Figure 5**).

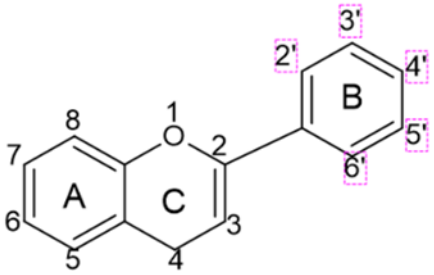
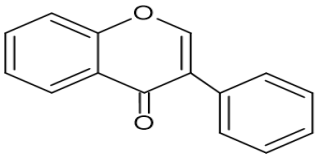
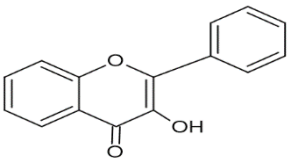
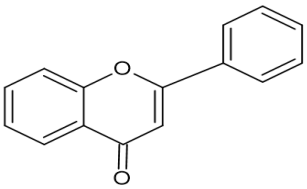
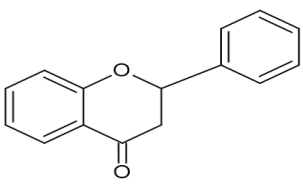
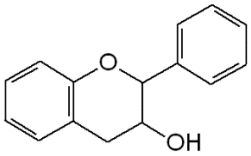
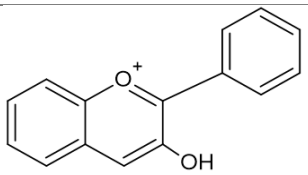


Figure 5: General flavan backbone of flavonoids

Flavonoids are further divided into isoflavones, flavan-3-ols, flavonols, flavones, flavanones and anthocyanidins (**Table 2**). These sub-groups are due to the level of oxidation or the different substitutions around the C-ring (Stalikas, 2007). The different R-group constituents (hydroxyl, hydrogen, methoxyl and *O*-glucosides) on the flavonoid backbone characterise their chemical activity including antioxidant activity. Omoba et al. (2015) reported the flavonoid content in orange peel as 12.49 mg/g for catechin, 14.03 mg/g for quercetin, 17.93 mg/g for rutin and 3.76 mg/g for kaempferol.

Table 2: Different classes of flavonoids

Flavonoid group	Chemical structure	Examples
Isoflavone		genistein, glycitein
Flavonol		quercetin, kaempferol, myricetin
Flavone		luteolin, apigenin
Flavanone		naringenin, hesperetin

Flavan-3-ol		catechin, epicatechin
Anthocyanidin		cyanidin, malvidin, petunidin, peonidin

2.3.3 Antioxidant activity of bioactive compounds

In the fresh produce industry, some important traits that affect consumers' acceptance of the products are sensory quality and appearance with regards to colour, overall flavour and texture (Fallik & Ilic, 2018). The shelf life of minimally processed fresh produce is limited due to exposure to aerobic conditions and therefore antioxidants inhibit a cascade of biochemical reactions that result in deterioration of the food products (Singh et al., 2018). Browning reactions occur as a result of mechanical injuries and tissue damage of plant cells during minimal processing causing the interaction between enzymes and phenolic compounds (Singh et al., 2018). Bioactive substances have been shown to exhibit antioxidant activity and are able to prevent enzymatic browning via a variety of pathways, making them a viable alternative to synthetic antioxidants (Saeed et al., 2019).

2.3.3.1 Proposed mechanisms of antioxidant activity

2.3.3.1.1 Free radical scavenging

The principal degrading process in foods is oxidation by molecular oxygen, which happens when electrons are transferred from a substrate to an oxygen atom present, resulting in free radicals. Phenolic compounds are antioxidants due to their ability to donate electrons and prevent oxidation. The antioxidant activity of phenolic compounds is related to their ability to scavenge radicals, chelation of metals like iron and their structures, particularly their side chains and substitutions on their aromatic ring structures (Rice-Evans et al., 1997).

Due to their unpaired electrons, free radicals are extremely reactive towards other molecules, increasing oxidation processes in vitamins, natural colours, and fatty components, resulting in nutritional value loss and the generation of unpleasant flavour and odour (Damodaran & Parkin, 2017). Antioxidants, which interact with free radicals and oxygen, are added to foods to avoid or reduce these reactions. The presence of phenolic hydroxyl groups is a crucial

structural characteristic for phenolic compounds' antioxidant action **(Figure 4)**. Generally, an increase in these hydroxyl groups in phenolic compounds, increases the antioxidant activity of the compounds (Rice-Evans et al., 1997). In comparison to Trolox, the antioxidant capacity of cinnamic acid derivatives showed that they are more effective than their benzoic equivalents. (Natella et al., 1999). This is because the double bond in the cinnamic acid derivatives contributes to the stabilization of any free radicals by resonance **(Figure 4)**. Likewise, the electron withdrawing carboxylic group has a negative influence on the H-donating ability of the phenolic ring.

2.3.3.1.2 Metal chelating ability

The efficiency of phenolic compounds in chelating of metals contributes to their antioxidant nature. Transition metals like iron and copper present in plants including fresh produce are involved in the generation of reactive oxygen species (Djenidi et al., 2020). Chelating these metals decreases the formation of reactive oxygen radicals.

$$Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^{\cdot -}$$

$$Fe^{2+} + O_2^{\cdot -} + 2H^+ \longrightarrow Fe^{3+} + H_2O_2$$

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{3+} + {\cdot}OH + OH^-$$

$$Fe^{3+} \longrightarrow Fe^{2+} \text{ (in the presence of a phenolic compound)}$$

Oxidation reactions of iron in the presence of phenolic antioxidants

Binding of iron to flavonoid antioxidants suppresses the accessibility of the iron to oxygen molecules. Binding of ligands to Fe^{2+} ions alter the redox potential due to the conversion of the ferrous ion to the ferric state thus inhibiting oxidative damage (Khokhar & Apenten, 2003). Phenolic compounds also reduce iron and form unreactive Iron (II)-polyphenol complexes. This explains why complexation of Fe^{3+} can inhibit oxidative damage even though the radical generating species is Fe^{2+}.

2.3.4 Antimicrobial activity

Amongst polyphenols, flavan-3-ols, flavonols and tannins have received a lot of attention due to their wide spectrum and antimicrobial activity in comparison with other polyphenols (Biharee et al., 2020; Prabakaran et al., 20018; Daglia, 2012). However, the antimicrobial activity mechanism of these phenolic compounds has not yet been fully determined. The differences in structure of polyphenols makes it difficult to predict the exact features of each

class of polyphenols that accounts for the antimicrobial activity (Papuc et al., 2017). The antimicrobial action of polyphenols (particularly flavonoids) is attributed to the hydroxyl groups attached to both the aromatic rings and the oxygen substituted ring, according to Kumar & Pandey (2013). Furthermore, microbicidal or inhibition effects are dependent on the dosage of polyphenols applied (Kao et al., 2010).

2.3.4.1 Proposed mechanisms of antimicrobial activity

2.3.4.1.1 Inactivation of microbial proteins

Polyphenols elicit their microbicidal or inhibitory effect through interactions with microbial membrane enzymes and proteins (Matsumoto et al., 2012). Kumar & Pandey (2013) suggests that antibacterial flavonoids have multiple cellular targets. Flavonoids work by forming complexes with proteins by nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as through covalent bond formation. As a result, their antimicrobial mechanism of action is linked to their capacity to inactivate microbial adhesins, enzymes and cell envelope transport proteins (González et al., 2016). Polyphenols, particularly flavonoids, reduce the fluidity of the outer and inner layers of the membranes, resulting from changes in the membrane fluidity within hydrophilic and hydrophobic regions of microbial cell membranes (Saidi Merzouk et al., 2017).

2.3.4.1.2 Binding of iron

Polyphenols interact with iron which is an essential nutrient for survival of most bacterial species (Papuc et al., 2017). Iron is required for the activity of enzymes and proteins involved in the neutralization of damaging oxidative species (superoxide dismutase), DNA synthesis, and ATP creation, all of which are essential for bacterial replication. Iron deficiency affects these activities in bacterial cells, which has a negative impact on the bacteria's capacity to produce virulence and survive (Haley & Skaar, 2012).

2.3.4.1.3 Interactions with microbial membranes

Matsumoto et al. (2012) and Cho et al. (2007) investigated the interaction of polyphenols, particularly catechins, with bacterial membranes. Polyphenols have been shown to interact with proteins and phospholipids in the lipid bilayer. Interaction with membrane proteins disrupts the lipid bilayer in both gram positive and gram negative bacteria, increasing membrane permeability, modifying membrane fluidity, slowing respiration and changing ion transport mechanisms (Nazzaro et al., 2013).

By using transmission electron microscopy to explore the antimicrobial activity of tea polyphenols, Yi et al. (2014) discovered that bacterial cell membranes are damaged, with increased outer and inner membrane permeability, ruptured cell membranes, and the release of tiny cellular content. Tian et al. (2012) discovered that phenolic chemical interactions with the fungal plasma membrane result in the formation of lesions inside the membrane. The development of granules in the cytoplasm of fungi, rupture of the membrane and inhibiting the synthesis of enzymes upon treatment with bioactive compounds is accountable for the antimicrobial mode of action (Tejirian & Xu, 2011).

2.3.4.2 Application of plant bioactive chemical substances or plant extracts as food preservatives

Owing to the safety and toxicological concerns regarding synthetic preservatives and consumer awareness, the demand for natural ingredients and food preservatives has increased. Plants provide a better option because of their abundance in bioactive compounds which can also act as natural preservatives in many food products thus leading to the development of clean label products (Aguilar et al., 2019). In this context plant extracts are gaining a wide interest in the food industry for their potential as antimicrobials and antioxidants since they are also Generally Recognised as Safe (Vinceković et al., 2017). Therefore, plant extracts including those from fruits and fruit by-products as preservatives have greater application potential in the food industry. Extracts from plant by-products have already been introduced within the food industry. Panza et al. (2021) showed how pomegranate by-products were used to prolong the shelf life of breaded cod stick. Okunowo et al. (2013) also showed that essential oils from grape fruit peel are potential antimicrobial preservatives in ham.

2.4 Papaya (*Carica papaya* L.)

2.4.1 Production and utilisation

Papaya (*Carica papaya* L.) is a “herbaceous plant grown in the tropical and sub-tropical regions of the world”, including South Africa. Papaya fruit can be described as an easy-to-cultivate and fast-growing crop in conditions such as good drainage, good aeration and rich in organic matter soils. Some species can also withstand the extreme humidity seen in tropical climates (Silva et al., 2007). In 2017, the global yearly output of papaya fruit was 13 016281 metric tonnes, according to Etim et al. (2021). Papaya fruit is consumed in a variety of ways including the fruit as a whole or as juice or can be dried and then milled into flour and added to porridges

(Yogiraj et al., 2014; Boshra & Tajul, 2013). The mature fruit is also used in jams, pickles, and sweets, while the unripe fruit is frequently used in Thai and Vietnamese cuisine and cooked as a vegetable (Silva et al., 2007). Papaya has a relatively high and fast post-harvest loss (Bantayehu & Bizuayehu, 2017). According to a study by Seid et al. (2013), "post-harvest loss of papaya is 1.5%, 1% and 3.3% at farmer's level, transportation and storage, respectively".

2.4.2 Bioactive compounds in *Carica papaya* and its peels

Both the pulp and the waste of papaya contain a number of beneficial bioactive compounds. The bioactive compounds present that have been reported in papaya and its by-products are summarised in **Table 3**.

Table 3: Bioactive compounds that have been identified in different parts of papaya fruit

Compound group	Compound extracted	References
Phenolics	**Pulp:** Ferulic acid, caffeic acid, gallic acid, vanillic acid, protocatechuic acid, artepillin C, kaempferol, quercetin **Peel:** Ferulic acid, caffeic acid, *p*-coumaric acid, rutin, quercetin, isorhamnetin, myricetin **Seed:** Caffeic acid, chlorogenic acid, gallic acid, *p*-coumaric acid, salicylic acid, vanillic acid, myricetin, hispidulin	Rodrigues et al., 2019; da Silva et al., 2014; Rivera-Pastrana et al., 2010
Glucosinolates	**Pulp, seed and peel:** Benzyl glucosinolate, benzyl isothiocyanate	Nguyen et al., 2013; Li et al., 2012
Carotenoids	**Pulp:** Lycopene, β-carotene, β-cryptoxanthin, lutein, α-cryptoxanthin **Peel:** Lutein, zeaxanthin, β-carotene, β-cryptoxanthin	Calvache et al., 2016
Organic acids	**Pulp:** Malic acid, fumaric acid, oxalic acid, tartaric acid, citric acid **Peel:** Ascorbic acid, propanoic acid, butanoic acid, butyric acid	Wu et al., 2019; Hernández et al., 2009

Papaya also has phenolic compounds that have antimicrobial and antioxidant properties. The most common phenolics in papaya fruit peel are rutin, ferulic, caffeic and ρ-coumaric acids (Rivera-Pastrana et al., 2010). "The ranges of content were 1.33-1.62 g/kg dry weight, 0.46-0.68 g/kg dw, 0.14-0.23 g/kg dw and 0.10-0.16 g/kg dw for ferulic acid, caffeic acid, ρ-coumaric acid and rutin, respectively" (Sacho et al., 2011; Rivera-Pastrana et al., 2010). Fruits have higher quantities of phenolic compounds in the peel than in the mesocarp and hydroxycinnamic acids are more abundant in fruits than hydroxybenzoic acids (Castillo-Munoz et al., 2009; Gancel et al., 2008).

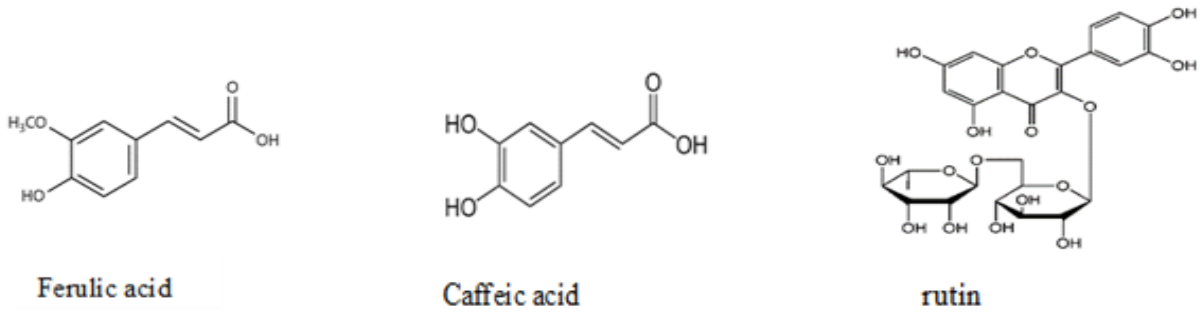


Figure 6: Structures of ferulic acid, caffeic acid and rutin

2.4.3 Toxicity of papaya waste products

There is concern about the toxicity of papaya by-products and the harm they can lead to upon consumption. Papaya seeds have raised more concern because they contain latex, a rich source of the four cysteine proteinases: papain, chymopapain, glycyl endopeptidase and caricain (Nunes et al., 2013). These enzymes are known to cause strong uterine contractions, explaining abortion (miscarriage) in pregnant women upon consumption of unripe papaya (Anuar et al., 2008). Ripening plays an essential role in the amount of latex within papaya. Peels from ripe papaya have been reported to be less toxic than unripe peels and seeds (Ang et al., 2012n an in vitro study conducted by Adebiyi et al. (2002), ripe papaya (0.1-0.8 mL) had no significant contractile effect on the uterine smooth muscles isolated from pregnant and non-pregnant rats; however, crude papaya latex (0.1-3.2 mg/mL) from unripe papaya induced sporadic contraction in the uterine muscles, similar to oxytocin and prostaglandin, hormones that play a vital role in uterine contractions Therefore, a concentration of papaya latex above 0.1 mg/mL in unripe papaya is reported to be toxic while ripe papaya contains no latex but has abundant bioactive compounds that have found application within the food industry.

2.5 Conclusion and gaps in knowledge

Beneficial bioactive compounds with regards to their health promoting properties are studied giving many reasons as to why fruits and vegetables need to be incorporated into our daily diets. Multitudes of waste and losses however occur due to various factors that occur during pre-harvest and post-harvest stages. These factors include but are not limited to processing and microbial deterioration. Furthermore, due to consumer demand for convenience, there has been a spike in the production of minimally processed fresh produce. This has led to by-products which result from the unit operations of minimal processing which contribute significantly to food waste. These by-products nevertheless can undergo further processing to obtain bioactive compounds which can be used as alternative preservatives for many food products. There is not much information available on how these natural compounds obtained can be incorporated into minimally processed fresh produce to prolong the shelf life of the food product while maintaining its quality. Papaya peel crude extract will therefore be used in this study as a source of bioactive compounds. As there are no significant studies on the application of bioactive compounds extracted from papaya into the preservation of minimally processed fresh produce, this study will aim at characterizing such properties.

CHAPTER 3: HYPOTHESES, AIM, OBJECTIVES AND EXPERIMENTAL DESIGN

3.1 Hypotheses

I. Crude extracts from *C. papaya* peel will show antioxidant activity and exert antimicrobial activity against pathogenic bacteria such as *E. coli* and *L. monocytogenes* because plant extracts have been shown to contain several chemical substances, including phenolic compounds(Mekonnen et al., 2018; Williams et al., 2013; Rivera-Pastrana et al., 2010) which have been found to exhibit antioxidant and antimicrobial properties. (Gómez-García et al., 2019; González et al., 2016; Allen, 2015; Borges et al., 2015; Matsumoto et al., 2012).

II. Crude extracts from *C. papaya* peel applied onto fresh cut produce will exert equal or higher antioxidant activity against enzymatic browning than ascorbic acid because they contain natural antioxidant compounds including phenolic compounds and organic acids which have been reported as inhibitors of PPO, a crucial enzyme in the enzymatic browning process(Moon et al., 2020; Singh et al., 2018; Hithamani et al., 2018; Wessels et al., 2014; Coultate, 2009).

3.2 Aim

The aim of the study is to investigate whether bioactive compounds in *C. papaya* peels have both antioxidant and antimicrobial properties and can preserve minimally processed produce.

3.3 Objectives

The objectives of the study were:

I. To determine the phytochemical quality (phenolic composition, antioxidant activity and antimicrobial activity) of the peel crude extracts.

II. To determine the activity and stability of the peel crude extracts as anti-browning agents on fresh cut apples and potatoes (due to the presence of PPO in these food models) under minimal processing conditions.

3.4 Experimental design

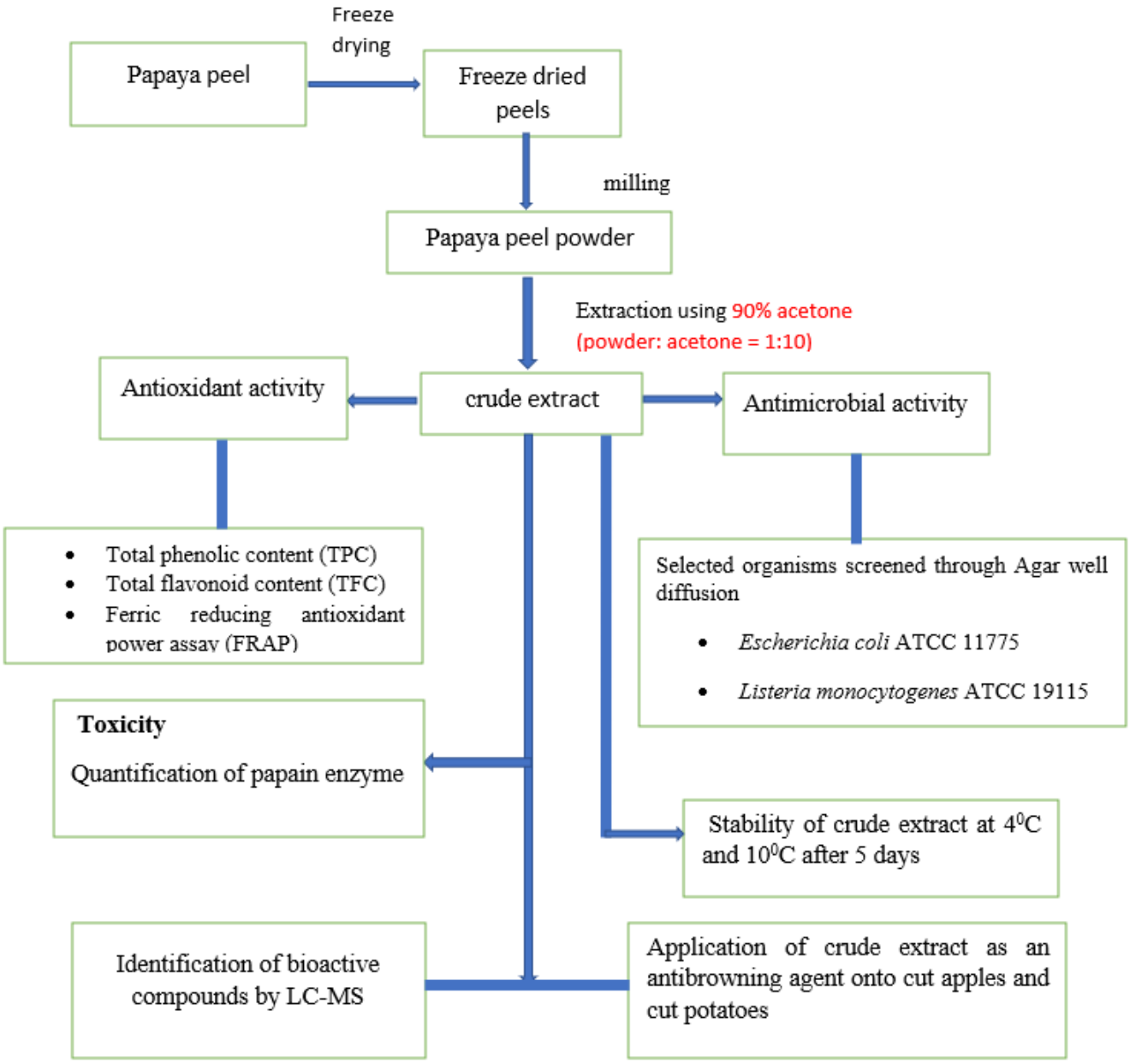


Figure 7: Experimental design for the determination of the antioxidant and the antibacterial activity of Carica papaya crude extracts in minimally processed apples and potatoes

CHAPTER 4: RESEARCH

The research conducted for this dissertation is organised in two chapters. The first chapter reports on the phytochemical composition of *C. papaya* peel crude extracts and screening of their antioxidant and antimicrobial properties. The second chapter reports on the application of the papaya peel crude extract in minimally processed produce.

4.1 Bioactive compounds, antioxidant and antimicrobial properties of *Carica papaya* peel crude extracts

Abstract

Carica papaya peels are a by-product obtained through processing of the fruit. These peels though considered waste can be utilised for their beneficial properties that include but are not limited to antioxidant activity, antimicrobial activity, anti-inflammatory, enzymatic and as colourants. *C. papaya* peel crude extracts were studied for antioxidant and antimicrobial properties. Their bioactive compounds were also identified and characterised and their toxicity investigated. The peel crude extract analysis for antimicrobial activity was done using the agar well diffusion method. The antioxidant properties of the crude extracts were analysed in terms of; the total phenolic content using the Folin-Ciocalteu assay, the total flavonoid content using the aluminium chloride calorimetric assay and metal chelating capacity through the Ferric Reducing Antioxidant Power assay. Quantification of the flavonoids and phenolic acids in the peel crude extracts was determined using Ultra-Performance Liquid Chromatography-Mass Spectrometry. The toxicity of the crude extracts was indirectly determined through the presence or absence of enzymes, chymopapain and papain which was done using the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. In the current study, analyses showed that the peel crude extracts obtained from peels of *C. papaya* had antimicrobial and antioxidant properties. Additionally, these crude extracts contained organic acids, phenolic compounds (phenolic acids and flavonoids) and benzyl glucosinolate that were identified. The absence of enzymes chymopapain and papain showed that the crude extracts were not toxic. The peel crude extracts also showed activity against *L. monocytogenes* and *E. coli* and were stable at 4°C and 10°C. Ultimately, the crude extracts showed no toxicity and possessed antimicrobial and antioxidant activity. These characteristics strongly suggest that the crude extracts could potentially be used within the food industry as antioxidants or antibacterial agents.

Key words: *C. papaya* peel crude extracts, antibacterial activity, antioxidant activity, toxicity, bioactive compounds

4.1.1 Introduction

Carica papaya (papaya) is an under researched and mostly underutilised tropical fruit in the Southern African region (Ngemakwe et al., 2017). Carbohydrates make up the majority of the dry matter in papaya fruit, with sucrose and glucose accounting for 80% of the total sugars. The remaining 20% of the carbohydrates comprise of insoluble and soluble dietary fibre (Martial-Didier et al., 2017). Micronutrients such as sodium, potassium, iron, calcium, zinc and vitamins A, B1, B2, B3, B6, C and K are found in the edible portion of the ripe papaya fruit (Khan et al., 2012).

Food scientists and the general public have been paying close attention to the role and positive effects of various phytochemicals derived from plant sources such as fruits in recent years. Phytochemicals are non-nutritive components in plants that exert protective or disease preventing effects. These bioactive compounds such as phenolic compounds are known natural antioxidants. Plant antioxidants possess antimicrobial (antibacterial, antifungal and antiviral) properties in addition to their free radical scavenging and metal chelating activities (Martial-Didier et al., 2017).

Due to a growing interest in recycling within the agro-food industry, studies are looking at substituting synthetic preservatives with natural based preservatives. Among the sources of natural preservatives, plant-based compounds are considered attractive due to their low cost and relative abundance as they can be obtained from the recycling of agro food waste. These agro-food by-products e.g. fruit peels and seeds from food processing are as useful as the edible portion (Sagar et al., 2018).

While fruit peels are considered inedible and therefore discarded as waste during fruit processing, studies show that peels of most fruits are rich sources of essential bioactive compounds such as phenolic compounds making them valuable sources as potential natural preservatives. As of 2019, about 10 000 tonnes of papaya were produced in South Africa for consumption (Abstract of Agricultural Statistics, 2020). Processing of papaya pulp necessitates a study to determine whether papaya peel can be utilised in human and/or animal diets (Martial-Didier et al., 2017). According to the South African Agricultural Research Council, the increasing volumes of fruit are due to the improved purchasing power of a large population of South Africa. As part of a study on the utilization of papaya peel, the chemical composition of the peels is now reported (Khor et al., 2021; Khan et al., 2012; Souza et al., 2008; Silva et al., 2007) with a viewpoint of evaluating nutrient and phytochemical content. This study therefore

sought to determine the bioactive compound composition of the peel crude extract and screen for the antioxidant and antimicrobial activities of the crude extract.

4.1.2 Materials and methods

All reagents were sourced from Merck Pty Ltd (Modderfontein, Gauteng Province, South Africa) and Sigma-Aldrich (Kempton Park, Gauteng Province, South Africa).

4.1.2.1 Carica papaya peel samples

One hundred and twenty mature papaya fruits (Sunrise Solo cultivar) were procured from Tshwane Fresh Produce market (Pretoria, South Africa). The fruits originated from Mpumalanga Province, South Africa. The papaya fruits selected based on the level of ripeness (yellow skin colour, reddish orange flesh colour and black seed colour). The fruits were washed under cold running water, manually peeled using a knife and cut into a uniform size of 10 mm and freeze dried (Instruvac lyophilizer model 13KL; Air & Vacuum Technologies, Midrand, South Africa) to a constant weight. The dried samples were milled with a hammer mill to powder form and packed in airtight Ziploc bags which were stored at -20°C until further analysis.

4.1.2.2 Preparation of Carica papaya peel crude extracts

A 10 g milled sample of the peel powder was extracted using 100 mL 90% acetone (Sigma-Aldrich, Gauteng, South Africa) for 1 h while stirring with a magnetic stirrer at room temperature. A Whatman 1 filter paper was used to filter the mixture. For more thorough extraction of bioactive compounds, the residue was re-extracted two more times with the same amount of solvent. After that, the filtrates were mixed and the solvent was evaporated using a rotary evaporator set at 45°C. The crude extract was then dried overnight at 45°C in an oven. Crude extracts were prepared in triplicates. The dried extract was stored at 4°C before it was used for further analyses.

4.1.2.3 Preparation of extracts for Ultra-Performance Liquid Chromatography-Mass spectrometry (UPLC-MS) analysis

The extracts for Ultra-Performance Liquid Chromatography-Mass spectrometry (UPLC-MS) analysis were prepared as reported by Apea-Bah et al. (2014) and Rivera-Pastrana et al. (2010) with modifications. “Extraction was done by soaking a 2 g sample of papaya peel powder in 15 mL volume of a combination of 50% (v/v) HPLC grade methanol and 1% (v/v) formic acid overnight”. “This was followed by extraction in an ultrasonic bath (0.5Hz, Integral systems,

SA) for 60 min at room temperature". "The extracts were centrifuged at 3000×*g* for 5 min (Hemle Z160m) and transferred to vials". The extracts for LC-MS analysis were prepared using methanol and not acetone since the latter is not conducive for use on this equipment.

4.1.2.4 Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

The chromatographic analysis was performed as described by Stander et al. (2017). "A Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) connected to a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS analysis". "Electrospray ionization was used in negative mode with a cone voltage of 15 V, desolvation temperature of 275°C, desolvation gas at 650 L/h, and the rest of the MS settings optimized for best resolution and sensitivity". "Data were acquired by scanning from m/z 150 to 1500 m/z in resolution mode as well as in MS^E mode". "In MS^E mode two channels of MS data were acquired, one at a low collision energy (4 V) and the second using a collision energy ramp (40−100 V) to obtain fragmentation data as well. Leucine enkaphalin was used as lock mass (reference mass) for accurate mass determination and the instrument was calibrated with sodium formate". "Separation was achieved on a Waters HSS T3, 2.1 × 100 mm, 1.7 µm column". "An injection volume of 2 µL was used and the mobile phase consisted of 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid as solvent B".

"Gradient elution was carried out as follows: 100% A for 1 min; 72% A and 28% B linear gradient for 22 min; 60% A and 40% B for 50 s; a wash step of 1.5 min using 100% B followed by re-equilibration to initial conditions for 4 min". "The flow rate was 0.3 mL/min and the column temperature was maintained at 55°C". Identification was done by comparing chromatograms and retention times of phenolic constituents, organic acids and the benzyl glucosinolate in the extracts with the MS/MS fragmentation data and UV spectra of the same compounds as reported in literature.

4.1.2.5 Determination of the antioxidant activity of Carica papaya peels

4.1.2.5.1 Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay modified for the use of a 96-well microplate as described by Apea-Bah et al. (2016). "A volume of 100 µL of *C. papaya* extracts in 90% acetone and gallic acid standard solutions of different concentrations (0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, 0.2, 0.225, 0.25, 0.275 and 0.3

mg/mL) were deposited in Eppendorf tubes and 200 µL of 10% v/v Folin-Ciocalteu reagent was added to each tube". A volume of 800 µL 700 mM Na_2CO_3 was then added to each tube and the reaction mixtures vortexed and incubated for 2 h in the dark at room temperature. "After incubation, 200 µL of the reaction mixture was placed into each well of a 96 well microplate and the absorbance read at 765 nm using a microplate reader (FLUOstar Omega, BMG LABTECH)". The TPC was expressed as milligrams gallic acid equivalents (GAE) per gram sample *db*.

4.1.2.5.2 Determination of total flavonoid content

The total flavonoid content (TFC) was determined using the aluminium chloride method modified for the use of a 96-well microplate as described by Kalita et al. (2013). "Briefly, 10 µL of the *C. papaya* extract in 90% acetone and quercetin standard solutions of different concentrations (0, 0.25, 0.5, 0.75 and 1 mg/mL) were each placed in a 96 well microplate". A 30 µL volume of 2.5% w/v $NaNO_2$ was then added to each well followed by 30 µL of 1.25% w/v $AlCl_3$ which was then followed by 100 µL of 2% w/v NaOH. The resultant mixture was read at 450 nm using a microplate reader (FLUOstar Omega, BMG LABTECH). The TFC was expressed as milligrams quercetin equivalents (QE) per gram sample *db*.

4.1.2.5.3 Ferric reducing ability of plasma assay for antioxidant activity

The ferric reducing plasma ability assay (FRAP) was performed according to the method reported by Moyo et al. (2020). "A fresh working solution of the FRAP reagent was prepared by sequentially mixing 25 mL 300 mM acetate buffer at pH 3.6, 2.5 mL 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 2.5 mL 20 mM $FeCl_3 \cdot 6H_2O$". This solution was kept at 37°C in a water bath before use. "To a 30 µL *C. papaya* extract sample and Trolox standard solutions of different concentrations (0, 0.1, 0.4, 0.6, 0.8 and 1 mg/mL) in Eppendorf tubes, 900 µL FRAP solution was added and the resultant mixture incubated in the dark for 30 min at room temperature". After incubation, 200 µL of the reaction mixture was placed into each well of a 96 well microplate and the absorbance read at 595 nm using a microplate reader (FLUOstar Omega, BMG LABTECH, software version 5.50 R4). Results were reported as millimoles Trolox (TE) equivalents per gram sample *db*.

4.1.2.6 Determination of the antimicrobial activity of Carica papaya peels

4.1.2.6.1 Bacteria cultivation

This study used *Escherichia coli* strain ATCC 11775 and *Listeria monocytogenes* strain ATCC 19115 as test organisms to determine the antibacterial effect of *C. papaya* crude extract. Single colonies for the *L. monocytogenes* and *E. coli* strains were prepared using the quadrant streaking method onto sterile Petri dishes with Palcam agar (Sigma-Aldrich) and MacConkey agar (Sigma-Aldrich), respectively. Both agar plates were incubated at 37°C for 24 h. Bacterial cultures were prepared by inoculating a single colony from the respective agar plates into test tubes containing 10 mL Mueller Hinton broth (Merck) and incubated for 24 h at 37°C. After 24 h, using the aseptic technique, each culture was streaked on Mueller Hinton agar (Merck) plates and incubated at 37°Cfor 24 h. McFarland standards were used to estimate the bacterial concentration, where a loopful of the grown culture was inoculated into a test tube containing 10 mL of 0.1% buffered peptone water (Biolab, Wadeville, SA) and vortex mixed. The culture was transferred into a test tube and vortexed until a McFarland Unit (MF-U) of 0.5 for *E. coli* and 2.0 for *L. monocytogenes* were reached on the densitometer (Grant bio, Shepreth, UK) giving a bacterial concentration of approximately 10^8 CFU/mL for both bacteria.

4.1.2.6.2 Agar well diffusion assay

The antibacterial activity of the extracts was determined using the agar well diffusion assay according to Burman et al. (2018) with modifications. A 100 µL aliquot of the bacterial culture suspended in buffered peptone water was added to a sterile Petri dish after which sterilised molten Mueller Hinton agar was poured to a depth of 5 mm into the Petri dish. The dish was then gently swirled and the media was allowed to solidify at room temperature. Using a sterile stainless steel cork borer, 6 mm diameter wells were punctured into the solidified agar and 100 µL of the crude extract (reconstituted in 50% DMSO) at different concentrations (50%, 40%, 30% and 25%) was inoculated into each well. A 100 µL volume of 1% citric acid and 50% DMSO were inoculated into designated wells as the positive control and negative control, respectively. Subsequently, the plates were incubated for 24 h at 37°C. The diameter of the clear zones (measured in mm) was used to interpret the antibacterial activity of the crude extract at different concentrations. The experiment was conducted in triplicates for both test microorganisms.

4.1.2.6.3 Effect of storage time and temperature on the stability of *Carica papaya* crude extracts

The stability of the *C. papaya* crude extracts was tested at temperatures 4°C and 10°C for 5 days. Aliquots of 5 mL of the crude extract were stored with the aforementioned conditions after which the agar well diffusion assay (as described above) was carried out to test for residual antibacterial activity of the crude extract.

4.1.2.7 Toxicity of the crude extract

4.1.2.7.1 Protein extraction of *Carica papaya* powder and crude extracts

Protein extraction was done according to the method of Chaiwut et al. (2010) with modifications. To *C. papaya* powder and crude extract, 50 mM of phosphate buffer at pH 7.0 was added to a ratio of 1:9 w/v followed by stirring for 30 min. "The extracted samples were then centrifuged at 9000 × *g* at 4°C for 20 min". The obtained supernatant was filtered through Whatman 1 filter paper to obtain protein extracts which were stored at 4°C to be used in further analysis.

4.1.2.7.2 Protein quantification using the Bicinchonic Acid (BCA) Assay

The protein content of the protein extracts was determined using the BCA assay following the manufacturers' (ThermoFisher, Waltham, USA) instructions. Briefly, 50 µL of the BSA standard solution at different concentrations (0, 125, 250, 500, 750, 1000, 1500 and 2000 µg/mL) and protein extracts from *C. papaya* peel and crude extracts were placed in Eppendorf tubes. A 1 mL volume of the working solution (prepared by mixing BCA Reagent A and BCA Reagent B at a 100:1 ratio) was added to the tubes and mixed immediately. The tubes were then incubated in a 37°C water bath for 30 min and returned to room temperature. The absorbance was read at 562 nm using a microplate reader and the results obtained expressed as µg/mL per gram sample *db*.

4.1.2.7.3 Identification and quantification of papain using SDS-PAGE under reducing conditions

SDS-PAGE was performed on the protein extracts, under reducing conditions using a method described by Anyango et al. (2013). The reducing buffer, the running buffer and staining solutions were prepared following instructions by Hycult Biotechnology. The reducing buffer was made by mixing 0.2 M Tris-HCl pH 6.8 buffer, 20% v/v glycerol, 10% SDS w/v, distilled water, 0.05% bromophenol blue and 10 mM mercaptoethanol. A 10% polyacrylamide gradient gel was used together with a vertical electrophoresis system (XCellSureLock TM Mini-Cell,

VersionTM). Protein extracts for loading were prepared by dissolving in reducing conditions to give a final concentration of 1 µg protein/µL. A volume of 10 µL of a 10-250 kDa standard protein marker (Bio-Rad laboratories, CA, USA) and protein samples was loaded into the gel wells using a 10 µm syringe. Electrophoresis was carried out at a constant voltage of 200 V, 80 mA and 10 W for 1 h. The gels were stained with Coomassie Brilliant Blue R-250 overnight, then rinsed with the destaining solution consisting of 40% ethanol and 10% acetic acid for 3 h. After destaining, the gels were scanned (Banik et al., 2018) to visualise the band markers of the protein on the gel.

4.1.2.8 Statistical analysis

All experiments were conducted in triplicates. A one-way analysis of variance (ANOVA) was conducted to test for significant differences in the antibacterial activity of *C. papaya* crude extracts at different concentrations. A Pearson's rank correlation was carried out between TPC, TFC and FRAP values. Analyses were done using STATISTICA, version 8 software (StatSoft Inc., Tulsa, OK, USA). Significant differences were determined at $p \leq 0.05$ using the least significant difference (LSD) test.

4.1.3 Results and discussion

4.1.3.1 Identification of bioactive compounds in Carica papaya peel crude extracts

Table 4 shows a summary of the bioactive compounds that were identified. A total of 18 phenolic compounds were identified from the crude extracts of the *C. papaya* peels. Mainly phenolic acids (vanillic acid, sinapic acid, protocatechuic acid-4-glucoside, acetyl salicylate derivative, 2-acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate, salicylic acid β-D-glucoside, caffeic acid glucoside, ferulic acid, ρ-coumaric acid), organic acids (citric acid, malic acid, ascorbic acid, gluconic acid), flavonoids (rutin, quercetin-3-*O*-rhamnosyl rutinoside, isoharmnetin-3-*O*-dirhamnosyl glucoside) and benzyl glucosinolate were identified. Compounds such as ferulic acid, protocatechuic acid-4-glucoside, caffeic acid hexoside and benzyl glucosinolate have been previously identified in *C. papaya* fruit (Ovando-Martínez & González-Aguilar, 2020; Rivera-Pastrana et al., 2010). **Figure 8** shows peaks of mostly phenolic compounds and organic acids that were identified in **Table 4** and the mass spectra showing the fragmentation patterns of all the identified compounds is shown in Appendix A.

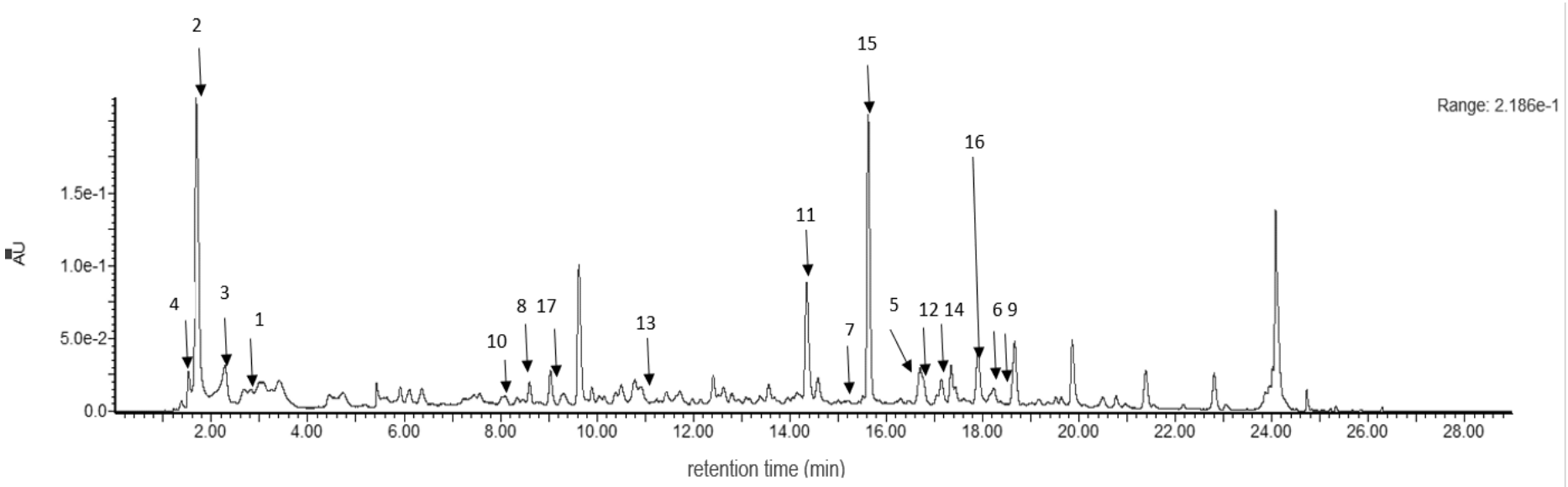


Figure 8: UPLC-MS chromatogram (λ = 280nm) of extracts from *Carica papaya* peel. 1 = Citric acid, 2 = Malic acid, 3 = Ascorbic acid, 4 = Gluconic acid, 5 = *ρ*-coumaric acid, 6 = Ferulic acid, 7 = Caffeic acid glucoside, 8 = Vanillic acid, 9 = Sinapic acid, 10 = Protocatechuic acid-4-glucoside, 11 = Acetyl salicylate derivative, 12 = 2-Acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate, 13 = Salicylic acid-β-D-glucoside, 14 = Rutin, 15 = Quercetin-3-*O*-rhamnosyl rutinoside, 16 = Isoharmnetin-3-*O*-dirhamnosylglucoside, 17 = benzyl glucosinolate

Table 4: Retention time (tR), UV-visible absorption maxima and mass spectral characteristics of organic acids, phenolic acids, flavonoids and glucosinolates identified in extracts from *Carica papaya* peel crude extracts

Peak number	***t_R (min)***	***λ_{max} (nm)***	***$[M-H]^-$***	***MS/MS fragments***	***Molecular formula***	***Proposed compound***
Organic acids						
1	2.96	280	191	111 (91)	$C_6H_8O_7$	Citric acid
2	1.84	245	133	115 (70), 89 (45)	$C_4H_6O_5$	Malic acid
3	2.35	243	175	115 (48), 87 (15)	$C_6H_8O_6$	Ascorbic acid
4	1.54	249	195	179 (5)	$C_6H_{12}O_7$	Gluconic acid
Hydroxycinnamic acids and derivatives						
5	16.79	265, 317	163	119 (29), 59 (23)	$C_9H_8O_6$	ρ-coumaric acid
6	18.22	323	193	149 (29), 134 (62)	$C_{10}H_{10}0_4$	Ferulic acid
7	15.33	282, 312, 338	341	179 (2), 85 (29)	$C_{15}H_{18}O_9$	Caffeic acid glucoside
Hydroxybenzoic acids and derivatives						
8	8.70	280	167	108 (11), 123 (12), 152 (29)	$C_8H_8O_4$	Vanillic acid
9	18.22	323	223	164 (29), 208 (6)	$C_{11}H_{12}O_5$	Sinapic acid
10	8.16	280, 312	315	153 (13), 109 (5)	$C_{13}H_{16}O_9$	

						Protocatechuic acid-4-glucoside
11	14.42	327	295	133 (100), 179 (33)	$C_{13}H_{11}O_8$	Acetyl salicylate derivative
12	16.84	265, 288	311	59 (56)	$C_{15}H_{20}O_7$	2-Acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate
13	11.05	274, 282, 289	299	254 (100), 137 (95)	$C_{13}H_{16}O_8$	Salicylic acid-β-D-glucoside
Flavonoids and flavonoid derivatives						
14	17.41	352	609	301 (8), 300 (8)	$C_{27}H_{30}O_{16}$	Rutin
15	15.70	254, 352	755	301 (1), 609 (1)	$C_{33}H_{40}O_{20}$	Quercetin-3-*O*-rhamnosyl rutinoside
16	17.51	287, 289, 302, 351	769	314 (2), 315 (2) 605 (2), 623 (1)	$C_{34}H_{42}O_{20}$	Isorhamnetin-3-*O*-dirhamnosyl glucoside
Glucosinolate						
18	9.17	302	408	97 (36), 96 (30)	$C_{14}H_{18}NO_9S_2$	Benzyl glucosinolate

4.1.3.1.1 Organic acids identified

The compound labelled as peak 1 with a retention time of 2.96 min, UV-vis absorption wavelength of 280 nm was identified as citric acid (**Table 4**). The main fragment ion produced by the compound had m/z 111 which corresponded to the loss of both a CO_2 unit (-44 amu) and two H_2O^- groups (-36 amu) (**Fig 9**). These results were similar to those reported by Al Kadhi et al. (2017).

The compound labelled as peak 2 with a retention time of 1.84 min, UV-vis absorption wavelength of 245 nm and an $[M-H]^-$ ion at m/z 133 was identified as malic acid (**Table 4**). The compound produced two main ionic fragments as illustrated in **Figure 9** at m/z 115 due to loss of a H_2O molecule (-18 amu) and m/z 89 due to loss of a CO_2 unit (-44 amu) (Al Kadhi et al., 2017).

The compound labelled as peak 3 with retention time 2.35 min, a UV-vis absorption wavelength of 243 nm and molecular ion at m/z 175 was identified as ascorbic acid (**Table 4**). The main fragment ion produced by the compound had m/z 115, corresponding to the loss of an HOC=CHO unit (-58 amu) and two hydrogen ions (-2 amu) (Boonpangrak et al., 2016) as illustrated in **Figure 9**.

The compound labelled as peak 4 (t_R = 1.54, λ_{max} = 249 nm) with molecular ion at m/z 195 (**Table 4**) was tentatively identified as gluconic acid. Fragmentation produced an ion at m/z 179 due to loss of an oxygen atom (-16 amu) as shown in **Figure 9**.

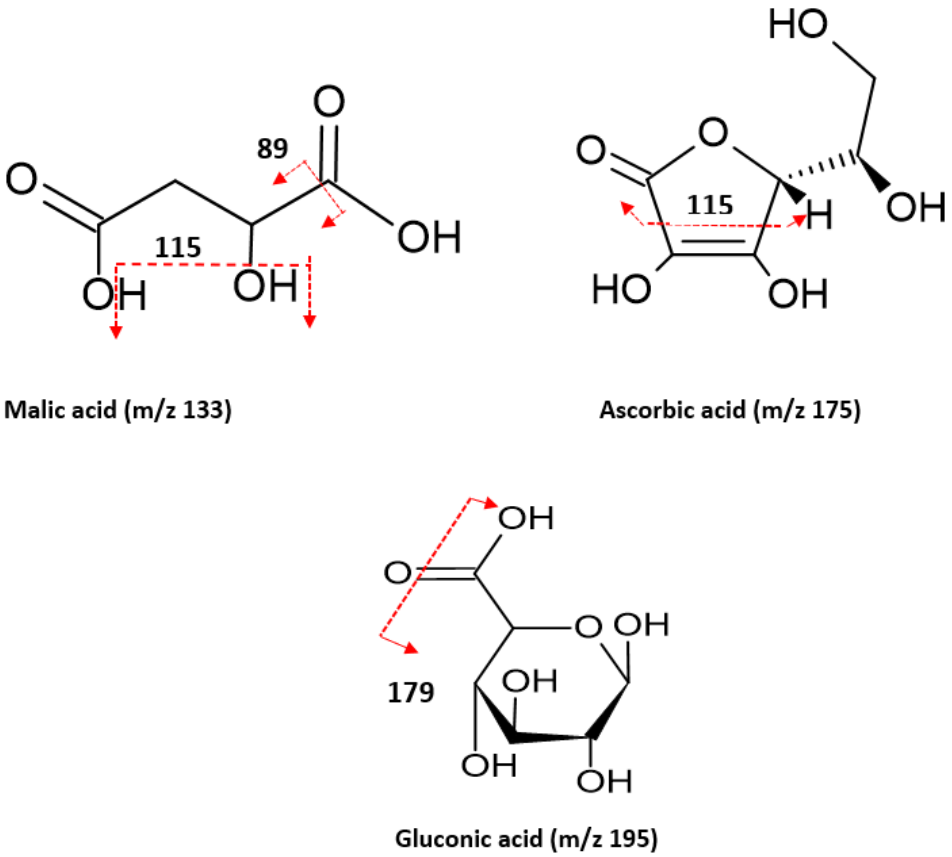


Figure 9: Proposed fragmentation patterns for organic acids and their derivatives identified in *Carica papaya* peel

4.1.3.1.2 Phenolic acids and their derivatives identified

The compound labelled as peak 5 with retention time 16.79 min, molecular ion at m/z 163 and maximum UV-vis absorption wavelengths at 265 and 317 nm (**Table 4**) was identified as ρ-coumaric acid. It produced a main fragment at m/z 119 due to loss of CO_2 (-44 amu) from the carboxylate group (Ibrahim et al., 2015). The fragmentation pattern is illustrated in **Figure 10**.

The compound labelled as peak 6 with retention time 18.22 min, molecular ion at m/z 193 and maximum UV-vis absorption wavelength 323 nm (**Table 4**) was tentatively identified as ferulic

acid. Fragmentation produced ions at m/z 149 corresponding to the loss of CO_2 (-44 amu) (Sinosaki et al., 2020), and m/z 134 which was due to the loss of a CH_3COO^- (-59 amu) (Xiang et al., 2019). The fragmentation pattern is illustrated in **Figure 10**.

The compound labelled as peak 7 with retention time 15.33 min, molecular ion at m/z 341 and maximum UV-vis absorption wavelengths of 282, 312 and 338 nm (**Table 4**) was identified as a caffeic acid glucoside. The compound produced fragment ions at m/z 179 which corresponded to loss of the glucose moiety ($C_6H_{11}O_6$) (Li et al., 2016) and m/z 85 which was due to loss of the propanoic acid ($CH_3CH=CHCOO^-$) unit as illustrated in **Figure 10**.

The compound labelled as peak 8 (t_R = 8.70, λ_{max} = 280 nm) with molecular ion at m/z 167 (**Table 4)** was tentatively identified as vanillic acid. Fragmentation produced ions at m/z 152 [due to loss of a CH_3 unit (-15 amu)], m/z 123 [due to the loss of CO_2 (-44 amu)] (Li et al., 2016) and m/z 108 due to loss of a CH_3COO^- unit (-59 amu) (Singh et al., 2019) as shown in **Figure 10**.

The compound labelled as peak 9 (t_R = 18.22, λ_{max} = 323 nm) with molecular ion at m/z 223 (**Table 4**) was tentatively identified as sinapic acid. Fragmentation produced ions at m/z 164 [due to loss of a CH_3 (-15 amu) and a COO^-unit (-44 amu)] (Lee et al., 2018) and m/z 208 due to loss of a CH_3 unit (-15 amu) (Oszmiański et al., 2013) as shown in **Figure 10**.

The compound labelled as peak 10 (t_R = 8.16, λ_{max} = 280 and 312 nm) with molecular ion at m/z 315 was tentatively identified as protocatechuic acid 4-glucoside (**Table 4**). Fragmentation produced ions at m/z 153 which corresponded to a protocatechuic acid [due to loss of a glucose ($C_6H_{11}O_6$) unit] and m/z 109 [due to loss of glucose ($C_6H_{11}O_6$) and CO_2] (Li et al., 2016) as shown in **Figure 10**.

The compound labelled as peak 11 (t_R = 14.42, λ_{max} = 327 nm) with molecular ion at m/z 295 (**Table 4**) was tentatively identified as a derivative of acetyl salicylate. Fragmentation produced a main ion at m/z 133 ($C_4H_5O_5^-$ unit) and at m/z 179 (acetyl salicylate; $C_9H_7O_4$) as shown in **Figure 10**.

The compound labelled as peak 12 (t_R = 16.84, λ_{max} = 265 and 288 nm) with molecular ion at m/z 311 (**Table 4**) was tentatively identified as 2-acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate. Fragmentation produced a main ion at m/z 59 (CH_3COO^- unit) as shown in **Figure 10**.

The compound labelled as peak 13 with retention time 11.05 min, molecular ion at m/z 299 and maximum UV-vis absorption wavelengths of 274, 282 and 289 nm (**Table 4**) was identified as salicylic acid-β-D-glucoside. The compound produced fragment ions at m/z 254 which

corresponded to loss of CO_2; -44 amu and m/z 137 which corresponded to a salicylic acid unit (Abu-Reidah et al., 2013) as shown in **Figure 10**.

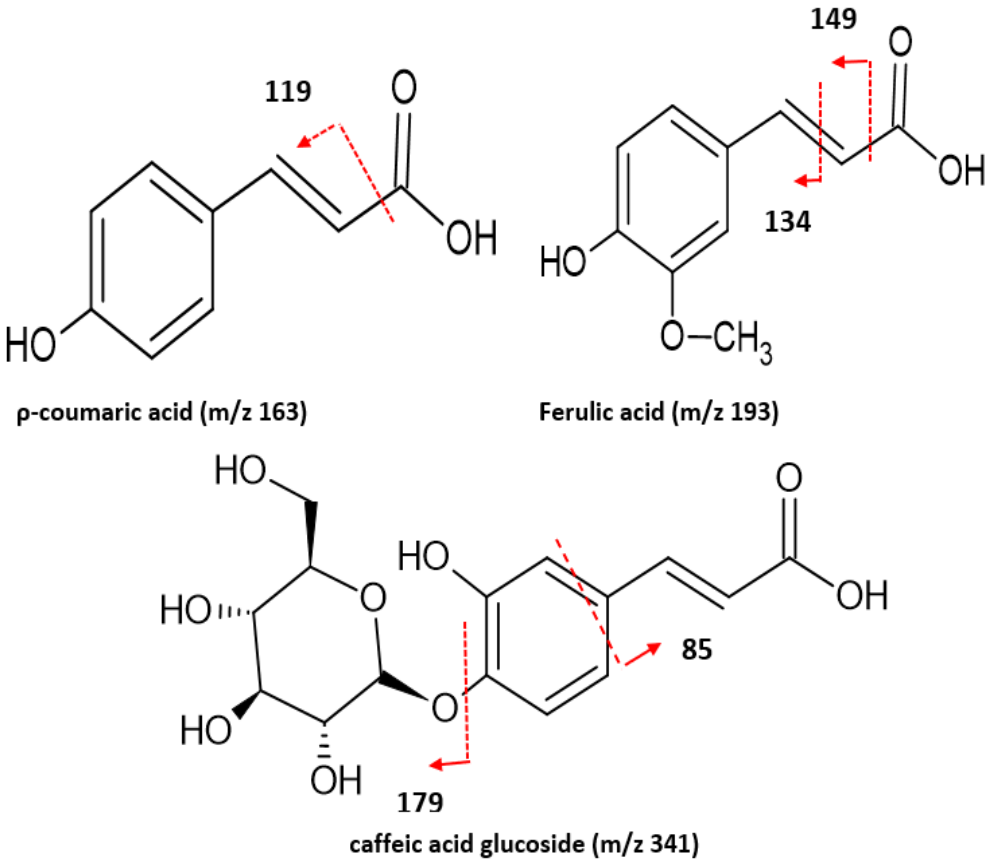


Figure 10: Proposed fragmentation patterns for phenolic acids and their derivatives identified in *Carica papaya* peel

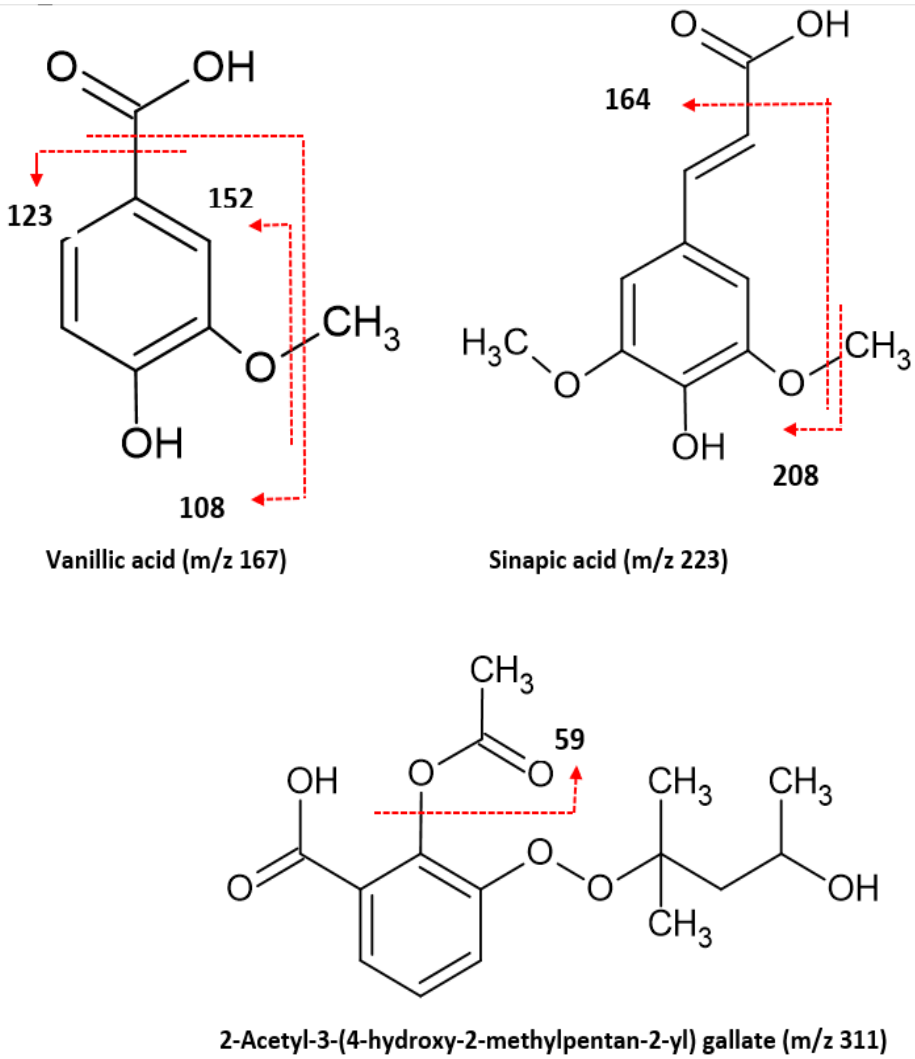
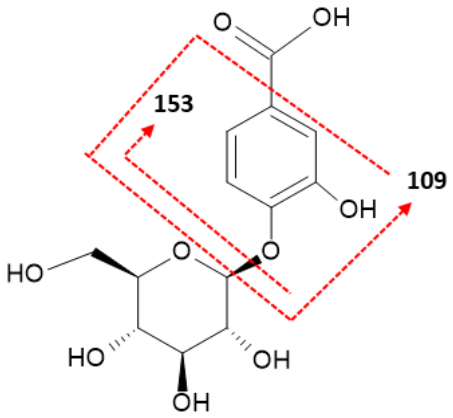
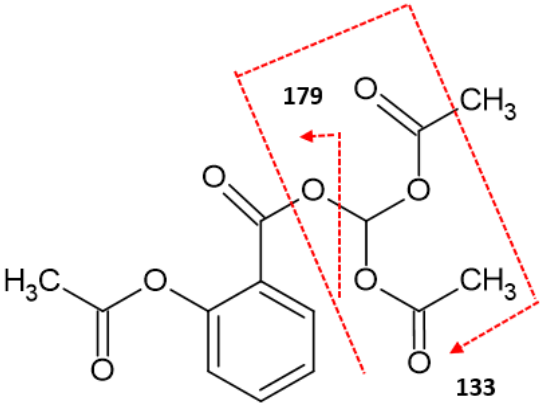


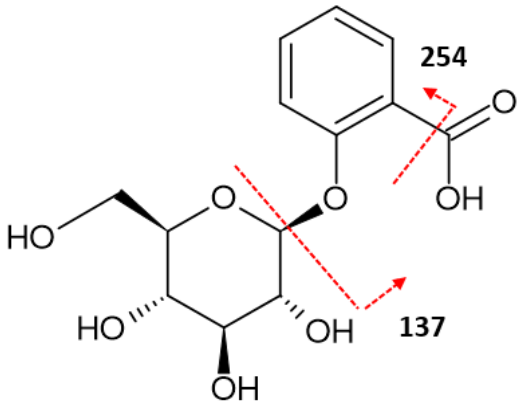
Figure 10 Continued: Proposed fragmentation patterns of phenolic acids and their derivatives identified in *Carica papaya* peel



Protocatechuic acid-4- glucoside (m/z 315)



Acetyl salicylate derivative (m/z 295)



Salicylic acid β-D-glucoside (m/z 299)

Figure 10 Continued: Proposed fragmentation patterns of phenolic acids and their derivatives identified in *Carica papaya* peel

4.1.3.1.3 Flavonoids and their derivatives identified

The compound identified as peak 14 with retention time 17.41 min, a UV-vis absorption wavelength of 352 nm and molecular ion at m/z 609 was tentatively identified as rutin (**Table 4**). Fragmentation produced ions at m/z 300 and 301 corresponding to a quercetin unit after loss of the two glucoside units (Kumar et al., 2017) as shown in **Figure 11**.

The compound identified as peak 15 with retention time 15.70 min, UV-vis absorption wavelengths of 254 and 352 nm and molecular ion at m/z 755 was tentatively identified as quercetin-3-*O*-rhamnosyl rutinoside (**Table 4**). Loss of the glucoside unit directly attached to the flavonoid skeleton produced the rutin fragment (m/z 609). Further loss of the two glucoside units of the rutin molecule produced a quercetin aglycone (m/z 301) (Won et al., 2018) as shown in **Figure 11**.

The compound at peak 16 with retention time 17.51 min, UV-vis absorption wavelengths 287, 289, 302 and 351 nm and exhibited a [M-H]⁻ion at m/z 769 was identified as Isoharmnetin-3-*O*-dirhamnosylglucoside (**Table 4**). The compound produced ion fragments at m/z 623 (Negri et al., 2018), due to loss of the rhamnose moiety. Fragmentation also produced another ion at m/z 605 which was due to the loss of rhamnose unit (**Figure 11**). Other fragment ions at m/z 315 corresponded to deprotonated isorhamnetin (due to loss of the glucosyl unit) and m/z 314 which was due to loss of the glycosyl groups (Ding et al., 2018).

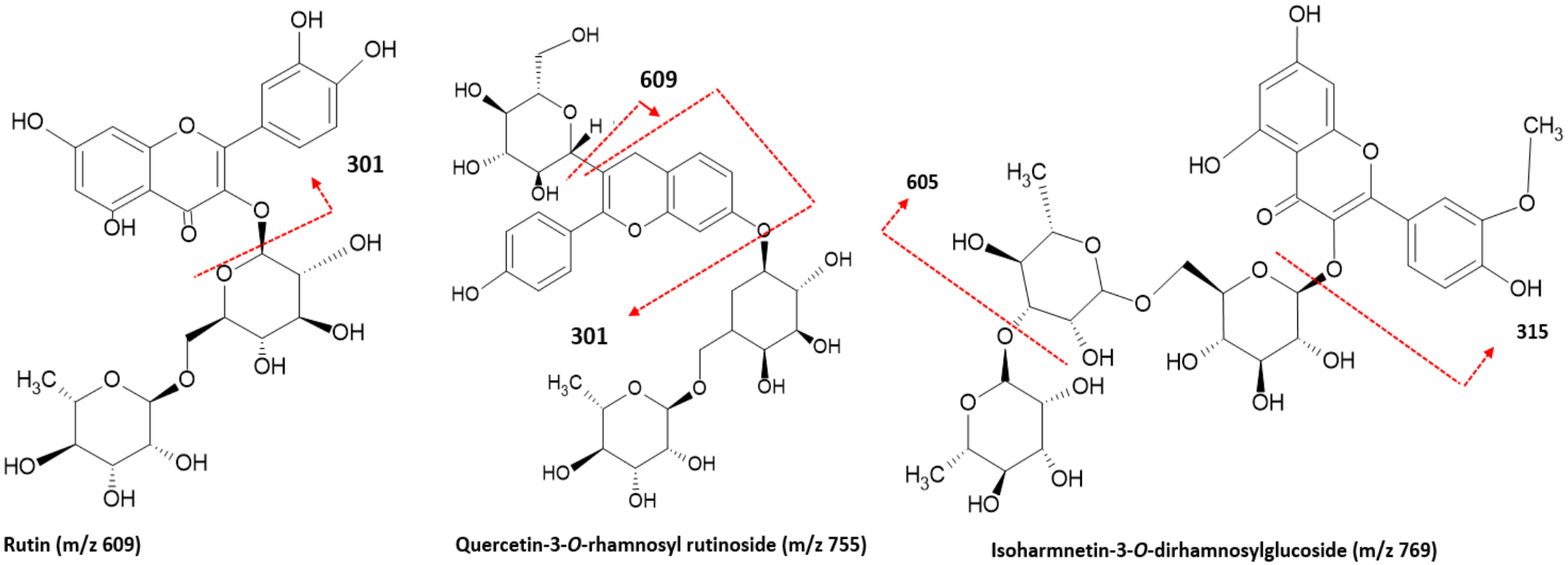
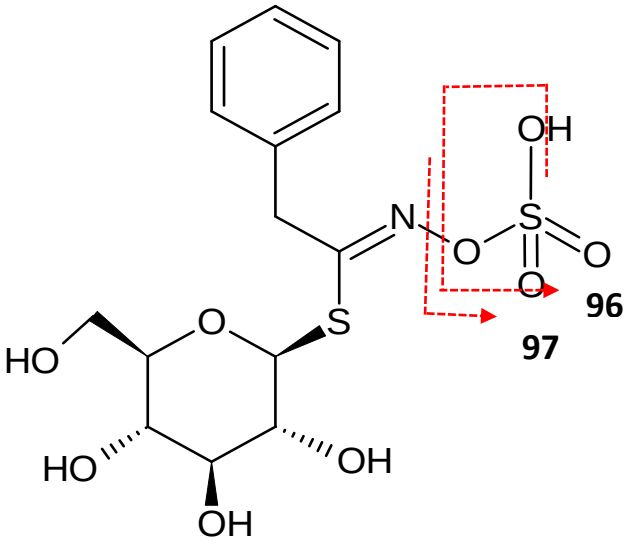


Figure 11: Proposed fragmentation patterns for flavonoids and their derivatives identified in *Carica papaya* peel

4.1.3.1.4 Glucosinolate identified

The compound identified as peak 17 with retention time 9.17 min, UV-vis absorption wavelength of 302 nm and molecular ion at m/z 408 was tentatively identified as benzyl glucosinolate (**Table 4**). Fragmentation produced two main ions at m/z 97 (corresponding to a hydrogen sulphate unit; HSO_4) and at m/z 96 which corresponded to a sulphate (SO_4^-) unit as shown in **Figure 12** (Castro-Vargas, Baumann & Parada-Alfonso, 2016).



Benzyl glucosinolate (m/z 408)

Figure 12: Proposed fragmentation patterns for benzyl glucosinolate identified in *Carica papaya* peel

4.1.3.1.5 How the structure of organic acids, phenolic compounds and glucosinolates influences their functionality

The structural differences in organic acids, phenolic compounds and glucosinolates play an important role in influencing their functionality as bioactive compounds. The metal chelating ability and radical scavenging activity of phenolic compounds depends on the number and position of hydroxyl and methoxy groups in the molecules (Mitra et al., 2010). From **Table 4**, among the identified phenolic acids, caffeic glucoside, protocatechuic acid-4-glucoside and salicylic acid β-D-glucoside with the most hydroxyl groups most likely had the strongest metal chelating and radical scavenging activities. This might be due to the structural advantage of the pyrogallol structure and its high H- donating capacity(Cai et al., 2006).

Flavonoids have diphenylpropanes (C6 + C3+ C6) as their fundamental structural skeletons, with varying oxidations of the core pyran ring. The flavonoids identified in **Table 4** belonged to

flavonols (a hydroxyl group at the 3-position) (Zhang et al., 2020). The number and configuration of phenolic hydroxyl groups, as well as glycosylation and the configuration of additional substituents, determine the bioactivity of flavonoids (Cai et al., 2006). Flavonols like quercetin and rutin are well known potent antioxidants. Flavonols have the 3-hydroxyl group in the C-ring and the catechol structure in the B-ring, as well as the 2,3-double bond in conjugation with the 4-oxo function in the C-ring, which are crucial structural factors for flavonols' powerful antioxidant action (Yi et al., 2011). Mitra et al. (2010) showed that quercetin and its glycosides (e.g. rutin) had the highest antioxidant activity compared to other flavonols and phenolic acids.

Organic acids have a generally simpler structure compared to phenolic compounds and glucosinolates. The functionality of antioxidants is based on their ability to donate protons though weak antioxidant activity has been reported for organic acids (Zhang et al., 2019). Organic acids however have stronger antimicrobial properties due to their ability to cross the cell membranes of microorganisms. Their simple structure enables them to cross the cell membrane and gain access into the cytoplasm in their undissociated forms where they increase the acidity of the cytoplasm (Le et al., 2021). This process enables organic acids to function as antimicrobial agents. On the other hand, glucosinolates have a more complex structure. Their functionality is based on their ability to act as antimicrobial agents and antioxidants. Glucosinolates can be hydrolysed to their more potent forms like isothiocyanates i.e. hydrolysis of benzyl glucosinolate yields isothiocyanates as one of the by-products (Nakamura et al., 2007). Bioactivity in isothiocyanates is due to the -N=C=S group reported to cleave disulphide bonds in proteins and scavenge free radicals (Tumer et al., 2015).

4.1.3.2 Quantification of the bioactive compounds identified in Carica papaya peel crude extracts

Table 5 shows the number of phenolic compounds expressed as µg/g GAE and µg/g QE for phenolic acids and flavonoids, respectively. Quantification of the different organic acids and glucosinolates was in relation to the compounds' peak areas. Benzyl glucosinolate had the highest peak area which may suggest that it occurred in high concentrations. Benzyl glucosinolate is a prominent compound found in different parts of *C. papaya* including the peels, seeds and leaves (Somanah et al., 2017). The amount of the different organic acids identified was also quantified in terms of the peak area with citric acid having the highest amount. Regarding phenolic compounds, there were more phenolic acids identified than the flavonoids and there were generally higher levels of phenolic acids than flavonoids. This means that the phenolic acids may have been rendered more extractable than flavonoids possibly due to the use of an acidified

methanolic solvent in combination with sonication for the extraction. Based on their molecular mass, the elution of phenolic acids is observed earlier than flavonoids although there is a definite chance of overlapping due to structural diversity of these compounds (Kumar, 2017). Most phenolic acids have a lower molecular mass than flavonoids and are found in different parts of plant cell walls.

The identification and quantification of these compounds within *C. papaya* is significant for further studies on *C. papaya* peel crude extract as a possible preservative in the food industry. This is because based on their structures, organic acids, phenolic compounds and glucosinolates are known to have a preservative effect in terms of antibacterial, antibrowning, flavouring and antioxidant properties (Fernandes et al; 2021; Zhang et al., 2020).

Table 5: Quantification of organic acids (expressed as peak areas), glucosinolates (expressed as peak areas), phenolic acids (expressed as GAE) and flavonoids (expressed as QE)

Compound		**Peak area**	**Equation of calibration curve**	**Determination coefficient (R^2)**	**Concentration µg/g *db***
Organic acids	Citric acid	6858.479			
	Malic acid	1732.121		N.D.	
	Ascorbic acid	3659.883			
	Gluconic acid	821.394			
Phenolic acids					**Expressed as GAE**
	ρ-coumaric acid	0.021			0.1 ± 0.1
	Ferulic acid	1116.327			577.0 ± 73.6
	Caffeic acid glucoside	754.036			330.8 ± 33.7
	Vanillic acid	193.518			87.4 ± 5.1
	Sinapic acid	277.578	y=17.189x	0.9995	126.0 ± 6.4
	Protocatechuic acid-4-glucoside	1678.991			770.5 ± 26.9
	Acetyl salicylate derivative	124.905			90.3 ± 44.7
	2-Acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate	1438.122			622.0 ± 76.8
	Salicylic acid-β-D-glucoside	7.679			3.7 ± 0.2
Total phenolic acids					**2607.8**
Flavonoids					**Expressed as QE**
	Rutin	786.102			41.5 ± 2.3
	Quercetin-3-*O*-rhamnosyl rutinoside	2870.582	y=147.25x	0.9963	157.3 ± 0.4
	Isorhamnetin-3-*O*-dirhamnosyl glucoside	311.983			16.7 ± 0.6
Total flavonoids					**215.5**
Glucosinolates	Benzyl glucosinolate	33459.469		N.D.	

GAE - gallic acid equivalent, QE - Quercetin equivalent, N.D. - not determined. Data reported as mean ± SD of two independent experiments

4.1.3.3 Total phenolic content (TPC), total flavonoid content (TFC) and ferric reducing ability of plasma (FRAP) assay of Carica papaya peel crude extracts

The TPC, TFC and FRAP results of the *C. papaya* peel crude extracts are shown in **Table 6**. The total phenolic content, total flavonoid content and ferric reducing capacity of the crude extracts were 6864 mg GAE/g, 3638 mg QE/g and 7968 mM TE/g, respectively. These are higher than the results reported by Suleria et al. (2020) and Addai et al. (2013). The differences in the values could have been due to the maturity stage of the fruit and type of cultivar where the peels in this research were at a higher ripening stage based on the levels of ripeness described by Addai et al. (2013). Addai et al. (2015) showed that the level of maturity has an influence on the bioactive compounds present and antioxidant activity in papaya fruit. A study done by Addai et al. (2013) on different papaya fruit at different maturity stages also showed that the TPC, TFC and FRAP values increased with an increase in the level of ripeness.

Table 6: Total phenolic content (TPC), total flavonoid content (TFC) and Ferric reducing ability of plasma (FRAP) assay of *Carica papaya* peel crude extracts

Antioxidant assay	Quantity in crude extract
TPC	6864 ± 153mg GAE/g *db*
TFC	3638 ± 251 mg QE/g *db*
FRAP	7968 ± 38 mM TE/g *db*

Results are expressed as dry matter weight (*db*), TPC - total phenolic content, TFC - total flavonoid content, FRAP - ferric reducing antioxidant power. GAE - gallic acid equivalent, QE - quercetin equivalent, TE - Trolox equivalent. All data reported as means ± SD (n = 6) of two experiments.

The correlation between TPC, TFC and FRAP of the papaya crude extracts is shown in **Table 7**. There was a strong positive correlation (r = 0.99) between the TPC and TFC values of the crude extract. A weak positive correlation (r = 0.43) between TFC and FRAP values of the crude extract was recorded. Similarly, a weak positive correlation (r = 0.30) between the TPC and FRAP values of the crude extract was observed. These results also show that the ferric reducing ability of the papaya peel crude extract is not entirely dependent on the phenolic compounds present. The non-phenolic compounds i.e. organic acids and benzyl glucosinolate identified in the crude extract (**Table 4**) could also offer ferric reducing ability. Mahattanatawee et al. (2006) reported on the contribution of ascorbic acid to antioxidant activity measured as total ascorbic acid content in different tropical fruits including *C. papaya* fruit (153.8 ± 12.1 mg/100g puree). The antioxidant potential of glucosinolates in cauliflower was reported by Cabello-Hurtado et al. (2012).

Table 7: Pearson correlation (r) between Total phenolic content (TPC), Total flavonoid content (TFC) and Ferric reducing ability of plasma (FRAP) for *Carica papaya* peel crude extracts

Crude extract	TPC	TFC	FRAP
TPC	1		
TFC	0.99	1	
FRAP	0.30	0.43	1

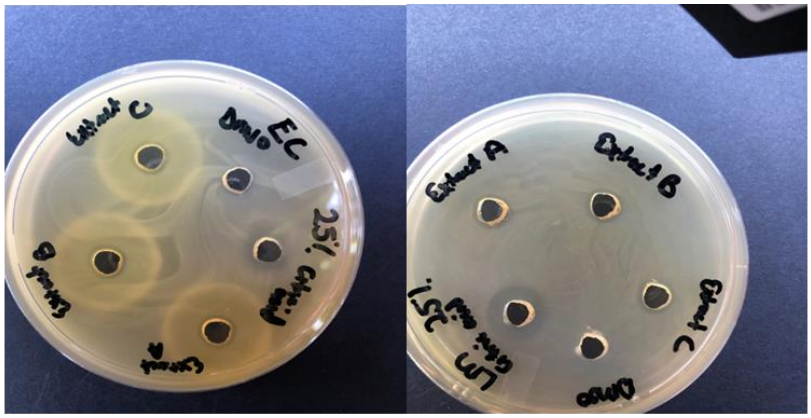
During the development of several fruits, organic acids build up in both their pulp and peel (Pande & Akoh, 2010). The results in **Table 4** show the presence of ascorbic, malic, gluconic and citric acids within the papaya peel crude extract as has been reported by Kelebek et al. (2015). Evidence suggests that organic acids such as ascorbic or malic acid have antioxidant properties because of their ability to chelate metals (Zafra-Rojas et al., 2018; Lopez-Bucio et al., 2000). Iron and copper are transition metals that are involved in the formation of reactive oxygen species. Reactions between these metals and organic acids alters the redox potential thereby decreasing the formation of reactive oxygen species (Khokhar & Apenten, 2003).

The chemical compound benzyl glucosinolate identified in the papaya peel crude extract (**Table 4**) had the highest peak in the chromatogram and is a compound that exists in all tissues of *C. papaya* except the mature pulp (Li et al., 2012). Benzyl glucosinolate is reported to possess antioxidant properties determined by its ferrous ion chelating ability (Chuanphongpanich et al., 2006).

4.1.3.4 Antibacterial activity of Carica papaya peel crude extract

4.1.3.3.1 Comparative study on antibacterial activity of *Carica papaya* peel crude extract against *Listeria monocytogenes* and *Escherichia coli*

The antibacterial potency of the crude extracts from against *L. monocytogenes* and *E. coli* was evaluated by the agar well diffusion method. It was evident from the respective zones of inhibition (**Table 8**) and seen in **Figure 13** that the crude extract exhibited a significantly higher ($p < 0.05$) inhibitory effect against *L. monocytogenes* than against *E. coli*. The inhibition zones were however much lower in diameter than those of citric acid which was used as a positive control.



LM=*L. monocytogenes*, EC=*E. coli*, positive control=1% citric acid, negative control= 50% DMSO

Figure 13: Susceptibility of the test organisms to *Carica papaya* peel crude extract

Table 8 also shows the effect of varying concentrations (250-500 mg/mL) of the crude extract on the inhibitory activity against both *E. coli* and *L. monocytogenes.* Concentrations of the crude extract lower than 250 mg/mL produced no antibacterial activity against the two bacterial species (data not shown). Increasing the concentration of the crude extract above 300 mg/mL, resulted in a significant increase ($p < 0.05$) in the zone of inhibition for both *E. coli* and *L. monocytogenes.*

Table 8: Evaluation of the antibacterial activity of different concentrations of *Carica papaya* peel crude extract

Extract concentration (mg/mL DMSO)	**Mean Diameter of inhibition zone (mm)**	
	E. coli	*L. monocytogenes*
500	$9.50^{Ac} \pm 0.84$	$11.75^{Bc} \pm 0.76$
400	$8.55^{Ab} \pm 1.14$	$10.83^{Bb} \pm 0.98$
300	$8.17^{Aab} \pm 0.26$	$9.33^{Ba} \pm 0.41$
250	$7.60^{Aa} \pm 0.15$	$8.92^{Ba} \pm 0.66$
Positive control (10 mg/mL) citric acid	$12.67^{Ad} \pm 0.52$	$14.92^{Bd} \pm 0.20$

Means with different superscripts are significantly different at $p<0.05$. Uppercase letters in show significant differences between the bacteria while lowercase letters represent significant differences within the microorganism. No zones of inhibition were observed for the negative control

The stability of the crude extract at 500 mg/mL on exposure to different temperature conditions and its effect on *L. monocytogenes* and *E. coli* is shown in **Figure 14**. The graph shows no changes in residual activity of the crude extract at 500 mg/ml after 5 days. The extract still showed inhibitory activity against both *L. monocytogenes* and *E. coli*. The crude extract was

also seen to maintain its activity irrespective of the temperatures it was exposed too (**Figure 14**). Overall, regardless of the storage temperature, the crude extract showed no changes in residual activity against *L. monocytogenes* and *E. coli* after the 5 day storage period.

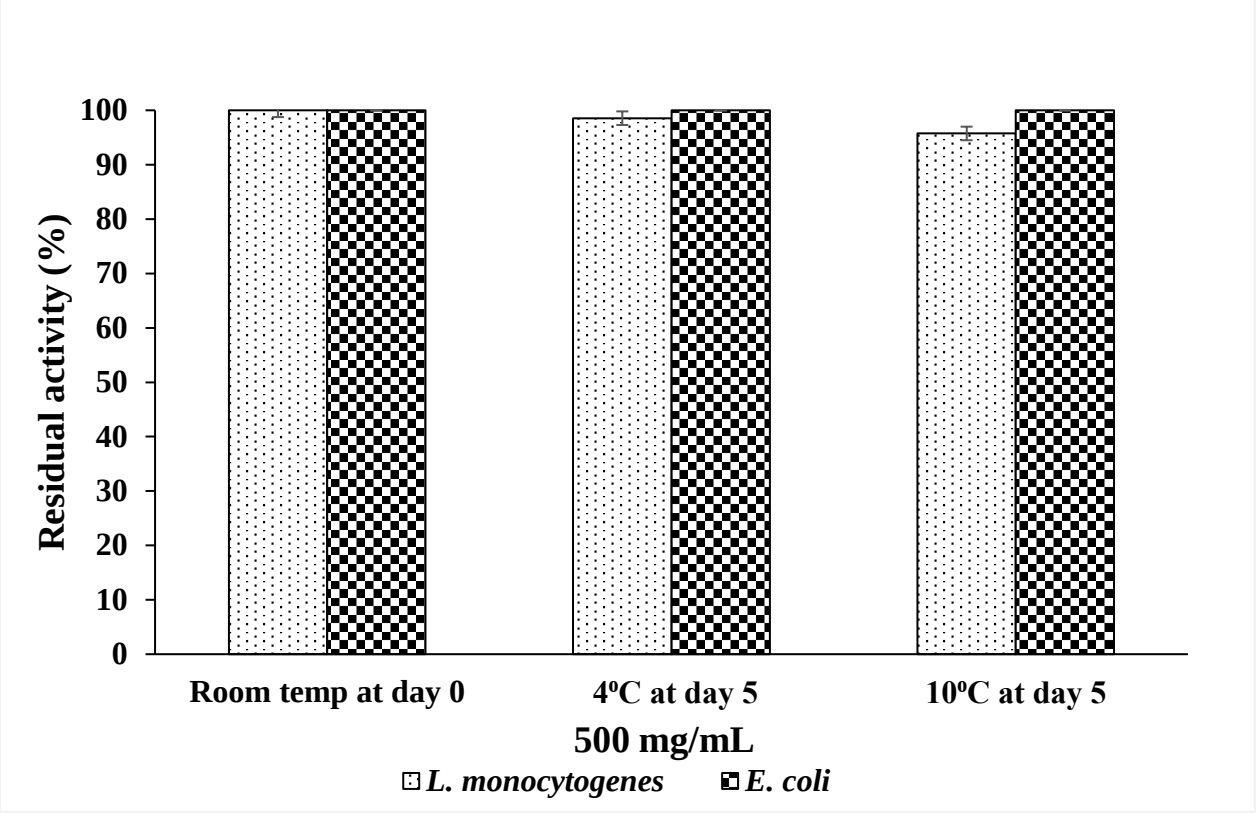


Figure 14: Stability of *Carica papaya* peel crude extract at 4°C and 10°C tested against *Listeria monocytogenes* and *Escherichia coli*

The antibacterial activity of papaya peel crude extract is linked to the active compounds within the crude extract. The active compounds identified within the crude extract were mainly organic acids, phenolic compounds and benzyl glucosinolate (**Figure 8**).

Organic acids' antimicrobial effect is based on their capacity to traverse cell membranes due to the hydrophobic nature of their undissociated state, causing changes in proton and related anion concentrations inside the microorganism's cytoplasm (Hirshfield et al., 2003). This influences the pH homeostasis of the bacterial cells and disrupts the proton motive force, an important electrochemical proton gradient that is responsible for a cells' vitality (Le et al., 2021). The undissociated form of the organic acid acts as a selective dissipater of the transmembrane pH gradient, that together with the membrane potential make up the proton motive force (PMF) of bacteria (Farha et al., 2017). The efficiency of the undissociated organic acids in consuming excess protons provides a mechanism to short circuit the bacterial transmembrane proton

gradient (Domenech et al., 2020). As a result, the purine bases and key enzymes are harmed, and bacterial viability suffers (Gómez-García et al., 2019).

The antibacterial mechanism of flavonoids is based on their formation of complexes with the cell membrane and transport proteins through hydrogen bonding and hydrophobic interactions, as well as by covalent bond formation (González et al., 2016). This leads to an alteration in membrane fluidity within hydrophilic and hydrophobic regions of the microbial cell membranes causing disruption in the membrane structure, increasing its permeability, inhibiting respiration and altering ion transport processes (Moravej et al., 2018). Intact benzyl glucosinolate does not offer antimicrobial activity. Hydrolysis of this compound produces bioactive compounds like isothiocyanates, nitriles, epithionitriles and thiocyanates (Eisenschmidt-Bönn et al., 2019) with isothiocyanates (ITCs) being of main importance. The most powerful inhibitors of microbial action are ITCs. ITCs attach to sulphydryl groups on the active sites of microbial enzymes, causing cellular levels of essential thiol groups to drop, resulting in the generation of oxygen and other free radicals, which reduces cell viability (Borges et al., 2015).

Escherichia coli is a gram negative bacterium, whereas *Listeria monocytogenes* is a gram positive bacterium. The differences in the cell wall structure of the two microorganisms explains the differences in the inhibitory activity of the crude extract against the two organisms. Quite commonly, plant extracts have been shown to be more effective against gram positive bacteria than against gram negative bacteria (Limsuwan et al., 2009). The cell walls of gram positive bacteria comprise mainly of a thick peptidoglycan layer and a single membrane in contrast to gram negative bacterial cells which are made up of an inner and outer membrane and a thin peptidoglycan layer embedded between the two membranes (Liu et al., 2018). Gram negative bacteria's outer membranes are the primary cause of resistance to a wide range of antimicrobial agents, including plant extracts. Many antimicrobial compounds gain access to their targets by passing through the outer membrane (Breijveh et al., 2020). This membrane acts as a permeability barrier to certain antimicrobial agents, preventing them from penetrating the cell (Exner et al., 2017). Gram positive bacteria are more vulnerable to antimicrobial treatments because they lack the outer membrane.

5.1.3.5 Toxicity of Carica papaya peel crude extract

The presence and quantity of *C. papaya* latex proteinases, papain, and chymopapain, which are powerful uterine contractants, influence the toxicity of the plant. **Table 9** gave an indication of

the amount of proteins present in both the crude extract and powder and further analysis using SDS-PAGE (**Figure 15**) confirmed whether proteins papain and chymopapain were present within both samples. The protein concentration of the crude extract and peel powder was determined to be 22.38 and 94.67 mg/g *db*, respectively (**Table 9**).

Table 9: Protein concentration in mg/g obtained using the Bicinchonic Acid (BCA) Assay

Sample	**Protein concentration** (mg/g *db*)
C. papaya peel crude extract	22.38 ± 0.84
C. papaya peel powder	94.67 ± 3.30

Results are expressed as dry matter weight (*db*), all data reported as means ± SD (n = 3) of two experiments.

The presence or absence of chymopapain and papain enzymes within the crude extracts was confirmed through protein bands of M_r 23 kDa for papain and M_r 27 kDa for chymopapain on the gel. The protein band corresponding to M_r 23-27 kDa (**Figure 15**) which was present in P represented proteins papain and chymopapain. Papain and chymopapain are reported to have molecular weights of 23 kDa and 27 kDa, respectively (Rojas et al., 2018; O'Hare &Williams, 2014). This same band was absent in CE. The number of proteins and their respective concentrations varied between extracts that were obtained from the crude extract (CE) and peel powder (P) of *C. papaya* (**Figure 15**), as defined based on the strength of the bands on the gel after staining. The absence of enzymes chymopapain and papain in CE could have been because of the extraction method that was used in order to obtain the crude extract. During extraction to obtain the crude extract, 90% acetone was used as the extraction solvent. This high concentration of acetone led to the denaturation of the enzymes. Szabó et al. (2006) shows that organic solvents at concentrations of 90% affect the activity and stability of papain. Denaturation of proteins or enzymes leads to their change in physical, chemical and biological properties. Mild denaturation may disrupt protein tertiary or quaternary structures, whereas harsher conditions may fragment the chain (Bhagavan & Ha, 2015). This explains the absence of chymopapain and papain enzymes in the crude extract.

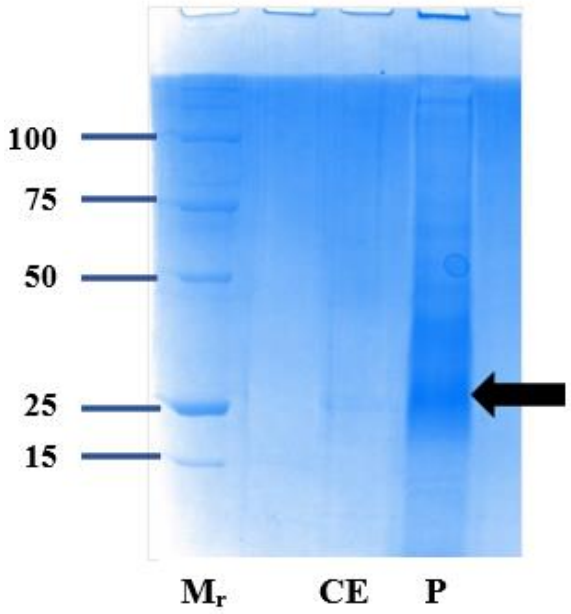


Figure 15: Staining of the SDS–PAGE separated protein profiles obtained from *Carica papaya* peel crude extract and *Carica papaya* peel powder extract. CE and P represent extracts from obtained from the crude extract and powder of the peel respectively. Proteins are visible only within the range of 20-50 kDa. A band of protein(s) representing 23-27 kDa present in the powder, as shown with the arrow is found to be absent in the crude extract

Ingestion of papaya latex (containing papain and chymopapain), which is commonly found in unripe papaya and papaya peels, causes uncontrolled uterine contractions in pregnant women, which can lead to abortion depending on oestrogen levels in the tissues, which could be due to uterotonic effects of a combination of enzymes rather than pure papain itself (Adaikan & Adebeyi, 2005). The results of the quantification of the total protein concentration in *C. papaya* peel powder, *C. papaya* peel crude extract and the results from the SDS-PAGE are enough to draw conclusions of absence of toxicity in the crude extract but not the peel powder. This is because SDS-PAGE showed absence of both enzymes chymopapain and papain in the crude extract. These enzymes were however detected to be present in the peel powder. This also suggests that the two enzymes contribute to the higher protein concentration seen in the powder compared to the crude extract (**Table 9**). Depending on the concentration, combination of chymopapain and papain induces uterine contractions upon ingestion (Adaikan & Adebeyi, 2005). A study by Adebiyi et al. (2002) showed that a combination of chymopapain and papain enzymes at concentrations between 0.01-0.64 mg/g produced uterine contractions. This

combination of enzymes even at low concentrations pose a health risk especially for pregnant women.

Within the active dyad of the enzyme, the cysteine proteinases chymopapain and papain include a catalytically active cysteine sulphydryl group (Cys-25) and a histidine imidazole group (His-159) (Adaikan & Adeyebi, 2005). These enzymes once ingested function in a similar way to oxytocin or prostaglandin F2α where they induce spasmodic myometrial contractions in both pregnant rats and guinea pigs (Odirichukwu et al., 2015). Oxytocin is a neuropeptide that induces the release of calcium ions which activate a muscular enzyme, myosin light-chain kinase causing muscular contractions (Ali et al., 2019). This is an important mechanism during labour in pregnant mammals but can also cause miscarriages (Annaházi et al., 2021).

4.1.3.6 Conclusion

The crude extracts from *C. papaya* peel powder have both antioxidant and antimicrobial properties. These observations are attributed to the bioactive compounds present which on analysis using the LC-MS method were found to be consisting of mainly organic acids, phenolic acids and flavonoids and the compound benzyl glucosinolate. The study also shows that the crude extract from *C. papaya* peel crude extract is not toxic due to the absence of enzymes papain and chymopapain upon analysis using SDS-PAGE. Based on these findings, *C. papaya* crude extract shows potential in its ability to serve as a preservative within the food industry.

4.2 Application of *Carica papaya* peel crude extract in controlling enzymatic browning in fresh cut apples and potatoes

Abstract

Carica papaya peel crude extract was tested for its potential to reduce enzymatic browning in fresh cut apples and potatoes. The treatments used in the study were T1 (control), T2 (distilled water), T3 (0.3% Sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid) T4 (5% crude extract) and T5 (1% crude extract, 0.1% ascorbic acid and 0.1% citric acid). The apple and potato pieces were immersed in these treatments for 30 minutes, stored at 4°C and 10°C and results recorded daily for 5 days. The colour changes of the apples and potatoes pieces were measured using a colorimeter (CR 400 chromameter) and by visual assessment to obtain a browning score. A general decrease in L* values was observed for both apples and potatoes for T1, T2, T4 and T5 during storage. The L* values of T3 for both apples and potatoes were higher than those of other treatments. The a* and b* values of T1, T2, T4 and T5 became more positive (an indication of redness for the a* value and yellowness for the b* values) for both apples and potatoes stored at 4°C and 10°C. The a* values of T3 showed that the apples and potatoes became greener while the b* values were less positive showing a tendency of a colour change from yellow to blue. The overall colour change (ΔE) was more pronounced in the apples and potatoes stored at 10°C. Potatoes also had lower ΔE values compared with apples. The browning index (BI) was also higher in apples than in potatoes. The BI for fresh cut produce at storage temperature, 10°C was higher than that for the produce at 4°C. Furthermore, the browning scores (BS) from apples were generally higher than those from potatoes. The BS values for apples and potatoes at 10°C were also higher than that of those stored at 4°C. Overall, sample treatments with the crude extracts showed inhibition of enzymatic browning in the fruit and vegetable models though were less effective than the standard treatment (T3). The crude extracts, therefore, have the potential to be used as preservatives that control enzymatic browning in fruits and vegetables.

Keywords: *C. papaya* crude extracts, apples, potatoes, enzymatic browning, colour, browning index, browning score, temperature

4.2.1 Introduction

Browning reactions in foods are a widespread occurrence and become evident when food material is processed or mechanically injured (Kong & Singh, 2016). Browning can be desirable or undesirable in foods and plays a vital role in altering their appearance, flavour and nutritive value (Wiley, 2017). Browning is desired (Maillard and Caramelisation) when it improves the look and flavour of products like coffee, maple syrup and bread toasting (Manickam & Pare, 2019). Browning is undesirable (enzymatic browning) in fresh fruit, frozen meals, and dried goods because it alters their appearance (Singh et al., 2018).

Browning of fresh produce is a result of injury caused by postharvest handling including minimal processing methods done on fresh produce to make them more convenient for consumers. Injury during minimal processing is due to unit operations like peeling, cutting, grating and slicing. These operations expose the injured fresh produce to air and the part that is exposed undergoes rapid darkening, a process known as enzymatic browning (Moon et al., 2020). This darkening reaction occurs when polyphenolase (PPO) reacts with phenolic compounds in the presence of oxygen to generate *O*-quinones, which then polymerize to form dark-coloured pigments (Singh et al., 2018).

Because to the presence of PPO, many fruits and vegetables, such as apples and potatoes, discolour quickly, posing a severe processing problem (Moon et al., 2020). A study by Cronjé et al. (2018) showed that apples and potatoes are among the most wasted fresh produce in South Africa due to their rapid browning during their processing. Common approaches to controlling the enzymatic browning process in fresh produce include the use of benzoates, sorbates and reducing agents like sulphites, citric and ascorbic acids (Impaprasert et al., 2020). These chemical compounds though effective, pause a health risk to consumers because they have been linked to toxicity potentially life-threatening effects such as allergies, asthma and cancer (Anand & Sati, 2013). Emphasis on replacing synthetic preservatives with natural based preservatives has therefore become a priority in the food industry. The use of plant-waste derived antioxidants therefore offers an alternative to prevention of enzymatic browning.

Papaya peel extracts contain phenolic antioxidants for example gallic acid, vanillic acid, ferulic acid and rutin (Jamal et al., 2017; Rivera-Pastrana et al., 2010). These compounds lower pH of a system below the optimal pH of the target enzyme, PPO whose pH optima is from 5 to 7.5 (Teng et al., 2017). Lower pH values retard the activity of the enzyme. Polyphenolic antioxidants also inhibit PPO activity (Losada-Barreiro & Bravo-Diaz, 2017) because binding

of these polyphenols to the enzyme changes the structure of PPO thus altering its functionality and activity (Adrar et al., 2019). The objective of this part of the study was to determine whether *C. papaya* peel crude extract can be used as a preservative with the aim of preventing browning in fresh cut produce.

4.2.2 Materials and methods

4.2.2.1 Fresh produce

Apples (Granny Smith cultivar) and potatoes (Sifra cultivar) were procured from Freshmark-Shoprite holdings located in Gauteng Province, South Africa.

4.2.2.2 *Chemicals*

Sodium metabisulphite, ascorbic acid and citric acid were all purchased from Sigma-Aldrich (Pty) limited located in Gauteng Province, South Africa.

4.2.2.3 Preparation of C. papaya peel crude extracts

Carica papaya peel crude extracts were prepared as described in **section 4.1**.

4.2.2.4 Procedure

Solutions of 250 mL containing 0.3% sodium metabisulphite, 0.1% w/v ascorbic acid, 0.1% w/v citric acid, 5% w/v crude extract, 1% w/v crude extract and water were measured in duplicate and poured into respective beakers. The pH of the solutions of ascorbic acid, citric acid, crude extract and sodium metabisulphite were measured and recorded. Fresh apples and potatoes were prepared separately by cutting them into similar thin slices (10 mm ×10 mm ×5 mm) and placed into each of the beakers for 30 min. The treatments were carried out according to the following **Table 10**.

Table 10: Treatments utilised for the enzymatic browning experiment on apple and potato pieces

	Treatment
T1	Fresh produce pieces were placed on top of cling film and left exposed to air.
T2	Fresh produce pieces were immersed in a beaker of water.
T3	Fresh produce pieces were immersed in a 0.3% solution of sodium metabisulphite, 0.1% citric acid and 0.1% ascorbic acid.

T4	Fresh produce pieces were immersed in a beaker containing 5% crude extract solution.
T5	Fresh produce pieces were immersed in a solution containing 1% crude extract, 0.1% citric acid, 0.1 % ascorbic acid.

The fresh produce pieces were then taken out of the treatments and immediately placed into Ziplock bags (Plastilon packaging, Gauteng Province, South Africa) The treated apple and potato samples were then stored at 4°C and 10°C for five days. The temperature parameters were chosen based on the conditions under which the unit operations of minimal processing of fresh produce occurs. The shelf life period of five days was based on the current market conditions of fresh cut produce in retail stores.

4.2.2.5 Determination of colour of apple and potato pieces

Colour changes in the apple and potato pieces were measured immediately after treatment and there after every 24 h for five days using a Minolta CR-400 Chroma Meter series version 1.07 (Minolta Corp., Tokyo, Japan). The instrument was calibrated before each measurement at 24h intervals with a white ceramic plate (L* = 94.18, a* = -0.66, b* = 4.05). The L*, a* and b* values were taken for the samples. "The L* value indicates lightness of the colour, which range from 0 (dark) to 100 (white)". "The positive value of a* indicates red colour, while negative value of a* indicates green colour". "The positive value of b* indicates yellow colour, while the negative value is an indication of the blue colour". The overall change in colour (ΔE) was also determined using the following equation:

"$\Delta E = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$"

where "ΔL* = L*- L, Δa * = a*- a and Δb* = b *- b"

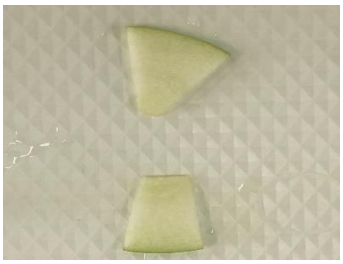
L, a, b are colour component values of control samples.

4.2.2.6 Determination of browning of apple and potato pieces

The browning of the apple and potato pieces was assessed using two different methods. The first method was by visualization of the total brown area of each sample (browning score - BS) using the following scale: 0 = deep brown, 1 = dark brown, 2 = brown, 3 = light brown, 4 = light yellow and 5 = very light yellow as shown in **Table 10**. The second method of determining browning involved calculating the Browning Index (BI) as follows: "BI = [100(x – 0.31)] /

0.17, where x = (a* + 1.75L*) / (5.645L* + a* - 0.3012b*)" (Ruangchakpet & Sajjaanantakul, 2007).

Table 11: Browning scores for apples and potatoes

Potato	Apple	Score
		5
		4
		3
		2

		1
Not applicable		0

NB: The end point of browning in potatoes was at 1

4.2.3 Results and discussion

4.2.3.1 Colour changes

The ability of the *C. papaya* extract to inhibit browning in fresh cut apples and potatoes was tested in a solution containing 5% of the crude extract (T4) and compared with four other control and standard treatments. These included T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% Sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid) and T5 (Sample immersed in 1% crude extract, 0.1% ascorbic acid and 0.1% citric acid). The data of L*, a*, b* and total colour change (ΔE) obtained from the experiments for potatoes are presented in **Figures 16** and **18** while those for apples are presented in **Figures 17** and **19**.

There was a general decrease in the L* values of potatoes for T1, T2, T4 and T5 for the 5 day storage period at both storage temperatures (4°C and 10°C) as shown in **Figure 16** The decrease in L* values was brought about by the browning of the potatoes. There was an initial increase in the L* value of T3 from day 0 to day 1 for the potatoes stored at 4°C. The increase in the L* value for the potatoes stored at 10°C (**Fig 16**) occurred between day 0 and day 3. The increase in L* values caused by the lightness in potatoes could have been due to the bleaching action of sodium metabisulphite. Sodium metabisulphite as a bleaching agent causes discolouration in foods through the pigment reduction action (Macedo et al., 2019). The L* values for T3 were also generally higher than those of the other treatments. A similar result was found for Asian

pears that were soaked in a solution containing sodium metabisulphite (Jiang et al., 2018). The combination of sodium metabisulphite, citric and ascorbic acids could have also produced a synergistic effect in terms of inhibiting browning of the potatoes. Sodium metabisulphite prevents browning in foods because it forms quinone-sulphite complexes thus preventing the polymerization of quinone compounds to form the brown melanoidin pigment (Haryani, 2017). Citric and ascorbic acids inhibit enzymatic browning through altering the pH conditions within the plant tissues which affects the activity of the enzyme (Zhou et al., 2018). Additionally, ascorbic acid is able to reduce quinones to diphenols thus stopping the browning reaction process (Lee, 2007).

There was a general increase in the a* values towards the positive in potatoes for T2, T3, T4 and T5 for the 5 day storage period at 4°C and at 10°C for T1, T2, T3 and T5 as shown in **Figure 16**. The increase in a* values is an indicator of browning. This is because an increase in a* values has been linked to either the development of reddish colour as a consequence of browning (Shrestha et al., 2020) or the reduction in green pigmentation (Castañer et al., 1999). The increase in a* values are more apparent for the potatoes stored at 10°C than those that were stored at 4°C. The a* values for the potatoes stored at 4°C for T1 and those stored at 10°C for T4 (**Fig 16**) however showed a decline in values after day 1 which indicated more greenness. This was because the potatoes contained photosynthetic tissue.

There was a general increase in b* values in potatoes for T1, T2, T4 and T5 for the 5 day storage period at 4°C and at 10°C (**Fig 16**). The increase in b* values indicated more yellowness of the potatoes (Tuyen et al., 2010). The increase in b* values is also an indicator of the browning process of the potatoes as a result of the yellowish colour formation (Shrestha et al., 2020). The b* values for T3 for both storage temperatures however decreased over the 5 day storage period.

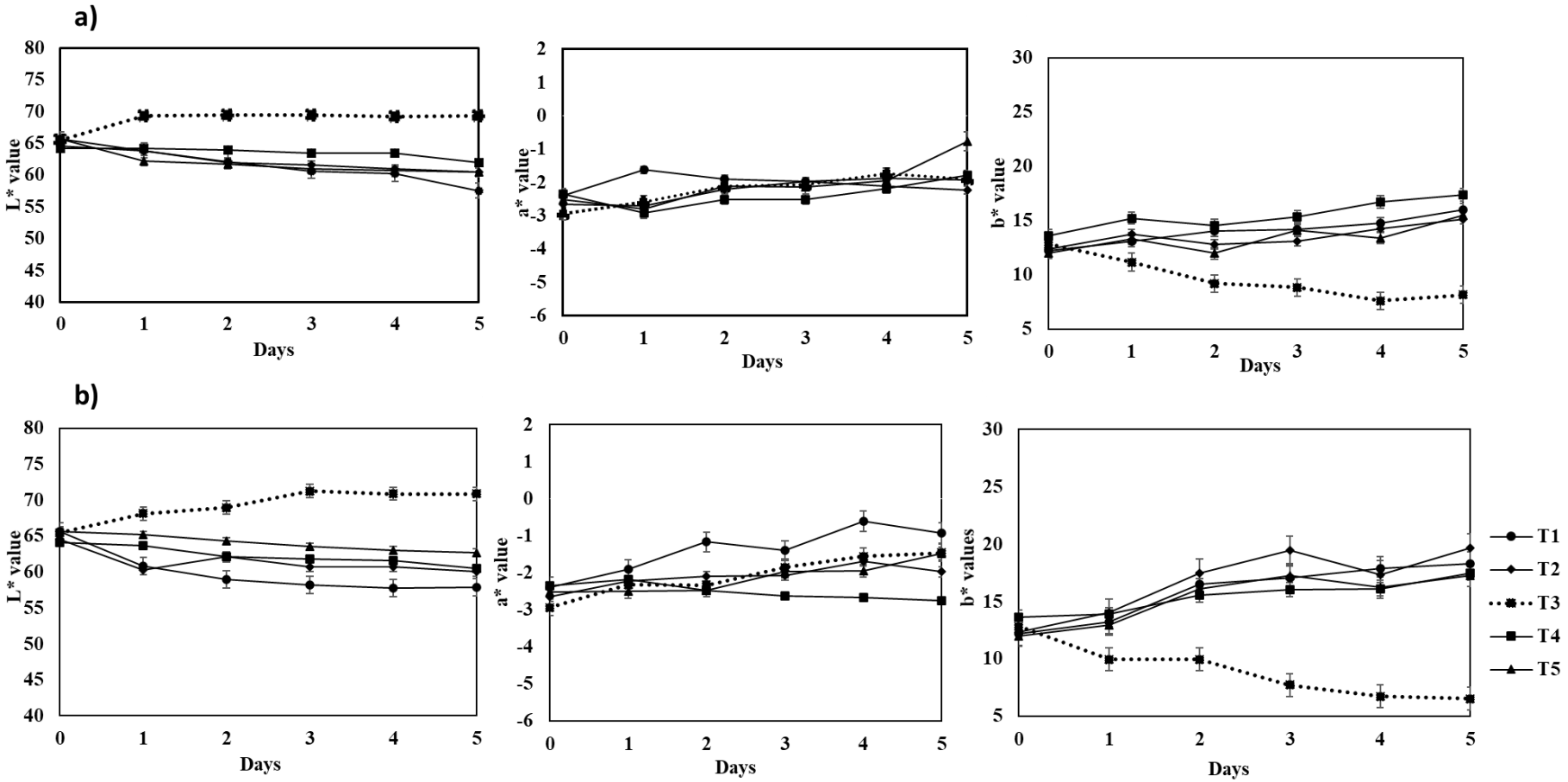


Figure 16: Effect of anti-browning agents on the colour of fresh cut potatoes measured as *Lab* values during storage at (a) 4°Cand (b) 10°C

T1 (Control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid

There was a general decrease in L* values of apples for T1, T2, T4 and T5 for the 5 day storage period at 4°C and 10°C (**Fig 17**). The decrease in L* values is an indication of browning. The L* values for T3 were higher than those of other treatments at both storage temperatures and also remained constant from the beginning to the end of the storage period. This was because of the bleaching effect of sodium metabisulphite. An overall increase in a* values was seen in apples for T1, T2, T4 and T5 for the 5 day storage period at 4°C and at 10°C for all treatments as shown in **Figure 17**. A general increase in b* values for apples was seen in all treatments from day 0 to day 5 except for T3 at 10°C as shown in **Figure 17**. T3 showed an initial increase in the b* value at day 1 and thereafter a decrease in values was seen up to day 5.

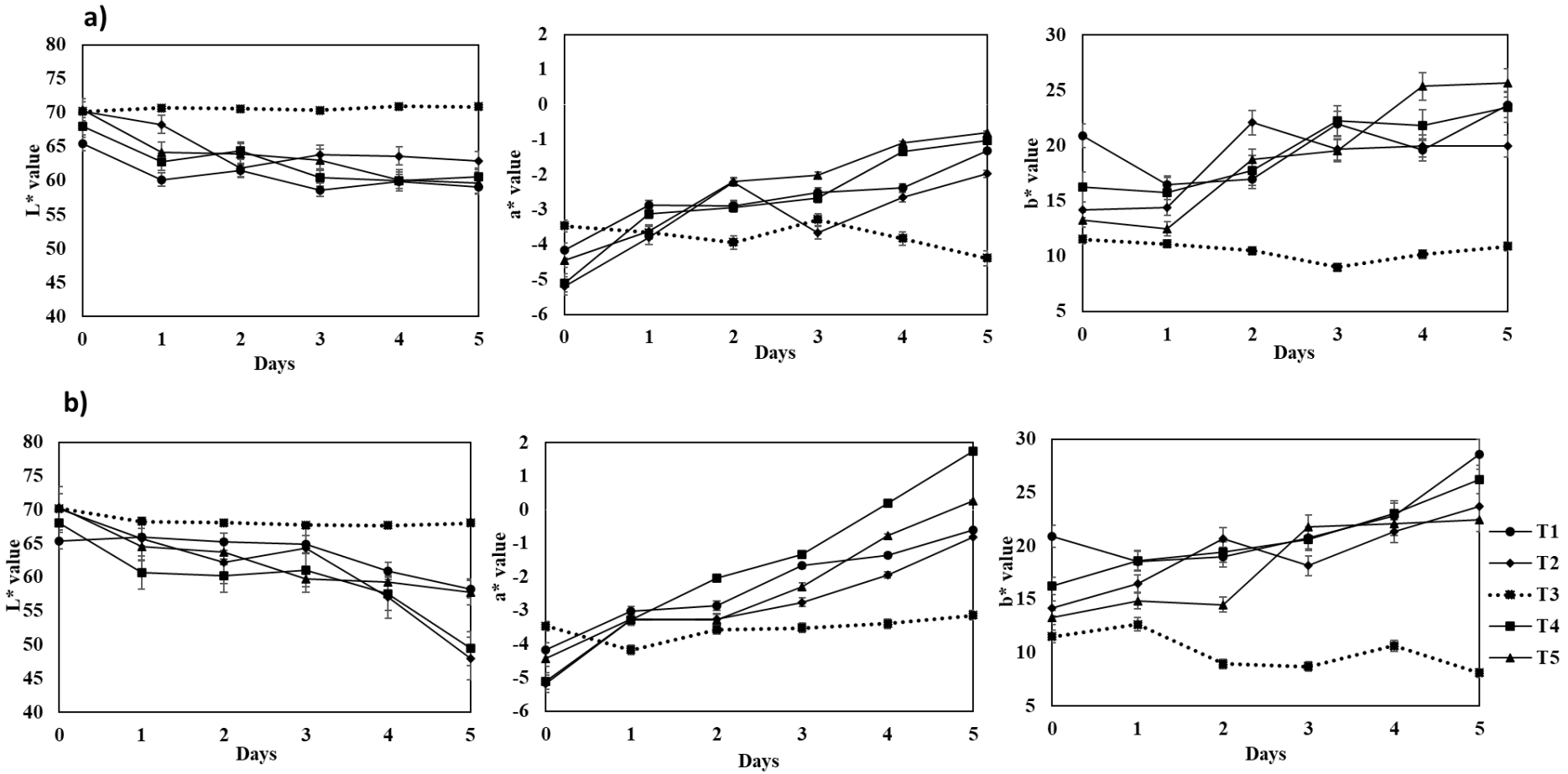


Figure 17: Effect of anti-browning agents on the colour of fresh cut apples measured as *Lab* values during storage at (a) 4°Cand (b) 10°C

T1 (Control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid.

The overall change in colour (ΔE) for the potatoes over the 5 day storage period at temperatures 4°C and 10°C is shown in **Figure 18**. There was a general increase in ΔE for all treatments at both storage temperatures as a result of the discolouration of the potatoes. The increase in ΔE was more apparent in potatoes stored at 10°C than those stored at 4°C because the higher temperature was more favourable to enzymatic browning than the lower temperature.

The overall change in colour (ΔE) for the apples over the 5 day storage period at temperatures 4°C and 10°C is shown in **Figure 19**. There was a general increase in ΔE for all treatments at both storage temperatures.

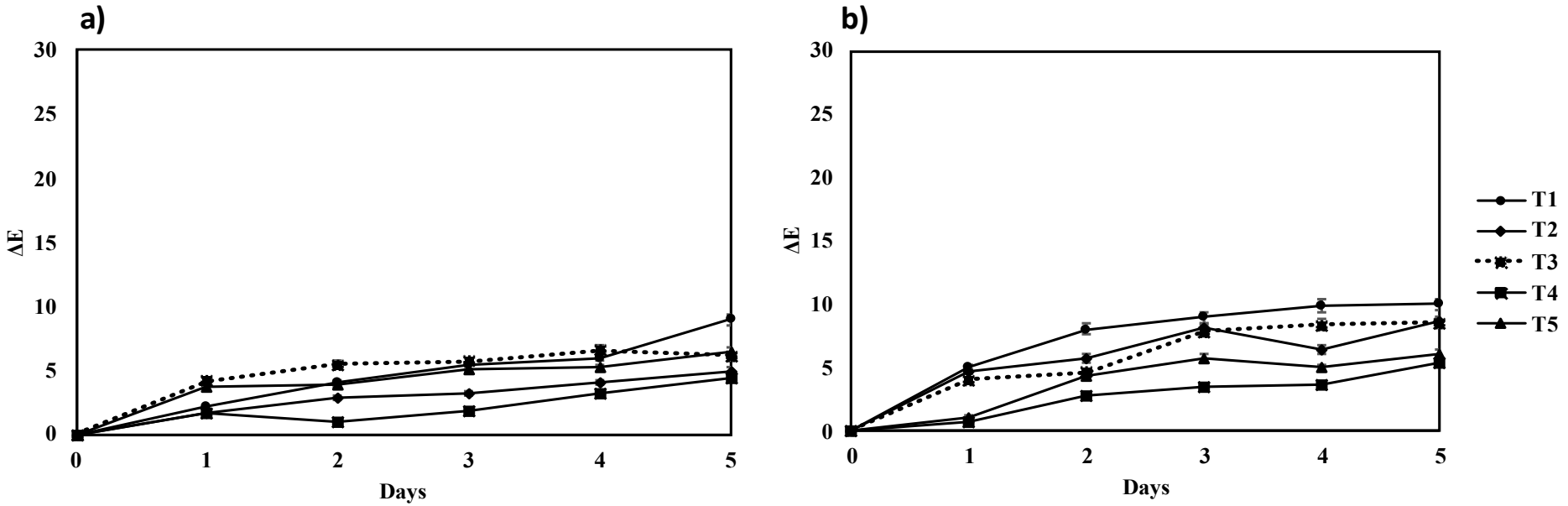


Figure 18: Effect of anti-browning agents on the overall colour change (ΔE) of fresh cut potatoes stored at (a) 4°C and (b) 10°C

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid).

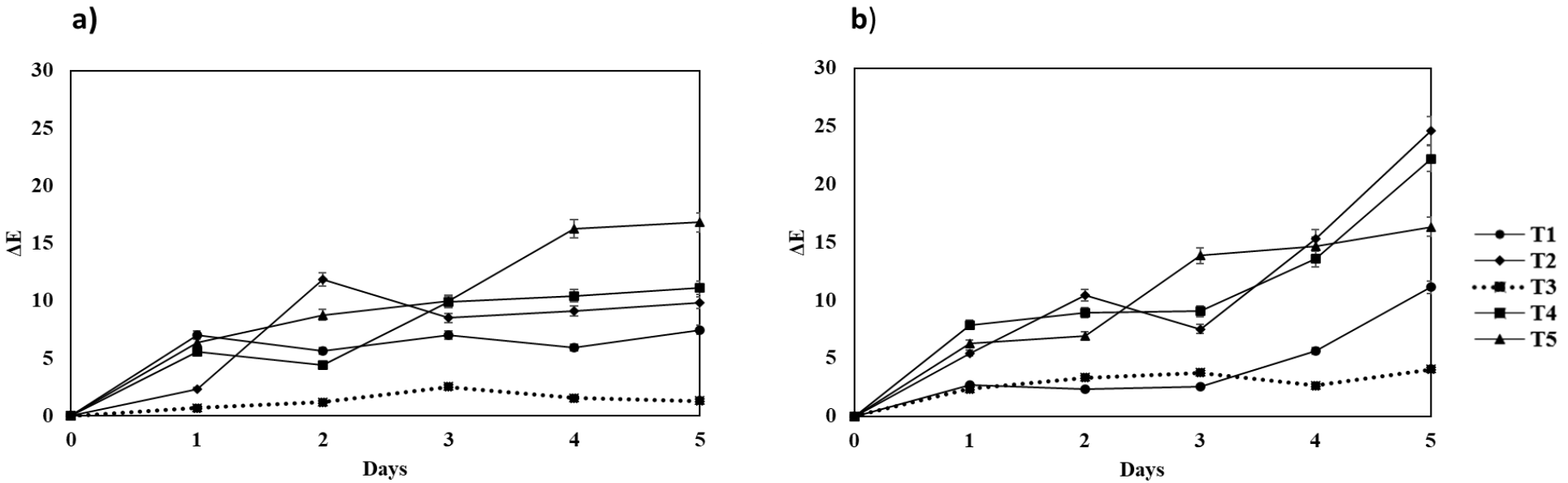


Figure 19: Effect of anti-browning agents on the overall colour change (ΔE) of fresh cut apples stored at (a) 4°C and (b) 10°C

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid).

Colour changes in fresh produce are an important sensory attribute from a consumers' perspective. The results from the L*, a*, b* values and total colour change (ΔE) showed that compared to the positive control samples (T3), the solutions that contained the crude extract (T4 and T5) were not as effective in controlling the change in colour of the potato and apple pieces. Regarding ΔE, the values higher than zero were observed suggesting that potatoes' and apples' colour changed as storage time increased. Zhang et al. (2021) showed that the ΔE values of kiwi fruits increased with increase in shelf life. According to Delgado et al. (2016), colour changes in fresh produce are related to the occurrence of enzymatic browning reactions due to PPO activity and this was further supported by the increase in a* values which represent redness, a typical colour of browning products. The increase in ΔE values was also more apparent in the fresh produce stored at 10°C than that stored at 4°C. Ultimately, the potatoes and apples stored at 10°C browned faster than those that were stored at 4°C.

4.2.3.2 Browning assessment

At day 0, there was no browning that was observed on the potatoes at both storage temperatures in all treatments (**Figures 20** and **21**). As storage progressed to day 5, browning became more evident in T1 and T2 stored at 4°C and 10°C. No browning was observed in T3 for the 5 day storage period at both storage temperatures. At storage temperature 4°C, no browning was recorded from day 0 to day 2 in T4 and T5 while at storage temperature 10°C, no browning was observed in T4 and T5 until day 3. At day 5, all treatments except T3 showed browning at both storage temperatures. Browning was more apparent in the potatoes stored at 10°C (**Figure 21**) than those that were stored at 4°C (**Figure 20**). The oxidative browning reactions in which PPO oxidizes phenolic compounds into a brown pigment called melanin resulted in the brownish colouration of the potatoes and apples (Ding & Ling, 2014).

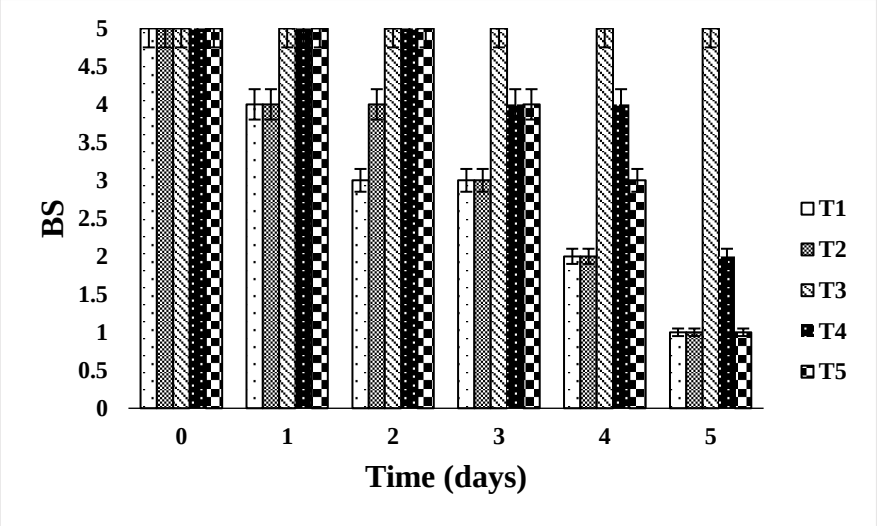


Figure 20: Effect of anti-browning agents on the browning score (BS) on fresh cut potatoes stored at 4°C for 5 days

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid)

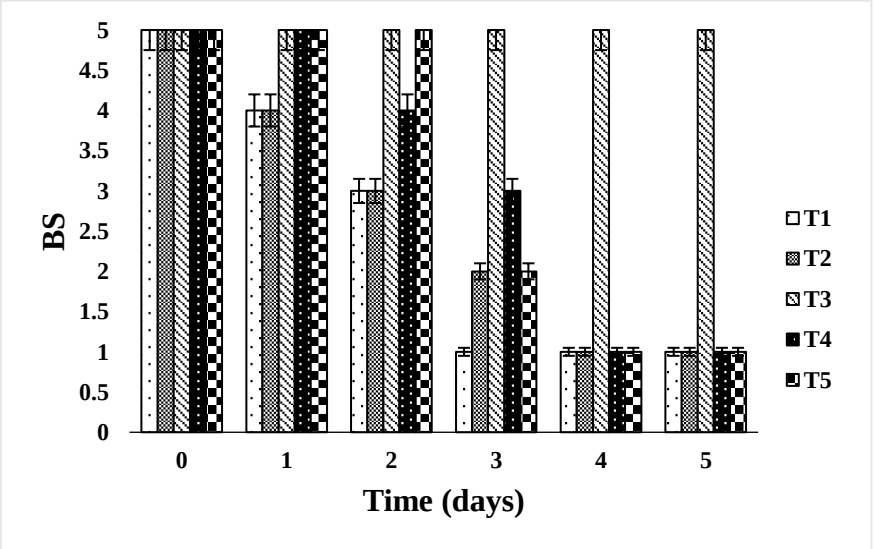


Figure 21: Effect of anti-browning agents on the browning score (BS) of fresh cut potatoes stored at 10°C for 5 days

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid)

At day 0, there was no browning that was observed on the apples at both storage temperatures in all treatments except T1 (**Figures 22** and **23**). By day 5, browning was evident in all treatments expect for T3 stored at 4°C and 10°C. No browning was observed in T3 for the 5 day storage period at both storage temperatures because of the synergistic effect of sodium metabisulphite, citric and ascorbic acids which are all anti-browning agents. T3 stopped the enzymatic browning process by inhibiting PPO enzyme and preventing the formation of the melanoidin brown pigments by either reducing the diphenols to phenols or forming complexes with the quinones (Haryani, 2017; Lee 2007). At day 1 and day 2, T4 (sample with the crude extract alone) was inhibiting browning more than when combined with other preservatives. This could have been because of the higher concentration of the crude extract used which most likely contributed to the higher amounts of phenolic compounds and organic acids (**Table 4**) that inhibited PPO. Browning was prominent in the apples stored at 10°C (**Figure 23**) than those that were stored at 4°C (**Figure 22**).

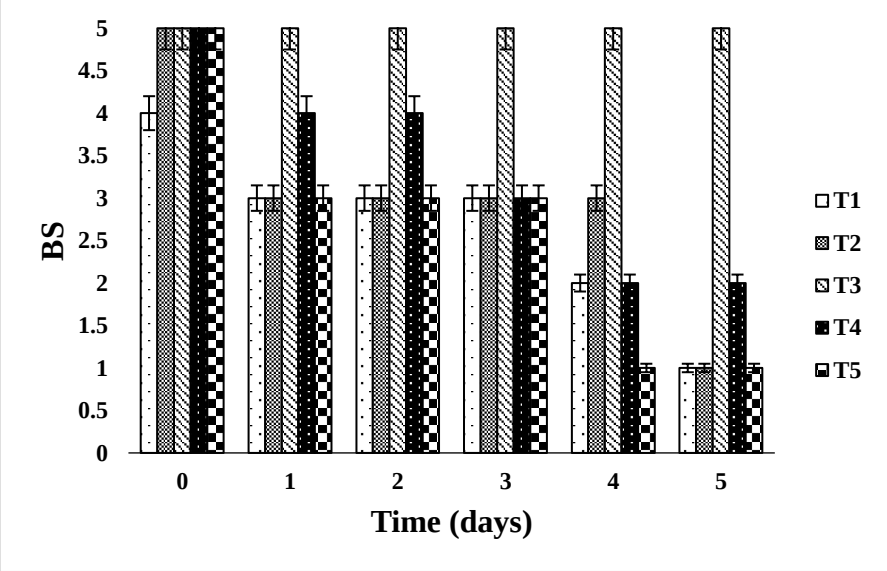


Figure 22: Effect of anti-browning agents on the browning score (BS) of fresh cut apples stored at 4°C for 5 days

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid)

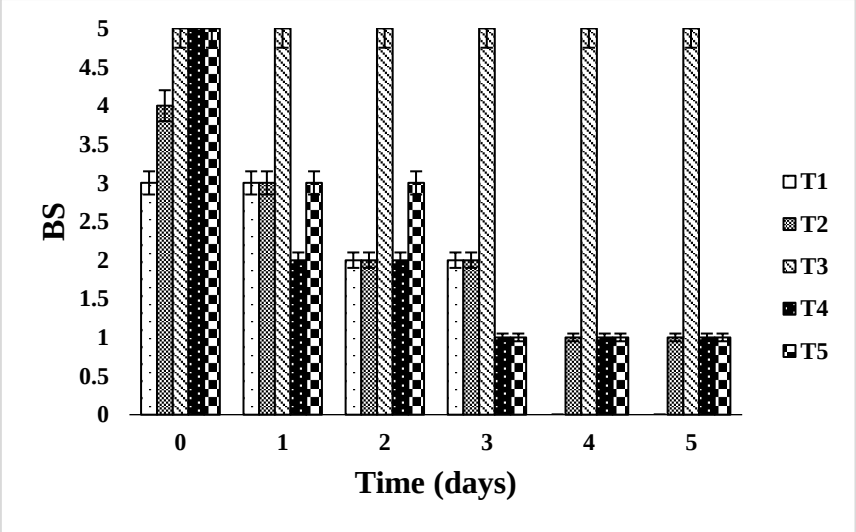


Figure 23: Effect of anti-browning agents on the browning score (BS) of fresh cut apples stored at 10°C for 5 days

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid)

The browning index (BI) of potatoes and apples derived from L*, a* and b* values is shown in **Figures 24** and **25**, respectively. There was an overall increase in BI from day 0 to day 5 during the storage period of potatoes at 4°C and at 10°C. Regarding the apples; T1, T2, T4 and T5 at both storage temperatures and T3 at 10°C showed a general increase in the BI. By day 3, there was a decrease in the BI of T3.

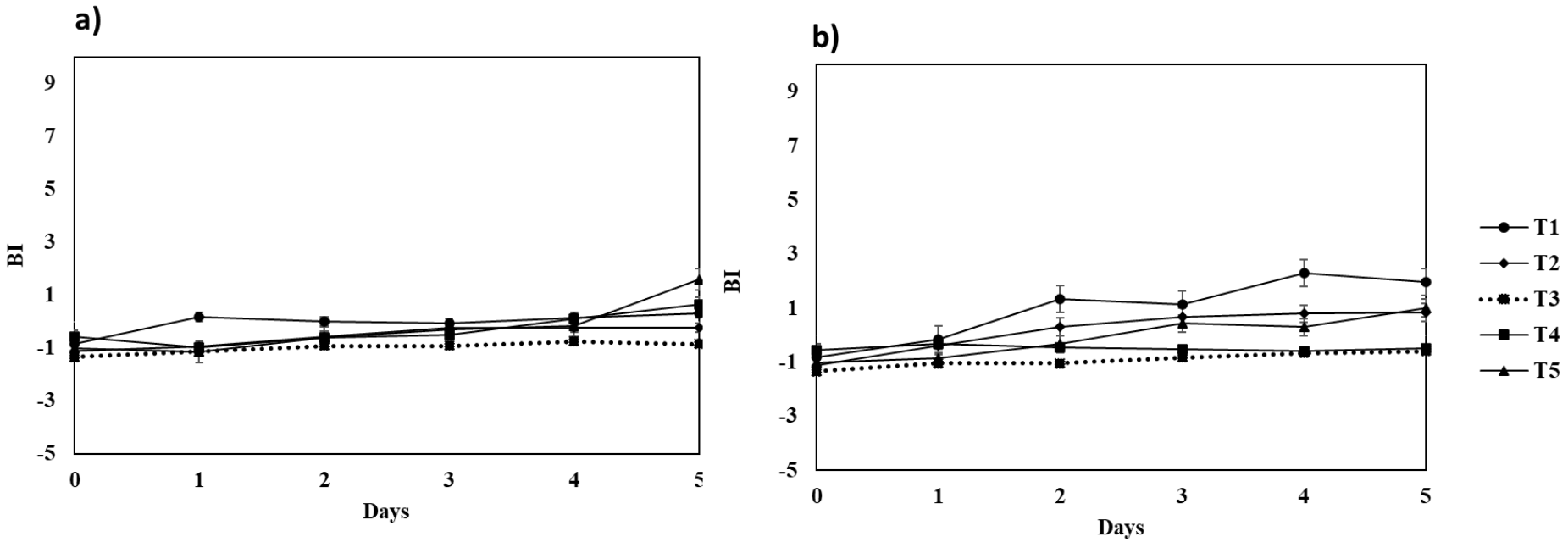


Figure 24: Effect of anti-browning agents on the browning index (BI) of fresh cut potatoes stored at (a) 4°C and (b) 10°C

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid)

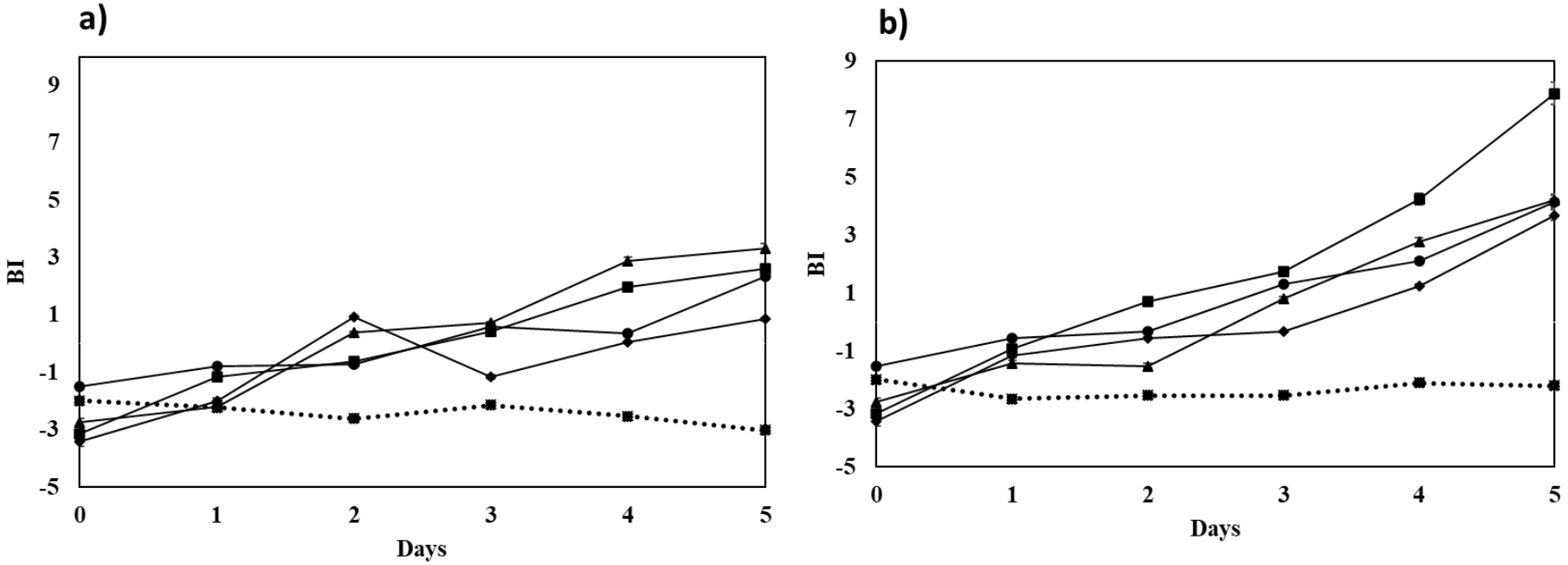


Figure 25: Effect of anti-browning agents on the browning index (BI) of fresh cut apples stored at (a) 4°C and (b) 10°C

T1 (control), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% Carica papaya crude extract) and T5 (Sample immersed in 1% Carica papaya crude extract, 0.1% ascorbic acid and 0.1% citric acid)

The results revealed that there was a positive relationship between the BI and BS. Increase in the BI was due to the increase in browning. These results were similar to those shown by Ruangchakpet & Sajjaanantakul (2007). From the results, it was also evident that besides the treatments, the rate of browning in the potatoes and apples was also influenced by the storage temperature conditions. Apples and potatoes stored at 4°C showed a lower rate of browning than those stored at 10°C. This is because temperature is an important factor in determining the activity of PPO, an enzyme that plays a vital role in enzymatic browning of fresh produce (Terefe et al., 2016). Lower temperatures below 5°C tend to inhibit enzyme activity because at these temperatures, the number of successful collisions between the enzyme and substrate is reduced due to decrease in their molecular movement (Li et al., 2018). In simpler terms, the enzyme is inactive. With regards to the treatments, the results show that T4 and T5 were not as effective as T3 in controlling browning in both apples and potatoes. However, T4 was generally more effective than T5 in controlling browning. This could be due to the differences in composition and concentration of the treatments.

4.2.3.3 Conclusion

Carica papaya peel crude extracts inhibited browning in both apples and potatoes but were not as effective as T3 which was the reference treatment used. The crude extracts were more effective in the apples and potatoes that were stored at 4°C than those stored at 10°C because the lower temperature (4°C) slowed down PPO activity more than the higher temperature (10°C). The crude extract treatments were also generally more effective against preventing enzymatic browning in potatoes than in apples. In reference to T3, the protective effect against enzymatic browning offered by T4 and T5 (samples that contained the crude extract) was much less since these treatments only prevented browning for an average of three days in potatoes and an average of two days in apples. The current shelf life of fresh cut apples is generally between 3 to 5 days while that for fresh cut potatoes is about 7 days. Based on this, findings in this research are significant within the minimally processed fresh produce industry because they show that the peel crude extracts have a potential to be used as antibrowning agents.

CHAPTER 5: GENERAL DISCUSSION

5.1 Discussion of research methodologies used

In this research study, peels from mature papaya fruit (fruit waste) were used. *Carica papaya* peels are reported to contain substantial amounts of phenolic compounds with antimicrobial and antioxidant properties (Muhamad et al., 2017).

The extraction parameters used in this study to obtain the crude extract namely, use of 90% acetone as the extraction solvent and extraction time of an hour were chosen based on a study done by Ng et al. (2012). Ng et al. (2012) studied the influence of different extraction parameters on the antioxidant properties of *C. papaya* peel and found that the best extraction conditions that yielded a high total phenolic content were 90% acetone for 1h. Acetone was therefore a suitable solvent because generally phenolic compounds are polar. Thus, to achieve extensive and efficient extraction of the bioactive compounds, the peels were freeze dried, milled to powder and the extraction process done three times on the same sample. After the extraction step, the samples were filtered using Whatman 1 filter paper to obtain a filtrate. The filtrates were then concentrated under vacuum at 45°C using a rotary evaporator. Concentration was done under a vacuum and at lower temperatures to remove acetone and to prevent any possible degradation of the bioactive compounds especially phenolic compounds that were present within the extracts. Temperatures 50°C and above favour thermal decomposition of bioactive compounds in plants especially the phenolic compounds (Badin et al., 2020; Başlar et al., 2014). It should however be noted that in food applications acetone would not be a suitable extraction solvent because it is not food grade. Further, suitable solvents can be used in the case where plant extracts to be used in food applications are needed, namely, water which is a universal solvent as well as commonly used material in food processing.

The agar well diffusion method was used to evaluate antimicrobial activity of the crude extracts. The assay utilised the pour plate method. Initial antimicrobial activity testing was done on both yeast and bacteria to determine which microorganism were susceptible to the crude extract. The crude extract was found to be active against the bacterial species (*Escherichia coli* and *Listeria monocytogenes*) as evidenced by the zones of inhibition.

To determine its stability, the crude extract was exposed to storage temperatures of 4°C and 10°C for five days. These are typical processing and storage temperatures of minimally

processed fresh produce (Banerjee et al., 2018). Upon exposure to these storage temperatures, the crude extract was tested for antimicrobial activity against the two bacteria: *L. monocytogenes* and *E. coli*. This was done to link the storage of produce at these temperatures with the survival of the bacteria at these temperatures.

The agar well diffusion method showed that the crude extracts from *C. papaya* peel were active against the bacteria but gave no indication of what bioactive compounds were responsible for this. To investigate this, it was necessary to use a chromatographic method for the characterisation and identification of the bioactive compounds in *C. papaya* peel. The LC-MS system comprising an ultra-performance liquid chromatograph (UPLC) and a quadrupole time-of-flight (QTOF) mass spectrometer with electrospray ionisation in the negative mode was used. The advantage of using the negative mode electrospray ionisation method for LC-MS is due to its ionisation efficiency and its high sensitivity and selectivity towards significant biological compounds (Xue et al., 2020). Generally, the LC-MS method allows for the identification and characterisation of bioactive compounds and gives information about the compound molecular formula and mass obtained from the mass-to-charge (m/z) ratio (Gupta et al., 2021).

The UPLC-QTOF mass spectrometry method utilises a 1.7 µm reverse phase packing column combined with a chromatographic system which operates at pressures between 6000-15 000 psi (Trenerry & Rochfort, 2010). This is different from the conventional high-performance liquid chromatography (HPLC) method that usually utilises a 5.0 µm column and runs at 5000 psi. Additionally, due to resistance to high back pressure, the UPLC-QTOF method provides a higher speed and resolution and sensitivity than HPLC (Chawla & Ranjan, 2016).

The extracts for the LC-MS analysis were prepared differently from those that were used for the antimicrobial and antioxidant assays. The extraction solvent used was 50% aqueous methanol and 1% formic acid instead of 90% acetone. This is because the 90% acetone would have damaged the columns of the UPLC. Using a different extraction solvent would most likely cause a variation in the composition of bioactive compounds obtained from the LC-MS analysis given that all experiments carried out during the research used acetone extracts. However, studies done by Sarikurkcu et al. (2015) and Tongur et al. (2018), showed that the phenolic compound composition of extracts from acetone and methanol was similar. The limitations in this study were lack of enough standards to validate and quantify all the phenolic compounds and as a result the phenolic acids were expressed as gallic acid equivalents and flavonoids as quercetin equivalents. Compounds such as organic acids and glucosinolates

known to possess bioactivity such as antioxidant activity were identified but could not be quantified due to lack of standards.

The total phenolic content (TPC) of the crude extract was determined using the Folin-Ciocalteu (F-C) method. The TFC assay also known as the aluminium chloride calorimetric assay is widely used for the quantification of flavonoids. It is based on the formation of flavonoid-aluminium chloride complexes and the spectrophotometric determination of the formed complex (da Silva et al., 2015). The F-C assay was originally for protein analysis but was later adapted to qualitatively detect phenolic compounds (Lamuela-Raventós, 2017). The principle of this method is based on the oxidation of phenolic hydroxyl groups (reducing agents) in a sample by the F-C reagent (oxidizing agent) (Soekamto & Syah, 2021). The F-C reagent ultimately reacts with any reducing agents within a sample; be they phenolic or not, implying that the F-C reagent is non-specific and can be affected by other non-phenolic reducing molecules like organic acids (citric, tartaric, ascorbic acid) and reducing sugars (e.g. fructose and glucose) (Cerulli et al., 2018). Even with this drawback, the F-C method produces several advantages such as convenience, reproducibility and simplicity and is widely preferred for the quantification of the total phenolic content of a sample (Messaoudene et al., 2018). Due to the non-specificity of the F-C method and its inability to identify phenolic compounds, it is always vital to carry out LC-MS method for further analysis. The LC-MS method as mentioned earlier can be used for the identification and characterisation of phenolic compounds within a sample.

This study utilised the Ferric Reducing Antioxidant Power (FRAP) assay to determine the metal chelating activity of the crude extract. The FRAP assay is based on the electron donating ability of antioxidants (Chen et al., 2010). It measures the ferric to ferrous reduction in the presence of antioxidants in a sample (Gupta et al., 2009). The reaction occurs at a low pH of 3.6 by the reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to the ferrous deep blue coloured form (Bibi Sadeer et al., 2020). The formation of chelates with Fe^{2+}, ferrozine can generate a complex that is a pale red colour. Nevertheless, the fading of red shade in ferrozine-Fe^{2+} complexes is only restricted in the presence of other chelating agents (antioxidants) present in a sample. The measurement of the reduced colour determines the chelating activity that competes with ferrozine for ferrous ion (Haida & Hakiman, 2019). The method is simple, relatively inexpensive and reproducible (Shah & Modi, 2015).

The toxicity of the peel and the peel crude extract with reference to the presence of papain and chymopapain enzymes was tested using the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. This method uses the principle of separation of proteins

based on molecular weight differences (Hidayat et al., 2018). Any variation of the SDS-PAGE assay is based on the complexes formed between SDS and proteins (Pavlova et al., 2018). Due to the presence of a twelve-carbon tail, SDS associates with non-polar regions of the protein molecule through hydrophobic interactions, while the polar head of the SDS molecule gives a net negative charge to the SDS–protein complexes (Hamdan & Righetti, 2005). In addition to this the Bicinchoninic acid (BCA) assay was used to quantify the proteins present in *C. papaya* peel and *C. papaya* peel crude extract. This method is based on the ability of amino acids in a sample to reduce Cu^{2+} ions to Cu^{+} by the amide backbone of polypeptide chains and result in a purple colour formation by bicinchoninic acid (Alhazmi, 2019). The reduction of copper is mostly induced by four amino acid residues found in protein molecules: cysteine, cystine, tyrosine, and tryptophan (He, 2011). Unlike the Coomassie dye-binding approaches, however, the universal peptide backbone also contributes to color production, reducing variability caused by changes in protein composition (Chalier et al., 2020). Normally, standard solutions of bovine serum albumin (BSA) are used to create an absorbance *versus*. mass concentration calibration curve. (Šafranko et al., 2019). The BSA method has been used for a long time to determine the concentration of extracted proteins in a sample and is one of the trusted assays (Brady & Macnaughtan, 2015).

The ability of the crude extract to inhibit the browning of fresh cut apples and potatoes was determined through colour changes measured using a colorimeter. In this study, colorimetric values were measured to add to the changes in colour of the apples and potatoes that were evaluated visually by the human eye. This is because visual discrimination of colour is very subjective to the viewer's unique perspective and therefore this alone could not be used as substantial evidence to determine colour changes in apples and potatoes for the browning experiment. Colour perception is based on two major theories, the trichromatic colour theory and the opponent process theory (Grzybowski & Kupidura-Majewski, 2019). The trichromatic hypothesis describes how colour vision works at the receptor level, with photoreceptors most sensitive to blue, green, and red colours (Ohta & Robertson, 2006). The opponent process theory describes specific colour pairings that are opposed to one another (green and red, black and white, blue and yellow) (Ohta & Robertson, 2006). The colorimeter equipment evaluates the reflected wavelength intensity to derive the colour perceived, similar to how the brain interprets the signal from the photoreceptors in human colour vision (Dunbar et al., 2017). Colorimetric devices with key components (illuminant and coloured filters that duplicate the

spectrum sensitivity of cones in the human eye) and a processor to correct for the standard observer are used to achieve objective colour quantification (Ly et al., 2020).

Colour quantification can be performed and represented using different systems. The CIELAB and XYZ colour systems describe components of a colour relative to the standardized reference wavelengths of monochromatic red, green and blue lights (Autelitano & Giuliani, 2019). The CIELAB system, a three dimensional colour system consists of three axes is the most widely used colour system (Durmus, 2020). The gray scale on the L* axis ranges from 0 (black) to 100 (white). The red/green axis is denoted by the letter a*; positive a* denotes green values, whereas negative a* denotes green values. The yellow/blue axis is denoted by the letter b*; positive and negative b* denote yellow and blue values, respectively. The asterisk (*) in the CIELAB units distinguishes the CIELAB system from other colour systems' units. (Edwards et al., 2016). Colorimeters have a wide range of uses, however they are restricted in their capacity to distinguish between colours that have the same perceived appearance but differ in spectral properties. Furthermore, in order to compare data acquired with different colorimetric devices, the fundamental standardization standards must be satisfied (Ly et al., 2020). Colorimetric measures, on the other hand, give objective and repeatable results while reducing biases and erroneous reporting that might occur when employing visual evaluation (Chien et al., 2016).

5.2 Summary and discussion of key research findings

5.2.1 Antioxidant and antimicrobial properties of *Carica papaya* peel crude extracts

Table 12: Summary of the main findings based on the bioactivity in *Carica papaya* peel crude extracts

Property	Property investigated	Observations
Antimicrobial activity	Antibacterial activity	Crude extracts were active against bacteria; *L. monocytogenes* and *E.coli* but did not possess antifungal activity.
Antioxidant activity	Total phenolic content	High total phenolic content present in the crude extracts than has been reported.
	Total flavonoid content	High flavonoid content present in the crude extracts.
	Ferric reducing antioxidant power (FRAP)	Ability of the crude extracts to reduce ferric ions to its ferrous state was determined
Identification and characterisation of compounds in the crude extracts		Glucosinolates, organic acids and phenolic compounds identified and characterised
Toxicity of the crude extract	Based on the presence of chymopapain and papain enzymes	Absent

Table 12 shows a summary of the main findings in this research study based on the bioactivity in *C. papaya* peel crude extracts. This study showed that *C. papaya* peel crude extracts possess antioxidant and antimicrobial properties due to the presence of bioactive compounds present in the crude extracts. Although the study only focussed on identifying organic acids, phenolic compounds and glucosinolates, carotenoids are also another group of bioactive compounds that could have been detected in the crude extracts. Carotenoids have been linked to *C. papaya* and have the ability to confer antioxidant (Calvache et al., 2016) and antimicrobial properties (Lydia et al., 2016). Carotenoids that have been found in *C. papaya* include lutein, zeaxanthin,

β-carotene and β-cryptoxanthin (Calvache et al., 2016). Of particular importance are carotenes (pure hydrocarbons) like β-carotene and carotenoids that contain oxygen as a functional group in its structure are referred to xanthophylls such as lutein, β-cryptoxanthin and zeaxanthin (Nabi et al., 2020). The existence of a polar group namely, hydroxyl, epoxy and keto in the structure affects the biological function of the carotenoid compounds (Rivera et al., 2014).

Figure 26 shows the basic chemical structure of plant carotenoids. The central biological function of carotenoids as antioxidants is their ability to inactivate singlet oxygen and to quench carboxy radicals (Keceli et al., 2013). Although the exact mechanism of carotenoids' antimicrobial action is unknown, literature shows that antioxidant and antibacterial properties of natural chemical components are linked (Shannon & Abu-Ghannam., 2016). Antibacterial action of antioxidants can be mediated in three ways: permeability of the outer membrane, cytoplasm leakage, and suppression of nucleic acid production (Zakynthino & Varzakas, 2016). **Figure 27** shows the possible antibacterial activity of bioactive compounds of plant origin. The mechanisms depicted in the figure are the inhibition of oxidation at several points within the bacterial cell membrane and this leads to formation of radicals that are detrimental. The cell ultimately diverts its energy in getting rid of these radicals there by depriving the cell of energy for essential metabolic activities. Another mechanism involves damaging of the membrane structure of the cell through the interaction of the compounds with the negatively charged cell membrane which modifies the membrane causing leakage of the cell's nutrients (Hintz et al., 2015). Additionally, natural chemical compounds also confer antibacterial properties through blocking binding sites of gyrase, an important enzyme in nucleic synthesis. This interrupts DNA coiling which retards the cell's growth (Naqvi et al., 2019)

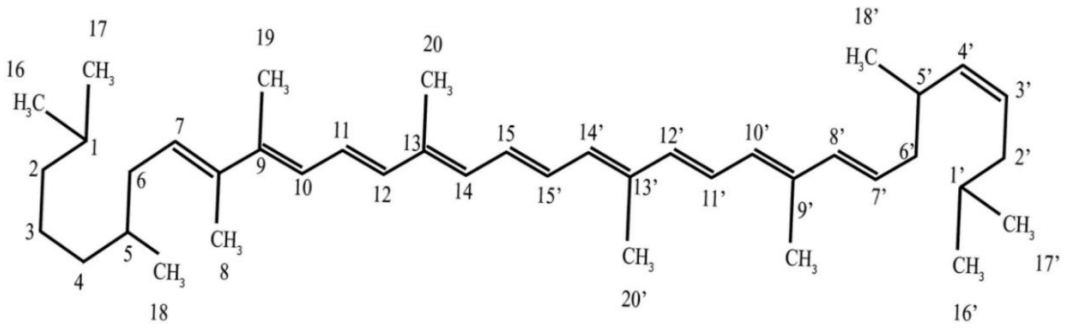


Figure 26: Basic structure of carotenoids (Adapted from Zakynthinos & Varzakas, 2016)

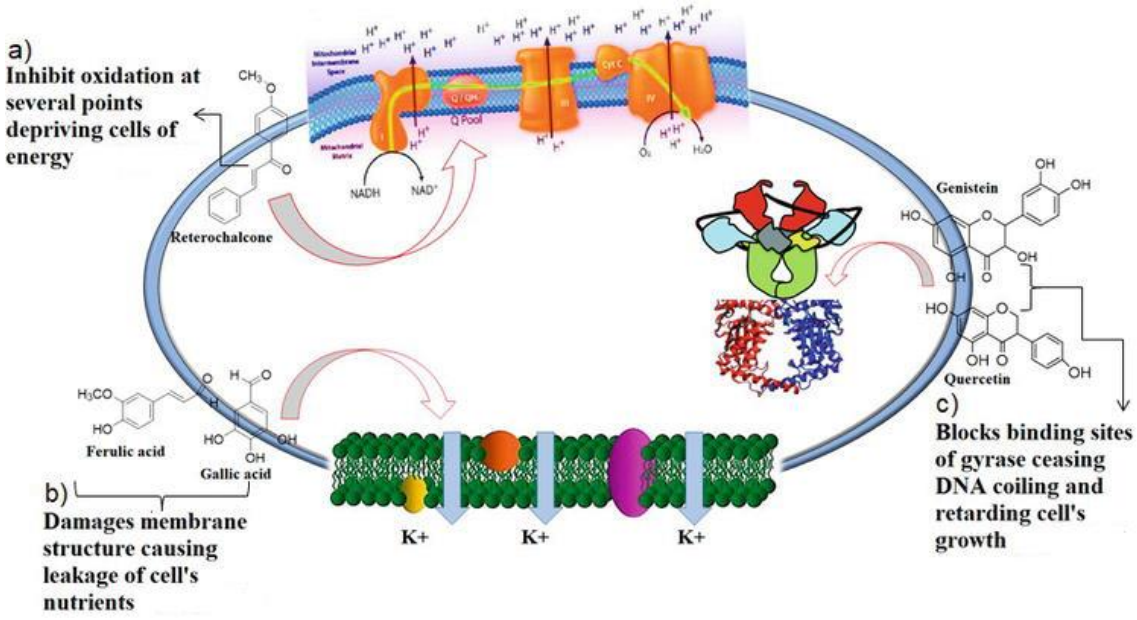


Figure 27: Schematic showing the natural antioxidant role as antibacterial: "(a)*inhibition of energy metabolism*; (b) *disruption of membranes* and (c) *interruption in nucleic acid synthesis"* (Adapted from Naqvi et al., 2019)

The crude extracts though effective on bacteria do not possess antifungal properties. *Carica papaya* crude extracts were initially screened for antimicrobial activity against bacteria (*L. monocytogenes* and *E. coli*) and yeast (*Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*) strains. The crude extracts showed antimicrobial activity against the bacteria but not the yeasts. Most plant extracts have shown more antimicrobial activity against bacteria compared to fungi (Gonelimali et al., 2018). A study by Khoo et al. (2018) showed that most if not all tropical fruits have no evidence of antifungal activity. Mohamed et al. (1994) supports this claim through their research which showed that most of the tropical fruit waste showed good activity against bacteria but poor activity against yeasts.

Inability of the crude extracts to be active against yeast could also have been concentration dependant. It is known that the antimicrobial activity of plant extracts increases with increase in their concentration. Therefore, had the concentration of the crude extract been higher than 500 mg/mL, the extracts could have been active against the yeast strains. Antimicrobial resistance to the crude extracts could also have been due to the cell wall structural differences between bacteria and yeasts (Sekyere & Asante, 2018; Pfaller, 2012). **Figure 28** shows some of the mechanisms by which fungi resist antimicrobial compounds.

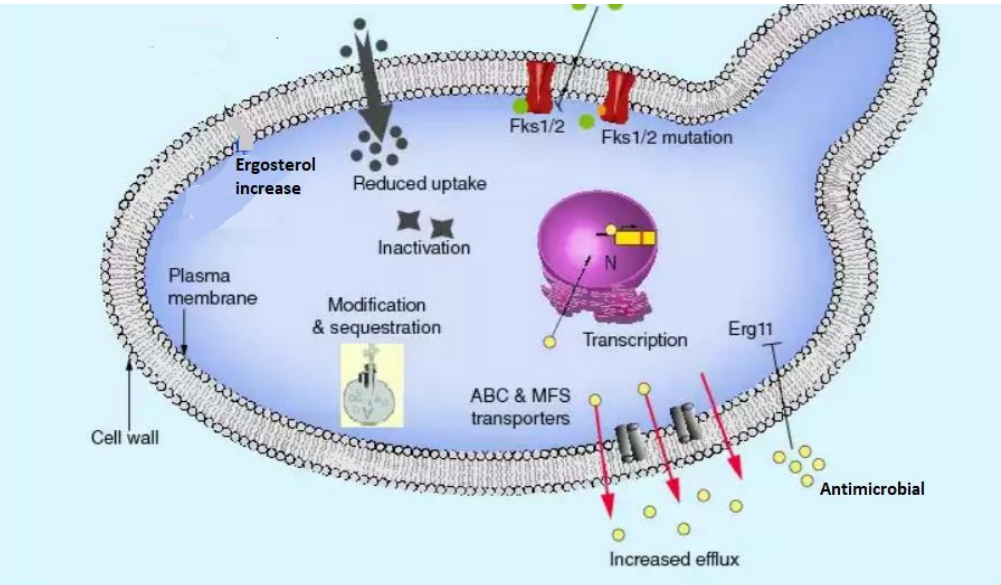


Figure 28: Antifungal mechanisms of resistance to antimicrobial compounds (Adapted from Scorzoni et al., 2017 and Tscherner et al., 2011)

5.2.2 Inhibition of enzymatic browning by *Carica papaya* peel crude extracts in fresh cut fruits and vegetables

Table 13: Summary of the general effect of treatments on the overall colour change (ΔE) and browning index (BI) of apples and potatoes stored for 5 days

	Fresh produce model	**Storage temperature**	**T1**	**T2**	**T3**	**T4**	**T5**
Overall colour change (ΔE)	Potatoes	4°C	Gradual increase	Slow increase	Constant	Gradual increase	Increase
	Apples		Increase	Increase	Constant	Increase	Increase
	Potatoes	10°C	Increase	Gradual increase	Increase	Gradual increase	Increase
	Apples		Increase	Rapid increase	Slow increase	Rapid increase	Rapid increase
Browning Index (BI)	Potatoes	4°C	Slow increase	Slow increase	Constant	Constant	Increase
	Apples		Increase	Gradual increase	Constant	Increase	Increase
	Potatoes	10°C	Gradual increase	Gradual increase	Constant	Constant	Increase
	Apples		Rapid increase	Rapid increase	Constant	Rapid increase	Rapid increase

T1 (untreated sample), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% *Carica papaya* crude extract) and T5 (Sample immersed in 1% *Carica papaya* crude extract, 0.1% ascorbic acid and 0.1% citric acid)

Table 14: Summary of the effect of treatments on the Browning score (BS) of apples and potatoes stored for 5 days at 4°C and 10°C

Fresh cut produce model	Storage temperature	T1	T2	T3	T4	T5
Potatoes	4°C	Browning observed from day 2 to day 5	Browning observed from day 3 until day 5	No browning observed for the 5 day storage period	Browning observed on day 5	Browning observed from day 4 to day 5
	10°C	Browning observed from day 2 to day 5	Browning observed from day 2 until day 5	No browning observed for the 5 day storage period	Browning observed from day 3 to day 5	Browning observed from day 1 to day 5
Apples	4°C	Browning observed from day 1 to day 5	Browning observed from day 1 until day 5	No browning observed for the 5 day storage period	Browning observed from day 3 to day 5	Browning observed from day 1 to day 5
	10°C	Browning observed from day 0 to day 5	Browning observed from day 1 until day 5	No browning observed for the 5 day storage period	Browning observed from day 1 to day 5	Browning observed from day 1 to day 5

T1 (untreated sample), T2 (Sample immersed in water), T3 (Sample immersed in 0.3% sodium metabisulphite, 0.1% ascorbic acid and 0.1% citric acid), T4 (Sample immersed in 5% *Carica papaya* crude extract) and T5 (Sample immersed in 1% *Carica papaya* crude extract, 0.1% ascorbic acid and 0.1% citric acid)

Table 13 shows a summary of the effects of treatments on the overall colour change (ΔE) and browning index (BI) of apples and potatoes stored for 5 days. Additionally, the summary of the effect of treatments containing *C. papaya* peel crude extracts in reference to other control and standard treatments on the browning score (BS) of apples and potatoes stored for 5 days at 4°C and 10°C is shown in **Table 14**. BS is indicative of the visual assessment of BS that was done on both apples and potatoes for the 5 day period. Treatments containing the crude extract showed an overall increase in ΔE, BI and BS which is directly proportional to the increase in browning as the storage period increased. The increase in these values was however more in the apples than the potatoes irrespective of the storage temperatures. Enzymatic browning in fresh produce is influenced by factors like the polyphenolic content, temperature conditions, level of maturity of fresh produce and polyphenolase (PPO) activity (Hithamani et al., 2018; Li et al., 2018).

The level of maturity of fresh produce influences the pH, polyphenolic content and PPO activity. Apples generally have a more acidic pH while pH of potatoes is slightly acidic. The pH of fresh produce does play a vital role on the influence of PPO activity on enzymatic browning. This is because enzymes work best when exposed to optimum pH conditions. A study by Li et al. (2018) showed the effect of pH on PPO activity in fresh cut potatoes as illustrated by **Figure 29**. Very high acidic and alkali conditions decrease PPO activity.

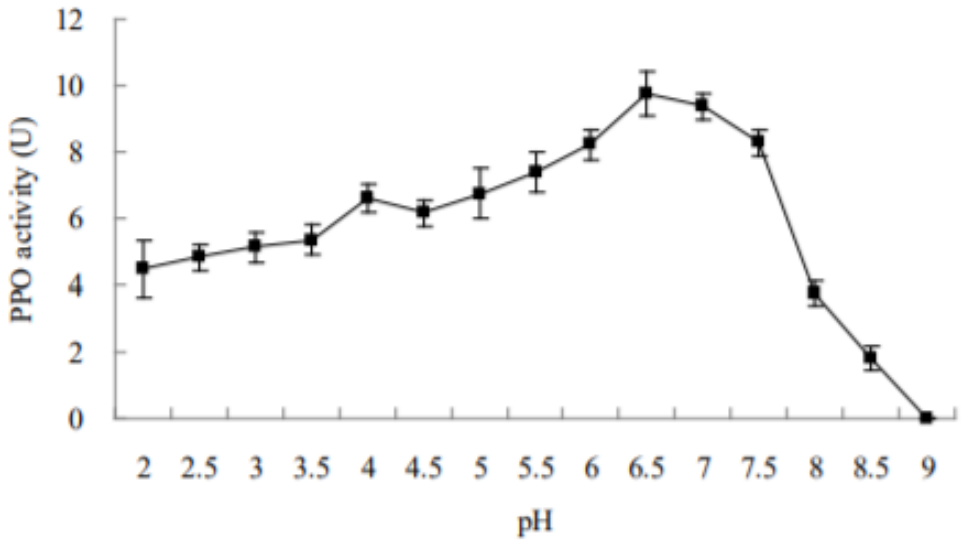


Figure 29: Effect of pH on PPO activity (Adapted from Li et al., 2018)

This shows that the potatoes would most likely experience enzymatic browning earlier than the apples however, it should also be noted that enzymatic browning is not affected solely by one factor but by a variety of factors. Another factor that plays a key role in PPO activity is temperature. From **Tables 13** and **14**, it is observed that the apples and potatoes stored at 10°C

browned faster than those at 4°C. Increase in temperature up to the optimum temperature of the enzyme increases PPO activity. **Figure 30** shows the effect of temperature on PPO activity.

The enzymatic browning process involves the oxidation of phenolic compounds in fresh produce to diphenols which are oxidized to orthoquinones and through a polymerisation reaction, melanoidins (brown pigments) are formed. Polyphenolic compounds act as substrates in this case. These compounds are found distributed within the plant cells and minimal processing processes like cutting bring the polyphenols in contact with the PPO enzyme. This therefore implies that some of the phenolic compounds within *C. papaya* peel crude extracts could have acted as substrates for the enzymatic browning process instead of inhibiting it. Holderbaum et al. (2010) investigated the link between enzymatic browning, PPO activity, and polyphenols in several apple cultivars and discovered that the total phenolic compounds (chlorogenic acid + epicatechin + procyanidin B2) and enzymatic browning had a strong positive correlation (**Figure 31**).

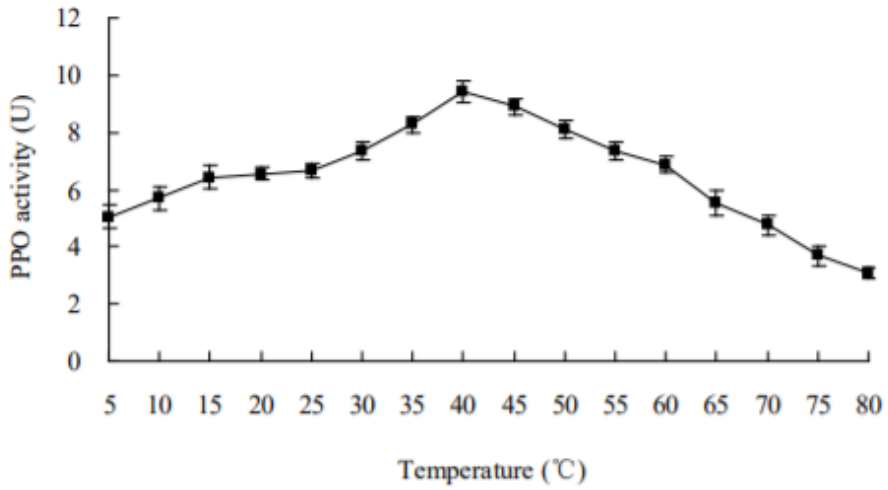


Figure 30: Effect of temperature on PPO activity (Adapted from Li et al., 2018)

The substrate concentration plays a key role in PPO activity. Increasing the substrate concentration increases the activity of the enzyme (**Figure 31**). In the current study, the concentration of phenolic compounds within the crude extracts could have been ineffective in controlling enzymatic browning in the apples and potatoes given the complexity of the food models (pH and sugar concentration) which might have led to dilution of the phenolic compounds lowering their quantity and antibrowning ability.

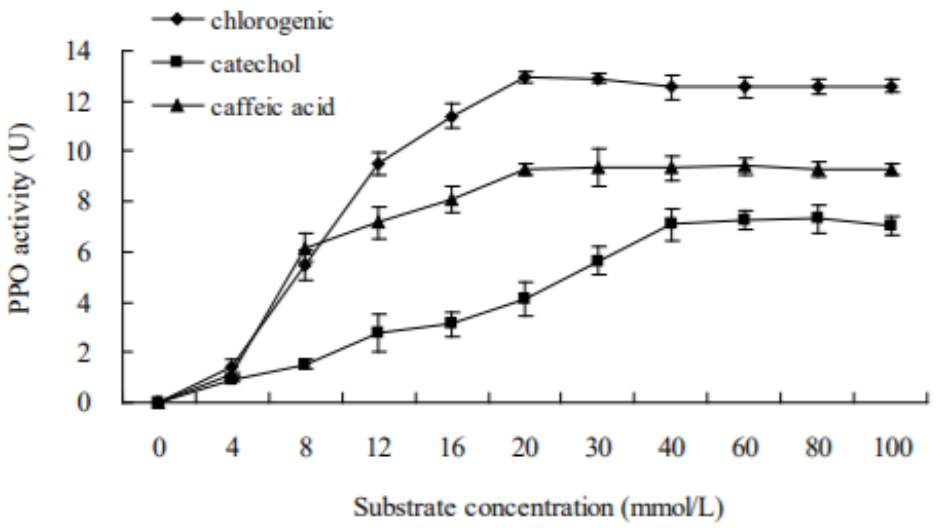


Figure 31: Relationship between phenolic compounds and substrate concentration on PPO activity (Adapted from Li et al., 2018 and Shinde et al., 2012)

5.2.3 Future prospects

Preservatives from natural sources are being developed to replace synthetic preservatives in the food industry. In a country where consumption of fruits and vegetables that are minimally processed is prevalent, the high amounts of fruit by-products from this processing could be used to extract bioactive compounds that can be used as preservatives. In Sub-Saharan Africa, *C. papaya* peel has potential use as a preservative source due to its antioxidant and antimicrobial properties. *C. papaya* peel crude extracts, the by-products from acetone extraction show a substantial number of organic acids, phenolic compounds and glucosinolates, and can be used as preservatives. Instead of using *C. papaya* peel only for enzyme extraction (Jain, 2020), it could be used as a raw material to extract phenolic compounds and organic acids. *C. papaya* peel crude extracts with their biological properties in terms of antioxidant and antimicrobial activity, could be used as natural preservatives. Such preservatives could be used in the making of edible films for coating fruits and vegetables such as apples, potatoes, pears and avocados (Rodríguez et al., 2020) or form part of treatments that inhibit enzymatic browning of minimally processed fresh cut produce to maintain their quality and extend the shelf life. Furthermore, the use of synthetic preservatives that are questionable to consumers due to their association with life threatening conditions could be reduced.

Overall, this research has shown that *C. papaya* peel crude extracts have bioactive compounds whose different properties can be utilised in the food industry. Research into application of these crude extracts as antimicrobial agents in food models is required.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

This study has shown that *Carica papaya* peel crude extracts possess antioxidant and antimicrobial properties. Based on their antioxidant properties, the crude extracts have potential to inhibit enzymatic browning in minimally processed fresh cut produce. The antioxidant and antimicrobial properties of these extracts is due to the presence of bioactive compounds (organic acids, phenolic compounds and benzyl glucosinolate).

The organic acids, phenolic compounds and benzyl glucosinolate identified by LC-MS in *C. papaya* peel are known to have antimicrobial activity and utilise different mechanisms. The ability of organic acids to gain access to the cytoplasm of microbial cells bringing about a change in the pH homeostasis and the disruption of the proton motive force in microorganisms could account for their antimicrobial effect. Phenolic compounds can form complexes with microbial cell membranes and their transport proteins through hydrophilic and hydrophobic bonding altering the cell's structures which is detrimental to its survival. Isothiocyanates which are produced from the hydrolysis of benzyl glucosinolate bind to sulphydryl groups within the active sites of microbial enzymes and reduce the viability of the microorganisms through the reduction of thiol groups which form free radicals.

Papain and chymopapain, enzymes that possibly contribute to toxicity, are absent in the crude extracts from *C. papaya* peel. The crude extract inhibits enzymatic browning in apples and potatoes though for a shorter time than the standard treatment used in the food industry during minimal processing. It is also evident that the crude extracts prevent enzymatic browning in potatoes than in apples.

Overall, this research shows that *C. papaya* peel crude extracts could have potential as a preservative in various food applications especially minimally processed fresh produce. *C. papaya* peel crude extracts can also possibly be used as alternatives to synthetic preservatives. Although the crude extract inhibits enzymatic browning in the apples and potatoes for shorter periods compared to the normal 5 day shelf life of minimally processed fresh produce, further purification of the crude extract could most likely improve its efficiency. A combination of the crude extract with organic acids or other preservatives produces a synergistic preservative effect on the fresh produce due to the improved efficiency of the preservatives in the mixture. The 5% crude extract solution in itself is a mix of other compounds as well thus activity can be affected

by the type of compounds present. The crude extract's ability as an antimicrobial preservative can be utilised in the food industry.

Research into applications of *C. papaya* peel crude extracts as antimicrobial preservatives in foods could be conducted to see whether these extracts within a food model can inhibit microbial growth. The refined extract could have better antioxidant and antimicrobial properties than the crude extract. Future research can also focus on the study of carotenoids in *C. papaya* peel. Carotenoids contribute to the orange colour of the peels and besides their application as colourants within the food industry, they also have known antioxidant properties.

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APPENDIX A

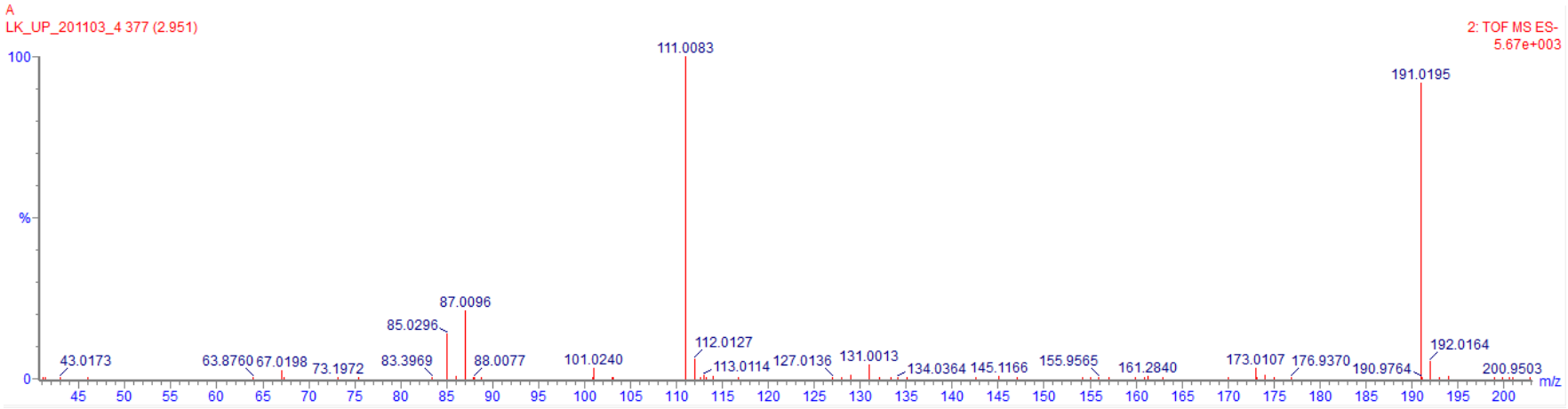

Figure A 1: Mass spectrum of citric acid (191 m/z) peak 1

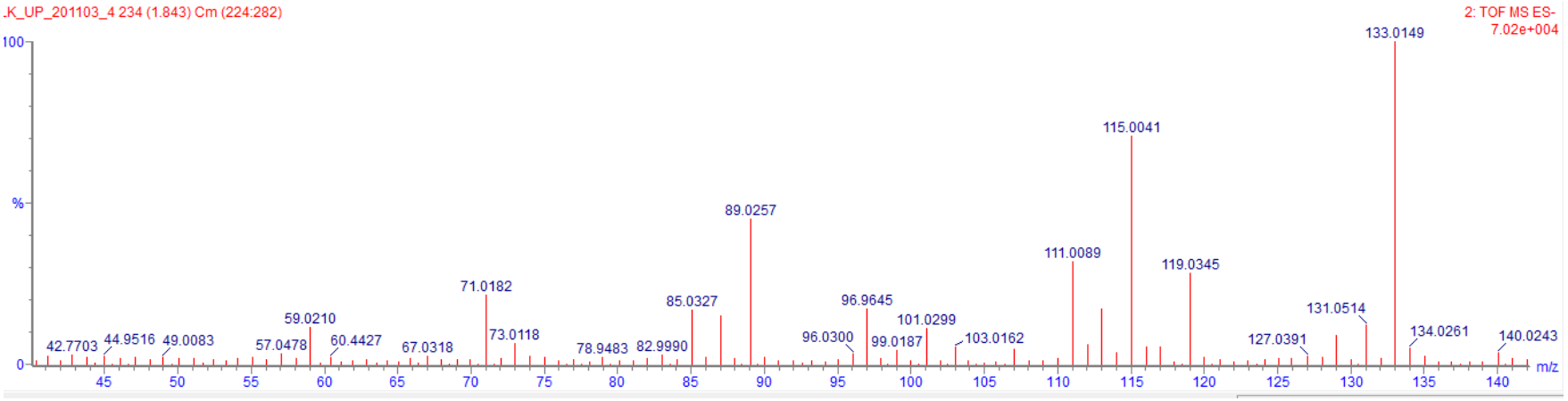

Figure A 2: Mass spectrum of malic acid (133 m/z) peak 2

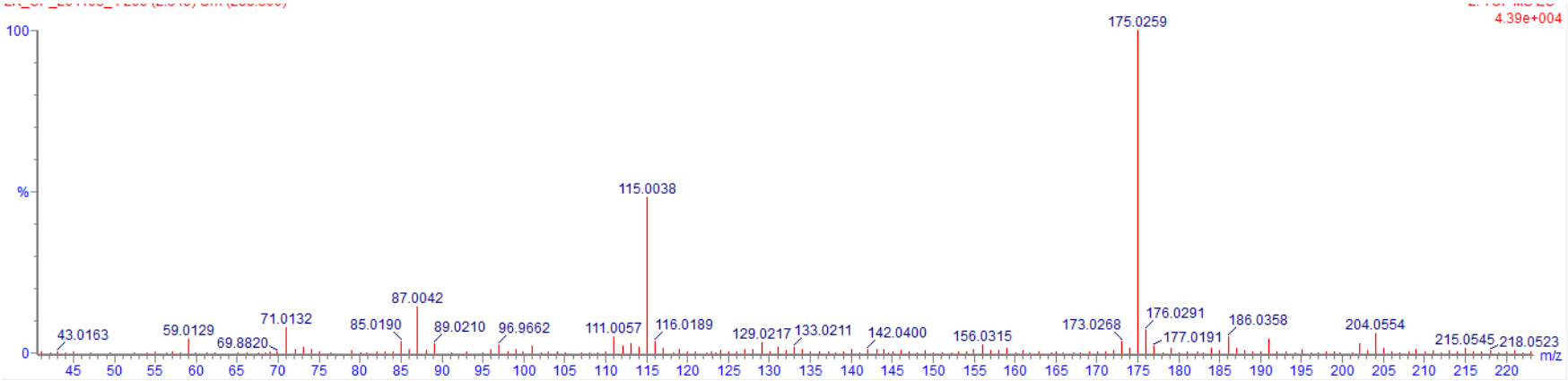

Figure A 3: Mass spectrum of ascorbic acid (175 m/z) peak 3

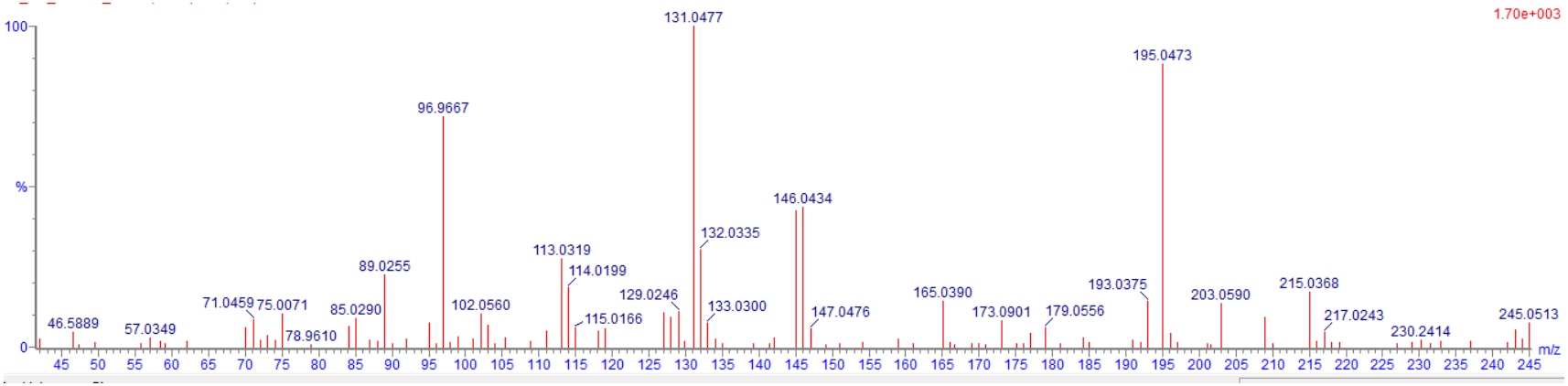

Figure A 4: Mass spectrum of gluconic acid (195 m/z) peak 4

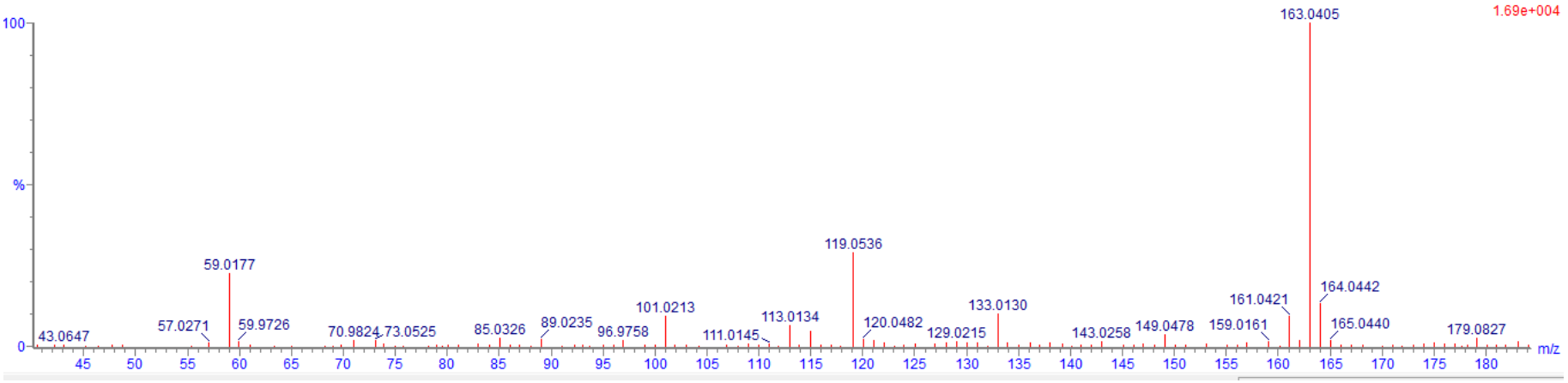

Figure A 5: Mass spectrum of ρ-coumaric acid (163 m/z) peak 5

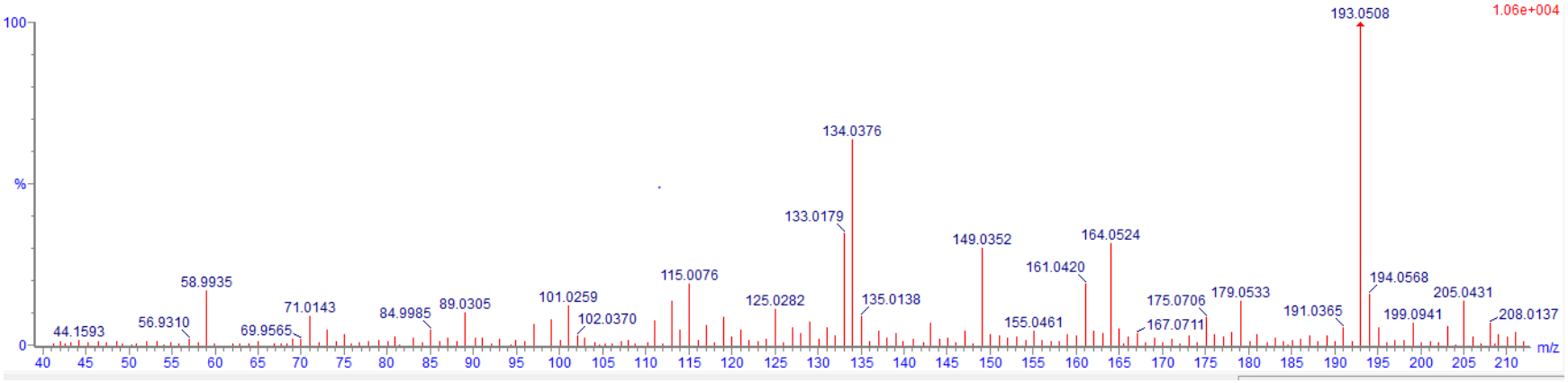

Figure A 6: Mass spectrum of ferulic acid (193 m/z) peak 6

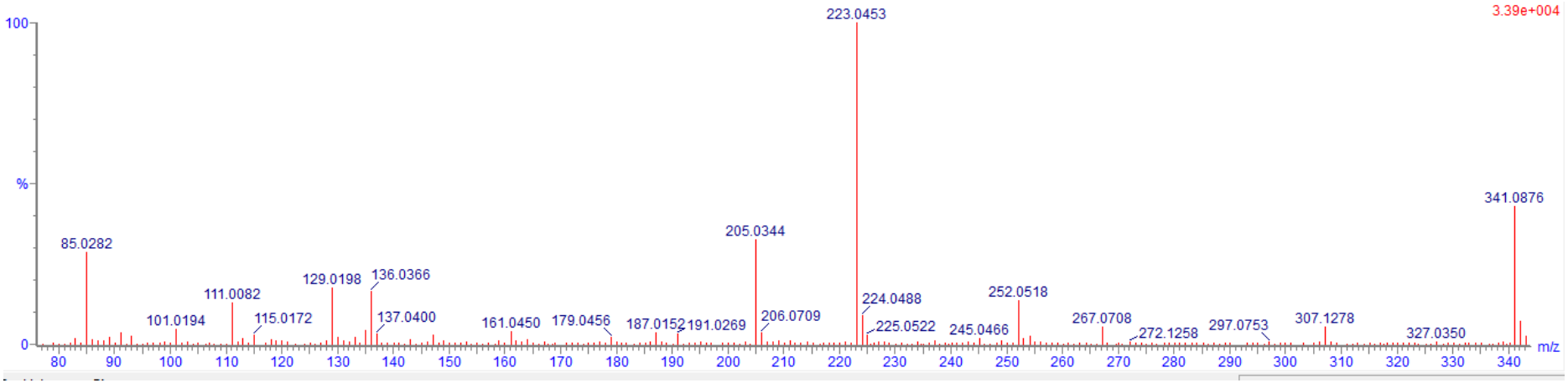

Figure A 7: Mass spectrum of caffeic acid glucoside (341 m/z) peak 7

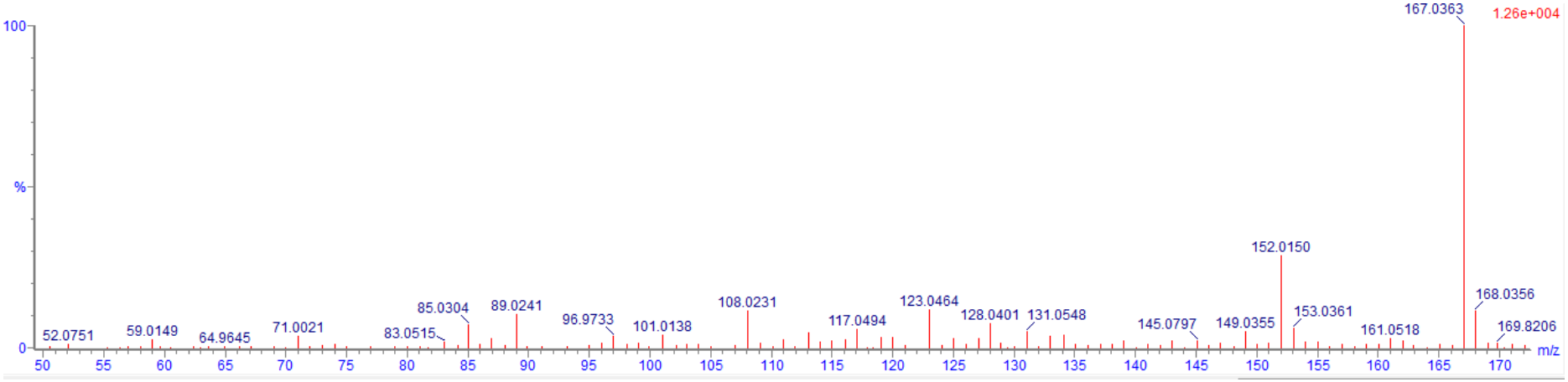

Figure A 8: Mass spectrum of vanillic acid (167 m/z) peak 8

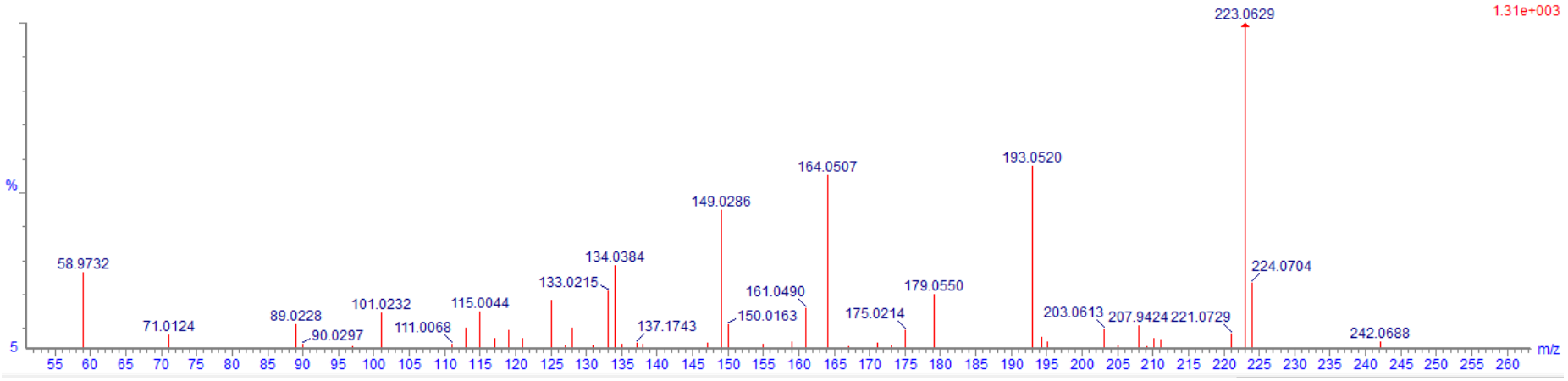

Figure A 9: Mass spectrum of sinapic acid (223 m/z) peak 9

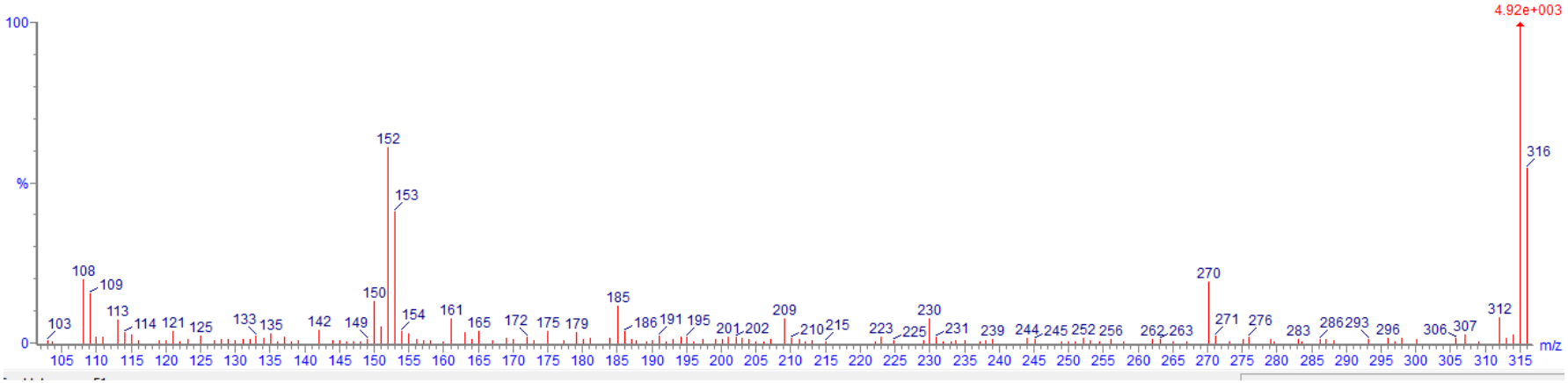

Figure A 10: Mass spectrum of protocatechuic acid-4-glucoside (m/z 315) peak 10

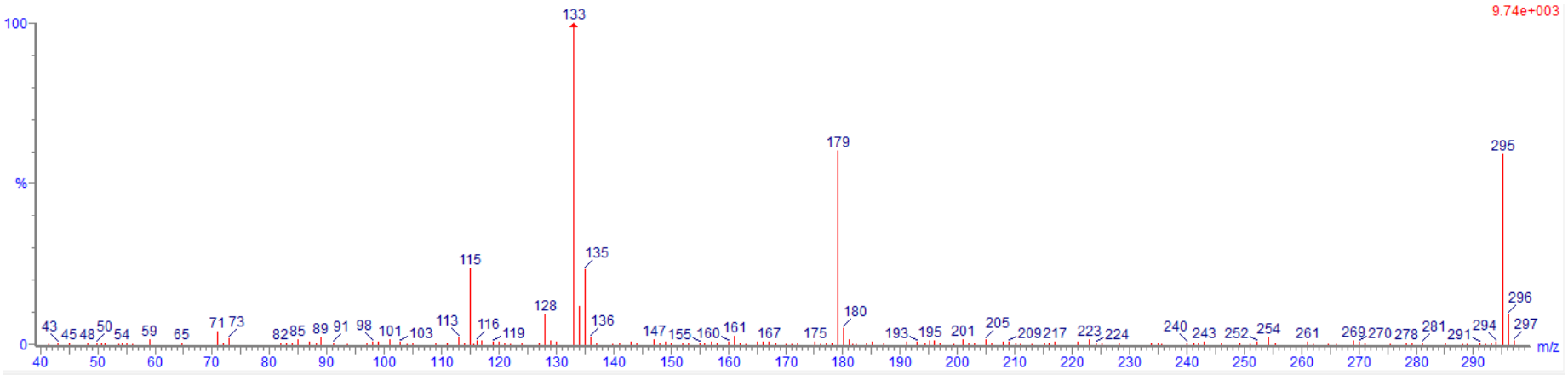

Figure A 11: Mass spectrum of acetyl salicylate derivative (295 m/z) peak 11

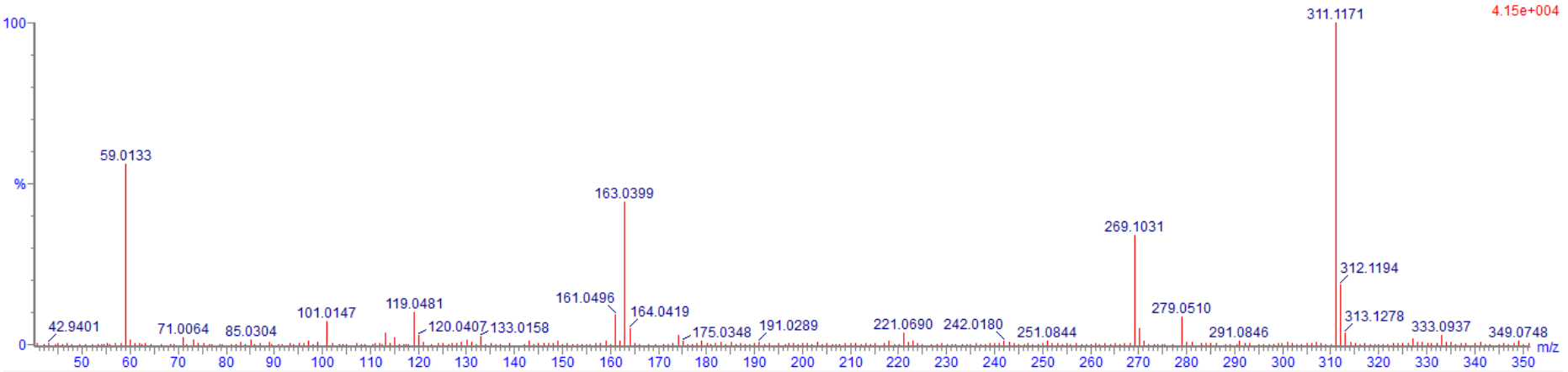

Figure A 12: Mass spectrum of 2-acetyl-3-(4-hydroxy-2-methylpentan-2-yl) gallate (311 m/z) peak 12

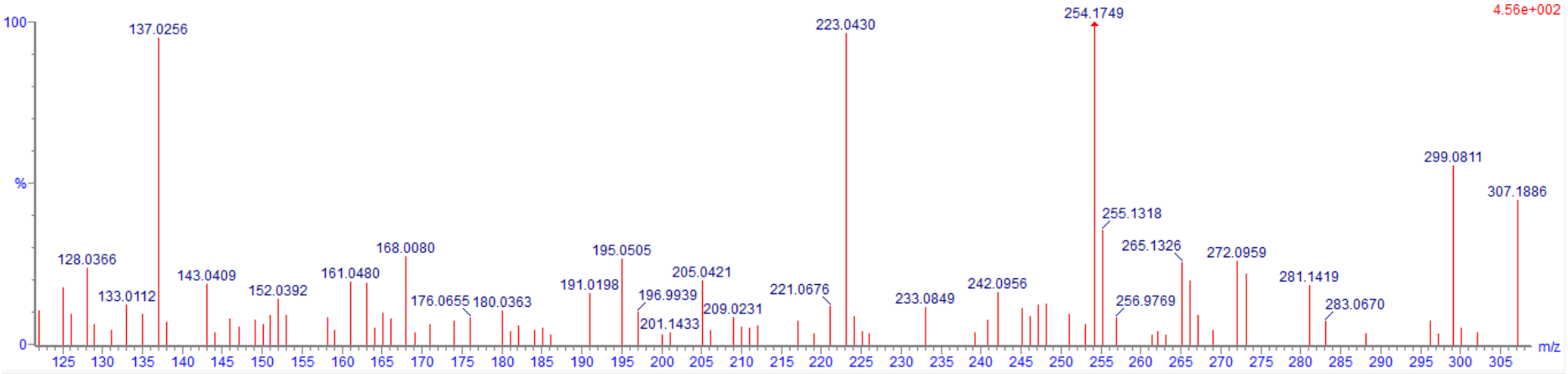

Figure A 13: Mass spectrum of salicylic acid β-D-glucoside (299 m/z) peak 13

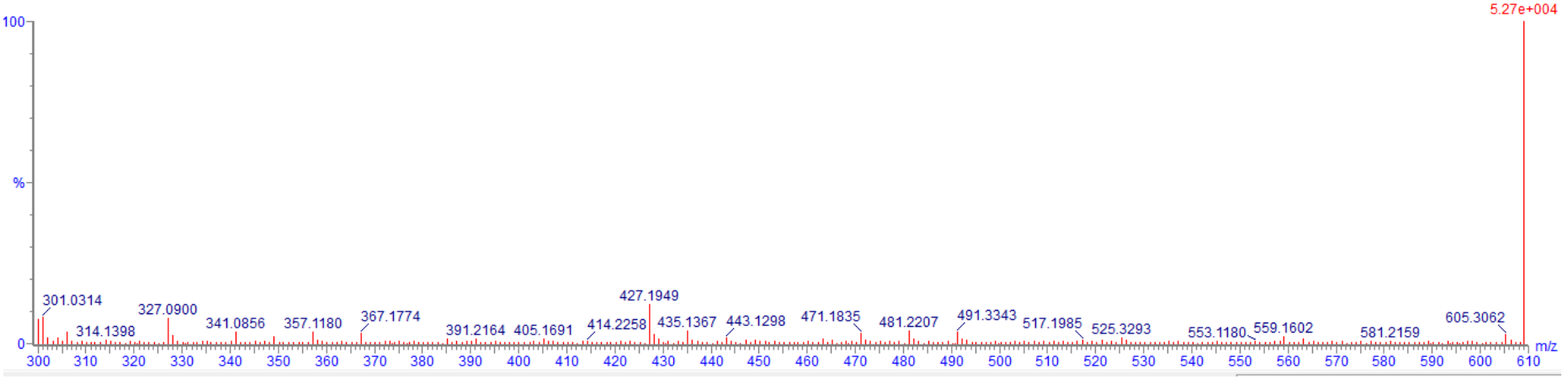

Figure A 14: Mass spectrum of rutin (609 m/z) peak 14

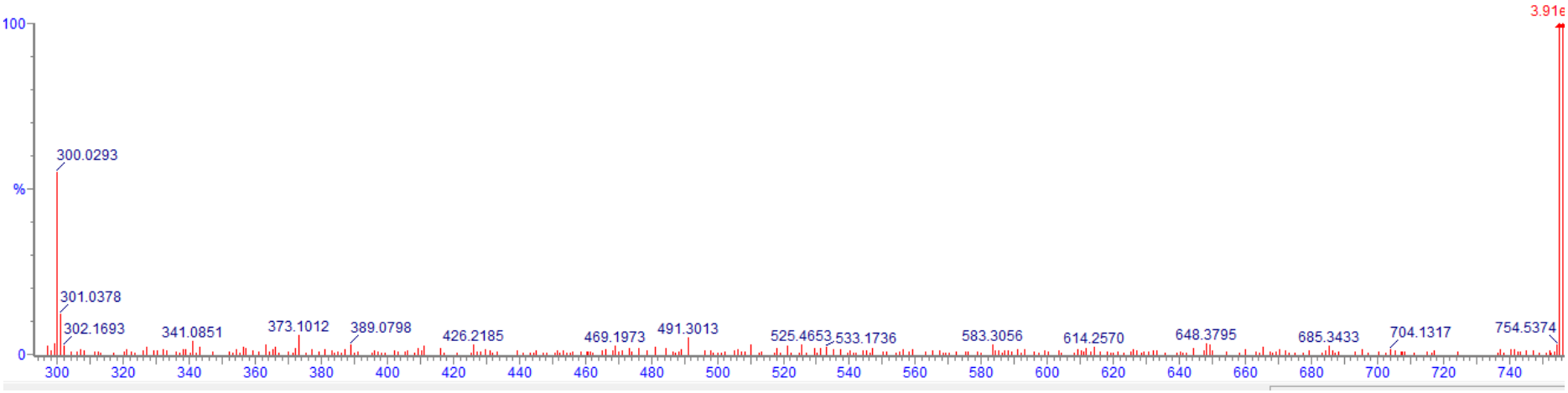

Figure A 15: Mass spectrum of quercetin-3-O-rhamnosyl rutinoside (755 m/z) peak 15

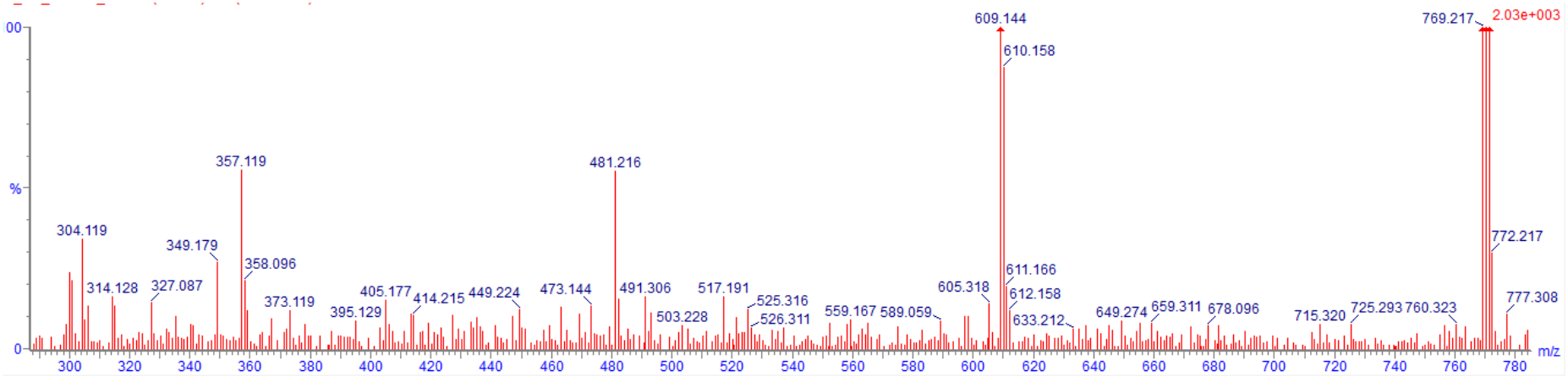

Figure A 16: Mass spectrum of Isorhamnetin-3-*O*-dirhamnosyl glucoside (769 m/z) peak 16

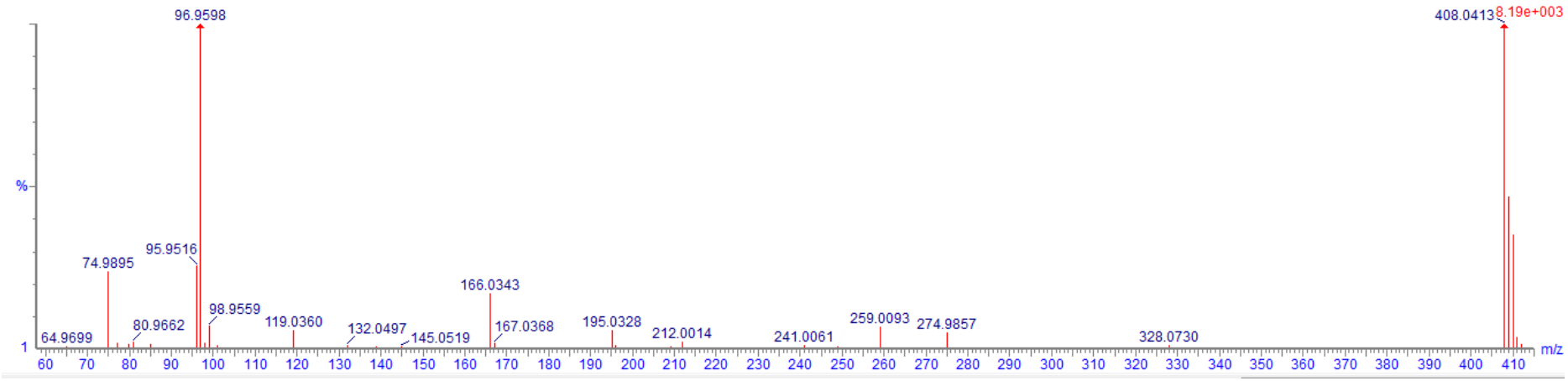

Figure A 17: Mass spectrum of benzyl glucosinolate (408 m/z) peak 17

APPENDIX B

Conference presentation:

L. Kafuko., K. G. Duodu., E. Kayitesi and N. N. Mehlomakulu., 2021. Screening for antioxidant and antimicrobial properties of bioactive compounds from *Carica papaya* peel crude extracts.

Oral presentation at the 24th Biennial International Virtual SAAFoST Congress, Paradigm Shifts – Food in the 21st Century. Hosted by SAAFoST KwaZulu Natal branch, South Africa, 20-22 September 2021.