COMPARATIVE STUDY ON PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF FERMENTED PAPAYA VINEGAR USING DIFFERENT RIPENESS OF EKSOTIKA PAPAYA AND THE INCOPORATION OF GINGER

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By

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ABSTRACT

COMPARATIVE STUDY ON PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF FERMENTED PAPAYA VINEGAR USING DIFFERENT RIPENESS OF EKSOTIKA PAPAYA AND THE INCOPORATION OF GINGER

YVONNE JONG CHING CHIN

Papaya (*Carica papaya*) is a tropical fruit widely grown in Malaysia, particularly the *Eksotika* variety. Papaya vinegar, produced by the fermentation of papaya fruit, is gaining niche popularity both locally and internationally due to its health benefits. Antioxidant properties of vinegar are always a key factor influencing consumers' purchasing decisions, since they are closely associated with human health. Meanwhile, ginger is a traditional medicine plant valued for its high nutritional and bioactive compound content. This study was conducted to investigate the total phenolic and total flavonoid content, DPPH radical scavenging assay, and reducing power assay of the fermented papaya vinegar using different papaya ripeness with the incorporation of ginger. This included the vinegars fermented from ripe papaya with and without ginger (R and RG), unripe papaya with and without ginger (U and UG), as well as a mix of ripe and unripe papaya with and without ginger (RU and RUG). Among the fermented papaya vinegars, a greater TPC was found in vinegar U (35.63 ± 1.07 mg GAE/100 g), RG (32.60 ± 0.47 mg GAE/100 g) and UG (31.91 ± 0.70 mg GAE/100 g). Meanwhile, TFC did not differ significantly ($p > 0.05$). In terms of DPPH radical scavenging activity, the vinegar RU (26.31 ± 4.38%) exhibited

a higher %RSA while vinegar U had a lower %RSA (4.35 ± 6.64%). Lastly, the vinegar R demonstrated the highest reducing power, which was 876.11 ± 20.37 μmol FS/L. In general, the results indicate that papaya vinegar is high in phytochemicals that beneficial to human health. Vinegar produced using ripe papaya has better physicochemical and antioxidant profiles, whereas the addition of ginger during vinegar fermentation had no significant impact on these profiles. Future analysis could be conducted on the sensory profiles, enzyme content and nutritional values of the papaya vinegars.

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DECLARATION

I hereby declare that this final year project report entitled "**COMPARATIVE STUDY ON PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF FERMENTED PAPAYA VINEGAR USING DIFFERENT RIPENESS OF EKSOTIKA PAPAYA AND THE INCOPORATION OF GINGER**" is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Yvonne Jong

YVONNE JONG CHING CHIN

APPROVAL SHEET

This final year project report entitled "**COMPARATIVE STUDY ON PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF FERMENTED PAPAYA VINEGAR USING DIFFERENT RIPENESS OF EKSOTIKA PAPAYA AND THE INCOPORATION OF GINGER**" was prepared by YVONNE JONG CHING CHIN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) Food Science at Universiti Tunku Abdul Rahman.

Approved by:

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Date: 07/10/2025

PERMISSION SHEET

I, **YVONNE JONG CHING CHIN** (ID No: **22ABD06785**) has completed this final year project report entitled "**COMPARATIVE STUDY ON PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF FERMENTED PAPAYA VINEGAR USING DIFFERENT RIPENESS OF EKSOTIKA PAPAYA AND THE INCOPORATION OF GINGER**" under the supervision of Dr. Lye Huey Shi and Dr. Tan Yen Nee from the Department of Agricultural and Food Science, Faculty of Science.

I hereby give permission to the university to upload the softcopy of my final year project report in pdf form into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

Yvonne Jong

YVONNE JONG CHING CHIN

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LIST OF ABBREVIATIONS

A. aceti	*Acetobacter aceti*
A. acidophilum	*Acetobacter acidophilum*
A. pasteurianus	*Acetobacter pasteurianus*
Acetyl-CoA	Acetyl-coenzyme A
ANOVA	Analysis of Variance
CVD	Cardiovascular diseases
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
F-C reagent	Folin-Ciocalteu's reagent
Fe^{2+}	Ferrous ions
Fe^{3+}	Ferric ions
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GRAS	Generally Recognized as Safe
H^{+}	Hydrogen ion
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
POD	Peroxidase
PPO	Polyphenol oxidase
QE	Quercetin equivalent
R	Vinegar fermented with ripe papaya
RG	Vinegar fermented with ripe papaya with ginger
ROS	Reactive oxygen species
RSA	Radical scavenging activity
RST	Ripening stage
RU	Vinegar fermented with ripe and unripe papayas
RUG	Vinegar fermented with ripe and unripe papayas with ginger
SAS	Statistical Analysis System
S. cerevisiae	*Saccharomyces cerevisiae*
-SH	Sulfhydryl group
$-S^{-}$	Thiolate anion

SmF	Submerged fermentation
TA	Titratable acidity
TFC	Total flavonoid content
TPC	Total phenolic content
TPC	Total phenolic content
TPTZ	2,4,6-tripyridy-s-triazine
TSS	Total soluble solids
U	Vinegar fermented with unripe papaya
UG	Vinegar fermented with unripe papaya with ginger
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Research Background

Vinegar, or “sour wine”, had a long history dating back to around 3000 B.C. The study of Bourgeois and Barja (2009) stated that fruits and date palm sap were initially utilized by the Babylonians to produce alcoholic beverages, which upon exposure to air, naturally transformed into fruit vinegar. Historical evidence also indicates that fruit vinegar was used in China as early as 1200 B.C. and in ancient Greece around 400 B.C. Nowadays, fruit vinegar is widely consumed around the world and has become part of the daily diet for its culinary versatility and potential health benefits. The large production of fruit vinegar is greatly due to the incorporation of various fermenter bacteria, including *Saccharomyces cerevisiae* and *Acetobacter* spp. that aid in increasing the efficiency of fermentation process (Bouatenin, et al., 2020; Kong, et al., 2018).

Papaya vinegar, on the other hand, does not have a well-documented ancient history. Nevertheless, it is believed that communities in tropical and subtropical regions, where papaya is abundantly cultivated, have traditionally transformed overripe or excess papayas into vinegar as a means of reducing postharvest losses (Kong, et al., 2018). Papaya vinegar exhibits sensory characteristics similar to other fruit vinegars, featuring a strong fruity aroma with pungent notes,

a sour flavour balanced by varying degree of sweetness, astringency mouthfeel and sometimes subtle spicy or peppery nuances (Es-Sbata, et al., 2023). Apart from that, papaya vinegar has an alcohol content of less than 0.5%, with pH values ranging from 2.4 to 3.9, titratable acidity between 0.24% to 6.2% and varying Brix values depending on sugar content (Ousaaid, et al., 2022). Papaya vinegar is typically served diluted with water to reduce its acidic strength, which otherwise may harm tooth enamel and irritate the throat (Weasler, 2025). It also serves well as a salad dressing or marinade, improving the flavours of the food.

Papaya vinegar is believed to have a positive impact on human health, particularly in terms of antioxidant, immunomodulatory, anticancer and anti-inflammatory properties (Leitão, et al., 2022). It typically contains abundant enzymes such as papain, along with alkaloids, phenolic compounds and carotenoids, all of which contribute to gut health and metabolic regulation. These bioactive components also offer protection against oxidative stress, helping to reduce cellular damage. Besides, papaya vinegar contains organic acids, primarily lactic acid and acetic acid, which provide potent antibacterial, antifungal and anti-infection activities, thereby enhancing overall immunity (Kong, et al., 2018). The combined action of these functional compounds supports cardiovascular health by reducing diabetes, cholesterol levels, blood pressure and more (Perumpuli and Dilrukshi, 2022).

Ginger (*Zingiber officinale*), on the other hand, is a popular traditional herb that is widely used as culinary spice and medicine nowadays. It is an herbaceous

perennial plant that belongs to the *Zingiberaceae* family, which comprises of 24 genera and more than 300 species (Moghaddasi and Kashani, 2012). It was originated in Maritime Southeast Asia and was later introduced to the Mediterranean region through ancient trade routes. Todays, ginger is widely cultivated in tropical and subtropical countries, particularly in Asia and Africa (Wang, 2020). Ginger is renowned for its bioactive compounds, including 6-gingerol and 6-shagaol, paradols and zingerone, which contribute to antiemetic, anticancer and inflammatory functions. Moreover, ginger plays a role in enhancing metabolic functions and has been reported to reduce chronic diseases such as diabetes and obesity (Anh, et al., 2020; Ballester, et al., 2022).

1.2 Problem Statement

Papaya is a fruit with a short shelf life and highly perishable nature, often leading to significant postharvest losses and reduced profitability for farmers. In Malaysia, where papayas are abundantly available throughout the year, overripe papayas are frequently discarded, contributing to significant food waste (Kong, et al., 2018). Vinegar production, on the other hand, is a traditional preservation method that believe to extend the shelf life of this perishable fruit as well as produce functional products with potential health benefits. However, there are limited scientific studies focusing on the antioxidant properties of papaya vinegar. Besides, there are also limited studies regarding the effect of ginger on the phytochemical content and antioxidant activities of papaya vinegar when it is incorporated during fermentation process. Last but not least, there is a lack of local manufacturers producing papaya vinegars. The majority

of papaya vinegars available in Malaysia market are imported and sold at high prices, typically ranging between RM 80 to RM 120 per 500 mL of vinegar (Shojikiya, 2025; Baizigui, 2025).

1.3 Objective(s)

The objectives of this study were:

i. to prepare and ferment papaya vinegars using different ripeness of papaya with or without ginger.
ii. to determine physicochemical and antioxidant properties of fermented papaya vinegars using different ripeness of papaya with or without ginger.
iii. to compare physicochemical and antioxidant properties of fermented papaya vinegars with commercial papaya vinegar.

CHAPTER 2

LITERATURE REVIEW

2.1 Papaya (*Carica papaya*)

2.1.1 Physical Characteristics and Sensory Attributes

Papaya, or *Carica papaya*, is a non-seasonal fruit widely cultivated across tropical and subtropical countries around the world. It is believed to have originated in tropical America and was introduced to Asian countries around 1598 (Silva, et al., 2007). In 2025, global papaya production reached approximately 13.8 million tons, with India recognized as the largest producing country. Malaysia, on the other hand, produced around 55,000 tons of papaya, accounting for about 0.40% of global production (Singh, 2025).

There are numerous varieties of papaya available in Malaysia, with the most popular is the Eksotika variety. Eksotika papaya was developed by the Malaysian Agricultural Research and Development Institute (MARDI) in 1987, as a result of a backcross breeding program that combined the superior eating qualities of the Hawaiian Sunrise Solo with the larger fruit size and local adaptability of the Subang 6 (Sekeli, et al., 2018). Eksotika papaya is well-regarded for its capability to grow across various soil types with complete drainage system. Besides, it had high productivity, capable of producing around 60 tons of fruit per hectare annually. Eksotika papaya typically weights between

400 to 800 g, featuring orange-red flesh with a pleasant aroma and firm texture. It also contains high sugar content ranging from 13 to 15%, thus it is highly favoured for fresh consumption as a dessert or processed into juices and jams (Panjaitan, et al., 2007). These appealing characteristics make it the main papaya variety cultivated for both local consumption and export purposes, creating an export value of RM 100-120 million each year (Shin, et al., 2011).

Nonetheless, Eksotika papaya is highly perishable. Overripe papaya exhibits undesired changes in appearance, flavour, texture and overall quality, leading to postharvest losses up to 40%. Papaya fruits are typically harvested at the pre-climacteric stage, a stage before the onset of ripening. This remains the papaya respiration at a basal level with low ethylene production, thereby delaying the storage life of papaya up to 6 days under room temperature (Tripathi, et al., 2015). However, this extension is still insufficient for long-distance market, and temperature fluctuations during transport can further accelerate ripening and shorten shelf life. Several approaches have been developed to prolong fruit shelf life, including modified atmosphere packaging, storage under low-temperature conditions, and the application of 1-methylcylopropene or carbon dioxide to inhibit the ethylene production (Sekeli, et al., 2018; Ding and Ng, 2008).

Figure 2.1: *Carica papaya* var Eksotika (Sekeli, et al., 2018).

2.1.2 Nutritional and Bioactive Profiles

Papaya is well-recognized as a nutrient dense fruit, indicating that it contains high nutrient contents per calorie. It is naturally low in calories, free of cholesterols and high in dietary fibres. It also contains numerous vitamins and minerals, including vitamins A and C, folate, calcium, magnesium and potassium, which collectively contribute to physiological functions. Apart from its nutritional value, papaya contains diverse bioactive compounds, such as tannins, saponins, carotenes, flavonoids and polyphenols, along with enzymes papain and chymopapain (Pinnamaneni, 2017; Nouman, et al., 2021). These phytochemicals and enzymes play a crucial role in diseases prevention, particularly in reducing the risk of cardiovascular diseases, cancers and metabolic syndromes. Moreover, it also exhibits therapeutic properties, such as anti-inflammatory, immune-modulatory, antibacterial, antioxidative and anti-amoebic properties (Ali, et al., 2011).

2.2 Ginger (*Zingiber officinale*)

2.2.1 Physical Characteristics and Sensory Attributes

Ginger, or *Zingiber officinale*, is an herbaceous perennial plant that had been utilized for culinary and medicinal purposes since ancient times. It is infertile and relies on rhizomes for vegetative propagation. Due to its unique refreshing aroma and spicy flavour, it is a frequently used in food preparation for both flavour enhancement and preservation properties (Yip, 2022). According to Rokade (2025), the global ginger market experiences a steady growth from 2023

and is expected to achieve a CAGR of 7.1% by 2033. This rising demand is largely driven by ginger's ability to prevent and cure certain illnesses.

Bentong Ginger, a local ginger variety originating from Pahang, Malaysia, is regarded as a premium cultivar and is often called the "*King of Ginger*." It is characterized by its bigger, dull yellow rhizomes and thinner, light brown peel. Additionally, the flesh is pale white, juicy and less fibrous, thereby it is classified as white ginger (Sundram, et al., 2019; Tan, 2017). Bentong ginger is reported to have more aromatic, pungent and spicy flavours than other ginger, making it an optimal choice for food processing (Kamaruddin, et al., 2023).

2.2.2 Nutritional and Bioactive Profiles

Ginger is a food that is naturally low in fat and high in minerals and vitamins, such as calcium, potassium, iron and ascorbic acids, that are crucial for maintaining normal physiological functions (Govindarajan and Connell, 2009). Apart from that, ginger contains a variety of bioactive compounds, including gingerols, shogaols, zingerone and paradols. These compounds are reported to have strong anti-inflammatory and antioxidant activities, that protect body against oxidative stress and cellular damage. Gingerols and shogaols, on the other hand, prevent cardiovascular diseases by lowering low-density lipoprotein (LDL) levels, which lowers the risk of heart attacks and strokes. Besides, ginger is also an anti-nausea and anticarcinogenic agent. These nutritional and bioactive profiles greatly boost human immunity and offer protection against diseases and cancers (Benzie and Wachtel-Galor, 2011; Cerdá, et al., 2022).

2.2.3 Previous Studies on the Incorporation of Ginger in Fermented Food

According to a study by Molaie, et al. (2022), the incorporation of ginger extract into fermented yogurt significantly enhances phytochemical contents and antioxidant activities in the final product. Another study by Shaukat, Nazir and Fallico (2023) explained that ginger improves the antioxidant properties, appearance and flavour of herbal yogurt. Similarly, adding ginger powder to bread greatly increases its total phenolic content, as well as improve the bread texture (Amjad, et al., 2022). These findings indicate that ginger greatly enrich the phytochemical profile of fermented products. Nonetheless, there is a lack of study regarding the incorporation of ginger into fermented vinegars. In particular, the impact of ginger addition on the phytochemical content and antioxidant capacity of fermented fruit vinegar has not been well explored, especially those vinegar made from tropical fruit like papaya.

2.3 Fruit Vinegar

2.3.1 Physical Characteristics and Sensory Attributes

Fruit vinegar is a sour beverage produced by using the fruit by-products that undergo two stages of fermentation, namely alcoholic and acetous fermentation, with the presence of naturally occurring fermenting bacteria or starter cultures (Luzón-Quintana, Castro and Durán-Guerrero, 2021). It is produced on a large scale for preservation purposes, turning overripe, second or third quality fruits into more valuable goods; otherwise, these fruits will be discarded as waste.

Fruit vinegar usually exhibits a pH range of 2.4 to 3.9 and a titratable acidity between 0.24% to 6.20%, depending on the fruit utilized and fermentation conditions. Besides, its residual alcohol content should remain below 0.5%, which is regulated by the standards established by Codex Alimentarius. The total sugar content of naturally fermented fruit vinegar is usually less than 3%. However, most commercial products with sugar being added has a broader Brix range of 8% to 64% (Chang, Lee and Ou, 2005; Ousaaid, et al., 2022). Fruit vinegar typically tastes sour with a sharp, pungent aroma and an astringent aftertaste. However, certain sensory attributes differ based on the types of fruits utilized due to the development of diverse flavour compounds during fermentation process (Ge, et al., 2025).

2.3.2 Therapeutic Potential

Fruit vinegar is reported to have significant impact on reducing the risk of cardiovascular diseases (CVD), which is one of the most deadly diseases in the world. The key components in fruit vinegar that lower the risk of CVD are acetic acids, chlorogenic acid and gallic acids. These acids act as an anti-obesity agent that significantly lower the LDL cholesterol levels and increase HDL cholesterol levels in plasma. Besides, these acids facilitate the acceleration of fatty acids beta-oxidation, converting fats and lipids into acetyl-CoA, which is then utilized to produce energy. This directly inhibit fats and lipids accumulation in the liver, which in turn reduce the formation of plaques in blood vessels, that can lead to heart attacks and strokes (Budak, et al., 2014).

Apart from that, these acids serve as effective antidiabetic agent by enhancing insulin sensitivity and accelerating glucose metabolism through the glucokinase pathway. This prevents the accumulation of sugars in the liver and bloodstream, which may lead to diabetes mellitus. In addition, acetic acid limits gastric acid secretion, delaying the complete digestion of complex carbohydrates, thereby avoiding sudden blood glucose surges that may lead to insulin resistance over time (Budak, et al., 2014). Vinegar consumption after eating meals allows a gradual rise in blood glucose levels, promoting sustained energy release and preventing excessive insulin production.

2.3.3 Processing

Fruit vinegar production usually begins with the extraction of juice from selected fruits. The fruits are first cleaned and peeled, followed by the removal of seeds or any unwanted materials. Next, the fruit bulb is blended or mechanically pressed to obtain fruit juice, which is subsequently pasteurized at mild temperature to inactivate endogenous enzymes and reduce viable microbial counts. This is followed by the adjustment of sugar concentration and cooling of pasteurized juice. Then, an appropriate yeast strain is added to initiate the alcoholic fermentation of juice at temperature ranging between 20°C and 30°C, depending on the yeast utilized. Upon the completion of alcoholic fermentation, mother of vinegar or acetic acid bacteria will be added to initiate the acetous fermentation (Luzón-Quintana, Castro and Durán-Guerrero, 2021; Adebayo-Oyetoro, et. al., 2017). Fermentation is considered complete when a stable titratable acidity is achieved.

2.4 Fermenter Microorganisms

2.4.1 *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is a food-grade yeast that has been granted Generally Recognized as Safe (GRAS) status by the United States Food and Drug Administration (FDA) (Belda, et al., 2019). It is widely used in food fermentation, such as breads, beers, wines and vinegars.

S. cerevisiae is the most widely used yeast during alcoholic fermentation as it exhibits a higher fermenting capacity, in which it metabolizes sugar more efficiently over other yeast strains (Bhat, Akhtar and Amin, 2014). This significantly affects the flavour and quality of the final product. During fermentation, the temperature is always controlled within a range of 20°C to 30°C to maximize yeast's activity. Embden-Meyerhof-Parnas (EMP) pathway is utilized by the yeast to first convert glucose or other fermentable sugars into pyruvate, followed by the generation of ethanol and carbon dioxide. Therefore, yeast is also considered chemoorganotrophic (Walker and Stewart, 2016). Study shows that the actual ethanol produced during alcoholic fermentation is around 60% of the initial glucose content, although theoretically it should be 65%. This condition is because of the utilization of sugar for cell maintenance, biomass and by-products production (Bhat, Akhtar and Amin, 2014).

Apart from that, numerous secondary metabolites are produced during yeast fermentation, including glycerol, succinic acids, and esters. These compounds

contribute to the unique flavours and aromas found in fermented products. For example, esters like ethyl acetate and isobutyl acetate impart flowery and fruity notes in the yeast fermented product (Soccol, et al., 2007). In addition, these metabolites offer certain health benefits. They exhibit antioxidant properties that reduce the risk of chronic diseases like atherosclerosis, cancer and respiratory diseases, and delay the aging process (Vilela, 2019).

2.4.2 *Acetobacter* spp.

Acetobacter spp. is the commonly employed acetic acid bacteria (AAB) in vinegar production. This includes *A*. *aceti*, *A*. *pasteurianus* and *A*. *acidophilum*. They are recognized as GRAS by FDA (FDA, 2018).

Acetobacter spp. are aerobes which require oxygen for metabolism at a temperature ranging between 25°C and 30°C (Bhat, Akhtar and Amin, 2014). They are inactivated during alcoholic fermentation that is conducted under anaerobic conditions. However, once the fermenting wine is exposed to oxygen, *Acetobacter* spp. start to grow and convert the ethanol in the wine into acetic acid through the action of the enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) (Hata, et al., 2023).

The secondary metabolites produced by *Acetobacter* spp. during acetous fermentation include gluconic acid and ascorbic acid. These organic acids serve as natural preservatives, as well as offer antioxidant activities that benefits

human health (Luzón-Quintana, Castro and Durán-Guerrero, 2021). Besides, compounds like acetoin, polyphenols, aldehydes and esters produced during fermentation contribute to the complex flavour and aroma of the finished product.

2.5 Antioxidant Compounds in Papaya Vinegar

2.5.1 Ascorbic Acid

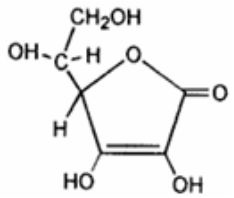

Figure 2.2: Molecular structure of ascorbic acid (Johnston, Steinberg and Rucker, 2001).

Ascorbic acid, also known as vitamin C, is a water-soluble compound that is vital to human health. It is naturally found in fruits and leafy vegetables, particularly around 70.9 mg per 100 g of papaya. It is recommended that adults should take at least 200 mg per day (MOH, 2022).

Ascorbic acid is recognized for its strong antioxidant properties that is ready to scavenge the reactive oxygen species (ROS) and free radicals. When exposed to these highly reactive species, ascorbic acid donates one hydrogen ion to neutralize them, transforming into L-ascorbate anions and then into a relatively

stable intermediate called the L-semi-dehydroascorbic acid radical. This radical undergoes further oxidation to form the non-radical L-dehydroascorbic acid (DHA), effectively terminating the chain reactions caused by ROS and free radicals (Johnston, Steinberg and Rucker, 2001; Njus, et al., 2020). A study by Martinez-Villaluenga, et al. (2009) explained that ascorbic acid level tends to decline during fermentation due to oxidation and microbial metabolism. Ascorbic acid may react with indole compounds in vegetables or fruits to form ascorbigen, as a mean of oxidation process. The decreased ascorbic acid levels during fermentation is also supported by the research on the fermentation of Gochu-jangachi by Jung, Kim, F.-E and Kim, S.-H (2001).

2.5.2 Flavonoids

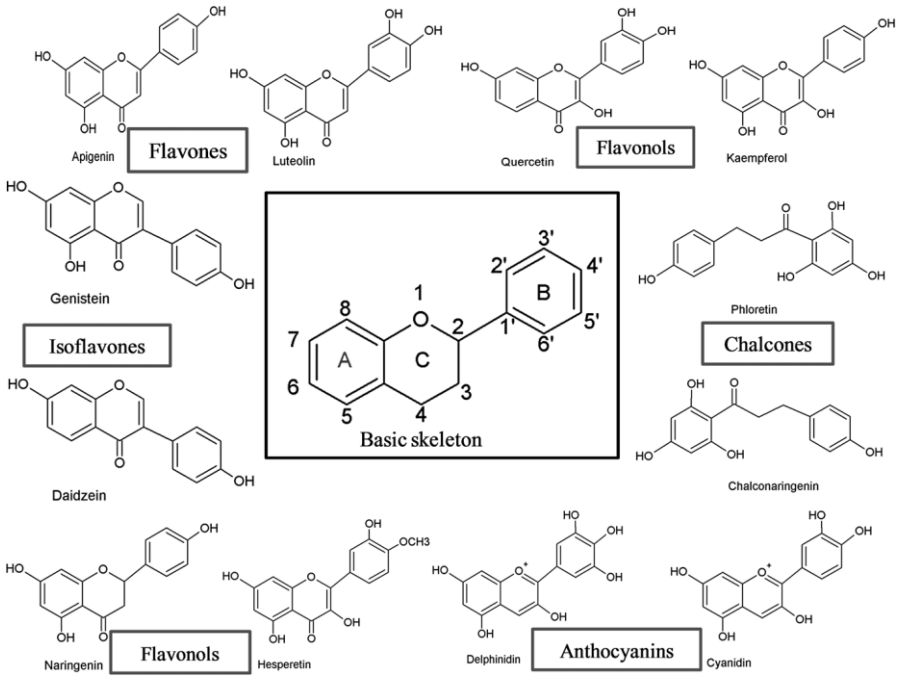

Figure 2.3: Different chemical structures of flavonoids and their subgroups (Panche, Diwan and Chandra, 2016).

Flavonoids, a group of functional substances that are naturally found in fruits and vegetables, grains, flowers and tea. It could be subdivided into flavones, flavonols, flavanols, flavanones, flavanonols, anthocyanins and chalcones (Panche, Diwan and Chandra, 2016).

The flavonoids primarily present in papaya pulp are myricetin, quercetin, kaempferol, apigenin and luteolin (Jeon, et al., 2022). Myricetin, quercetin and kaempferol belongs to flavonols, while apigenin and luteolin are flavones. A study by Kingori, Ochanda and Koech (2021) suggested that fermentation causes a significant decline in myricetin levels, while quercetin and kaempferol levels are less impacted. Besides, a study highlighted that rutin and isoquercitrin typically found in jujubes will degrade into smaller quercetin molecules during fermentation, which possess stronger antioxidant and anti-inflammatory properties (Yang, et al., 2023). This implies that quercetin is more stable than its glycoside derivatives and fermentation enhances its bioavailability.

2.5.3 Carotenoids

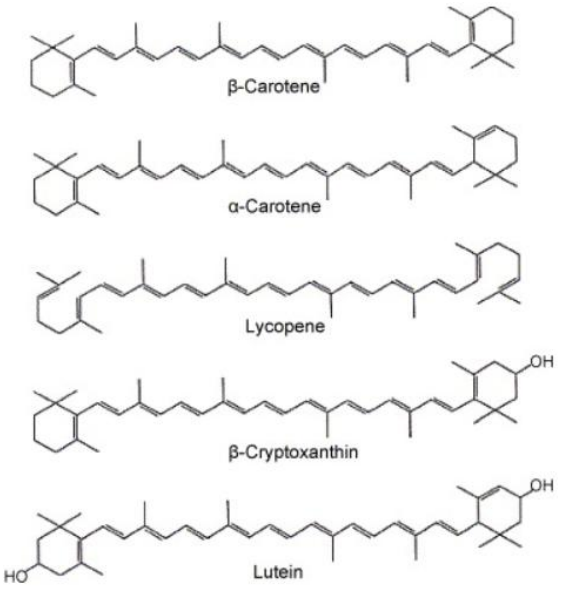

Figure 2.4: Different chemical structures of carotenoids (Rao, A.V. and Rao, L.G., 2007).

Carotenoids, on the other hand, are naturally occurring lipid-soluble isoprenoid pigments that give fruits, vegetables and flowers their yellowish-orange or red colour. The frequently seen carotenoids include σ- and β-carotene, lycopene, β-cryptoxanthin and lutein (Rao, A.V. and Rao, L.G., 2007). Among all, σ- and β-carotene and β-cryptoxanthin are provitamin A carotenoids, meaning that the body may metabolize them for vitamin A production, while the others are not.

Papayas are rich in β-cryptoxanthin, σ and β-carotene, while lycopene is only found in red-flesh papaya (Chandrika, et al., 2003). These compounds mainly attack singlet oxygen ($^1O_2$) by absorbing the energy released by the singlet oxygen to form a triplet exited carotene. The energy is then eliminated into

surroundings as heat. Besides, they also attack peroxyl radicals by neutralizing the radicals with electrons or hydrogen atoms (Stahl and Sies, 2003). A study on the alcoholic fermentation of orange juice explained that the total carotenoid content of the fermenting orange juice increases due to the release of carotenoid molecules from the food matrix during fermentation (Cerrillo, et al., 2014). Nonetheless, another study by Cheng, et al. (2012) highlighted that carotenoids are susceptible to acidic conditions, especially when the pH falls below 3.0, resulting in a lower carotenoid concentration in the vinegar.

2.6 Methods to Study Antioxidant Activity

Determination of phenolic compounds present in a vinegar sample, as well as the assessment on the antioxidant activity are the two crucial aspects that estimate the functional value of vinegars. Several methods have been developed for the antioxidant study, including metal chelating activity, Folin-Ciocalteu colorimetric method, TFC, DPPH, ABTS, FRAP and ORAC (Rumpf, Burger and Schlze, 2023). Each method had its own strengths in antioxidant determination. In this project, only F-C method, TFC, DPPH and FRAP assays were utilized.

2.6.1 Total Phenolic Content (Folin-Ciocalteu Assay)

The Folin-Ciocalteu (F-C) colorimetric method is widely used to determine the total phenolic content in food, plant extract and biological samples. For example, simple phenols, phenolic acids and flavonoids (Blainski, Lopes and De Mello, 2013). The F-C reagent is a yellowish solution containing phosphomolybdic and

phosphotungstic acids. When this reagent reacts with antioxidants, a redox reaction occurs where the antioxidants are oxidized while the reagent is reduced, turning the yellow solution into blue, indicating the formation of molybdenum-tungsten complexes. The resulting mixture is then measured at 760 nm using a spectrophotometer (Pérez, Dominguez-López and Lamuela-Raventós, 2023). The intensity of blue colour implies the phenolic content in the sample. The reference standards that are normally used in F-C assay includes gallic acid and catechin for their stability and reliable reaction with the F-C reagent.

However, there had been reported that non-phenolic reducing molecules could be detected by this assay, leading to an overestimation of TPC. In papaya, compounds like ascorbic acids, proteins and amino acids, saponins and carotenoids are potentially to cause this overestimation (Chen, Cheng and Liang, 2015; Rover and Brown, 2013).

2.6.2 Total Flavonoid Content (Aluminium Chloride Colorimetric Assay)

The aluminium chloride colorimetric method employs the principle that stable Al (III)-flavonoid complexes could be formed through the reaction of aluminium (III) cations with specific functional groups of flavonoids. These key groups include the C-4 keto group, the C-3 or C-5 hydroxyl group as well as vicinal hydroxyl groups on the B-ring of flavonoids. Upon complex formation, the reaction mixture exhibits a yellow colour, which is then spectrophotometrically measured at 415 nm. The intensity of yellow colour directly correlates with the amount of flavonoid in the sample (Sultana, et al.,

2024). Quercetin and rutin are the two most commonly used reference standards due to their ability to form stable complexes with aluminium chloride.

In addition, several modifications have been made to increase the efficiency of this method, such as the introduction of sodium nitrite and sodium hydroxide. Sodium nitrite is added prior to aluminium chloride, acting as a selective nitrating agent for aromatic vicinil diols to generate flavonoid-nitrosyl derivatives. Sodium hydroxide is also utilized to create an alkaline environment that enhance the formation of complexes (Shraim, et. al., 2021).

2.6.3 DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging assay operates based on the neutralization of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent when it is interacting with the antioxidants. DPPH, a purple-coloured radical with an unpaired valence electron on its nitrogen atom, exhibits maximum UV absorption at 515 nm. Antioxidant, a scavenger molecule, donates one hydrogen atom to the DPPH radical, reducing it into a stable hydrazine form (DPPH-H). This reduction results in a colour change from purple to yellow, reducing the absorbance at 515 nm when measured by spectrophotometer (Mishra, Ojha and Chaudhury, 2012). A greater yellow intensity of reacting mixture indicates a higher amount of antioxidant, thus having higher radical scavenging activity. The reference standards usually employed in DPPH assay include Trolox and ascorbic acid for their strong antioxidant properties.

2.6.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP reagent is a colourless solution composed of acetate buffer, 2,4,6-tripyridy-s-triazine (TPTZ) and iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in a ratio of 10:1:1. It is required to warm to 37°C before use to optimize its reaction with target molecules. FRAP reagent consists of ferric (Fe^{3+}) ions, which are easily reduced to ferrous (Fe^{2+}) ions, producing a blue-coloured ferric-tripyridyltriazine (Fe^{2+}-TPTZ) complex in the assay (Guo, et al., 2003). This reduction is driven by the donation of hydrogen atoms from antioxidants. The reacting mixture is determined spectrophotometrically at 593 nm. The intensity of the blue colour reflects the antioxidant capacity of the sample. Ascorbic acid, Trolox and catechin are the commonly used reference standards in FRAP assay.

Nonetheless, antioxidant concentration in samples does not always directly correlate with the reducing power. In fact, different antioxidants possess varying reducing power, with the compounds like ascorbic acid, quercetin and catechins are recognized as strong reducing agents (Nurul and Asmah, 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Carica papaya var Eksotika, Bentong Ginger, Mauri-pan Bakers' yeast (*Saccharomyces cerevisiae*), table sugar and Bragg's raw unfiltered apple cider vinegar (mother of vinegar) were purchased from the local supermarkets, Lotus's Kampar and Econsave Kampar. The commercial papaya vinegar was purchased from Shopee.

3.2 Equipment

Table 3.1: Lists of equipment used with their manufacturers.

Equipment	Manufacturers
Autoclave	Hirayama, Japan
Brix refractometer	Atago, Japan
Electronic balance	Mettler Toledo, United States
Food blender	Panasonic, Japan
Incubator	Memmert, Germany
Micropipettes	Gilson, United States
Microplate reader	BMG Labtech, Germany
pH meter	Mettler Toledo, United States
Vortex mixer	Scientific Industries, United States
Water bath	Memmert, Germany

3.3 Chemicals

Table 3.2: Lists of chemicals used with their manufacturers.

Chemicals	Manufacturers
Aluminum chloride anhydrous	Friendemann Schmidt, United States
Ascorbic acid	HmbG, Malaysia
DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) powder	Sigma-Aldich, United States
Ferric chloride hexahydrate	Merck, Germany
Folin-Ciocalteu's phenol reagent	Chemiz, Malaysia
Gallic acid	Alfa Aesar, United States
Methanol	Emsure, Germany
Phenolphthalein	Merck, Germany
Quercetin	Sigma-Aldrich, United States
Sodium acetate	Merck, Germany
Sodium carbonate	Merck, Germany
Sodium hydroxide pellet	Merck, Germany
Potassium ferricyanide	HiMedia, India
Phosphate buffer	Bendosen, Malaysia
Trichloroacetate acid	Bendosen, Malaysia

3.4 Overview of Methodology

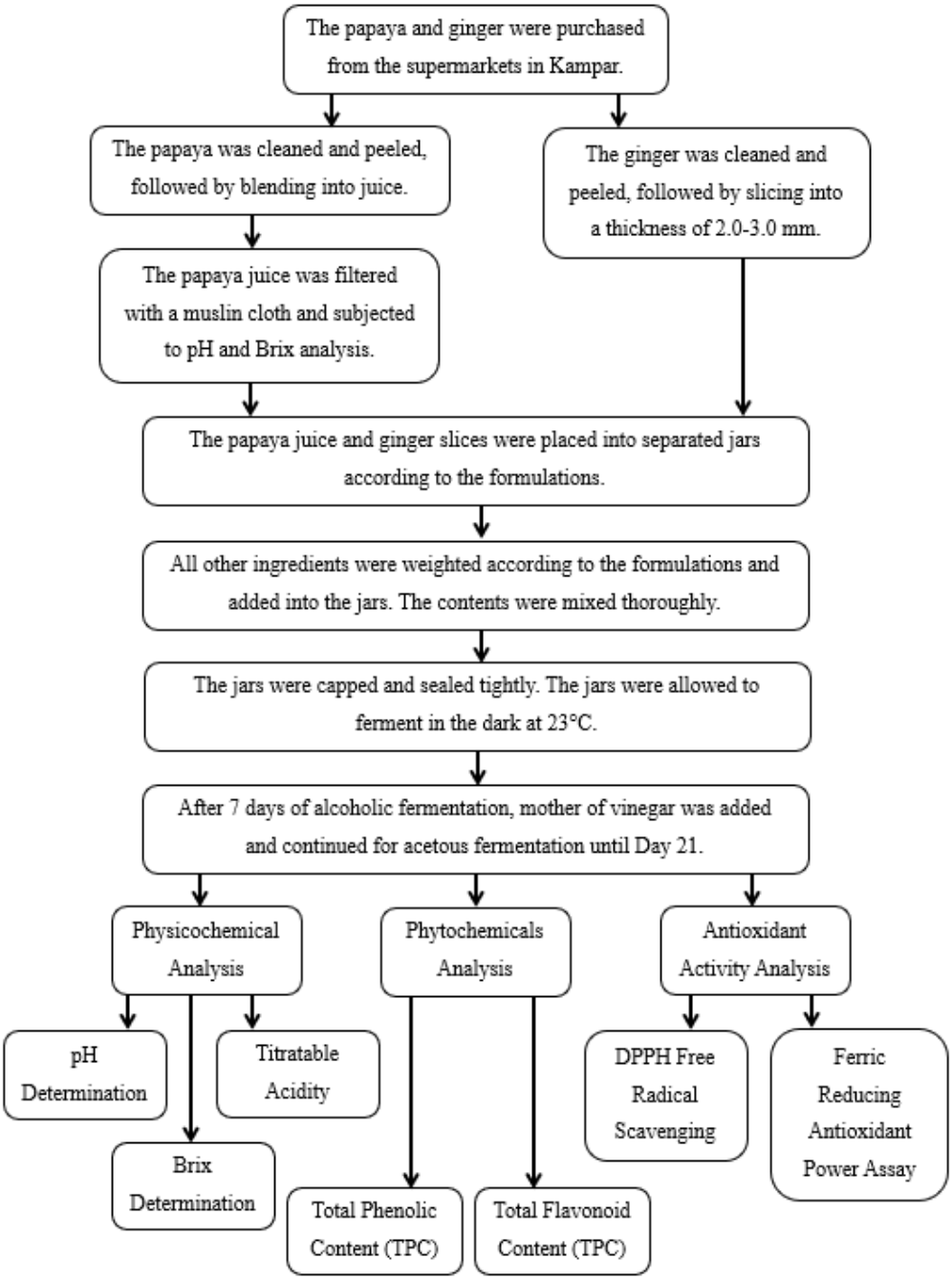

Figure 3.1: Overview of methodology**.**

3.5 Preparation of Papaya Vinegar Samples

* All processes were performed aseptically.

Both ripe and unripe Eksotika papayas and Bentong gingers were purchased from the supermarkets in Kampar. The degree of ripening of papayas was examined based on the ripeness indices (Barragán-Iglesias, Méndez-Lagunas and Rodríguez-Ramírez, 2018; Chan and Sim, 2019). The fruits were first cleaned with water, followed by the removal of peels and seeds. The bulbs were blended into juice and filtered through muslin cloth (Bouatenin, et al., 2020). The Brix value of papaya juice was adjusted to 18°Brix by adding table sugar. Meanwhile, the gingers were cleaned, peeled, and then cut into 1.0-2.0 mm slices. Next, the pasteurized papaya juice (200.0 mL) and dried powered *S. cerevisiae* (2.0 g) were filled into six separate jars. Ginger slices (0.3% w/w) were added according to the formulations. The jars were covered with cheese cloth and parafilm to generate anaerobic conditions for alcoholic fermentation. The papaya juice was fermented in a dark environment at 23°C. Sampling for pH and Brix determination was performed every two days until Day 7 (Kong, et al., 2018). After alcoholic fermentation, the papaya wine was filtered from the must, and 10% of mother of vinegar was added (Song, et al., 2019). The papaya wine was aerobically fermented at 23°C to produce papaya vinegar. The determination of pH, Brix and titratable acidity was carried out every two days until Day 21, when stability was achieved. The papaya vinegars were kept at 4°C for future analyses.

3.6 Physicochemical Analysis

3.6.1 Determination of Papaya Ripeness

The ripeness of papaya was evaluated based on two different aspects (colour and pH), following the methods described by Barragán-Iglesias, Méndez-Lagunas and Rodríguez-Ramírez (2018) as well as Chan and Sim (2019). Papaya ripeness is typically categorized according to its ripening stages on-tree (RST). At RST 1, the fruit reaches physiological maturity and appears completely green, with a pH of approximately 4.32. As ripening progresses through RST 2, 3, 4 and 5, the skin colour gradually changes from green to yellowish-orange. The pH also increases throughout the ripening process. The over-ripe stage (ORF) is characterized by a fully yellow appearance and a pH value of around 4.87. In this study, colour of papaya was visually evaluated while the pH measurements were obtained by using a pH meter.

3.6.2 pH Value Determination

All papaya juice (ripe papaya, unripe papaya and a mix of ripe and unripe papaya in 1:1 ratio), fermented vinegars and commercial papaya vinegar were placed at room temperature for 1 h to achieve a similar temperature, followed by thorough mixing to prevent sedimentation. Then, 30 mL of each sample was poured into separate 50 mL breakers, followed by pH determination using a pH meter (Jun, Lee D.H and Lee S.M., 2016).

3.6.3 Brix Value Determination

All fermented papaya vinegars and commercial papaya vinegar were placed at room temperature for 1 h to achieve a similar temperature, followed by thorough mixing to prevent sedimentation. The samples were dropped into the refractometer's well until the screen was fully covered (Jun, Lee D.H and Lee S.M., 2016).

3.6.4 Titratable Acidity (TA) Determination

Titratable acidity of fermented papaya vinegars and commercial papaya vinegar was determined using the method described by Bouatenin, et al. (2020) with slight modifications. Approximate 1 mL of papaya vinegar was diluted with 4 mL of diluted water to achieve a 1:5 dilution. Then, 3 drops of 1% phenolphthalein indicator were added to the diluted vinegar, followed by titration using 0.1 N sodium hydroxide until the solution turned pink. The titratable acidity (%) was calculated using the formula:

$$\mathrm{TA}(\%) = \frac{\text{Volume of titrated NaOH} \times 0.1\ \text{N} \times 0.067 \times \text{Dilution factor}}{\text{Volume of sample}} \times 100\%$$

3.7 Phytochemical Content Analysis

3.7.1 Total Phenolic Content (TPC)

The TPC of papaya vinegars and commercial papaya vinegar were determined using the methods published by Zuhair, et al. (2013) with minor modifications. To prepare the gallic acid standards, 2 mg of gallic acid powder was dissolved in 10 mL of distilled water to prepare a 200.00 mg/L gallic acid stock solution.

Then, serial dilution was performed to prepare 100.00, 50.00, 25.00, 12.50 and 6.25 mg/L gallic acid standards. To prepare 10% Folin-Ciocalteu reagent, 100 µL of Folin-Ciocalteu reagent was diluted with 900 µL of distilled water. Meanwhile, 750 mg of sodium carbonate powder was dissolved in 10 mL of distilled water to create a 7.5% sodium carbonate solution.

For the assay, 20 µL of diluted vinegar samples were injected into Elisa 96-well plate, followed by the addition of 100 µL of 10% Folin-Ciocalteu's reagent. The mixture was incubated in the dark for 5 min at room temperature. Next, the mixture was mixed with 80 µL of 7.5% sodium carbonate and again incubated in the dark for 2 h at room temperature. The absorbance was measured using a microplate reader at a wavelength of 765 nm, with distilled water was used as a blank. The steps were repeated for gallic acid standards to produce gallic acid calibration curve for the calculation of TPC of the samples in mg GAE/ 100 g.

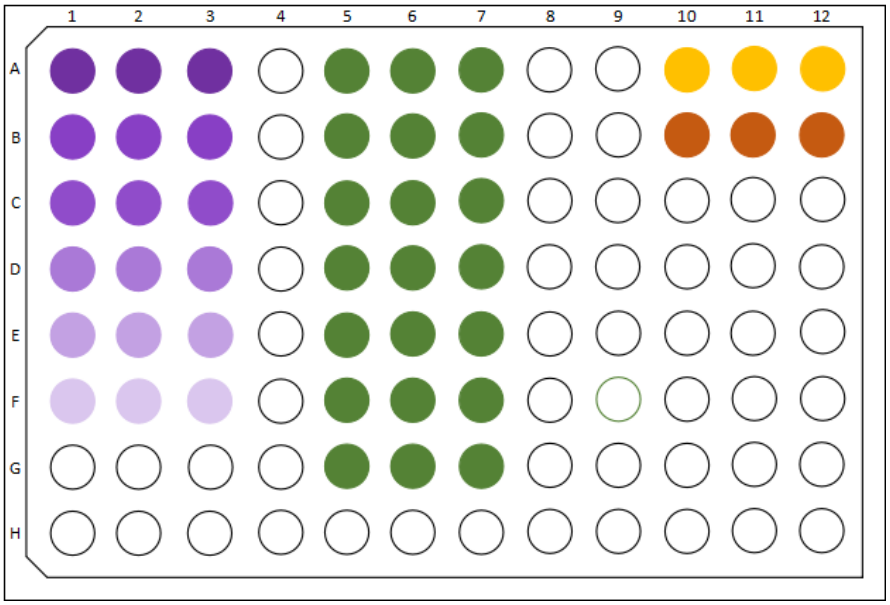

Figure 3.2: Layout of 96-well plate for Folin-Ciocalteu Assay.

Key:

 : Gallic Acid (6.25 to 200 mg/L) + F-C Reagent + Sodium Carbonate

 : Samples + F-C Reagent + Sodium Carbonate

 : F-C Reagent + Sodium Carbonate + Distilled Water (Negative control)

 : Distilled Water (Blank)

3.7.2 Total Flavonoids Content (TFC)

The TFC of papaya vinegars and commercial papaya vinegar were determined using the methods published by Suksamran, et al. (2022) with minor modifications. A 200.00 mg/L quercetin stock solution was prepared by dissolving 2 mg of quercetin powder in 10 mL of distilled water. Then, serial dilution was performed to prepare 100.00, 50.00, 25.00, 12.50 and 6.25 mg/L standards. To prepare 10% aluminium chloride, 10 g of aluminium chloride powder was dissolved in 100 mL of distilled water, while 82.04 g of sodium acetate powder was dissolved in 10 mL of distilled water to produce 0.1 M sodium acetate.

For the assay, 100 µL of vinegar samples were mixed with 300 µL of 99% methanol, 20 µL of 10% aluminium chloride, 20 µL of 0.1 M sodium acetate and 560 µL of distilled water. This is followed by the incubation in the dark for 30 mins at room temperature. The absorbance was measured using a microplate reader at a wavelength of 415 nm, with methanol was used as a blank. The steps

were repeated for quercetin standards to produce quercetin calibration curve for the calculation of TFC of the samples in mg QE/ 100 g.

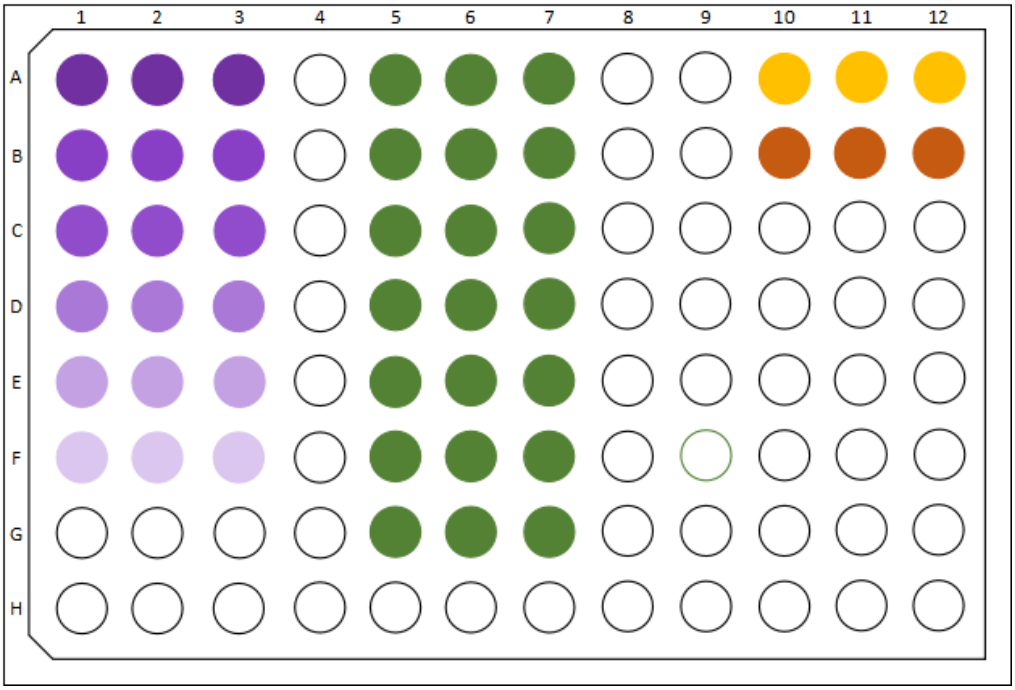

Figure 3.3: Layout of 96-well plate for TFC Assay.

Key:

: Quercetin (6.25 to 200 mg/L) + Methanol + Aluminium Chloride + Sodium Acetate + Distilled Water

: Samples + Methanol + Aluminium Chloride + Sodium Acetate + Distilled Water

: Methanol + Aluminium Chloride + Distilled Water (Negative control)

: Methanol (Blank)

3.8 Antioxidant Activity Analysis

3.8.1 DPPH Free Radical Scavenging Activity Assay

The DPPH free radical scavenging activity of papaya vinegars and commercial papaya vinegar were determined using the methods published by Zuhair, et al. (2013) with slight modifications. A total of 10 mg of ascorbic acid powder was dissolved in 100 mL of distilled water to produce a 100.00 mg/L ascorbic acid stock solution. Then, serial dilution was performed to obtain 50.00, 25.00, 12.50 and 6.25 mg/L ascorbic acid standards. Similarly, the vinegar samples were prepared at concentrations of 50.00, 25.00, 10.00 and 5.00 mg/L. Meanwhile, 3.94 mg of DPPH powder was dissolved in 10 mL of 99% methanol to produce 1 mM DPPH stock solution, then diluted with methanol to obtain a concentration of 0.2 mM DPPH reagent.

For the assay, 100 µL of vinegar samples were mixed with 100 µL of 0.2 mM DPPH reagent, then incubated in the dark for 45 mins at room temperature. The absorbance was measured using a microplate reader at a wavelength of 516 nm, with methanol was used as a blank. The steps were repeated for ascorbic acid that served as positive control. The %RSA was calculated using the formula:

$$\%RSA = [1 - (\frac{Average\ absorbance\ of\ sample}{Average\ absorbance\ of\ negative\ control}) \times 100\%]$$

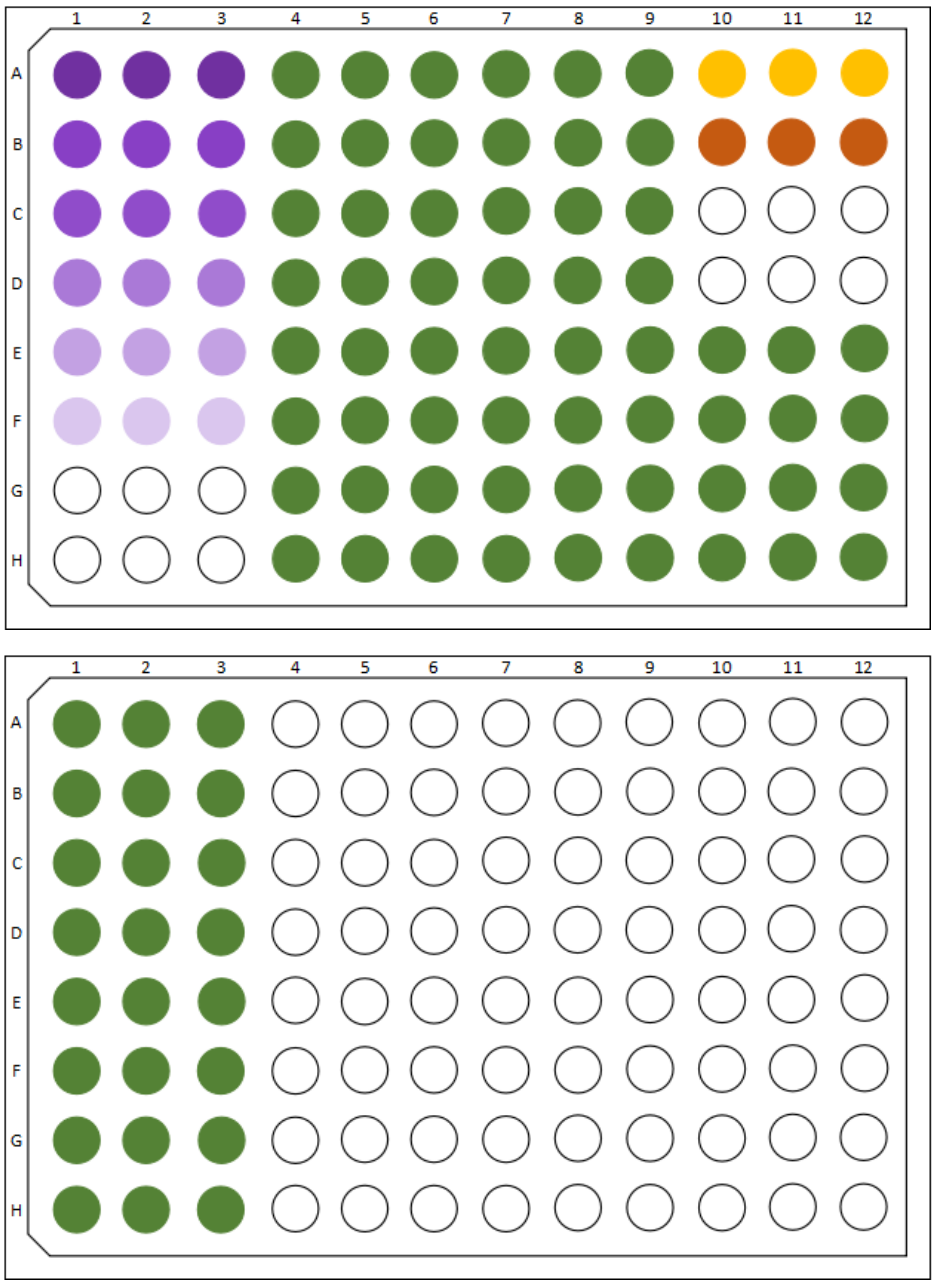

Figure 3.4: Layout of 96-well plate for DPPH Free Radical Scavenging Activity Assay (Plate 1&Plate 2).

Key:

: Ascorbic Acid (6.25 to 200 mg/L) + Methanol + DPPH Reagent

: Samples (6.25 to 200 mg/L) + Methanol + DPPH Reagent

 : Methanol + DPPH Reagent (Negative control)

 : Methanol (Blank)

3.8.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of papaya vinegars and commercial papaya vinegar were determined using the methods published by Madhuranga and Samarakoon (2023) with minor modifications. A total of 17.6 mg of ascorbic acid powder was dissolved in 100 mL of distilled water to produce a 1000 µmol/L stock solution, and was serially diluted to produce 800, 600, 400, 200 and 100 µmol/L ascorbic acid standards. A 1% potassium ferricyanide (III) was prepared by dissolving 1 g of potassium ferricyanide powder in 100 mL of distilled water, whereas 10% trichloroacetic acid (TCA) was prepared by dissolving 10 g of TCA powder in 100 mL of distilled water. To prepare 1% ferric chloride solution, 1 g of ferric chloride powder was dissolved in 100 mL of distilled water.

For the assay, 0.4 mL of vinegar samples were mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide, followed by incubation in the dark for 20 minutes at 50°C. Then, 1 mL of 10% TCA, 2 mL of distilled water and 0.4 mL of 1% ferric chloride solution were added to the mixture. The mixture was again incubated in the dark for 30 minutes at 37°C. The absorbance was measured using a microplate reader at a wavelength of 593 nm, with distilled water was used as a blank. The steps were repeated for ascorbic acid standards to produce ascorbic acid calibration curve for the calculation of reducing power of the samples in µmol/L.

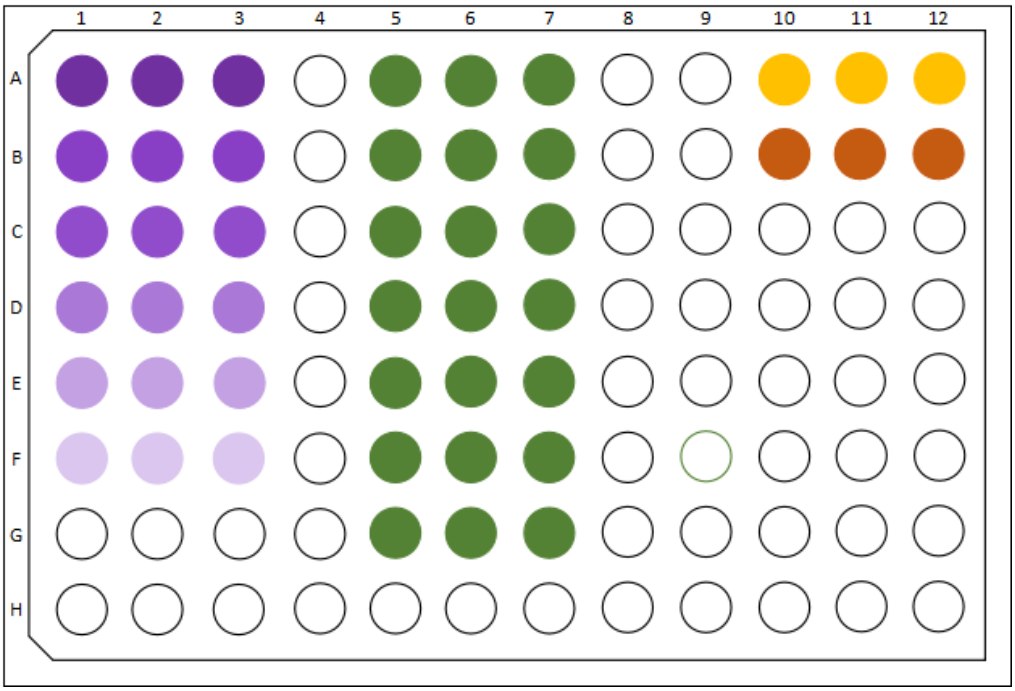

Figure 3.5: Layout of 96-well plate for FRAP Assay.

Key:

: Ascorbic Acid (100 to 1000 µmol/L) + Phosphate Buffer + Potassium Ferricyanide + Trichloroacetic Acid + Distilled Water + Ferric Chloride Solution

: Samples + Phosphate Buffer + Potassium Ferricyanide + Trichloroacetic Acid + Distilled Water + Ferric Chloride Solution

: Phosphate Buffer + Potassium Ferricyanide + Trichloroacetic Acid + Distilled Water + Ferric Chloride Solution (Negative control)

: Distilled water (Blank)

3.9 Statistical Analysis

All measurements were conducted in triplicate for two separate runs (n=6). Statistical Analysis System (SAS) Software with a 9.4 version and Microsoft Excel were used for the analysis of data. All collected data was presented as

mean ± standard deviation. The significant difference in pH, Brix, TA, phytochemical contents and antioxidant activities of papaya vinegars prepared with different papaya ripeness with or without incorporation of ginger, as well as the commercial papaya vinegar were analysed using one-way analysis of variance (ANOVA) and Tukey's HSD post-hoc test at a significant level of $p = 0.05$.

CHAPTER 4

RESULTS

4.1 Physicochemical Analysis

4.1.1 Determination of Papaya Ripeness

Papaya Sample	Colour	pH
1	Fully green	4.30 ± 0.01^{a}
2	Yellow fruit with the region near the stalk remained slightly green	4.65 ± 0.01^{b}

Table 4.1: Observation and pH measurements of the papaya.

Figure 4.1: The fermenting papaya vinegar in the jar covered with cheese cloth and parafilm.

4.1.2 pH

Table 4.2: pH of fermented papaya vinegars with different ripeness of papayas with incorporation of ginger on Day 21 and commercial papaya vinegar.

Papaya Vinegar	pH
R	2.98 ± 0.01^{d}
RG	3.02 ± 0.01^{c}
U	3.30 ± 0.01^{a}
UG	3.32 ± 0.01^{a}
RU	3.26 ± 0.01^{b}
RUG	3.25 ± 0.01^{b}
Commercial papaya vinegar	2.38 ± 0.01^{e}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Referring to **Table 4.2**, a higher pH was observed for papaya vinegars UG (3.32 ± 0.01) and U (3.30 ± 0.01). Meanwhile, the commercial papaya vinegar (2.38 ± 0.01) showed the lowest pH and differed significantly ($p < 0.05$) from other vinegars. There was no significant difference ($p > 0.05$) between U and UG as well as between RU and RUG. However, significant difference ($p < 0.05$) was observed among other papaya vinegars.

4.1.3 Brix

Table 4.3: Brix values of fermented papaya vinegars with different ripeness of papayas with incorporation of ginger on Day 21 and commercial papaya vinegar.

Papaya Vinegar	Brix
R	2.23 ± 0.00^{b}
RG	2.00 ± 0.00^{d}
U	2.11 ± 0.01^{c}
UG	2.30 ± 0.00^{b}
RU	2.18 ± 0.00^{bc}
RUG	2.13 ± 0.01^{c}
Commercial papaya vinegar	35.03 ± 0.06^{a}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

According to **Table 4.3**, the commercial papaya vinegar (35.03 ± 0.06) showed the highest Brix value and differed significantly ($p < 0.05$) from other samples, followed by papaya vinegars UG (2.30 ± 0.00), R (2.23 ± 0.00), RU (2.18 ± 0.00), RUG (2.13 ± 0.01), U (2.11 ± 0.01) and RG (2.00 ± 0.00). Besides, papaya vinegar RG also exhibited a significant difference ($p < 0.05$) from other samples. However, no significant difference ($p > 0.05$) was observed between R and UG, R and RU and U, RU and RUG.

4.1.4 Titratable Acidity (TA)

Table 4.4: TA of fermented papaya vinegars with different ripeness of papayas with incorporation of ginger on Day 21 and commercial papaya vinegar.

Papaya Vinegar	TA (%)
R	3.63 ± 0.26^{ab}
RG	3.29 ± 0.10^{ab}
U	3.91 ± 0.10^{ab}
UG	4.02 ± 0.00^{ab}
RU	3.46 ± 0.19^{ab}
RUG	3.18 ± 0.73^{b}
Commercial papaya vinegar	4.24 ± 0.18^{a}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Table 4.4 showed that commercial papaya vinegar (4.24 ± 0.18%) had a higher TA, while vinegar RUG (3.18 ± 0.73%) had a lower TA. There was significant difference ($p < 0.05$) existed between papaya vinegars RUG and commercial papaya vinegar. However, the two vinegars did not differ significantly ($p > 0.05$) from the other papaya vinegars.

4.2 Phytochemical Content

4.2.1 Total Phenolic Content (TPC)

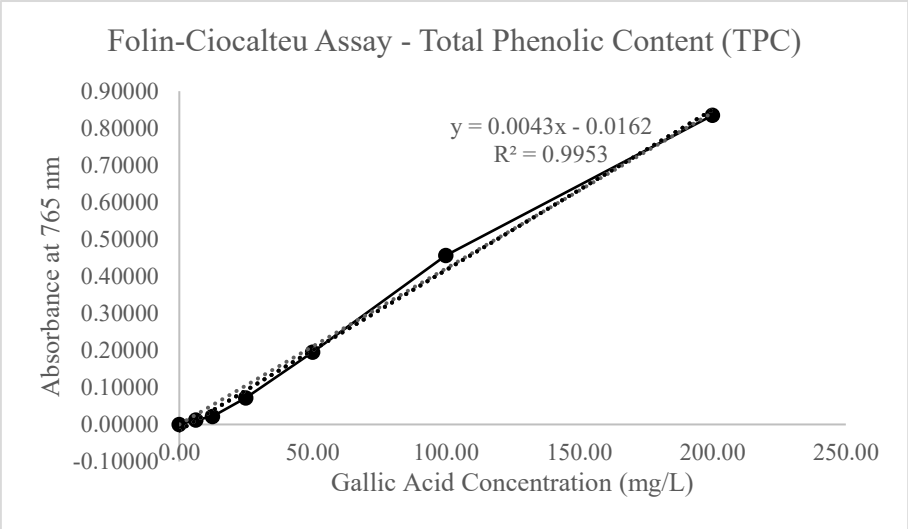

Figure 4.2: Standard curve of gallic acid.

Table 4.5: TPC of fermented papaya vinegars with different ripeness of papayas with incorporation of ginger on Day 21 and commercial papaya vinegar.

Papaya Vinegar	TPC (mg GAE/100 g extract)
R	29.58 ± 1.07^{bc}
RG	32.60 ± 0.47^{ab}
U	35.63 ± 1.07^{a}
UG	31.91 ± 0.70^{abc}
RU	28.50 ± 0.75^{bc}
RUG	32.29 ± 4.72^{abc}
Commercial papaya vinegar	27.18 ± 0.36^{c}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Figure 4.2 illustrated the gallic acid standard curve used for determining the TPC of fermented papaya vinegar samples and commercial papaya vinegar. A linear equation of $y = 0.0043x - 0.0162$ with a regression coefficient of $R^2 = 0.9953$ was obtained using 0.00, 6.25, 12.50, 25.00, 100.00 and 200.00 mg/L gallic acid. Papaya vinegars U (35.63 ± 1.07 mg GAE/100 g extract), RG (32.60 ± 0.47 mg GAE/100 g extract) and UG (31.91 ± 0.70 mg GAE/100 g extract) had a higher TPC, whereas commercial papaya vinegar (27.18 ± 0.36 mg GAE/100 g extract) had a lower TPC, as shown in **Table 4.5**. There was significant difference ($p < 0.05$) between papaya vinegars U with R, RU and commercial papaya vinegar, as well as between papaya vinegar RG with commercial papaya vinegar. However, papaya vinegars UG and RUG had no significant difference ($p > 0.05$) from all other vinegars.

4.2.2 Total Flavonoid Content (TFC)

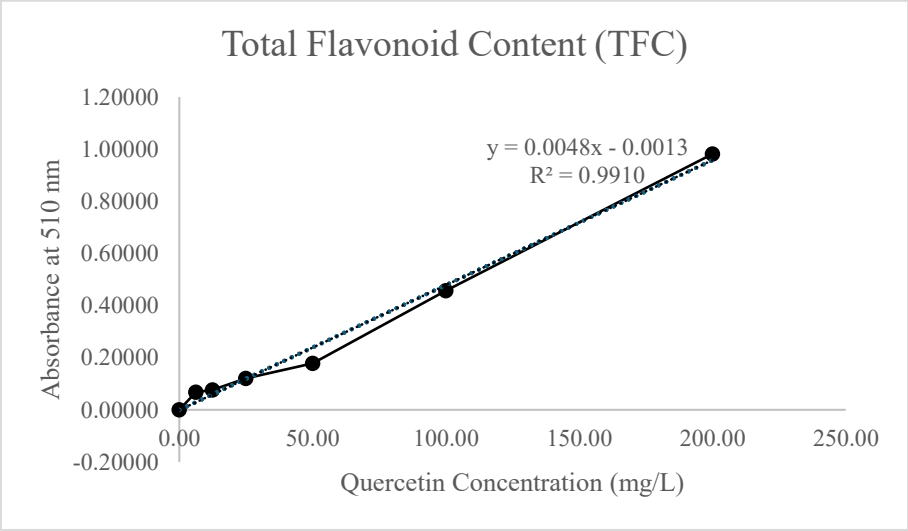

Figure 4.3: Standard curve of quercetin.

Table 4.6: TFC of fermented papaya vinegars with different ripeness of papayas with incorporation of ginger on Day 21 and commercial papaya vinegar.

Papaya Vinegar	TFC (mg QE/100 g extract)
R	20.34 ± 4.64^{a}
RG	18.59 ± 0.38^{a}
U	18.26 ± 0.96^{a}
UG	17.84 ± 0.94^{a}
RU	16.38 ± 0.33^{a}
RUG	16.87 ± 1.22^{a}
Commercial papaya vinegar	16.50 ± 0.49^{a}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Figure 4.3 demonstrated the quercetin standard curve used to assess the TFC of fermented papaya vinegar samples and commercial papaya vinegar. A linear equation of $y = 0.0048x - 0.0013$ with a regression coefficient of $R^2 = 0.9910$ was obtained using 0.00, 6.25, 12.50, 25.00, 100.00 and 200.00 mg/L quercetin. According to **Table 4.6**, papaya vinegar R (20.34 ± 4.64 mg QE/100 g extract) exhibited a higher TFC, while papaya vinegar RU (16.38 ± 0.32 mg QE/100 g extract) had a lower TFC. There was no significant difference ($p > 0.05$) existed among all papaya vinegars.

4.3 Antioxidant Activity

4.3.1 DPPH Free Radical Scavenging Activity Assay

Table 4.7: %RSA of fermented papaya vinegars with different ripeness of papayas with incorporation of ginger on Day 21 and commercial papaya vinegar.

Papaya Vinegar	%RSA
R	18.96 ± 2.06^{ab}
RG	24.37 ± 2.10^{a}
U	4.35 ± 6.64^{c}
UG	9.38 ± 0.84^{bc}
RU	26.31 ± 4.38^{a}
RUG	18.96 ± 1.93^{ab}
Commercial papaya vinegar	7.834 ± 7.26^{bc}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Table 4.7 showed the %RSA of fermented papaya vinegar samples and commercial papaya vinegar. A lower %RSA was observed in papaya vinegar U (4.35 ± 6.64%), whereas papaya vinegars RU (26.31 ± 4.38%) exhibited a higher %RSA. There was no significant difference ($p > 0.05$) existed among papaya vinegars R, RG, RU and RUG, as well as among papaya vinegars U, UG and commercial papaya vinegar. However, papaya vinegars R was differed significantly ($p < 0.05$) from U and RU.

4.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

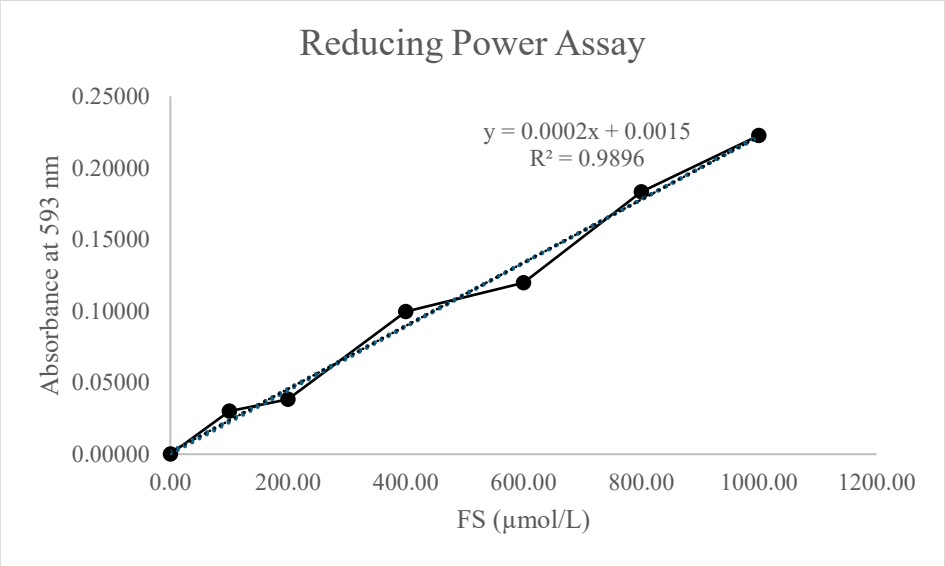

Figure 4.4: Standard curve of ascorbic acid.

Table 4.8: Fe^{2+} (µmol/L) of fermented papaya vinegars with different ripeness of papayas with incorporation of ginger on Day 21 and commercial papaya vinegar.

Papaya Vinegar	FS (µmol/L)
R	876.11 ± 20.37^{a}
RG	697.22 ± 36.72^{b}
U	677.22 ± 12.62^{b}
UG	636.11 ± 55.81^{b}
RU	657.22 ± 13.47^{b}
RUG	677.22 ± 8.39^{b}
Commercial papaya vinegar	365.00 ± 44.85^{c}

Data are expressed as mean ± standard deviation. Means with different lowercase superscripts indicate significant differences at $p < 0.05$.

Figure 4.4 illustrated the ascorbic acid standard curve used for determining the Fe^{2+} content (μmol/L) of fermented papaya vinegar samples and commercial papaya vinegar. A linear equation of $y = 0.0002x + 0.0015$ with a regression coefficient of $R^2 = 0.9896$ was obtained using 0.00, 100.00, 200.00, 400.00, 600.00, 800.00 and 1000.00 μmol/L ascorbic acid. According to **Table 4.8**, papaya vinegar R (876.11 ± 20.37 μmol/L) had the highest Fe^{2+} content, whereas the commercial papaya vinegar (365.00 ± 44.85 μmol/L) had the lowest Fe^{2+} content. The papaya vinegar R and commercial papaya vinegar were differed significantly ($p < 0.05$) from all papaya vinegars. However, the papaya vinegars RG, U, UG, RU and RUG exhibite no significant difference ($p > 0.05$).

CHAPTER 5

DISCUSSIONS

In this study, the Eksotika papaya with two ripening stages, RST2 and RST4, were defined by the ripeness indices from Barragán-Iglesias, Méndez-Lagunas and Rodríguez-Ramírez (2018) and the Malaysia Ministry of Water, Land and Natural Resources (Chan and Sim, 2019). The phytochemical contents and antioxidant activities of papaya vinegars fermented with different ripeness of papaya with or without added ginger, were evaluated through TPC, TFC, DPPH free radical scavenging activity and FRAP assay. These results were compared against a commercial papaya vinegar, which only consists of papaya and organic brewed enzyme, according to its ingredient list as shown in **Figure 5.1**.

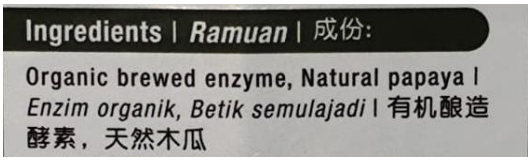

Figure 5.1: Ingredient lists of commercial papaya vinegar.

5.1 Physicochemical Analysis

5.1.1 pH

The pH refers to a measure of acidity or alkalinity of an aqueous solution based on the hydrogen ions (H^+) concentrations. It is an essential indicator in terms of food safety and quality, where most spoilage microorganisms are unable to grow

in high acidic environments (Helmenstine, 2025). For fruit vinegar, pH indicates the strength of the vinegar as a natural preservative, as well as its overall quality. A study by Ousaaid, et al. (2021) claims that the pH of fruit vinegars typically falls within the range of 2.4 to 3.9.

As presented in **Table 4.2**, both vinegars U and UG exhibited relatively higher pH values, implying a greater presence of weak acid in the vinegar. Weak acids only partially dissociate into H^+ ions in an aqueous solution, contributing to a limited amount of free moving H^+ ions (Munegumi, 2013). The decrease in pH was followed by RU, RUG, RG and R. In contrast, commercial papaya vinegar exhibited the lowest pH, indicating the highest concentration of free H^+ ions due to the complete dissociation of strong acids. The results also displayed no significant impact of ginger on the pH of fermented vinegar.

Acetic acid, an acid produced by the metabolism of *Acetobacter* spp. during acetous fermentation, is the predominant acid in vinegar and plays a critical role in determining the product pH. The study by Pednekar and Mangaonkar (2020) highlighted that unripe papaya usually contains lower simple sugar concentration as most are stored in complex polysaccharides. As the fruit ripens, enzymatic breakdown of polysaccharides increases the amount of glucose and fructose (Chung, et al., 2023). Higher sugar concentrations lead to a faster fermentation rate, resulting in a greater acetic acid production and a lower pH. However, the Brix values of the papaya juices were standardized in this study,

thereby allowing an assessment on how fruit ripeness alone affected the fermentation changes.

The enzymes such as amylase and glucosidase contribute to the degradation of carbohydrate during ripening process. These enzymes actively convert complex polysaccharides into simple sugar, primarily glucose and fructose. The activities of these enzymes are significantly higher in ripe papaya, thereby enhancing the release of fermentable sugars for more efficient fermentation. This results in lower pH values in vinegar R and RG (Garcia and Lajolo, 1988). In contrast, unripe papaya exhibits much lower concentration of these enzymes, which greatly slows down the fermentation process, leading to a lower acids production and higher pH in vinegar U and UG.

5.1.2 Brix

Brix is a parameter that measures the total soluble solids (TSS) in a solution. It primarily represents the sugar concentration, that directly correlates with the perceived sweetness of a sample (Kleinhenz and Bumgarner, 2012). However, the compounds like phenols and organic acids also contribute to Brix value. In vinegar fermentation, the initial Brix value is essential for the estimation of fermentable sugars while the final Brix value indicates the balance between sourness and sweetness in the fermented vinegar. Normally, fruit vinegars display Brix values ranging from 8% to 64% (Chang, Lee and Ou, 2005).

According to **Table 4.3**, apart from the commercial papaya vinegar, vinegar UG exhibited a higher Brix value, whereas vinegar RG showed the lowest. Nonetheless, no significant impacts exhibited by the ginger on vinegar Brix.

The high residual sugar concentrations in vinegar suggest a possible incomplete or stuck fermentation. During alcoholic fermentation, sugars are utilized by *S. cerevisiae* for the production of ethanol and carbon dioxide. However, in unripe papaya, the limited activity of polysaccharides-degrading enzymes restricts the release of fermentable sugars, resulting in a slower sugar consumption rate and increasing the likelihood of incomplete fermentation (Fairbairn, 2012). In contrast, ripe papaya contains more fermentable sugars that are continuously released through enzymatic breakdown, accelerating the fermentation rate and thus leaving minimal residual sugar in the vinegar.

5.1.3 Titratable Acidity (TA)

Titratable acidity, also known as total acidity, quantifies the total acid content in a food sample, whether in dissociated or undissociated forms (Sadler and Murphy, 2003). In fruit vinegar, the most commonly present organic acids include acetic, lactic, quinic, tartaric, propanedioic, malic, succinic, citric and ascorbic acids (Ren, et al., 2015). TA always reflects the concentration of these organic acids present in a particular vinegar. This parameter provides a more comprehensive evaluation of acidity than pH alone as it accounts for the buffering effect of weak acids. The titratable acidity of fruit vinegar is reported to fall within the range of 0.24% to 6.20% (Ousaaid, et al., 2022).

As shown in **Table 4.4**, the commercial papaya vinegar, along with vinegars UG and U, exhibited relatively higher TA, while no significant differences were observed among the other papaya vinegars. There is no noticeable impact of ginger on vinegar TA.

The higher TA in vinegars UG and U might be due to organic acid composition of unripe papaya. Unripe papaya generally contains lower levels of ascorbic acids than the ripe papaya (Chung et al, 2023; Bron and Jacomino, 2006). Ascorbic acids are particularly unstable and highly susceptible to pH changes and oxidation. During vinegar fermentation, gradual drop in pH and oxidation process accelerate ascorbic acid degradation, especially in ripe papaya, thereby resulting in a lower total acid composition (Jung, Kim, F.-E and Kim, S.-H, 2001). In contrast, unripe papaya naturally contains more malic acid, which is relatively stable and less affected by fermentation conditions. This stability explains the relatively higher TA observed in vinegars U and UG.

On the other hand, the high TA observed in the commercial papaya vinegar could be explained by the fermentation method employed. Submerged fermentation (SmF), also known as liquid fermentation, is a widely used industrial technique for large-scale vinegar production. In SmF, microorganisms are cultivated in a nutrient-rich liquid medium, where sugars can be continuously supplied to sustain microbial activity. Besides, a well-controlled condition is generated to maximize the fermentation efficiency (Subramaniyam and Vimala, 2012). For example, the continuous sugar supply maximizes the

production of ethanol during alcoholic fermentation, while well-aeration system optimizes the conversion of ethanol into acetic acid by *Acetobacter* spp. Thus, incomplete fermentation is avoided, yielding a high-quality vinegar with elevated organic acid concentrations that contribute to high TA.

5.2 Phytochemical Content

Phytochemical compounds refers to the bioactive, non-nutritive chemicals that are naturally present in plants. They are strong antioxidants that perform neutralization and scavenging activities, thus blocking the chain reaction of free radicals and ROS (Altemimi, et al., 2017). Given the critical involvement of phytochemicals in antioxidant activity, TPC and TFC assay were thus conducted. The reported TPC and TFC range of fermented papaya by Nurul and Asmah (2012) were 20 to 40 mg GAE/100 g and 15 to 35 mg QE/100 g, respectively.

5.2.1 Total Phenolic Content (TPC)

Table 4.4 demonstrated that papaya vinegars U and RG exhibited higher TPC while the commercial papaya vinegar had a lower TPC. It could be observed that RG and RUG exhibited a higher TPC compared to those without the incorporation of ginger. All results obtained in **Table 4.4** were within the normal range of TPC for fermented papaya.

The higher TPC observed in vinegar U than RG could be explained by the greater initial phenolic content in unripe papaya fruit. Maisarah, et al. (2013)

highlighted that unripe papaya contains significantly higher TPC (339.91 mg GAE/100 g dry weight) than ripe papaya (272.66 mg GAE/100 g dry weight). This is due to the fact that phenolic compounds are gradually degraded during ripening process through enzymatic oxidation, primarily triggered by polyphenol oxidase (PPO) and peroxidase (POD). This is supported by the research by Othman (2014), that explained an increase in PPO activity during papaya ripening process, which converts polyphenols into quinones. Additionally, Rana (2008) reported a general decrease in organic acids and phenolics in fruits and vegetables as they are ripe.

Apart from that, Liang, et al. (2023) suggested that phenolic compounds may decrease during fermentation due to enzymatic degradation by fermentative bacteria. For example, ferulic acid and cyanidin-3-*O*-glucoside, two phenolic compounds abundantly found in papaya, will be broken down by decarboxylase and glucosidases that secreted by *S. cerevisiae*, respectively. The same observation was also reported by Nurul and Asmah (2012) that pickled papaya had lower TPC than fresh papaya.

However, Yang, et al. (2023) reported that enzymatic degradation during fermentation enhances the bioavailability and bioactivity of phenolic compounds. This enzymatic degradation, also known as biotransformation, is a process that transforms a compound into a structurally modified form. For instance, biotransformation of cyanidin-3-*O*-glucoside produces simpler anthocyanins or cyanidin that are more easily absorbed by human body. These

biotransformed product also exhibit stronger antioxidant, antimicrobial and anti-inflammatory activities.

5.2.2 Total Flavonoid Content (TFC)

Flavonoids are a group of substances that categorized under phenolic compounds. Therefore, the TFC of a sample must be lower than its TPC. However, a higher TPC does not necessarily correspond to a higher TFC. Phenolic compounds are broadly divided into two main subgroups, namely flavonoids and non-flavonoids phenolic acids (Altemimi, et al., 2017). The relative proportions of these subgroups vary among different fruits.

According to **Table 4.5**, papaya vinegars R and RG exhibited higher TFC, whereas vinegar RU showed a lower TFC. However, there was no significant difference between the TFC of the fermented vinegars and the commercial papaya vinegar. All results obtained fell within the normal range established.

A higher TFC observed in papaya vinegar R is mainly due to the greater flavonoid levels present in ripe papaya. Zuhair, et al. (2013) highlighted that ripe papaya with RS5 (36.36 mg QE/100 g) exhibited a relatively higher TFC than papaya with RS1 (18.45 mg QE/100 g). Similarly, this observation was supported by the study conducted by Maisarah, et al. (2013). In contract, papaya vinegar RU exhibited a lower TFC among all samples. This may be due to the reason that the mixing of ripe and unripe papaya activates the PPO in the unripe

fruit, thereby accelerating PPO activity to degrade polyphenols and flavonoids (Cano, Ancos and Lobo, 2006).

Furthermore, the study by Nurul and Asmah (2012) reported a decrease in TFC during fermentation, with fresh papaya containing approximately 57.80 mg rutin /100g, while pickled papaya had only 19.71 mg rutin /100 g dry samples. This reduction could be explained by the biotransformation of flavonoid compounds during fermentation. Specifically, glucosidases produced by *S. cerevisiae* hydrolyze quercetin into aglycone, a bioactive substance that could not be detected by TFC assay (Yang, et al., 2023).

5.3 Antioxidant Activity

Antioxidant activity refers to the effectiveness of antioxidants to prevent the oxidation of free radicals and ROS. Antioxidant plays an important role in prolonging the food shelf life by inhibiting lipid oxidation, as well as enhancing the health-promoting potential of functional food, protecting human against chronic diseases (Shahidi and Zhong, 2015). DPPH free radical scavenging activity and FRAP assessments were carried out to evaluate the antioxidant activities of bioactive compounds found in papaya vinegar.

5.3.1 DPPH Free Radical Scavenging Activity Assay

As shown in **Table 4.6**, papaya vinegars RU exhibited a higher %RSA while vinegar U had the lowest %RSA. Additionally, papaya vinegars RG and UG

displayed slightly higher %RSA values compared to vinegars R and G, although there was no significant differences observed between the two pairs.

A higher %RSA observed in papaya vinegar RU is primarily due to the presence of polyhydroxylated phenolic compounds. As reported by Jing, at al. (2012), these compounds contain multiple hydroxyl groups, allowing them to donate more than one hydrogen atom to neutralize free radicals. In contrast to monohydroxyacids that can donate only one hydrogen atom at a time, polyhydroxylated compounds scavenge reactive species more efficiently, resulting in higher antioxidant activity. Caffeic, coumaric, chlorogenic, neochlorogenic and ferulic acids are the examples of polyhydroxylated compounds commonly found in papaya (Kumarasinghe, et al., 2024; Jeon, et al., 2022). Nevertheless, there exact composition and concentration of these compounds in ripe and unripe papaya still remain unclear.

On the other hand, the low %RSA observed in papaya vinegar U may be due to the presence of weaker radical scavengers, such as amino acids like cysteine and tryptophan, as well as citric acids (Prior, Wu and Schaich, 2005). These compounds, which are abundantly found in unripe papaya, donate electrons or hydrogen atoms to reduce DPPH radicals to DPPH-H. However, their lower radical scavenging capacity compared to polyphenolic compounds results in a reduced overall %RSA. A similar observation was reported by Zuhair, et al. (2012), in which papaya at the RS1 stage exhibited the lowest %RSA, with radical scavenging activity increasing progressively over the ripening process.

Nevertheless, it could be observed that the TPC of papaya vinegar U was relatively higher than other samples. The studies by Chen, Cheng and Liang (2015) and Rover and Brown (2013) stated that certain non-phenolic reducing molecules like ascorbic acids able to reduce the F-C reagent during TPC assay, leading to an overestimation of phenolic content. The same reduction process also occurs in DPPH assays, yet, in a weaker manner. Therefore, a higher TPC value does not necessarily correspond to greater radical scavenging activity.

5.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Table 4.7 described that papaya vinegar R had the highest reducing power, while the commercial papaya vinegar displayed the weakest reducing power. There was no significant difference among the reducing power of other samples.

The highest reducing power observed in papaya vinegar R is mainly due to the presence of reducing agents capable of effectively reducing ferric ion (Fe^{3+}) to ferrous ions (Fe^{2+}). For example, ascorbic acid is one of the antioxidants that exhibit strong ferric reducing capacity. Apart from that, Zuhair, et al. (2013) claimed that ripe papaya contained higher FRAP values than unripe fruit, which likely contributes to the elevated reducing power in its derived product. In contrast, the lowest reducing power exhibited by the commercial papaya vinegar may be due to the presence of weaker ferric reducing agents, such as proteins.

By comparing the results of vinegar RU in the DPPH and FRAP assays, it was observed that it exhibited a higher %RSA but relatively low ferric reducing power. This is because there are some compounds that effectively reduce DPPH radicals but are unable to reduce Fe^{3+} ions in FRAP assay. For instance, proteins and glutathione that contain sulfhydryl (-SH) group in their structures (Malta and Liu, 2014). The FRAP assay is conducted under an acidic pH, which is around pH 3.6. However, -SH groups remain in their protonated form, making them less reactive to reduce the Fe^{3+} ions. In contrast, DPPH assay is carried out under a near-neutral pH, where a more reactive thiolate anion ($-S^-$) donate its electron to reduce the DPPH radicals (Rumpf, Burger and Schulze, 2023).

5.4 Limitations of Study

Despite providing valuable insights, this study has several limitations that should be taken into account. First and foremost, the lack of sensory evaluation restricts the understanding of consumer acceptance and preference in terms of taste, aroma and mouthfeel. This significantly affects the estimation of vinegars' overall quality as well as their potential for commercialization. Moreover, the lack of nutritional profiling limits the information on the macro- and micronutrients content of the product, leaving their additional functional value unclear. In addition, the absence of safety assessments raises uncertainty about the microbial safety of the product. Lastly, this study did not include a detailed identification and quantification of specific phytochemical compounds. This limits the understanding of the bioactive composition present in papaya at different ripening stages, as well as in ginger.

5.5 Recommendations for Future Studies

It is recommended to conduct sensory evaluations in future studies to assess consumer perceptions of flavor, aroma and overall acceptability on the papaya vinegar. This provides a crucial insight into product development and market potential evaluation. Besides, proximate analysis is recommended to determine the nutritional composition, especially the minerals and vitamins available in the product. In addition, safety assessments should be performed to ensure the vinegar meets food safety standards and regulatory requirements. Last but not least, advanced analytical techniques such as HPLC should be used for the detailed identification and quantification of phytochemicals, enabling a deeper understanding of how bioactive compounds vary with papaya ripeness and how they contribute to antioxidant activity

CHAPTER 6

CONCLUSION

In this study, vinegars were fermented using Eksotika papaya at RST2 and RST4 stages, both with and without the incorporation of ginger. A commercial papaya vinegar was served as a reference to compare antioxidant activities. The phytochemical content of the vinegars was determined using TPC and TFC analyses, whereas antioxidant activities were evaluated using DPPH and FRAP assays. The results demonstrated that vinegars made from ripe papaya exhibited higher phytochemical content and stronger antioxidant properties compared to all other samples. On the other hand, the addition of ginger showed no significant impact on the phytochemical content and antioxidant activity of the papaya vinegar. Future studies are recommended to evaluate the sensory attributes of the vinegar, as well as their nutritional profiles and microbial safety. Moreover, it is recommended to identify and quantify phytochemicals present in the vinegars using HPLC method. In short, papaya vinegar produced from ripe papaya exhibited better antioxidant properties, while no significant impact was observed with the addition of ginger. Thus, it is recommended to add ginger during papaya vinegar fermentation based on personal preference.

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