EVALUATION OF DYNAMIC PHYSICOCHEMICAL PROPERTIES CHANGES OF PURPLE SWEET POTATO (*Ipomoea batatas* (L.) Lam) VINEGAR THROUGH STEPWISE FERMENTATION

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By

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ABSTRACT

EVALUATION OFDYNAMIC PHYSICOCHEMICAL PROPERTIES CHANGES OF PURPLE SWEET POTATO (*Ipomoea batatas* (L.) Lam) VINEGAR THROUGH STEPWISE FERMENTATION

Tan Sin Yun

Vinegar is a health-beneficial condiment produced from two-stage fermentation involving alcohol conversion and subsequent acetification using high-sugar materials. However, vinegar production from starchy materials, such as purple sweet potato and cassava, has been rarely studied due to the limited conversion of starch to sugar. Since agarwood leaves are an underutilized by-product of agarwood cultivation, their infusion has been incorporated to enhance the physicochemical properties of vinegar, such as total phenolic content. This study aimed to evaluate the effects of different substrates and the presence of agarwood leaf infusion on the physicochemical and biochemical properties of vinegar production. Purple sweet potato and cassava obtained from the local market were saccharified using *Aspergillus oryzae*, followed by alcohol and vinegar fermentation. During this process, dynamic changes in the biochemical properties of vinegar were monitored, alongside phytochemical qualitative screening and organic acid analysis. Purple sweet potato showed a sugar content of 28.40 %wb, but the amino acid content after saccharification was lower compared to cassava. Vinegar incorporated with agarwood leaves

displayed a similar trend in alcohol content as the water-treated samples. Dynamic changes in vinegars showed an increase in pH and a decrease in alcohol content during acetic acid fermentation. The total phenolic content of agarwood-treated vinegar was higher than that of water-treated vinegar. Quantitative analysis revealed that the acetic acid ranged from 1.176 mg/mL to 4.536 mg/mL for both vinegars. Besides, similar acetic acid production was observed in the water-treated and agarwood-treated vinegar at Day 10. The study showed higher potential for purple sweet potato for vinegar production. In addition, the incorporation of agarwood tea improved the biochemical properties of the vinegar while not affecting the alcohol content throughout the fermentation process and acetic acid level at the end of acetic acid fermentation.

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DECLARATION

I hereby declare that this final year project report 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.

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This final year project report entitled "**EVALUATION OF DYNAMIC PHYSICOCHEMICAL PROPERTIES CHANGES OF PURPLE SWEET POTATO (*Ipomoea batatas* (L.) Lam) VINEGAR THROUGH STEPWISE FERMENTATION"** was prepared by TAN SIN YUN and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) Food Science at Universiti Tunku Abdul Rahman.

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It is hereby certified that **TAN SIN YUN** (ID No: **2207451**) has completed this final year project report entitled "EVALUATION OF DYNAMIC PHYSICOCHEMICAL PROPERTIES CHANGES OF PURPLE SWEET POTATO (*Ipomoea batatas* (L.) Lam) VINEGAR THROUGH STEPWISE FERMENTATION" under the supervision of Dr. Chang Ying Ping from the Department of Agricultural and Food Science, Faculty of Science.

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Yours truly,

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(TAN SIN YUN)

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
B.C.	Before Christ
Cfu	Colony Form Unit
DNS	3,5-Dinitrosalicylic Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
$FeCl_3$	Ferric Chloride
GAE	Gallic Acid Equivalent
H_2SO_4	Sulphuric Acid
HPLC	High Performance Liquid Chromatography
HSD	Honestly Significant Difference
KOH	Potassium Hydroxide
NaOH	Sodium Hydroxide
SD	Standard Deviation
$SnCl_2$	Tin Chloride
UV	Ultra-Violet
wb	Wet Basis

CHAPTER 1

INTRODUCTION

Vinegar has been present since as early as 3000 to 5000 B.C. In 3000 B.C., the Babylonians produced vinegar by exposing alcoholic drinks made from fruits and sap of date palms to air, allowing the alcohol to naturally turn into vinegar. This vinegar was commonly used as a food or ingredient preservative, or pickling agent (Bourgeois and Barja, 2009). Today, modern science recognizes vinegar as a sour, acidic liquid that rich in organic acids, especially acetic acid, and other nutrients that contribute to various health benefits. A study had highlighted that regular consumption of vinegar can improve metabolic health, such as regulating blood glucose levels, cholesterol levels, and promoting softening of blood vessels, thus lowering the risk of cardiovascular diseases (Wu et al., 2017). In addition, it was found to help in preventing diabetes, hyperlipidemia, cancer, and boosting immune function (Ousaaid et al., 2021).

Vinegar can be produced from sugar or carbohydrate-rich material. Modern industries often rely on fruits such as apples, grapes, and berries, or on grains such as rice for vinegar production (Chen et al., 2023). In contrast, the starchy materials, like purple sweet potatoes, are less commonly used. Purple sweet potatoes are carbohydrate-rich materials that are high in nutrition and functional compounds. One of their key bioactive compounds in purple sweet potato is anthocyanin, which is a group of phytochemical that deep purple colour. Anthocyanin was linked to health benefits such as

antioxidant activity and protection against cardiovascular disease. This natural pigment is a strong antioxidant that can scavenge free radicals and have anti-inflammatory effects in the body. Besides, sweet potatoes also contain various nutrients such as fibers, vitamins, and minerals that are essential to human health (Laveriono-Santos et al., 2022). Hence, using purple sweet potato as a raw material for vinegar production can broaden the variety of vinegar types available and also provide the opportunity to develop a functional vinegar with enhanced nutritional and health-promoting properties.

In addition, Gupta et al. (2023) mentioned that the significance of the composition and nutrition of food become more apparent to consumers who are health-conscious. Hence, manufacturers are more concentrated on fortified beverages or products that are innovative and have higher bioavailability of bioactive compounds, as well as their associated health benefits. Phenolic compounds, minerals, vitamins, amino acids, and other bioactive ingredients are used in functional beverages, and these ingredients can be obtained from plants. However, the combined effect of the infused agarwood leaves in vinegar on these properties remains untested. Agarwood is a known fragrant resinous wood that derived from the *Aquilaria* tree. It has been widely used in perfumery and traditional medicine (Shicanand et al., 2022). However, the leaves of agarwood are generally considered an underutilized by-product of agarwood cultivation. Historically, these leaves were often discarded, but it was highlighted that they contain phytochemical compounds that contribute to potential health-promoting properties (Wangiyana, 2020). There is a study that revealed that agarwood leaves are rich in bioactive compounds such as flavonoids and 2-(2-phenethyl)

chromone derivatives (Xie et al., 2017). Furthermore, agarwood leaves have been reported to exhibit a wide range of beneficial effects, including antioxidant, anti-inflammatory, and antimicrobial activities (Rashed et al., 2024). These findings suggested that agarwood leaves are a valuable functional ingredient that can be used to develop new health-beneficial products.

In a previous study, agarwood leaves were incorporated as an ingredient in herbal kombucha, a fermented tea beverage. The dried agarwood leaves were brewed together with other herbs, such as roselle hibiscus and peppermint, during kombucha fermentation. The resulting drink had significant functional improvements, including a higher total phenolic content and the ability to inhibit harmful bacteria (Suwanposri, 2025). This demonstrates that the antimicrobial and antioxidant properties of agarwood leaves can be retained and even be enhanced by fermentation. In addition, there was also research that explored lactic acid fermentation of agarwood leaf infusions to increase their bioactive compound yield (Lieu et al., 2024).

However, no studies have so far reported the incorporation of agarwood leaves in vinegar production. This leaves a gap and an opportunity for innovation. Thus, the unexplored approach has the possibility to produce a vinegar with enhanced antioxidant and antimicrobial properties that offer consumers a new health beverage option. The aims of this study were:

1. To evaluate the effect of different tuber crops such as cassava and purple sweet potato on fungal-induced saccharification rate.

2. To investigate the effect of infused agarwood leaves on the biochemical profile of vinegar produced.
3. To determine the biochemical properties underlying vinegar production by monitoring dynamic changes across different fermentation stages.

CHAPTER 2

LITERATUREREVIEW

2.1 Vinegar

Vinegar is a sour liquid that is produced by two-step fermentation process (Mota and Vilela, 2024). Different types of vinegar produced from a variety of raw materials, such as wine vinegar produced using grape wine and inoculated with *Acetobacter*, rice vinegar using rice wine, and fruit vinegar using fermented juice from various fruits such as berries, apples, and so on (Bhat, Akhtar and Amin, 2014). Fruit-based vinegars generally have a pH ranged from 2.40 to 3.90 (Ousaaid et al., 2022). According to Sengun, Kilic and Ozturk (2019), most of the commercial vinegars have a pH range of pH 2.4 to 3.5. Most of the vinegar contains acetic acid ranging from 1.1% to 5.6%. The regulatory standards in the U.S. or other countries require a bottled vinegar to contain more than or equal to 4% v/v of acetic acid (Sengun, Kilic and Ozturk, 2019).

In addition to acetic acids, vinegar also contains other organic acids such as malic, oxalic, succinic, tartaric, citric, lactic, and acetic acid (Figure 2.1) that serve as the primary constituents that enhance the health benefits of vinegar (Wang et al., 2023). Figure 2.2 also shows a HPLC chromatogram of a vinegar contains various organic acids such as malic, citric, and succinic acid (Liu et al., 2019).

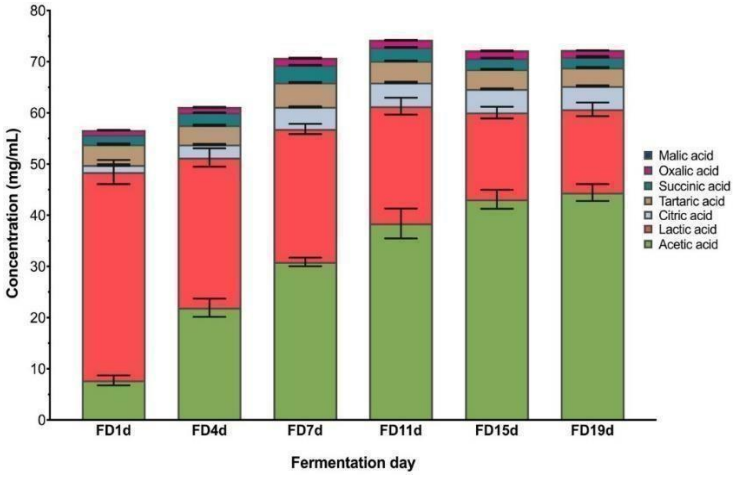

Figure 2.1: Organic acid content in Chinese grain vinegar (Wang et al., 2023).

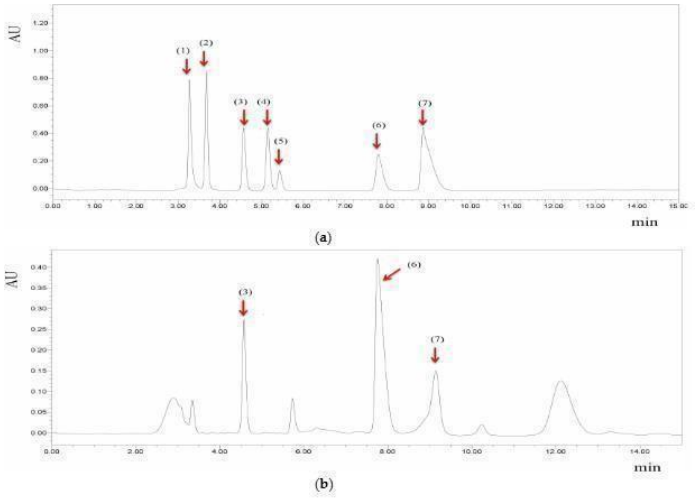

Figure 2. The chromatograms of the organic acid standards (**a**) and apple vinegar (Cu Bo Shi) (**b**) under 210 nm. Peak identification and retention time: (1) oxalic acid, 3.268 min; (2) tartaric acid, 3.677 min; (3) malic acid, 4.565 min; (4) ascorbic acid, 5.150 min; (5) lactic acid, 5.429 min; (6) citric acid, 7.795 min; (7) succinic acid, 8.869 min.

Figure 2.2: HPLC results showing malic acid, citric acid, and succinic acid content in apple vinegar (Liu et al., 2019).

In addition, it was found that vinegar may contribute various health benefits to humans upon consumption. For example, a previous study had reported that apple cider vinegar and grape vinegar contain phenolic compounds such as gallic acid, catechin, caffeic acid, and vitamins that can help to combat oxidative stress due to their strong antioxidant properties. It has been indicated that such compounds may

lower the risk of degenerative disease by exerting antioxidant effects (Budak et al., 2014). Besides, Ho et al. (2017) revealed that vinegar can enhance digestion, stimulate appetite and lower the blood lipid level, and regulate blood pressure. Perumpuli and Dilrukshi et al. (2022) also mentioned that the polyphenol, particularly resveratrol, contained in vinegar has anticancer properties. Resveratrol is a dietary polyphenol that can interfere with all three stages of carcinogenesis and inhibit cancer cell proliferation. In addition, it was also reported that the weak acids, including acetic acid also exhibit anticancer effects.

2.2 Fermentation Process of Vinegar

Fermentation can be divided into solid-state and liquid-state fermentation, which is also known as submerged fermentation, based on the type of substrate used. Solid-state fermentation is a process where the microorganisms grow on a moist solid substrate without the presence of free-flowing water. Solid substrates like bran and bagasse are typically used for solid-state fermentation. The advantage of this method is that the nutrient-rich waste material can be recycled as a fermentation substrate, but the substrate utilization occurs slowly in this method. Hence, a longer time of fermentation was needed (Ravichandran and Vimala, 2012). The example that used solid-state fermentation is the production of Chinese vinegar, in which the saccharification and alcohol fermentation may be either solid-state fermentation or submerged fermentation, while the other stages, like koji preparation and acetic acid fermentation, happen via solid-state fermentation (Dengru, Zhu and Beeftink, 2004). On the other hand, submerged fermentation used free-flowing liquid substrates like molasses or broth. In this process, the

bioactive compounds can be released into the fermentation medium, and the substrates are consumed rapidly (Ravichandran and Vimala, 2012).

The two consecutive fermentations of vinegar involve an initial alcoholic stage driven by yeast, followed by an acetic stage where acetic acid bacteria are playing the main role (Luzón-Quintana, Castro and Durán-Guerrero, 2021). The first phase of vinegar fermentation is known as alcoholic fermentation. During this step, yeast, primarily *Saccharomyces cerevisiae*, the dominant microbe, will initiate the process by converting simple sugars into alcohol under anaerobic conditions (Luzón-Quintana, Castro and Durán-Guerrero, 2021). In fermentation, *S. cerevisiae* converts glucose or fructose into two pyruvate molecules via the ATP-producing Embden-Meyerhof-Parnas (EMP) glycolytic pathway, generating 2 ATP and 2 NADH per glucose. Each pyruvate is decarboxylated by pyruvate decarboxylase, releasing acetaldehyde and CO_2 as byproducts. Then, acetaldehyde is reduced to ethanol by alcohol dehydrogenase, using the NADH produced earlier (Aalst et al., 2023). During alcohol fermentation, microorganisms such as *Hanseniaspora*, *Metchinikowia*, *Pichia*, and *Candida*, which are classified as semi-fermentative genera, produce secondary metabolites that can significantly influence the flavor and quality of vinegar. These effects can either enhance or impair vinegar characteristics (Mota and Vilela, 2024). The optimal temperature for alcohol fermentation is typically 20 to 30 ℃ (Sengun, Kilic and Ozturk, 2019). Additionally, the sugar concentration for vinegar production usually measures around 15 °Brix (Divyashree, Narayanaswamy and Tejashwini, 2022). Generally, the final alcohol content in yeast fermentation ranges from 5 to 10% v/v (Walker and Stewart, 2016).

The second stage of fermentation is acetic acid fermentation, which is also known as acetification (Luzón-Quintana, Castro and Durán-Guerrero, 2021). The microbes that are responsible for this stage of fermentation are obligate aerobes in the family of *Acetobacteraceae*. The key genera include *Gluconacetobacter* and *Acetobacter,* which can tolerate high acidity (Qiu, Zhang and Hong, 2021). These microbes can be obtained from unfiltered apple cider vinegar as it contains various beneficial microbes, including the acetic acid bacteria (Yetiman and Kesmen, 2015). During the acetic acid fermentation, the two membrane-bound enzymes, which are alcohol dehydrogenase and aldehyde dehydrogenase on the cytoplasmic membrane of these bacteria, will convert ethanol to acetic acid. The action of conversion is carried out by oxidizing the ethanol to acetaldehyde by alcohol dehydrogenase, followed by aldehyde dehydrogenase oxidizes it to acetic acid. In addition, the other microorganisms, such as lactic acid bacteria, may be involved in this stage and influence the fermentation process and quality. The activity of lactic acid bacteria can help regulate pH and enhance the flavour of vinegar. Besides, the nutritional quality of vinegar can be improved by providing a probiotic effect and supporting a stable fermentation process (Mota and Vilela, 2024). However, certain acetic acid bacteria like *Lactobacillus brevis* and *Pediococcus* can cause off-flavours and develop haziness, thus degrading the quality of vinegar (Xu et al., 2020). Aeration is required for acetic acid fermentation. Shaking is better performed to force the aeration that causes the oxygen to be better supplied to acetic acid bacteria. The optimal growth temperature for most of the acetic acid bacteria is 20 to 30 ℃. Besides, the alcohol fermentation should be maintained below 6 %

v/v as the level above this will inhibit the activity of acetic acid bacteria (Song et al., 2022).

2.3 Koji

Koji refers to grains or beans that are inoculated with mold such as *Aspergillus oryzae*, *Aspergillus sojae*, and *Aspergillus kawachii* that are able to perform fermentation. It is a basis for producing sake, miso, and soy sauce. During the koji preparation, *A. oryzae* grows in the substrates such as rice, barley, or soybean that produces hydrolytic enzymes, notably amylase, including α-amylase and glucoamylase, and proteases that break the starch and proteins into simple sugar and amino acids, respectively. Koji also enhances the colour, flavour, and scent of fermented foods, which are essential for their overall characteristics (Kitamoto, 2002). Besides sake, miso, and soy sauce production, koji is also used for rice vinegar to perform saccharification before the two-step fermentation of vinegar (Tawwekasemsombut et al., 2021).

Koji was generated from the history of sake brewing. It is known that the main component in sake is alcohol, and it is produced from wheat or rice that is high in carbohydrates. For fruit wine, yeast can directly convert the naturally present reducing sugar in fruits to produce alcohol. However, the sake production requires an additional saccharification step as it uses wheat or rice that is rich in starch but low in simple sugar (Yamashita, 2021). Saccharification is a process that hydrolyzes starch into simple sugars that can be consumed by yeast. It is a critical step in the vinegar fermentation of grains, rice, or starch-rich substrates

(Kitagaki, 2021). Typically, the koji will be added to substrates used to make vinegar. Then, the α-amylase and glucoamylase in *A. oryzae* obtained from koji will hydrolyze the starch into glucose, which is needed for the alcoholic fermentation (Takefuji, Ninomiya and Morita, 2016).

2.4 Purple Sweet Potato

Purple sweet potato, with the scientific name of *Ipomoea batatas* (L.) Lam is a dicotyledonous plant and herbaceous perennial vine specific to the Neotropics. It is a member of the *Ipomoea batatas* series that belongs to the Convolvulaceae family, which is classified under the *Ipomoea* genus (Rosell et al., 2024). Sweet potato originated from Latin America and was brought to Europe, Asia, and Africa in the 16th century. In 2016, Asia produced 74.7% which was about 79 million tons of the world's sweet potatoes (Im, Kim and Lee, 2021). In today's world, the sweet potato is a desirable starch crop that can help resolve global food and environmental issues (Kim et al., 2018). After potatoes, it is considered the third most significant crop in the world (Ngcobo et al., 2024).

Rosell et al. (2024) reported that purple sweet potatoes have defense against several illnesses due to their composition. It contains a high concentration of bioactive substances, including phenolic acids, anthocyanins, vitamins, dietary fiber, and starch. Numerous studies have found that purple sweet potato exhibits anti-inflammatory, antioxidant, anti-tumor, and neuroprotective effects. It was found that purple sweet potato contains 72.10 g of carbohydrates per 100 g on a dry weight basis, with reducing sugars such as glucose and fructose being the

predominant types (Triasih and Utamin, 2020). The other types of sugar, like maltose and sucrose also present depending on their species and stage of maturity. Furthermore, the protein content of purple sweet potato is low at 2.33 g per 100 g of dry weight. Glutamic acid, aspartate, arginine, alanine, and leucine are among the amino acids that are abundant in the crude protein extract of purple sweet potato. In addition, Wan, Wang and Xiao (2024) revealed that purple sweet potato has succinic acid 202.04 times higher than white flesh sweet potato and 128.93 times higher than orange flesh sweet potato. Other organic acids found in sweet potato include malic, citric, and succinic acids (Picha, 1985).

One of the chemicals that is abundant in purple sweet potato is anthocyanin. Anthocyanin is the pigment that is responsible for the purple colour of the sweet potato. (Kurata et al., 2024). Im, Kim and Lee (2021) highlighted that Peonidin 3-sophoroside-5-glucoside and cyanidin 3-sophoroside-5-glucoside are the main anthocyanins in purple sweet potatoes. They are mono- or di-acylated with p-hydroxybenzoic, ferulic, and caffeic acids, with acylated anthocyanins constitute over 98% of the total anthocyanin content of purple sweet potatoes. Besides, the pigment anthocyanin in purple sweet potatoes is more stable than anthocyanin in red corn, cabbage, and blueberries (Ngcobo et al., 2024). Herawati et al. (2020) highlighted the potential of anthocyanins in sweet potato to reduce the oxidative stress caused by hyperglycemia. This previous study demonstrated that the intake of anthocyanins from purple sweet potato drastically reduced blood, liver, and kidney malondialdehyde levels in hyperglycaemic rats. Furthermore, it may lower creatinine and urea levels, which represent the markers of better renal function. In addition, Tena, Martín, and Asuero (2020) also describe

anthocyanins as a significant group of flavonoids in plants. It can avoid oxidation by scavenging free radicals and decreasing oxidative stress by acting as a hydrogen donor. Consequently, it serves as an important source of antioxidants. Additionally, Rosell et al. (2024) reported that purple sweet potatoes contain other phytochemicals, such as carotenoids. Laveriano-Santos et al. (2022) stated that the phenolic phytochemicals in purple sweet potato have demonstrated *in vitro* antitumor activity in bladder cancer. The phytochemicals can diminish the viability of bladder cancer cells in a dose-dependent manner, thereby enhancing the apoptosis rate and inhibiting the cell cycle (Li et al., 2018).

2.5 Cassava

Cassava, scientifically known as *Manihot esculenta*, is a starchy root crop domesticated initially in the southwestern Amazon. Nowadays, cassava is widely used in commercial agriculture for fresh and starch-based foods, animal fodder, and industrial starch products (Cock and Connor, 2021). Besides these, cassava is a valuable ingredient for the manufacturing of alcohol due to its high starch content (Kawano, 1980). Falade and Akingbala (2010) stated that cassava can be processed into fermented and unfermented products. Cassava bread, fermented cassava flour, and starch are examples of fermented products. On the other hand, cassava chips and pellets are unfermented products.

Besides, cassava roots contain high amounts of carbohydrates but are low in vitamins and protein, as highlighted by Montagnac, Davis and Tanumihardjo (2009). Borku (2025) mentioned that cassava root is predominantly composed

of carbohydrates, with a relatively low protein content, ranging from 1 to 3%. It also contains essential minerals, including potassium, magnesium, calcium, iron, and zinc. In addition, cassava also contains organic acids such as succinic, malic, and fumaric acids (Uarrota and Maraschin, 2015).

Furthermore, cassava offers several health benefits, including the prevention of high blood pressure by promoting blood vessel relaxation and aiding in the elimination of excess sodium from the body due to its mineral content, such as potassium and magnesium (Borku, 2025). In addition, the study by Mohidin et al. (2023) revealed that cassava exhibits a wide range of therapeutic activities, including antibacterial, anticancer, antidiabetic, antidiarrheal, anti-inflammatory, and wound-healing capabilities.

2.6 Agarwood Leaves

Agarwood leaves are the leaves that come from the *Aquilaria* tree from the family *Thymelaeaceae*. It is naturally found in Asian countries, particularly in Southeast Asia, including Thailand, Malaysia, China, Cambodia, Indonesia, and India. These trees typically grow in cultivated plantations or grow naturally in forests (Rashed et al., 2024). Nowadays, it is widely planted as farmers utilize the leaves from the cultivated *Aquilaria* tree to produce agarwood tea. Agarwood leaves have been considered a minor byproduct of agarwood cultivation, and they have still been receiving little attention until recent years. Traditionally, farmers would cut and remove the leaves throughout the cultivation process, with the primary focus on the economically valuable heartwood. For instance,

the agarwood leaves were classified as disposal waste after trimming the *Aquilaria* tree (Adam, Lee and Mohamed, 2017). However, several studies highlighted that agarwood leaves contain valuable medicinal compounds such as flavonoids and phenolic compounds, which are well-known for their potent antioxidant, anti-inflammatory effect that contributes to many potential health benefits (Adam et al., 2018). Muthalib et al. (2025) conducted a study on the sensory properties of agarwood leaf extract powder. The results of this previous study show that chocolate incorporating the agarwood leaf extract powder achieved the second-highest mean score for overall acceptability compared to other leaf-based chocolates. Thus, it is proven that agarwood leaves may serve as a valuable component for functional foods.

Agarwood leaves contain numerous phytochemicals such as flavonoids, sesquiterpenoids, and chromones (Eissa et al., 2022). Adam, Lee and Mohamend (2017) also reported that agarwood leaves are rich in chromones, terpenoids, and alkene that are related to advantageous pharmacological effects. Tannins and phenolic compounds are also substantial bioactive compounds that are present in the agarwood leaves (Lee et al., 2017). Agarwood leaf extracts have been known to exhibit high effectiveness in antioxidants, anti-inflammatory properties, and metabolic effects. For instance, flavonoids, phenolic acids, chromones, and terpenoids are bioactive compounds and free radical scavengers that reduce inflammation (Xie et al., 2024). Additionally, both agarwood infusions and extracts are reported to contain a high level of phenolics, which contribute to a considerable radical-scavenging power, thereby providing a desirable antioxidant capacity (Batubara et al., 2018). Furthermore, agarwood

leaf extract has antimicrobial activity against bacteria and fungi. A study has proven that incorporating agarwood into food products, such as kombucha, can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* (Suwanposri et al., 2025). In addition, it has also been reported that the ingestion of agarwood leaf tea could contribute to hypoglycemic activity by lowering the blood glucose level (Pranakhon, Pannangpetch and Aromdee, 2011).

Agarwood leaves have now gradually become an ingredient in functional foods such as herb beverages. One of them is the best-known agarwood tea. In some parts of Asia, dried agarwood leaves were used as an aromatic herbal beverage known as Chengxiang tea or agarwood leaf tea, which is known to be beneficial to health and protect the body against some illnesses (Adam, Lee and Mohamed, 2017). Besides tea, agarwood leaves have been incorporated in a wide range of applications, such as the fermentation of agarwood leaf infusions into kombucha. Suwanposri et al. (2025) blended agarwood tea with roselle and peppermint to produce a kombucha with a fruity flavour. On the other hand, the agarwood leaves have also been utilized as a flavouring or functional ingredient in soups, ice cream, or coffee-based drinks (Adam, Lee and Mohamed, 2017).

2.7 Vinegar Produced by Other Tuber Roots

Galapia et al. (2018) studied the development and acceptability of cassava vinegar. The cassava vinegar was produced by combining cassava with yeast, sugar, and water. The sugar content was adjusted until it reached 15 to 28 °Brix. After inoculation with dry yeast, alcoholic fermentation was allowed to occur, followed by 21 days of acetic acid fermentation in a covered container under ambient conditions. Subsequently, the pH of the cassava vinegar was approximately 4.0, with a titratable acidity of around 4%. High scores were achieved on the overall acceptability of cassava vinegar.

The other starchy material used to make vinegar was potato pulp, the by-product of the starch industry that is mainly disposed of in Hokkaido (Tamura et al., 2004). Tamura et al. (2004) produced vinegar from fermented potato pulp to produce vinegar which was obtained by fermenting potato pulp with *Rhizopus oryzae* NBRC 4707. After fermentation, it was found that approximately 1% lactic acid was produced and exhibited notable antioxidant activity in the water-soluble fraction of potato pulp. The resulting fermented potato pulp vinegar contained 3.5% acetic acid, with a total acidity of 5.6%. It exhibited a grain vinegar-like color and a mild, mellow acidity. Moreover, this vinegar showed significantly stronger antioxidant activity compared to commercial vinegar (Tamura et al., 2004).

Additionally, it was discovered that taro has also been used to produce vinegar. Yang et al. (2018) conducted a study to analyze the composition and antioxidant properties of taro vinegar. This previous study highlighted that taro vinegar has

a rich biochemical profile. Taro vinegar comprises 14 types of amino acids, in which arginine and histidine are the most abundant, and the essential amino acids are also 38.93% of the total amino acid content. Moreover, taro vinegar was also observed to have a high content of acetic acid with 6 g per 100 mL. The total contents of protein, polysaccharide, and polyphenol were significantly higher than those in other vinegars, such as rice vinegar, white vinegar, and apple cider vinegar, which contributed to the substantial nutritional value of taro vinegar. Additionally, its total acid content was lower than that of rice vinegar. These findings suggest that taro vinegar has the potential to be a high-quality vinegar with notable health-promoting properties (Yang et al., 2018).

2.8 Sweet Potato Vinegar

Several studies investigated the production of vinegar using sweet potato as the substrate. Chun et al. (2014) had conducted a two-stage fermentation to produce purple sweet potato *makgeolli* vinegar. The purple sweet potato was first cooked and mashed prior to being mixed with the hard-boiled rice and water to proceed with further steps. After the mixture was done, *S. cerevisiae* was added to perform alcohol fermentation for 7 days. After 7 days, the purple sweet potato *makgeolli* was added with *Acetobacter aceti* ATCC 15973 to convert the alcohol into vinegar. The liquid was incubated at 30℃ to produce vinegar. It was found out that the *makgeolli* made with purple sweet potato contained 5.57% of alcohol. The aroma and taste scores of purple sweet potato *makgeolli* were higher than rice *makgeolli*. Furthermore, purple sweet potato *makgeolli* vinegar exhibited superior functional properties compared to rice *makgeolli* vinegar, as it contains

higher total phenolic content with 327.14 mg GAE/L compared to 24.73 mg GAE/L. It exhibits strong antioxidant activity, as its DPPH free radical scavenging activity was significantly higher (67.63%) than that of rice makgeolli vinegar (6.72%). On the other hand, purple sweet potato *makgeolli* vinegar displayed a slightly higher pH and lower total acidity compared to rice *makgeolli* vinegar.

Wu et al. (2017) also produced vinegar from purple sweet potatoes using a liquid fermentation process, and the antioxidant activity of the resulting product was investigated. *Acetobacter pasteurianus* JST-S, obtained from solid fermented vinegar grains in China, was used for the acetic acid fermentation. After 4 days of fermentation, the purple sweet potato vinegar was produced. It was found that the acetic acid level reached 4.2% in 3.5 days. This previous study also revealed that the anthocyanins from purple sweet potato showed strong DPPH scavenging activity. Besides, the acetic acid and other non-phenolic antioxidants may also contribute to the antioxidant activity.

Cakrawati and Hongsprabhas (2024) had used purple sweet potato as a substrate to produce vinegar by first processing it into flour prior to fermentation. A consecutive fermentation technique was used, involving *Aspergillus* for saccharification, *Saccharomyces cerevisiae* for alcohol fermentation, and *Aspergillus pasteurianus* for acetic acid fermentation. During starter preparation, several starchy materials were incorporated to maintain the viability of *A. pasteurianus* byensuring the performance and stability. Among these materials,

rice flour showed the highest number of *A. pasteurianus* (10^8cfu/g). This previous study suggests that vinegar production can be simplified by using a defined starter culture consisting of fungi, yeast, and acetic acid bacteria.

2.9 Incorporation of Other Leave Extract into Fermented Product

Jia, Yu, and Zhang. (2019) utilized the leaves of *Eucommia ulmoides* tree with medical properties to produce vinegar by optimizing fermentation conditions. The process involved adding 8% bran, 8% sugar, 0.3% leaven yeast, and 12% acetic acid to create the desired fermentation environment (Hussain et al., 2016). The resulting vinegar contained a high acetic acid level of about 45.5 mg/mL. It was found that the vinegar contains bioactive compounds such as caffeic acid (around 0.98 mg/mL), which are associated with antioxidant, antimicrobial, and neuroprotective benefits. (Kadar et al., 2021). In comparison with commercial Zhenjiang vinegar, *Eucommia ulmoides* leaves vinegar demonstrated a stronger antibacterial effect. This effect was mainly due to the combination of acetic acid and plant compounds. These combined effects can damage the bacterial cell wall and membrane, leading to leakage of intercellular contents and ultimately cell death.

Additionally, Pencak et al. (2024) utilized oak leaves as the main source in the production of an alcoholic fermented beverage. The resulting product initially showed a high antioxidant capacity and phenolic content, which gradually reduced during fermentation. However, other compounds, such as catechin, gallic acid, and gallocatechin, were found to increase over the course of

fermentation. The reducing sugar, such as glucose and fructose, which served as substrates for alcohol fermentation, were steadily decreased from Day 10 to Day 60. Besides, sensory evaluation indicated there was higher acceptability with the beverage made only from oak leaves compared to the beverage enriched with orange juice and peel. These findings demonstrated the potential of utilizing underused plant material, such as leaves, in the development of fermented beverages.

Furthermore, Freitas et al. (2024) investigated the fermentation of grapevine leaves using two methods, which are natural fermentation and fermentation using *S. cerevisiae*. The ability of these fermented leaves to enhance the antioxidant and overall functional properties was examined. In addition, the bioactivity and shelf life of yogurt incorporated with fermented and non-fermented grapevine leaves were compared to those of yogurt preserved with potassium sorbate (control sample). The findings revealed that grapevine leaves could naturally undergo fermentation on their own, as evidenced by increased phenolic content and antioxidant activity compared to the unfermented control. Conversely, the phenolic content and antioxidant activity were slightly higher when *S. cerevisiae* was used as a starter culture. These findings suggest that grapevine leaves can naturally promote fermentation, which can be further enhanced by *S. cerevisiae* to maintain quality. Moreover, the yogurt enriched with fermented grapevine leaves also had the potential to inhibit microbial growth. This suggests that grapevine leaves may be used as a natural preservative to replace potassium sorbate.

CHAPTER 3

MATERIAL AND METHOD

3.1 Procedure Flowchart Overview

Sweet potato and cassava were used to perform the saccharification by adding koji. The reducing sugar and amino acid content of the samples were determined after saccharification. The saccharified material with the highest reducing sugar content was chosen to proceed with further fermentation. Agarwood leaves treatment and water treatment were applied during alcohol and acetic acid fermentation. After 14 days of alcohol fermentation, mother vinegar was added to each sample and proceeded with 10 days of acetic acid fermentation. Alcohol content, titratable acid, organic acid, total phenolic content and pH of samples were determined at different durations. Phytochemical screening was carried out for alcoholic mixture at Day14 and the final product of acetic acid fermentation.

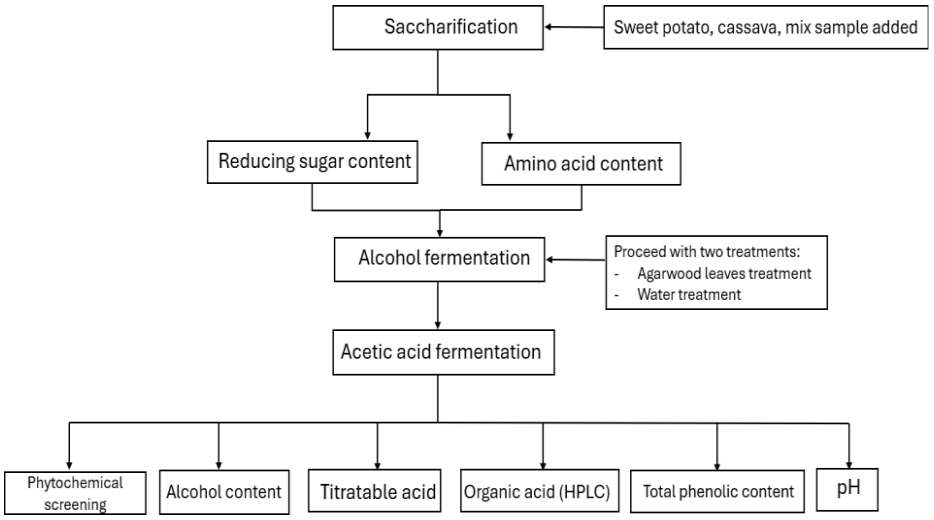

Figure 3.3: Experimental workflow of vinegar production.

3.2 Sample Material

Purple sweet potato (*Ipomoea batatas* (L) Lam.) and cassava (*Manihot esculenta*) were purchased from the local market in Kampar. Koji was obtained from a soy sauce manufacturer (Kicap Bentong) near Bentong, Pahang. Baker's yeast (Saf- Instant) was purchased from a bakery ingredient shop in Kampar, while the unfiltered apple cider vinegar (Bragg) was obtained from a local market in Ipoh.

3.3 Chemicals

Chemicals and Reagents	Brand
3,5-Dinitrosalicyclic acid	Qrec
Absolute ethanol, 99.9%	Chemsoln
Acetic Acid Glacial, 99.8%	Bendosen
Ammonia solution, 30%	Merck
Chloroform	Merck
Ferric Chloride	ACROS Organic
Folin-Ciocalteu's phenol reagent	Merck
Gallic acid	R&M Chemical
Glycine	Chemiz
Gram's Iodine	Fisher Scientific

Hydrochloric acid, 37%	Fisher Scientific
Ninhydrin	Merck
Organic acids kit	Supelco
Phenolphthalein	Bendosen
Potassium dichromate	R&M Chemical
Potassium hydroxide	Qrec
Potassium iodate	Qrec
Potassium sodium tartrate 4-hydrate	Chemsoln
Sodium Acetate 3-Hydrate	Bendosen
Sodium carbonate anhydrous	Chemiz
Sodium hydroxide	Fisher Scientific
Sulphuric acid, 98%	R&M Chemical
Tin Chloride	R&M Chemical
Tri-n-butyl Phosphate	Chemsoln

3.4 Equipment

Equipment	**Brand**
Analytical Balance	Mettler Toledo
Autoclave Machine	Hirayama HVE50
Centrifuge	Newton Scientific

Drying Oven	Memmert
High Performance Liquid Chromatography	Shimadzu
Incubator shaker	Grant Scientific
Incubator Shaker with cooling	INFORS HT
Microplate Reader	Thermo Scientific
pH meter	Mettler Toledo
Thin Layer Chromatography Visualizer	CAMAG
Vortex Mixer	Scientific Industries
Water bath with Shaker	Memmert

3.5 Selection of Tuber Crop with High Saccharification Rate

3.5.1 Sample Preparation

Peeled purple sweet potato and cassava were washed and cut into equal-sized pieces. Thirty gram of cut sweet potato, cassava, and a mixture of purple sweet potato and cassava at a 1:1 ratio with 15 g each were weighed and placed into sterile conical flasks. All conical flasks containing samples were autoclaved. After autoclave, 0.2 g of koji (*Aspergillus oryzae* used in soy sauce manufacturing) was added to each sample and mixed thoroughly (Tawwekasemsombut et al., 2021). The mouths of the conical flasks were covered with sterile cheesecloth and saccharified for 2 days under aerobic conditions. After saccharification, the samples were dried in the drying oven at 50°C for 12 h for further analysis (Zulkifi et al., 2021).

3.5.2 Reducing Sugar Content Determination

Reducing sugar was extracted by mixing 10 mL of distilled water and 1 g of dried sample in a centrifuge tube and incubating in a hot water bath at 35℃ for 40 min. The mixture was then centrifuged at 5000 rpm, and the supernatant was collected for the analysis of reducing sugar content. DNS method was used to determine the concentration of reducing sugar (Martínez-Avila, Llenas and Ponsá, 2022).

DNS reagent was prepared by mixing 30 g of potassium sodium tartrate, 1 g of 3,5-dinitrosalicylic acid, and 20 mL of NaOH (1M) into 40 mL of distilled water. The final mixture was then topped up to 100 mL with distilled water in a volumetric flask (Saquib and Whitneym, 2011). Glucose standard solutions were prepared at concentrations ranging from 0.2 to 1.0 mg/mL. Three milliliters of DNS reagent was added to 1 mL of the supernatant samples and the glucose standard solutions. The mixture was then incubated in a hot water bath at 100 ℃ for 10 min to allow for colour development. After heating, the mixtures were cooled to room temperature in an ice bath. The absorbance of the mixture was measured at 550 nm in a 96-well plate reader (Deshayath et al., 2020). Lastly, a standard calibration curve of absorbance against the sugar concentration was constructed, and the reducing sugar concentration ofeach sample was calculated.

3.5.3 Free Amino Acid Determination

The method for amino acid extraction was modified from Jones, Owen and Farrar (2003). Four gram of dried sample was added to 20 mL of potassium chloride (2M). The mixture was shaken at 200 rpm for 15 minutes using an incubator shaker. The mixture was then centrifuged at 7830 rpm, and the supernatant obtained was used for further analysis.

The amino acid of the sample was determined using the ninhydrin method. Acetate buffer (4M) with pH 5.5, stannous chloride ($SnCl_2$) solution, and ninhydrin stock solution were prepared according to Abernathy, Spedding and Starcher (2009). Glycine standard solution was prepared at concentrations ranging from 0.1 to 0.5 mg/mL. The ninhydrin working solution was freshly prepared by mixing 1 mL of the ninhydrin stock solution with 25 µL of $SnCl_2$ solution. Then, 200 µL of the working solution was added to 30 µL of each sample and glycine standard solution. The mixtures were incubated in a boiling water bath for 10 minutes to allow colour development. After that, 2.8 mL of distilled water was added to each mixture and mixed thoroughly using a vortex mixer. Lastly, 200 µL of the mixture was transferred to a 96-well plate reader to measure the absorbance at 575 nm (Abernathy, Spedding and Starcher, 2009). A glycine standard curve was constructed, and the concentration of the amino acid was calculated.

3.6 Alcohol Fermentation

3.6.1 Agarwood Leaf Tea Infusion

Agarwood leaf tea bags containing 100% dried agarwood tea leaves were purchased from the online platform. The methods for preparing the agarwood leaf infusion were adapted from Delica-Balagot et al. (2024). The agarwood leaves tea bags with 4 g were added to 240 mL of distilled water. The mixture was boiled at 100°C for 5 min. After boiling, the infusion was allowed to cool to room temperature for 50 min.

3.6.2 Process forAlcoholic Fermentation

The substrate with the highest reducing sugar content was selected for repeated saccharification again, followed by alcoholic fermentation. A total of 0.24 g of baker's yeast was added to a conical flask containing 30 g of saccharified purple sweet potato. The setup was performed in duplicate. Then, 200 mL of distilled water was added to one flask, while the other conical flask was filled with 200 mL of agarwood leaf infusion (Lay *et al.*, 2012; Wu *et al.*, 2017). The flasks were labelled as water-treated vinegar and agarwood-treated vinegar. The sugar concentration of each solution was adjusted to 10% (w/v) by adding glucose (Srivatsava, Modi and Garg, 1997). The conical flask's mouth was covered with a cotton plug, and the mixtures were fermented for 14 days. The concentration of sugar in saccharified purple sweet potato was calculated as follows:

$$\text{Reducing sugar (\%db)} = \frac{total\ reducing\ sugar\ extracted\ from\ DNS\ method\ (g)}{Sample\ weight\ (g) \times (100\% - moisture\ content)} \times 100\%$$

3.7 Acetic Acid Fermentation

After 14 days of alcoholic fermentation, 4 mL of mother vinegar obtained from unfiltered apple cider vinegar was added to the alcoholic purple sweet potato mixtures. The conical flasks were then covered with autoclaved cheesecloth and allowed to undergo acetic acid fermentation for 10 days (Ezemba et al., 2021). The pH, alcohol content, total titratable acidity, organic acid, and total phenolic content were determined for the vinegar mixtures at Days 0, 2, 3, 4, 5, 10 and the alcoholic mixture at Day 14 from alcohol fermentation. Besides, phytochemical screening was carried out on both the alcoholic mixture at Day 14 and the final product.

3.8 pH determination

The pH meter was calibrated using standard buffer solutions. Then, the pH of the samples as mentioned in 3.7 was determined using the calibrated pH meter.

3.9 Total Titratable Acid

3.9.1 NaOH Standardization

Standardization of NaOH solution (approximately 0.2M) was conducted with 5 mL of standardization H_2SO_4 (0.25 M) using phenolphthalein indicator. The NaOH solution was added dropwise until the H_2SO_4 solution turned from colourless to pink colour which persisted for 30 seconds (Kingsborough Community College, 2013). The volume of NaOH that was used to reach the endpoint was recorded. The molarity of NaOH was calculated as shown below:

$$\text{Molarity of NaOH} = \frac{volume\ of\ \text{H2SO4} \times (molarity\ of\ \text{H2SO4} \times 2)}{volume\ of\ NaOH\ used}$$

3.9.2 Total Titratable Acid Determination

The standardized NaOH solution was filled into a burette. Five milliliter of the vinegar sample was added with 2 or 3 drops of phenolphthalein. The vinegar sample was then titrated with NaOH solution until the sample turned pink colour, which persisted for 30 seconds (Kingsborough Community College, 2013). The volume of NaOH used was recorded, and the concentration of total titratable acid was calculated with the equation as shown below:

$$\text{Molarity of acid} = \frac{volume\ of\ \text{NaOH used} \times molarity\ of\ \text{NaOH}}{volume\ of\ sample}$$

Concentration of total titratable acid (g/L) = molarity of acid × molar mass of acetic acid

3.10 Alcohol Content Determination

The method for alcohol content determination was modified from Sriayiyanyn et al. (2019). The dichromate reagent was prepared by adding 26.65 mL of concentrated sulphuric acid to 50 mL of water. The solution was then allowed to cool to room temperature and subsequently diluted to a final volume of 100 mL with distilled water. A total of 10 g of potassium dichromate was dissolved slowly in the solution. The ethanol standard solutions ranging from 2% to 8% (v/v) were prepared. Then, 1 mL of the vinegar sample or ethanol standard was mixed with 1 mL oftri-n-butyl phosphate and centrifuged at 7830 rpmfor 5 min.

After that, 500 µL of the supernatant was added to 500 µL of dichromate reagent and vortexed for 1 min. The mixture was left to stand for 10 min. After 10 min, 100 µL of the lower layers of the solution was transferred and mixed with 900 µL of water. The absorbance of the solutions was measured in a 96-well plate reader at 595 nm (Sriayiyanyn et al., 2019). An ethanol standard curve was constructed, and the alcohol concentration of the samples was determined.

3.11 Total Phenolic Content Determination

Folin-Ciocalteu method was used to determine the total phenolic content of the sample. Gallic acid standard solutions ranging from 20 to 100 mg/L were prepared. Then, 0.2 mL of the vinegar sample or standard solution was mixed with 0.8 mL of deionised water and 0.1 mL of Folin-Ciocalteu reagent. The mixture was left to stand for 3 min. After that, 0.3 mL of Na_2CO_3 (20 %w/v) was added to the mixture and then incubated for 120 min at room temperature under dark conditions. The absorbance of the mixture was measured in a 96-well plate reader at 765 nm (Chai and Wong, 2012). A standard curve of gallic acid was constructed, and the total phenolic content of each sample was calculated.

3.12 Organic Acids Determination

The concentration of acetic acid, phytic, malonic, succinic, maleic, propionic, and tartaric acids of the samples was determined using High Performance Liquid Chromatography (HPLC). The range of stock solutions prepared is shown in Table 3.1. The samples were filtered through a 0.22 µm membrane before injection. An injection volume of 20 µL was used for both samples and the

standards. The HPLC system used was equipped with a Rezex ROA-Organic Acid (H^+) column (300 × 7.8 mm). The analysis was performed at a wavelength of 210 nm under isocratic conditions. The mobile phase consisted of 0.005 N sulphuric acid with a flow rate of 0.5 mL/min, and then the column temperature was maintained at 35℃ (Yildiz, 2023).

Table 3.1: Range of standard solutions of each organic acid.

Standard	**Concentration range (µmole/20µL)**
Acetic acid	0.5 to 4.0
Phytic acid	0.05 to 0.75
Malonic acid	0.15 to 1.2
Succinic acid	0.15 to 4.0
Maleic acid	0.0050 to 0.0300
Propionic acid	1 to 6
Tartaric acid	0.03 to 0.2

3.13 Phytochemical Screening

The phytochemical screening was carried out for the alcoholic mixture at Day 14 and the final product of acetic acid fermentation. The analysis included the detection of phytochemicals such as alkaloids, flavonoids, tannins, triterpenoids, phlobatannins, coumarin, quinone, and antroquinones.

Table 3.2: Procedure ofphytochemical screening.

Phytochemical Group	Reagent Preparation:	Assay:	+ Results:	References:
Alkaloids	Wagner's reagent (0.44M):1g of iodine was added to 3g of potassium iodate were dissolved in 50mL water	A few drops of Wagner's reagent were added to 2 mL of extract from the side of the test tube.	Reddish Brown Precipitate	Aziz, 2015
Flavonoids	NaOH (5%): Add 2.5 g of NaOHin 50mLwater HCl (1M): Add 4.106mL of conc HCl to 50mL of water	A few drops of 5% NaOH were added to 1 mL of extract. After reaction, add a few drops of diluted HCl.	After addition of NaOH, turn deep yellow colour. Colour lost after HCl added	
Tannins	Ferric Chloride (5%): Dissolve 2.5g of $FeCl_3$ in 50 mL water	A few drops of 5% $FeCl_3$ were added to 5mL of extract	Bluish Black Colour	
Triterpenoids	Chloroform concentrated H_2SO_4	2mg sample was added to 1mL Chloroform (final concentration 2mg/mL). The chloroform layer was taken and was added with conc H_2SO_4 to the side of the tube.	Red Brown colour at the interface	
Phlobatannins	HCl (1%): Add 1 mL of conc HCl to 99mL of water (or top up to 100mL)	A total of 2mL of extracts was added to 2mL of HCl (1%). Boil the mixtures.	Red Colour precipitates formed	Sabri et al., 2019
Coumarin	NaOH (1M) Filter Paper	A total of 2mL of extract was heated in a tube using a boiling water bath while covering the tube with Filter paper moistened with NaOH (1M). The paper was removed and visualized under UV light.	Yellow fluorescence observed.	Jabeen et al., 2023
Quinone	KOH in Ethanol (5%)	An aliquot of 2 to 3 mL of extract was added to 1 mL of alcoholic KOH.	Blue-green, red or purple indicate quinone presence	
Anthraquinones	Chloroform Ammonia solution	A 100mg/mL of extract was added to 5mL of chloroform and shaken for 5 minutes. Then, 1 mL of the chloroform layer was transferred to 1 mL of concentrated ammonia solution (100%).	Ammonia lower layer turn pink voilet or red	Tamene and Endale, 2019

3.14 Descriptive and Statistical Analysis

Means and standard deviation were calculated for all results except for phytochemical screening. Normality of data was verified using Normality Test. Pairwise comparison among groups or treatments and durations was conducted

using Tukey’s HSD analysis followed by two-way ANOVA at 95% confidence level for reducing sugar content, amino acid content, pH value, total titratable acidity, total phenolic content, and acetic acid.

CHAPTER 4

RESULTS

4.1 Effect of Saccharification: Reducing Sugar Content

Table 4.1 presents the reducing sugar content of purple sweet potato, cassava, and mixed samples at three different stages: blank (control), before saccharification, and after saccharification. The reducing sugar content was calculated using the equation (y= 0.5929x) with R^2 = 0.9942, based on the glucose standard curve shown in Figure 4.1. As shown in Table 4.1, the reducing sugar content of the three samples increased steadily from the control to before and after saccharification. Among the samples, purple sweet potato exhibited the highest reducing sugar content, while cassava showed the lowest across all stages.

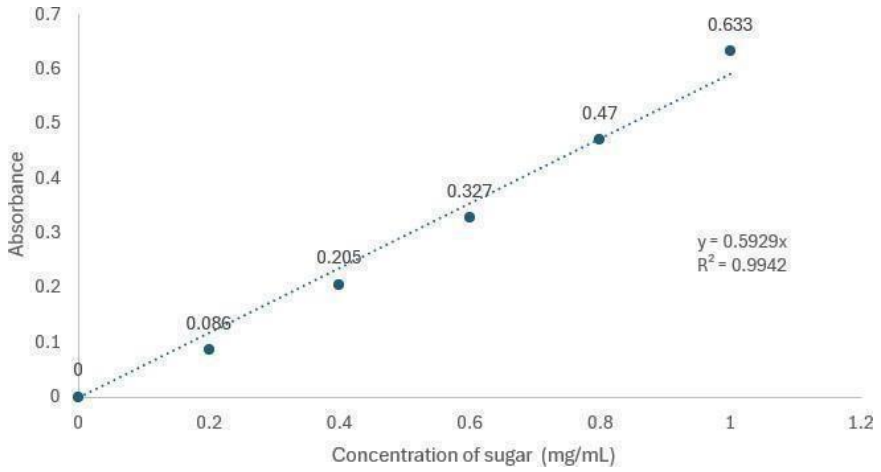

Figure 4.1: Standard curve ofreducing sugar content constructed using glucose.

Table 4.1: Reducing sugar content ofdifferent samples (blank, before, and after saccharification).

Sample	Reducingsugar (%wb)		
	Blank	Initial	Saccharified
Purplesweet potato	$7.51 \pm 0.31^{a*}$	$10.98 \pm 0.33^{a**}$	$28.40 \pm 2.00^{a***}$
Cassava	$0.78 \pm 0.18^{b*}$	$1.19 \pm 0.06^{c*}$	$11.98 \pm 0.61^{c**}$
Mix	$1.91 \pm 0.15^{b*}$	$2.87 \pm 0.40^{b*}$	$18.28 \pm 1.02^{b**}$

*wb represents wet weight basis.

*Valuesrepresent mean ± SD (n=4).

*Means with different letters (a, b, c) within the same column are significantlydifferent ($p<0.05$)

*Means with different numbers of asterisks within the same row are significantly different ($p<0.05$).

4.2 Effect of Saccharification: Free Amino Acid Content

Figure 4.2 shows the colour change with different concentrations of glycine solutions after reacting with the ninhydrin working solution. The intensity of purple colour increased as the concentration of the glycine solutions increased from low to high. Table 4.2 shows the free amino acid content for purple sweet potato, cassava, and mixed samples at different stages, which are blank, before, and after saccharification. The free amino acid content was calculated using the equation ($y = 1.7511x$, $R^2 = 0.9997$) generated from the glycine standard curve shown in Figure 4.3. According to Table 4.2, the free amino acid content of the cassava was the highest after saccharification among the samples. Its amino acid level remained constant at blank and before saccharification. Besides, purple sweet potato showed the lowest free amino acid content after saccharification among all samples, and its amino acid content increased after the koji was added. The amino acid level for the mixed sample was gradually increased from blank, before, and after saccharification.

Figure 4.2: Colour of glycine standard solutions after reacting with the ninhydrin working solutions.

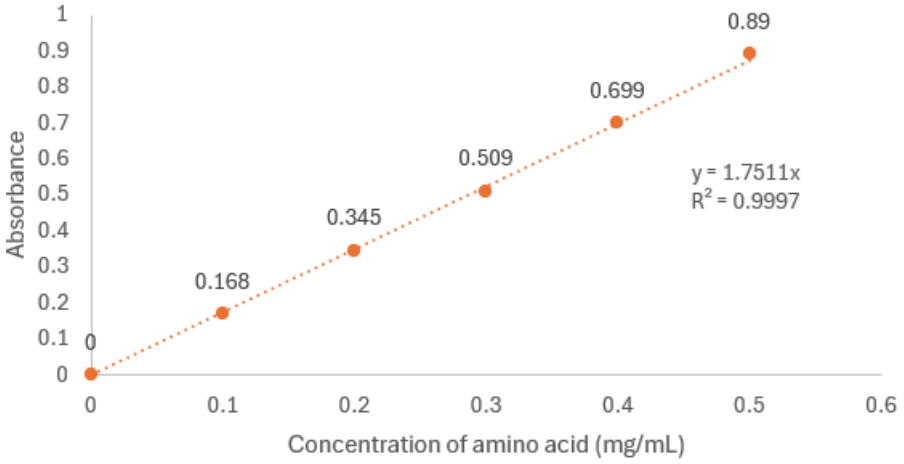

Figure 4.3: Standard curve of amino acid content constructed using glycine.

Table 4.2: Free amino acid content ofdifferent samples (blank, before, and after saccharification).

Sample	Free amino acid content (%wb)		
	Blank	Initial	Saccharified
Sweet potato	$0.07 \pm 0.000^{c*}$	$0.11 \pm 0.002^{b**}$	$0.07 \pm 0.003^{c*}$
Cassava	$0.11 \pm 0.002^{a*}$	$0.11 \pm 0.002^{b*}$	$0.25 \pm 0.001^{a**}$
Mix	$0.09 \pm 0.005^{b*}$	$0.13 \pm 0.002^{a**}$	$0.14 \pm 0.004^{b***}$

*wb represents wet weight basis.

*Valuesrepresent mean ± SD (n = 2).

*Means with different letters (a, b, c) within the same column are significantl different ($p<0.05$)

*Means with different numbers of asterisks within the same row are significantly different ($p<0.05$).

4.3 Effect of Agarwood Leaf Infusion on Acetic Acid Fermentation: Alcohol content and pH

The colour change of ethanol standard solutions with different concentrations were illustrated in Figure 4.4. The solutions shifted progressively from yellow to green and finally to blue as the concentration increased from low to high. Table 4.3 presents the alcohol content of vinegars from different treatments over various durations. The concentration of alcohol was calculated using the equation $y = 0.0885x$, with $R^2 = 0.998$ from the ethanol standard curve shown in Figure 4.5. There was no significant difference observed between the two treatments. The alcohol content of both treatments remained relatively stable from the blank control (alcoholic mixture at Day 14) to Day 5. However, they showed a sharp decrease at Day 10, reaching 2.27 ± 0.02 %v/v for agarwood leaves treatment and 2.03 ± 0.21 %v/v for the water treatment.

Table 4.3 displayed the pH values of vinegars with different treatments over various durations. The pH value for agarwood leaves treatment was significantly higher than water treatment from Day 2 to Day 5. Both treatments showed a gradual increase in pH from the blank until Day 10, with maximum values of 4.03 ± 0.05 for agarwood leaves treatment and 4.06 ± 0.039 for water treatment.

Figure 4.4: Colour of ethanol standard solutions after reacting with potassium dichromate.

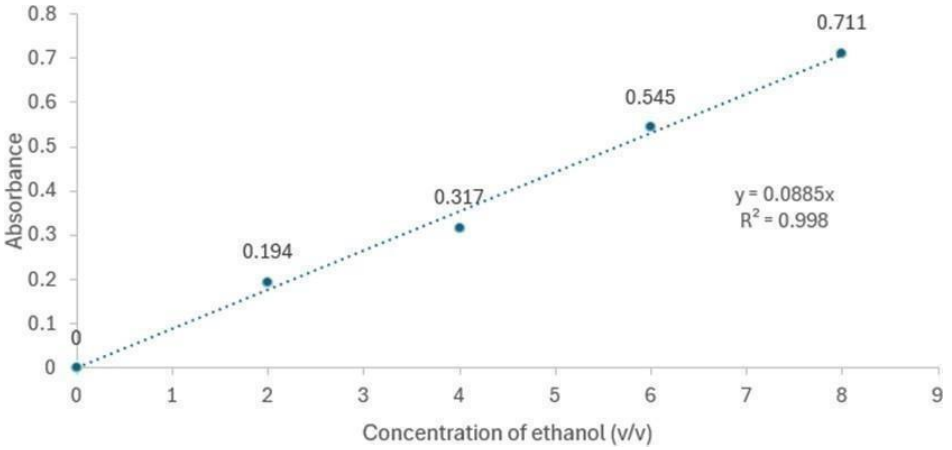

Figure 4.5: Standard curve of alcohol content constructed using ethanol.

Table 4.3: Alcohol content and pH value of samples fermented with and without agarwood leaves extract as a function of fermentation duration.

Duration	Alcohol content (%v/v)		pH value	
	Water	Agarwood	Water	Agarwood
Blank	4.93 ± 0.02[a]	5.26 ± 0.39[a]	3.74 ± 0.05[e]	3.87 ± 0.01[cd]
Day 0	-	-	3.72 ± 0.01[ed]	3.82 ± 0.00[d*]
Day 2	4.47 ± 0.20[a]	4.33 ± 0.50[a]	3.85 ± 0.00[c]	3.90 ± 0.01[c*]
Day 3	4.46 ± 0.31[a]	4.18 ± 0.32[a]	3.85 ± 0.00[c]	3.90 ± 0.01[c*]
Day 4	4.50 ± 0.52[a]	4.18 ± 0.66[a]	3.85 ± 0.02[c]	3.96 ± 0.03[b*]

Day 5	4.51 ± 0.29^{a}	4.10 ± 0.21^{a}	3.92 ± 0.02^{b}	$3.97 \pm 0.01^{b*}$
Day 10	2.27 ± 0.02^{b}	2.03 ± 0.21^{b}	4.03 ± 0.05^{a}	4.06 ± 0.04^{a}

*Values for alcohol content and pHrepresent mean ± SD (n=4).

*Means with different letters (a, b, c) within the same column are significantlydifferent ($p<0.05$)

*Means with asterisk within the sameroware significantlydifferent ($p<0.05$).

4.4 Effect of Agarwood Leaf Infusion on Acetic Acid Fermentation: Total Phenolic Content and Titratable Acid

Figure 4.6 illustrates the colour change of different concentrations of gallic acid standard solutions after reacting with Folin-Ciocalteu reagent and sodium carbonate. The concentration of blue colour increased with the concentration of gallic acid. Table 4.4 showed the total phenolic content of vinegar with agarwood and water treatment at different durations. The concentrations of total phenolic content were calculated using the equation ($y = 0.0077x$) with $R^2 = 0.9998$ derived from the gallic acid standard curve as shown in Figure 4.7. The total phenolic content of the agarwood leaves treatment was significantly higher ($p<0.05$) than water treatment from the blank stage in all durations. The phenolic content of water treatment showed fluctuation and reached a maximum yield of 40.097 ± 6.366 mg/mL by Day 10. Besides, the phenolic content of agarwood leaves treatment remained stable from the blank stage to Day 10.

Table 4.4 presents the titratable acid content of samples with and without agarwood leaves extract at varying fermentation durations. There was no significant difference in titratable acid content between the treatments from the blank stage to Day 5. However, the titratable acid of sample fermented with

agarwood leaves extract reached 2.252 ± 0.000 g/L, which was significantly higher than water treatment at 1.689 ± 0.159 g/L by Day 10.

Figure 4.6: Colour of gallic acid standard solutions after reacting with Folin-Ciocalteu reagent and sodium carbonate.

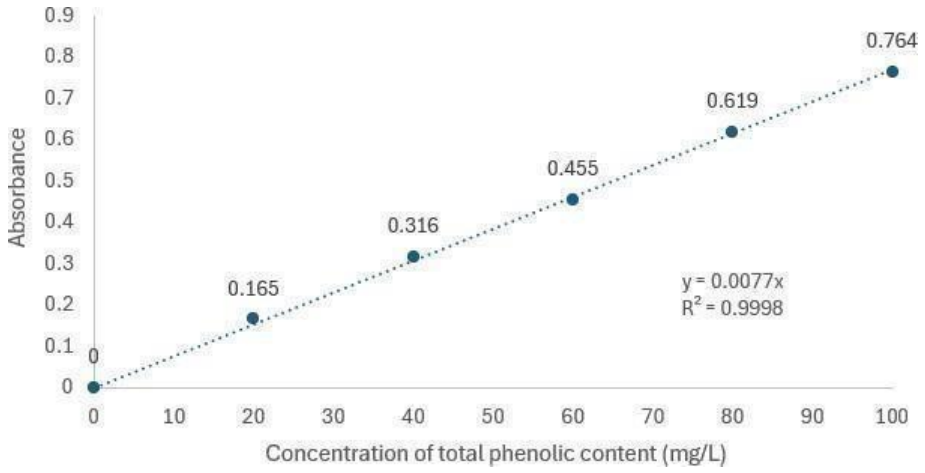

Figure 4.7: Standard curve of phenolic content constructed using gallic acid.

Table 4.4: Totalphenolic content and titratable acid from vinegar with water and agarwood leaves treatment in different durations.

Duration	Total titratable acid (g/L)		Total phenolic content (mg/mL)	
	Water	Agarwood	Water	Agarwood
Blank	2.252	2.502	33.247 ± 0.184[ab]	45.130 ± 3.765[a*]
Day 0	2.928 ± 0.000	3.828 ± 0.318	30.650 ± 7.573[b]	44.773 ± 4.755[a*]

Day 2	3.040 ± 0.159	3.153 ± 0.318	31.958 ± 7.496^{ab}	44.416 ± 2.037^{a*}
Day 3	3.040 ± 0.159	3.040 ± 0.478	28.474 ± 3.551^{ab}	47.338 ± 5.660^{a*}
Day 4	3.040 ± 0.159	3.040 ± 0.159	26.494 ± 4.765^{b}	44.870 ± 0.546^{a*}
Day 5	2.590 ± 0.159	2.477 ± 0.000	33.636 ± 1.403^{ab}	44.156 ± 1.605^{a*}
Day 10	1.689 ± 0.159	2.252 ± 0.000*	40.097 ± 6.366^{a}	51.234 ± 1.297^{a*}

*Values for total phenolic content represent mean ± SD (n=4).

*Values for titratable acid represent mean ± SD (n=2); Post hoc test is not performed due to limitation of sample size; Standard deviation not performed for blank due to the limitation of sample size (n=1).

*Means with different letters (a, b, c) within the same column are significantlydifferent ($p<0.05$)

*Means with different numbers of asterisks within the same row are significantly different ($p<0.05$).

4.5 Effect of Agarwood Leaf Infusion on Acetic Acid Fermentation: Organic Acid Content of Vinegar with Agarwood and Water Treatment at Different Durations

Table 4.5 depicts the organic acids, including acetic acid, phytic acid, and maleic acid, present in samples fermented with and without agarwood leaves extract over different fermentation durations. Besides, Table 4.6 shows the other organic acids, including tartaric acid and malonic acid content, while Table 4.7 presents succinic acid and propionic acid of both vinegars at different durations.

4.5.1 Acetic Acid Content

The acetic acid content ofvinegar with different treatments is presented in Table 4.5. The concentration of acetic acid for agarwood leaves treatment was significantly different at Days 0, 2, 3, 4, and 5. The acetic acid content of both

treatments gradually increased throughout the duration and reached a maximum yield by Day 10, with 4.302 ± 0.357 mg/mL for the water treatment and 4.536 ± 0.263 mg/mL for the agarwood leaf treatment.

4.5.2 Other Organic Acid Content

Tables 4.5, 4.6, and 4.7 show the phytic acid, maleic acid, tartaric acid, malonic acid, succinic acid, and propionic acid of vinegar with water and agarwood leaves treatment at different durations. Phytic acid showed the highest concentration among the organic acids, with a maximum yield of 35.188 ± 0.408 mg/mL for water treatment and 47.463 ± 1.087 mg/mL for agarwood leaves treatment. In contrast, the concentration of maleic acid, tartaric acid, malonic acid, succinic acid, and propionic acid ranged from 0.055 ± 0.003 mg/mL to 4.464 ± 0.458 mg/mL for water treatment, while agarwood leaves treatment ranged from 0.070 ± 0.001 mg/mL to 4.213 ± 0.335 mg/mL. Propionic acid was not present at the blank stage and on Day 0 for water treatment.

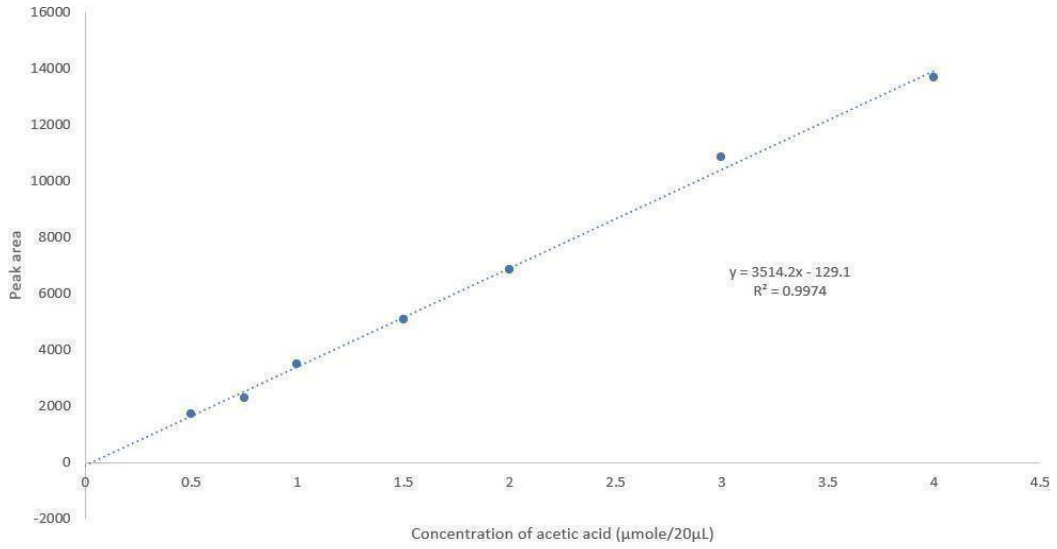

Figure 4.8: Standard curve of acetic acid.

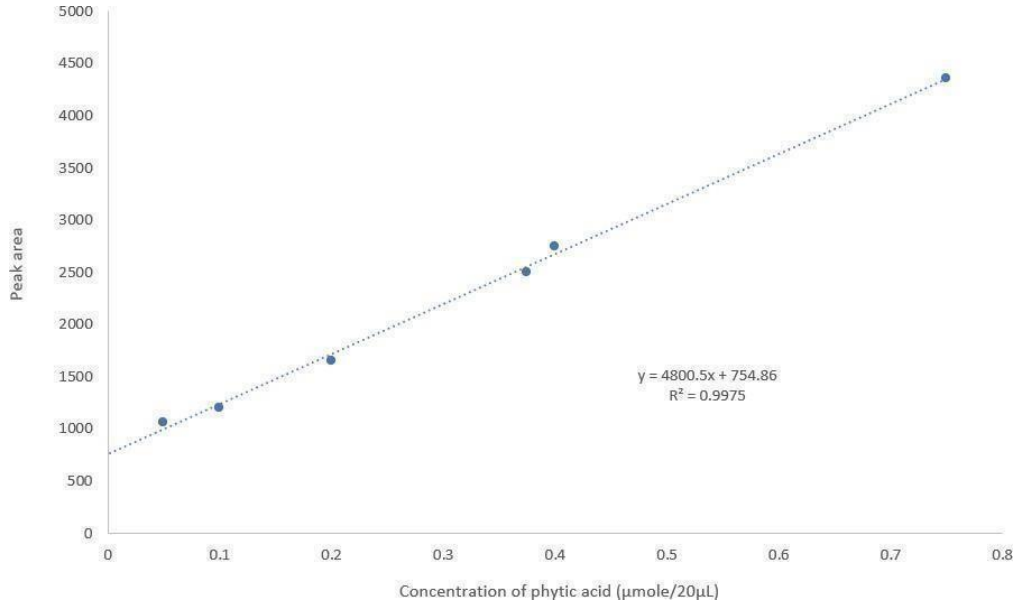

Figure 4.9: Standard curve of phytic acid.

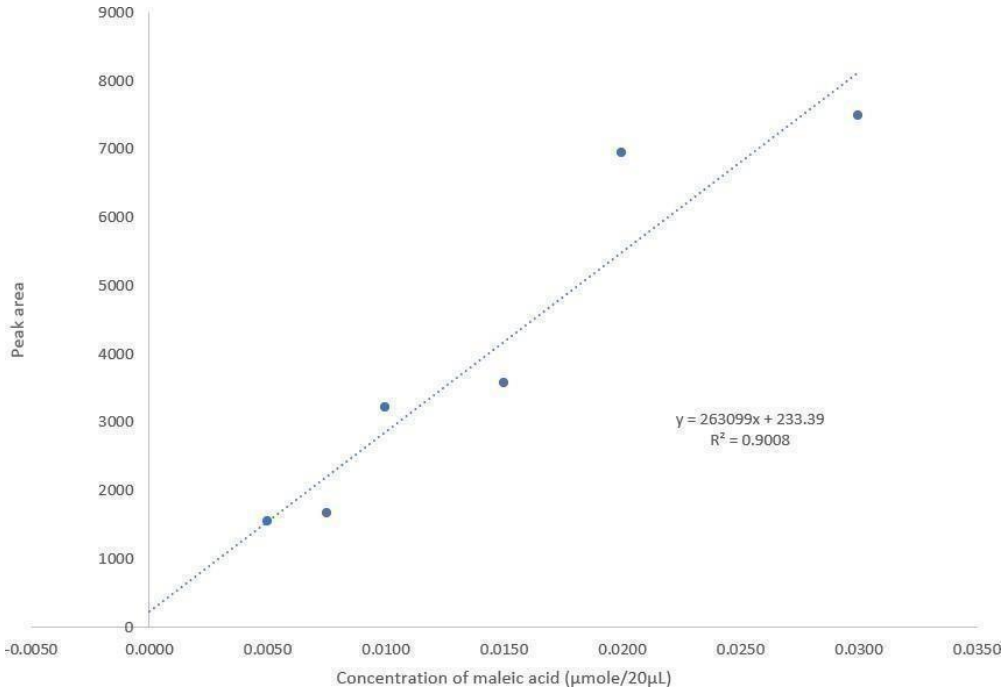

Figure 4.10: Standard curve of maleic acid.

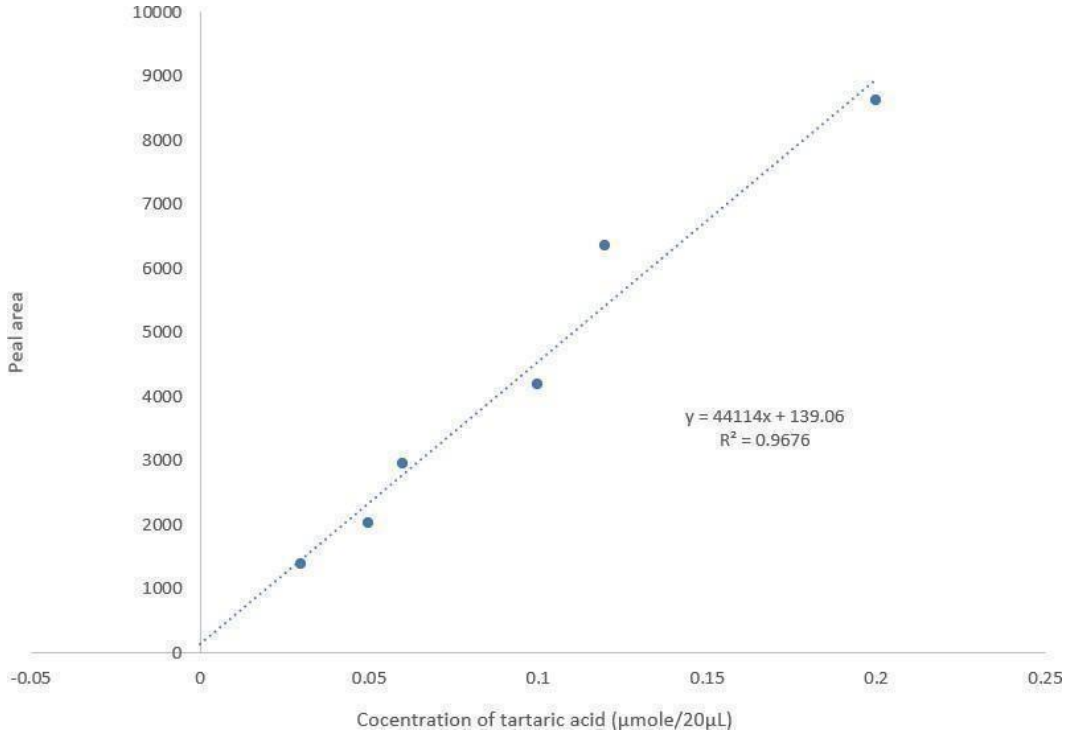

Figure 4.11: Standard curve oftartaric acid.

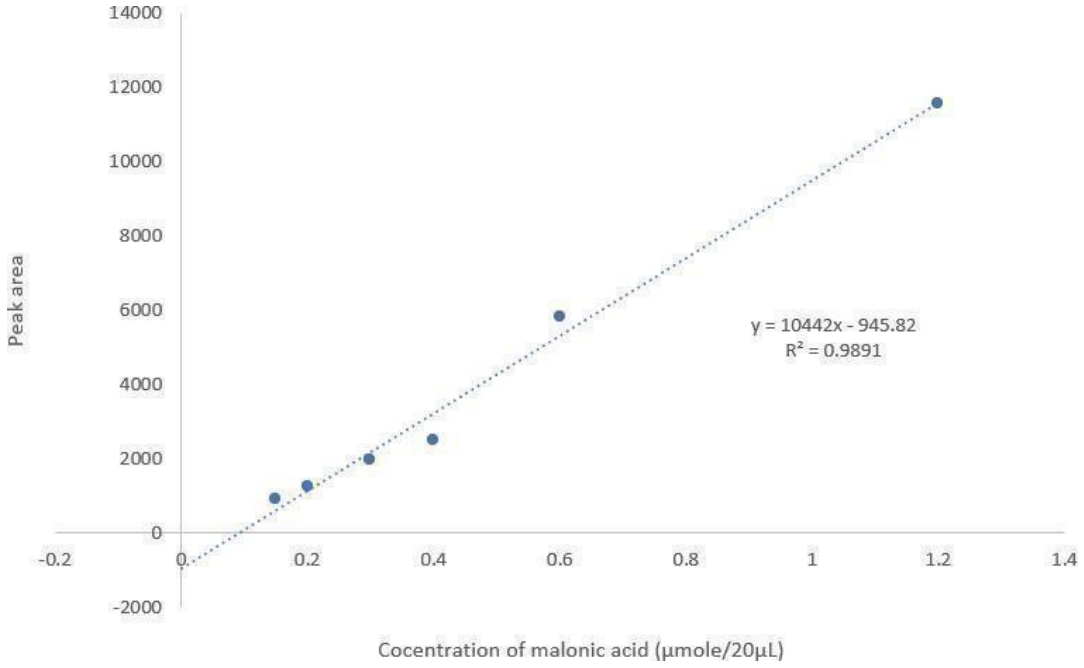

Figure 4.12: Standard curve of malonic acid.

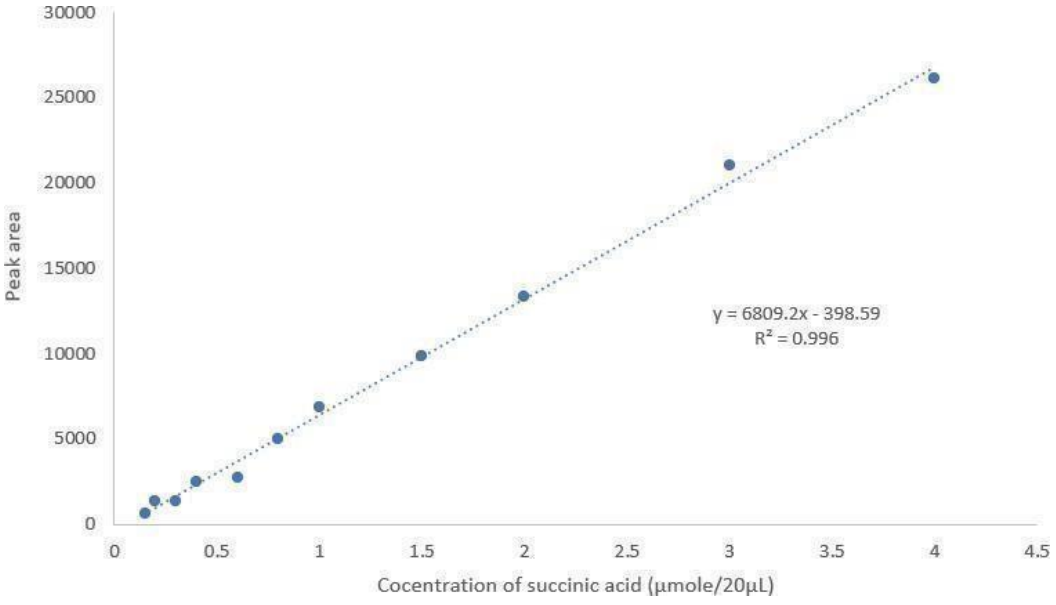

Figure 4.13: Standard curve of succinic acid.

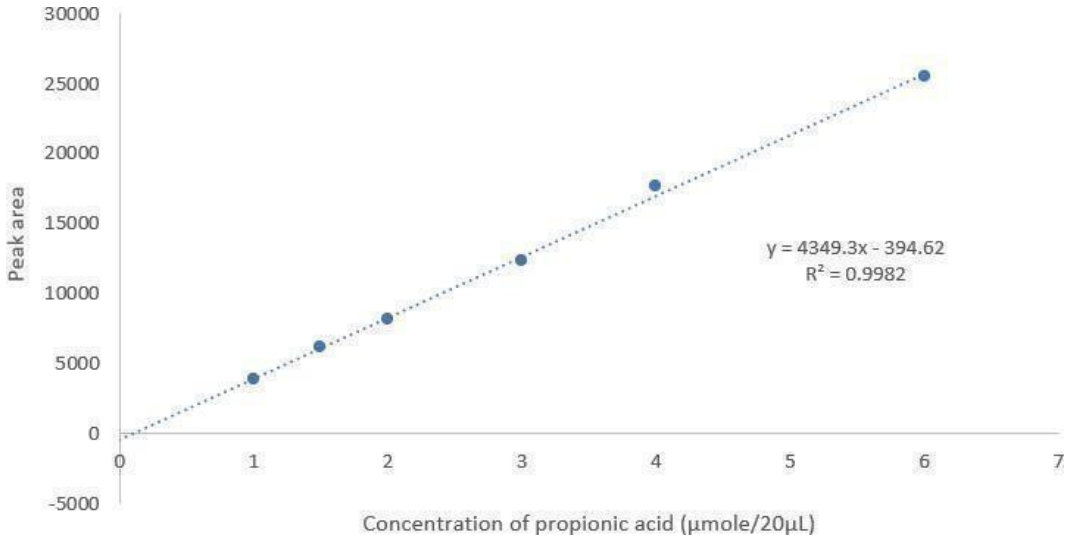

Figure 4.14: Standard curve of propionic acid.

Table 4.5 Contents of acetic acid, phytic acid, and maleic acid from vinegar with water and agarwood leaves treatment at different fermentation durations.

Duration	Acetic acid content (mg/mL)		Phytic acid content (mg/mL)		Maleic acid content (mg/mL)	
	Water	Agarwood	Water	Agarwood	Water	Agarwood
Blank	1.214 ± 0.008^{e}	1.529 ± 0.277^{cd}	31.184± 0.318	47.463 ± 1.087	0.054 ± 0.003	0.073 ± 0.001
Day 0	3.258 ± 0.125^{b}	2.974 ± 0.516^{d*}	33.387 ± 0.136	44.347 ± 0.330	0.057 ± 0.001	0.073 ± 0.001
Day 2	2.663 ± 0.495^{bcd}	3.089 ± 0.205^{c*}	32.991 ± 0.044	44.961 ± 0.233	0.056 ± 0.000	0.072 ± 0.004
Day 3	2.376 ± 0.165^{cd}	2.213 ± 0.063^{c*}	32.981 ± 0.332	44.482 ± 0.123	0.058± 0.002	0.070 ± 0.001
Day 4	2.819 ± 0.133^{bc}	2.059 ± 0.263^{b*}	32.256 ± 0.340	45.059 ± 0.330	0.061 ± 0.001	0.071 ± 0.002
Day 5	2.005 ± 0.220^{d}	1.176 ± 0.023^{b*}	34.329 ± 0.351	45.767 ± 0.298	0.060 ± 0.001	0.072 ± 0.001
Day 10	4.302 ± 0.357^{a}	4.536 ± 0.263^{a}	35.188 ± 0.408	47.368 ± 0.410	0.075 ± 0.001	0.089 ± 0.005

*Valuesrepresent mean ± SD (n=3).

Table 4.6: Contents of tartaric acid and malonic acid from vinegar with water and agarwood leaves treatment at different fermentation durations.

Duration	Tartaric acid content (mg/mL)		Malonic acid content (mg/mL)	
	Water	Agarwood	Water	Agarwood
Blank	0.575 ± 0.020	0.303 ± 0.005	1.199 ± 0.214	1.165 ± 0.071
Day 0	0.572 ± 0.005	0.283 ± 0.010	1.535 ± 0.040	1.197 ± 0.029
Day 2	0.405 ± 0.002	0.171 ± 0.022	1.558 ± 0.022	1.181 ± 0.115
Day 3	0.342 ± 0.012	0.085 ± 0.003	1.473 ± 0.212	1.197 ± 0.014
Day 4	0.342 ± 0.007	0.162 ± 0.011	1.599 ± 0.263	1.167 ± 0.062
Day 5	0.218 ± 0.010	0.066 ± 0.001	1.688 ± 0.080	1.127 ± 0.033
Day 10	0.147 ± 0.006	0.224 ± 0.032	1.358 ± 0.039	1.403 ± 0.192

*Values for represent mean ± SD (n=3).

Table 4.7: Contents ofsuccinic acid and propionic acid fromvinegar with water and agarwood leaves treatment at different fermentation durations.

Duration	Succinic acid content (mg/mL)		Propionic acid content (mg/mL)	
	Water	Agarwood	Water	Agarwood
Blank	3.884 ± 0.373	4.022 ± 0.150	-	0.610 ± 0.013
Day 0	3.757 ± 0.069	4.213 ± 0.335	-	0.663 ± 0.114
Day 2	3.706 ± 0.038	3.787 ± 0.753	0.432 ± 0.003	2.001 ± 0.106
Day 3	3.988 ± 0.369	3.714 ± 0.098	0.705 ± 0.006	1.681 ± 0.066
Day 4	4.464 ± 0.458	3.503 ± 0.388	0.995 ± 0.032	1.511 ± 0.113
Day 5	3.938 ± 0.138	3.722 ± 0.214	0.807 ± 0.030	1.802 ± 0.018
Day 10	3.892 ± 0.650	2.867 ± 0.375	1.898 ± 0.237	2.066 ± 0.015

*Valuesrepresent mean ± SD (n=3).

4.6 Phytochemical Screening

Table 4.8 shows the results of phytochemical screening, for compounds such as anthraquinones, triterpenoids, alkaloids, flavonoids, tannins, quinones, coumarins, and phlobatannins. The alcoholic mixture at Day 14 (Alcohol control) and final product of acetic acid fermentation (Vinegar control) of water and agarwood leaves treatment show positive results for flavonoids, tannins, and coumarin. A positive result for quinones was also observed in the vinegar control of water treatment.

Figures 4.15 to 4.18 show that a yellow-green colour developed in the samples after the addition of 5% NaOH. When diluted HCL was added, a pink colour, the original colour of purple sweet potato vinegar was observed, indicating that the presence of flavonoids. Table 4.10 presents the colour changes observed in the samples after reaction with 5% $FeCl_3$. As shown in Figure 4.19 to 4.22 a bluish-black colour developed in all samples, indicating the presence of tannins. Figure 4.24 displays three distinct colour layers: a yellowish-brown upper layer, a thin greenish middle layer, and a red bottom layer, confirming the presence of quinone. In contrast, Figures 4.23, 4.25, and 4.26 show yellowish-brown colour developed in samples after alcoholic KOH was added, which indicates that quinone was absent. Figure 4.27 shows the result of phytochemical screening of coumarin. It showed that light yellow fluorescence was observed under UV light in all samples indicating that coumarin was present.

Table 4.8: Phytochemical content of water and agarwood leaves treatment with alcohol and vinegar control.

Phytochemical	Treatment			
	Water		Agarwood leaves	
	Alcohol control	Vinegar control	Alcohol control	Vinegar control
Anthraquinones	-	-	-	-
Triterpenoids	-	-	-	-
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Quinone	-	+	-	-
Coumarin	+	+	+	+
Phlobatannins	-	-	-	-

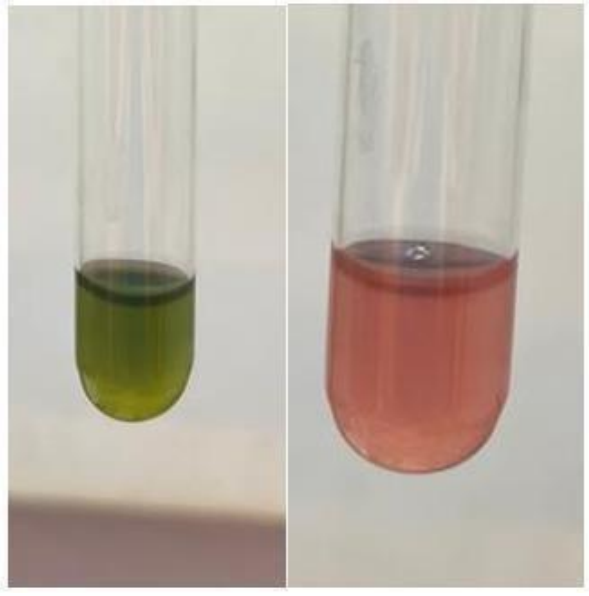

Figure 4.15: Yellowish green developed after adding 5% NaOH (left test tube). The sample shows the original colour of purple sweet potato vinegar after adding diluted HCL (right test tube). Presence of flavonoid: water-treated fermented sample (alcohol control).

Figure 4.16: Yellowish green developed after adding 5% NaOH (left test tube). The sample shows the original colour of purple sweet potato vinegar after adding diluted HCL (right test tube). Presence of flavonoid: water-treated fermented sample (vinegar control).

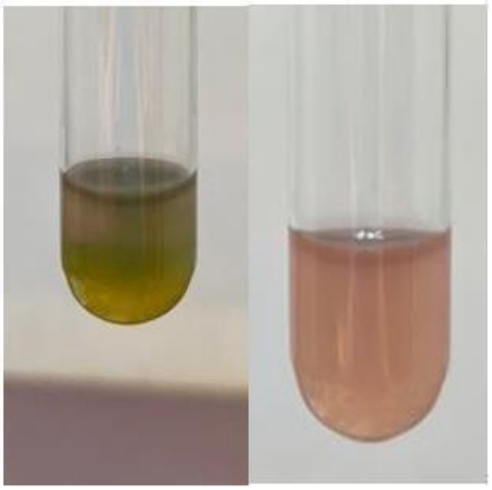

Figure 4.17: Yellowish green developed after adding 5% NaOH (left test tube). The sample shows the original colour of purple sweet potato vinegar after adding diluted HCL (right test tube). Presence of flavonoid: agarwood- treated fermented sample (water control).

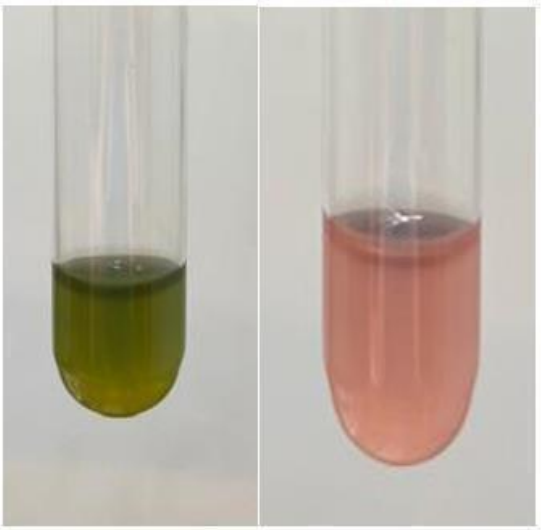

Figure 4.18: Yellowish green developed after adding 5% NaOH (left test tube). The sample shows the original colour of purple sweet potato vinegar after adding diluted HCL(right test tube). Presence of flavonoid: agarwood- treated fermented sample (vinegar control).

Figure 4.19: Black colour was developed after 5% $FeCl_3$ was added. Presence of tannins: water-treated fermented sample (alcohol control).

Figure 4.20: Black colour was developed after 5% $FeCl_3$ was added. Presence of tannins: water-treated fermented sample (vinegar control).

Figure 4.21: Black colour was developed after 5% $FeCl_3$ was added. Presence of tannins: agarwood-treated fermented sample (alcohol control).

Figure 4.22: Black colour was developed after 5% $FeCl_3$ was added. Presence of tannins: agarwood-treated fermented sample (vinegar control).

Figure 4.23: Yellowish-brown colour was developed after alcoholic KOH was added. Absence of quinone: water-treated fermented sample (alcohol control).

Figure 4.24: Yellowish-brown upper layer, a thin greenish middle layer and a red bottom layer were observed after alcoholic KOH was added. Presence of quinone: water-treated fermented sample (vinegar control).

Figure 4.25: Yellowish-brown colour was developed after alcoholic KOH was added. Absence of quinone: agarwood-treated fermented sample (alcohol control).

Figure 4.26: Yellowish-brown colour was developed after alcoholic KOH was added. Absence of quinone: agarwood-treated fermented sample (vinegar control).

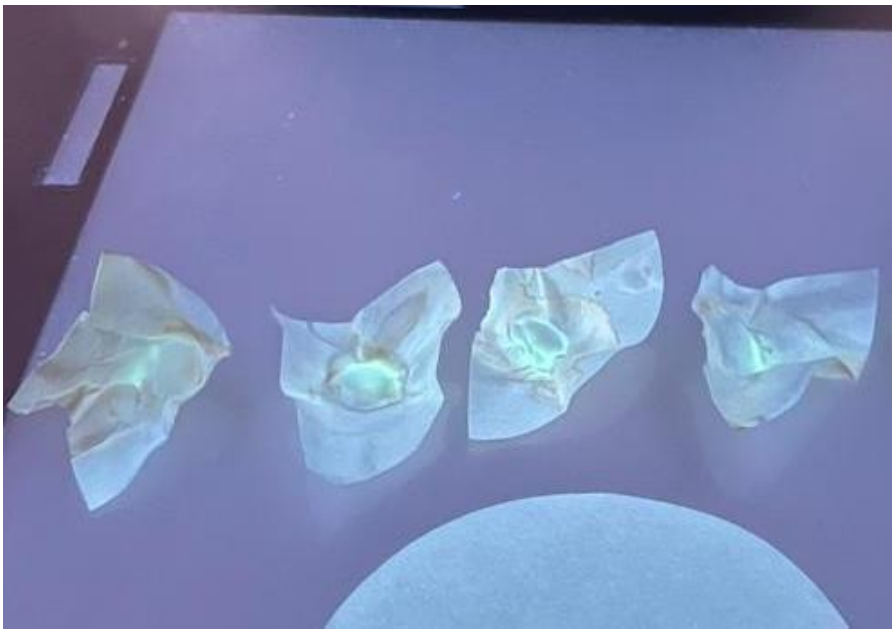

Figure 4.27: Phytochemical screening result of coumarin.

CHAPTER 5

DISCUSSION

5.1 Reducing Sugar Changes during Saccharification

Koji is produced from grains such as rice, soybeans, or wheat that are inoculated and fermented with *Aspergillus oryzae* (Zhu and Tramper, 2013). The key enzymes found in koji are α-amylase and glucoamylase. There are two forms of α-amylase present during the koji preparation: acid-labile and acid-stable α-amylase. Both forms contribute to hydrolysis of starch into reducing sugar by cleaving the internal α-1,4-glycosidic bonds. In addition, α-amylase, glucoamylase present in koji plays a role in hydrolyzing starch by cleaving both α-1,4 and α-1,6-glycosidic linkages. According to Table 4.1, the reducing sugar content of the saccharified purple sweet potato, cassava, and mixed samples differed significantly. Among the three samples, saccharified purple sweet potato exhibited the highest reducing sugar content. This result aligns with the findings of Muhiddin et al. (2019), who reported that 3:1 ratio of sweet potato to cassava produced higher sugar levels through local tapai ragi containing *A. oryzae* when compared to the ratio of 1:3. This may be due to the different composition of polysaccharide chains in sweet potato and cassava starch. Peroni et al. (2006) suggested that sweet potato starch has a higher amylose content compared to cassava starch, resulting in a greater number of α- 1,4-glycosidic bonds. Hence, this leads to a higher sugar conversion efficiency of starch due to its higher amylose content, resulting in a higher reducing sugar content after saccharification compared to cassava.

On the other hand, purple sweet potatoes have significantly higher reducing sugars compared to cassava and the mixed sample prior to inoculation with koji. These findings align with an earlier study by Wanjekeche and Keya (2010), which indicated that the sugar content of cassava is lower than that of sweet potato. Most of the reducing sugars found in raw sweet potato are fructose and glucose (Rezvanian, Jafarinejad and Bovell-Benjamin, 2023). In contrast, cassava is primarily composed of starch. Raw cassava typically has a substantially lower reducing sugar content compared to sweet potatoes, as cassava actively accumulates starch rather than free sugars (Pan et al., 2021).

According to the results shown in Table 4.1, the reducing sugar content of the samples increased after the addition of koji. This may be caused by the activity of *A. oryzae* in koji. The sugars in koji are the result of the enzymatic reaction between *A. oryzae* and the substrate used for koji. Furthermore, koji also contains glycosylceramides that are primarily composed of glucose and galactose (Hamajima et al., 2016). Wisman et al. (2020) have indicated that koji contains several simple sugars, including glucose and fructose. The distributions and concentrations of these sugars may vary depending on fermentation conditions and substrate, such as rice, soybean, or wheat.

5.2 Amino Acid Changes during Saccharification

Apart from α-amylase and glucoamylase, koji also contained protein-digesting enzymes such as proteases (Daba, Mostafa and Elkhateeb, 2021). Referring to the results obtained fromTable 4.2, the amino acid content ofsaccharified purple

sweet potato was similar to that of the blank but significantly lower than the initial treatment. This can be explained by the previous study conducted by Son et al. (2018). It is found that glucoamylase has higher enzymatic activity compared to the protease. In this previous study, koji with *A. oryzae* was inoculated into rice to produce the Korean alcoholic drink, *Makgeolli*, followed by the identification of metabolites in it. According to Nile (2015), *Makgeolli* had lower carbohydrate content compared to protein. However, after identifying the metabolites in *Makgeolli* using gas chromatography, it is found that the peak area of amino acids such as proline, glycine is substantially lower than galactose. This suggests that koji is highly specialized in starch conversion rather than protein breakdown. Hence, the inoculation of koji in sweet potato and cassava does not significantly affect the content of amino acids (Son et al., 2018).

According to the study by Chandrasekara and Kumar (2016), it was found that raw sweet potato and cassava have similar amino acids content, ranging from 1.4 g to 1.6 g per 100 g. However, Chen et al. (2015) studied the major constituents contained in the storage protein of sweet potato, which were sporamins. It contained up to 80% of the total soluble protein of sweet potatoes. Sporamins are proteins that play an important role in the defense mechanisms of plants as they inhibit the proteases from insects and pathogens (Senthilkumar and Yeh, 2012). Thus, the proteases from other organisms are unable to digest it into free amino acids. On the other hand, a previous study reported that approximately 50% of the free amino acids in cassava roots contributed to a higher total nitrogen content compared to sweet potato (Chikezie et al., 2016).

This explained whythe amino acid content of purple sweet potatoes was lower than that of cassava.

Additionally, the mixed sample and cassava exhibited higher levels of amino acids after adding koji, as koji itself contains some free amino acids. For instance, the studyof Wisman et al. (2020), stated that there were amino acids distributed onthe edges or inside the structureofkoji. This may be caused bythe proteolytic enzyme contained in *A. oryzae,* which can break down the protein in rice, the substrate used to make koji.

5.3 Phytochemical Changes throughout Fermentation

According to Table 4.8, there were positive results of flavonoid, tannin, and coumarin observed in alcoholic mixture at Day 14 and final product of acetic acid fermentation of both treatments. This demonstrated that both vinegars have comparable phytochemical content. Alam, Kurnianingsih, and Fatchiyah (2022) studied that purple sweet potato contains flavonoid and tannin that also occur in agarwood leaves. Besides, Brown (2005) reported that purple and red tuber roots have higher flavonoid content than the other colours of flesh. These may be caused by the purple colour of the flesh of the sweet potato can influence the composition of these phytochemicals. Furthermore, a prior study had proposed that the phytochemical content in purple sweet potato, including flavonoids and tannins, is linked to the antioxidant activity (Sendangratri, Handayani and Elya, 2019).

In addition, Biswas and Madhu (2016) revealed that coumarin can be found in a broad range of plants. According to the study of Zaynab et al. (2024), coumarins have played an important role in plant defense against pathogens due to their antibacterial and antioxidant properties. It has been widely utilized in sustainable agricultural practices that prioritize ecologically friendly integrated pest management procedures. Matsumoto et al. (2012) stated that purple sweet potato accumulates coumarins, particularly scopoletin and umbelliferone. Coumarins are produced through an enzyme-catalyzed reaction called *ortho*-hydroxylation. Firstly, the enzymes, 2-oxoglutarate-dependent dioxygenases (2OGD), which are encoded by five cloned cDNAs designated to Ib1 and Ib2 in the sweet potato, will add an OH group to the side of cinnamate molecules, a component that is found naturally in sweet potato (Franková et al., 2022). Ib1 will turn the feruloyl-CoA, which is the derivative of cinnamate, into scopoletin, while Ib2 will convert either feruloyl-CoA or p-coumaroyl-CoA into scopoletin or umbelliferone, respectively (Matsumoto et al., 2012). On the other hand, Saadati et al. (2024) revealed that coumarins have a strong anti-inflammatory effect via changing signaling pathways and preventing pro-inflammatory mediators, thus providing a potential health benefit to vinegar.

Furthermore, there is a positive result of quinone was observed in the vinegar control of the water treatment. Similar observations was documented by Tarko et al. (2020). Tarko et al (2020) revealed that quinones can be produced when wine is in contact with oxygen. When oxygen comes in contact with wine, the phenolic compounds will be oxidized to their corresponding quinones, which are subsequently polymerized to produce yellow-brown pigments. The atoms

that catalyze oxidation reactions, such as iron and copper, are involved in this reaction. Besides, Chen et al. (2019) investigated the stability of anthocyanins in purple sweet potato. The previous study revealed that anthocyanin was suggested to be retained at pH 3. In addition, Cheng et al. (2023) reported that anthocyanins can be oxidized into ortho-quinones that are unstable and susceptible to brownish polymeric complexes. In contrast, quinone was not present in the vinegar with agarwood leaves treatment. This may be due to the high stability of phenolic content in agarwood leaves. Delica-Balagot et al. (2024) had studied the effect of drying method on the phenolic compounds of agarwood leaves. It was found that the phenolic content of agarwood leaves was well preserved after applying drying methods.

5.4 Total Phenolic Content Comparison between Agarwood and Water-treated Vinegar

Table 4.4 shows that both treatments showed a substantial amount of phenolic content, with the water-treated vinegar contained 40.10 mg/mL while agarwood-treated vinegar contained 51.23 mg/mL by Day 10 of acetic acid fermentation. This is because the anthocyanin naturally found in purple sweet potatoes contributes to the phenolic content in the vinegars (Li et al., 2019). Anthocyanins are a specific category of flavonoid compounds characterized by numerous hydroxyl groups. Besides, Franková et al. (2022) reported that purple sweet potato contains several compounds, such as phenolic compounds, carotenoids, and anthocyanins. These compounds are the secondary metabolites ofplants that

are constructed with one or more aromatic rings that linked with hydroxyl groups that can be detected in the Folin-Ciocalteu assay (Zhang et al., 2022).

The total phenolic content of the vinegar with agarwood leaves treatment was significantly higher than the water treatment vinegar throughout the fermentation from Day 0 to Day 5. Agarwood leaves treated and water-treated vinegar consisted of 44.156 mg/mL to 51.234 mg/mL and 26.636 mg/mL to 40.097 mg/mL of total phenolic content, respectively. This trend agreed with the observations of Suwanposri et al. (2025) who studied the biological properties of the herbal kombucha treated with the agarwood leaves. The results from their study showed that the kombucha incorporated with the agarwood leaves had the highest statistically significant total phenolic content. This can be explained by the phenolic content that is naturally found in the agarwood leaves. According to Batubara et al. (2021), hot-water extracts of agarwood leaves are rich in plant secondary metabolites such as flavonoids, tannins, triterpenoids, and glycosides. Indeed, agarwood leaf tea is noted for its high antioxidant content, as there is a previous study indicating very strong antioxidant activity in hot-water extract. Thus, when infused agarwood leaves were added, these phytochemicals probably dissolved into the fermentation. In contrast, plain water does not contain such beneficial chemicals or nutrients. Xie et al. (2024) study reported that agarwood leaves are rich in sesquiterpenes, flavonoids, flavones, terpenoids, and phenolic acids, which are a group of polyphenolic compounds. Furthermore, Kahlil et al. (2013) also state that agarwood leaves contain phenolic compounds such as tannins and saponins. Hence, the presence

of these compounds that elevate the total phenolic content of vinegar with agarwood leaves treatment.

James et al. (2022) had mentioned that the microbial enzymes, including β-glucosidase, amylase, and lipases, are activated and mobilized during the fermentation process. Besides, this previous study suggested that the release of the phenolic compound bound to the complex protein or carbohydrate was due to the late microbial fermentation. Eventually, enable the phenolic content easier to be extracted. On the other hand, there are fluctuations of the total phenolic content shown in the water treatment. A previous study had reported that *Acetobacter* could produce a variety of flavouring chemicals, including phenols, which are essential for influencing the scent of vinegar (Ge et al., 2025). In contrast, it is found that the activity of the *Lactiplantibacillus plantarum* secretes β-glucosidase that could hydrolyze the phytochemicals, particularly flavonoids and phenolics. As a result, the activities of this microbial species may lead to an increase or decrease in flavonoids, as well as the phenolic content (James et al., 2022).

5.5 Alcohol Content of Agarwood and Water-treated Vinegar

Referring to Table 4.3, the result shows that there is no significant difference between the alcohol content of agarwood-infused vinegar and water-treated vinegar. Batubara et al. (2022) stated that agarwood leaf extract contains soluble compounds, including phenolic acids, flavonoids, tannins, and chromones. They might supplytrace antioxidants or nutrients, but do not add sugar to support yeast

metabolism. Besides, since the amount of purple sweet potatoes and the saccharification conditions utilized in both treatments are the same, the fermentable sugar load for both treatments was also approximately the same (Lareo et al., 2013). The similar trend of alcohol content for both treatments is likely because the agarwood infusion only adds negligible extra sugar, hence it was not sufficient to increase the alcohol level.

On the other hand, the results from Table 4.3 shows that the alcohol content of both treatments remained unchanged from Day 2 to Day 5 and subsequently fell quickly at Day 10. The result does not tally with the research of Song et al. (2019) and Wang et al. (2023). Song et al. (2019) monitored the dynamic changes of alcohol content during apple vinegar fermentation. The alcohol content decreased gradually from Day 3 to Day 14. Wang et al. (2023) investigated the quality changes of vinegar during the fermentation process. It was found that the alcohol content also gradually decreased from 22.44 to 3.51 %v/v throughout the 15 days of fermentation. However, the results from Table 4.3 follow a similar trend to that of Won et al. (2024). In this research, the unripe *Citrus unshiu* was used to produce vinegar using 3 different acetic acid bacteria strains as inoculum. The fermentation was undergone for 30 days. The results from this study showed a slow reduction of alcohol content in the vinegar inoculated with *Acetobacter pasteurianus* from Day 0 to Day 10, which was probably due to the conversion of alcohol to organic acid during the fermentation process (Won et al., 2024). Besides, Table 4.5 also shows that the production of acetic acid was elevated at Day 10 for both treatments. These suggest that the sudden decrease in alcohol

concentrations indicates that acetic acid fermentation had onset by Day 10 (Maske et al., 2024).

5.6 Dynamic Change of Microbial Community

5.6.1 Microbes involved in Fermentation Process

The results in Table 4.3 indicate a sudden drop in alcohol content by Day 10 for both treatments. This result could be linked to the result shown in Table 4.5 as the acetic acid content had reached a maximum yield by Day 10 for both treatments. According to the study conducted by Song et al. (2019), it is stated that are wide variety of microorganisms present during the dynamic fermentation process of apple vinegar. The pre-fermentation phase, which corresponds to alcoholic fermentation of vinegar, was dominated by *Lactococcus* and *Oenococcus*. Notably, *Oenococcus* was exclusively found at Day 3, indicating that this early stage was associated with alcoholic fermentation (Song et al., 2019). Furthermore, the research of Maske et al. (2024) observed that there were several bacterial species, including *Cronobacter*, *Luteibacter*, and *Saccharomyces*, present during the early stages of fermentation. *S. cerevisiae* plays a key role in converting sugars into alcohol during this phase (Topaloğlu et al., 2023).

When the fermentation reaches its final stage, the *Acetobacter* takes control and converts ethanol into acetic acid, the main ingredient in vinegar (Wang et al., 2023). Song et al. (2019) reported that *Acetobacter* had proliferated throughout acetic acid fermentation, indicating that it was the secondary dominating

bacterium in vinegar fermentation. Particularly, it was found to be the most numerous on Day 14 of fermentation. Maske et al. (2024) stated that during the stable microbial community phase, which spans from Days 17 to 70, *Leuconostoc* and *Gluconobacter* are prevalent, maintaining a steady metabolic process and producing organic acids that lower the pH, thereby creating optimal conditions for *Acetobacter*. Additionally, the growth of *S. cerevisiae* was inhibited by the depletion of sugar, which may further contribute to the domination of *Acetobacter* by reducing competition for resources (Mota and Vilela, 2024). This aligns with the study by Topaloğlu et al. (2023), which highlights that the conversion of ethanol to acetic acid occurs only at the late fermentation stage, suggesting why the alcohol concentration remains relatively constant before Day 10.

Furthermore, acetic acid bacteria can dominate the fermentation process due to their adaptation to the fermentation environment. As mentioned earlier, other bacteria, such as *Lactococcus* and *Oenococcus*, dominate the fermentation process at the early stages of fermentation. However, acetic acid bacteria had adapted to the fermentation environment and dominated the process at the late stage of fermentation. Adaptation is the process by which microorganisms gradually change to increase their tolerance in a challenging environment. During the adaptation process, microorganisms employ several mechanisms to enhance stress tolerance, ultimately increasing their ability to adjust for growth and survival (Tan et al., 2022). In this case, acetic acid bacteria apply the oxidative fermentation mechanism to dominate the late stage of fermentation by inhibiting the growth ofother bacteria. This distinct mechanism enables them to

effectively transfer electrons and produce energy through specialized respiratory chains on their cell membranes, thereby increasing the amount of reactive oxygen species (ROS) in the environment, including hydrogen peroxide, hydroxyl radicals, and superoxide anions. The pH value of both treatments was suspected to be elevated due to the ROS created by acetic acid bacteria may actively inhibit or kill the competitive microbes, as ROS can destroy the cell wall and cellular molecules like lipids, proteins, and DNA of coexisting microorganisms. This inhibitory action may reduce the competitiveness of other microorganisms and allow acetic acid bacteria to outcompete them (Han et al., 2024).

5.6.2 Possibility of Spoilage Microbe Involvement

The study's findings suggest that, in addition to alcohol-producing and acetic acid bacteria, microbial contaminants or spoilage microbes may have been involved during fermentation. Table 4.3 shows that the pH level had significantly increased after Day 4 for agarwood leaves treatment and Day 5 for water treatment. Besides, the titratable acid of both treatments began to decrease after Day 4. These trends were negatively correlated with the study of Li et al. (2022). Li et al. (2022) monitored the pH and titratable acid of *Hongqu* aromatic vinegar during acetic acid fermentation, in which *Hongqu* is a red yeast rice produced by fermenting *Monascus purpureus* with rice and liquor that is usually used to make rice wine (Park et al., 2016). The previous study showed that the pH was decreased from 4.5 to 3.4 while the total titratable acid

was increased from 0 to 80 g/L during the 38 days of fermentation. Besides, the trend of pH level also does not tally with the study of Onuorah, Lina and Obika (2016). Onuorah, Lina and Obika (2016) produced a vinegar using oil-palm wine and *Acetobacter aceti*. It was found that the pH level began at 6.9 at Day 1 was decreased to 4.6 at Day 7. These findings indicate that the low pH promotes the production of acids, like acetic acid, and suppresses the proliferation of contaminating bacteria. This suggests that the reduction of titratable acid and elevation of pH may be due to the activity of spoilage yeast, *Zygosaccharomyces bailii* in vinegar. According to Palma, Guerreiro, and Sá-Correia (2018), *Z. bailii* is a spoilage yeast commonly found in shelf-stable and acidic foods such as wine and fermented products. It is known to cause serious economic losses in the food industry because of its high tolerance to weak acid (Rodrigues et al., 2012). Battey, Duffy and Schaffner (2002) reported that *S. cerevisiae* is another spoilage yeast commonly found in ready-to-drink beverages. During alcohol fermentation, *S. cerevisiae* primarilyconverts reducing sugars into ethanol. In comparison with *Z. bailii*, *S. cerevisiae* is unable to oxidize the acetic acid when glucose is present. This is because the glucose will suppress the genes and genes that are needed for the acetate metabolism. Hence, when glucose and acetic acid co-exist, which is when the middle stage of the fermentation, *S. cerevisiae* will utilize glucose first and produce alcohol. At the late stage of fermentation, sugars such as glucose become depleted. Regarding this, *S. cerevisiae* will start to consume the acetic acid, and this process is called diauxic growth.

In comparison with *S. cerevisiae*, *Z.bailii* does not contain the specific acetate export system. It will consume the acetic acid and glucose at the same time. This

is due to its ability to regulate both acetic acid membrane transport and acetyl-CoA synthetase activity. Both of this system helps to avoid toxicity as the intracellular acetate levels are low because the acetic acid is taken in and converted to acetyl-CoA immediately. Once the acetate is converted to acetyl-CoA, it is fed into the tricarboxylic acid (TCA) cycle immediately. The process is supported by the enzymes related to carbohydrate metabolism, energy generation, and stress response. Hence, this suggests that *Z.bailii* can consume glucose and acetic acid at the same time, which gives it a characteristic of high tolerance in an acidic environment (Palma, Guerreiro and Sá-Correira, 2018).

5.7 pH Value and Total Titratable Acidity Comparison between Agarwood and Water-treated Vinegar

Referring to the results in Table 4.3, it was shown that the pH of vinegar incorporated with agarwood was significantly higher than that of the control from Day 0 to Day 5. This may be caused by the constituents that are contained in the leaves. Min et al. (2023) reported that agarwood leaves contain various amino acids such as lysine, leucine, glutamate, and alanine. These amino acids contain a basic group that can take up a proton in the medium, thus increasing the pH value. Hence, this factor may cause an elevation of the pH value for agarwood-treated vinegar. However, the results from Table 4.4 showed that the titratable acid of agarwood leaves treatment vinegar was significantly higher than water treatment vinegar at Day 10. This may be due to the bioactive compound contained in the agarwood leaves. According to Xie et al. (2024), agarwood leaves contain the sesquiterpenes, which are bioactive compound that

contributes to the effect of antibacterial and antifungal effects. It is found that sesquiterpenoids block acetylcholinesterase and other enzymes necessary for microbial growth. The presence of agarwood leaf phytochemicals may cause inhibitory and bacteriostatic effects on the spoilage microbes mentioned above, causing a reduction in acid consumption (Li et al., 2016).

5.8 Organic Acid Changes throughout Fermentation and Their Effect on the Vinegar

5.8.1 Acetic Acid

The acetic acid concentration of the agarwood leaves treatment was shown to be significantly higher than water treatment at Days 4 and 5. This may be due to the microbial community in the vinegar. According to a previous herbal kombucha study, it was proven that adding agarwood leaf tea increased the ethanol yield and yeast count. The yeast cell counts of kombuchas made with agarwood leaves were greater than the control, which ranged from 7.59 ± 0.04 to 7.89 ± 0.017 log CFU/mL (Suwanposri, 2025). This indicates that the agarwood leaves contain some components that significantly enhance the yeast viability and activity during fermentation. Ciesarová, Šmogrovičová and Dömény (1996) reported that magnesium encouraged yeast growth during fermentation. Furthermore, several minerals including magnesium, zinc, potassium, and iron are found in agarwood leaf tea (Tin, 2023). The study of Dombek and Ingram (1986) suggested that adding magnesium to fermentations accelerated exponential growth, thus leading to greater yeast cell mass. The presence of the minerals may help in increasing the efficiency and yield ofalcoholduring fermentation. Hence,

the growth of the yeast may prolong the lag phase of acetic acid bacteria and delaythe onset ofvigorous acetic acid production (Phathanathavorn et al., 2019).

5.8.2 Other Organic Acids

Referring to Table 4.5, it is found that phytic acid was the predominant acid in both vinegars. Mitiku and Teka (2017) reported that purple sweet potato contains phytic acid, ranging from 0.24 to 0.31 mg per 100g. Besides, the study of Bhadram, Pardhe and Nagappan (2024) stated that 60.83 mg per 100 g of phytic acid was identified in the purple sweet potato. Hence, the content in purple sweet potato had contributed to a high concentration of phytic acid in the vinegar. According to Feizollahi et al. (2021), it is stated that phytic acid has significant health effects. For example, phytic acid can help to prevent heart disease. Phytic acid has negative charges at physiological pH, hence, phytic acid can serve primarily as a storage mineral chelating agent. Thus, it can protect human body from coronary heart disease by chelating copper and zinc, which alter the equilibrium in the body that results in hypercholesterolemia. Besides, Onomi, Okazaki, and Katayama (2014) state that even 0.02% of phytic acid included in the diet can reduce blood triglyceride and cholesterol levels. Thus, this indicates that low concentrations of phytic acid could promote anticancer characteristic (Feizollahi, 2021). Furthermore, phytic acid can serve as an energy source and antioxidant as it is a donor of the phosphate group. In addition to being antioxidants, dietary phytates have been reported to reduce kidney stone development, protect the human body against diabetes, caries, and atherosclerosis (Pires et al., 2023).

The concentration of phytic acid in water treatment was lower than in agarwood leaves treatment. There is a previous study showed that food ingredients, such as dough inoculated with lactic acid bacteria, exhibit phytase enzyme that can degrade phytic acid. The dough inoculated *with Lactiplantibacillus plantarum, Levilactobacillus brevis,* and *Lactilactobacillus curvatus* has similar phytase activity. A proper pH condition could cause of high degradation rate of the phytic acid (Reale et al., 2015). The phytic acid of agarwood leaves treatment was higher because it had an antimicrobial effect that derived from phytochemicals such as sesquiterpenes that inhibit the growth of lactic acid bacteria (Xie et al., 2024).

According to Table 4.7, it was observed that both vinegars contain propionic acid and succinic acid. Yildiz (2023) proposed that vinegar samples consist of various organic acids such as acetic acid, propionic acid, and succinic acid. Besides, Ge et al. (2025) studied that the main flavouring and essential components of vinegar are organic acids. The volatile organic acids, which are mainly produced during the fermentation process, include acetic and propionic acids, and the non-volatile organic acids such as succinic acid and citric acid that make up 0.5% to 2% of total acid content in vinegar. A previous study investigated the mechanism leading to the formation of succinic acid in a liquid culture containing 15% glucose under both aerobic and anaerobic conditions by using different *Saccharomyces cerevisiae* strains. This previous finding showed that succinic acid is mostly produced via the TCA cycle of *Saccharomyces cerevisiae* in a medium solution supplemented with a glucose level of 15% (Arikawa et al., 1999). Besides, Wan, Wang and Xiao et al. (2024) reported that succinic acids

were found abundantly in purple sweet potato. Insani and Wikandari (2024) had studied the potential fermentation of purple sweet potato pickle using the *Lactobacillus plantarum.* It was found that propionic acid was the second-highest organic acid detected in the pickles. This suggests that the presence of propionic acid can be due to the presence of lactic acid bacteria.

On the other hand, malonic acid and tartaric acid content were also observed in Table 4.6 while Table 4.5 showed the content of maleic acid. This trend was comparable with the research of Yildiz (2023). This previous finding had detected three of these acids in fruits vinegar. It was reported that the isomer of fumaric acid is maleic acid (Ugarte, Bustos and Moreno-Villoslada). Thus, fumaric acid in vinegar may be converted to maleic acid and detected in purple sweet potato vinegar (Abdali et al., 2023). It was discovered that tartaric acid is a naturally occurring component in some fruits, such as grape berries (Tang and Li, 2017). Bentley (1952) had reported that malonic acid has been documented in leaves of lucerne and green plants. Their biosynthesis in the fermented system is poorly understood. It is possible that they are not actively created during fermentation but can be derived from natural compounds in the raw material in which has not been well studied in sweet potato.

There was no lactic acid detected in the results of HPLC. However, the lactic acid bacteria still be possible to be present in the vinegar. This is because Yetiman and Kesmen (2015) had reported that lactic acid bacteria were detected in the vinegar samples and their mother. It was likely introduced throughthe raw

materials or contamination from processing equipment. Hence, the lactic acid not detected may be due to the other acid such as succinic acid masking the peak of lactic acid and preventing the peak unable to being seen. The retention time of succinic acids (15.46 min) was close to the of lactic acids (15.76 min) (refer to Appendix E). Besides, Ma et al. (2019) stated that the lactic acids and succinic acids had retention times at 37.76 min and 38.83 min, respectively.

5.8.3 Buffering Effect of Each Organic Acid

On the other hand, the elevation of the pH level throughout fermentation may be caused by the buffering effect of organic acid. According to Table 4.5, 4.6, and 4.7, there were acetic acid, succinic acid, maleic acid, malonic acid, tartaric acid, propionic acid, and phytic acid were contained in vinegar. The predominant acid in this vinegar, phytic acid, has several phosphate groups, which give significant buffering capacity over a wide pH range from pH 2 to 11 (Gupta, Gangoliy and Singh, 2013). According to Konietzny and Greiner (2003), phytic acid has three weak acid protons with pKa ranging from 5.2 to 8.0, three very weak acid protons that pKa ranging from 9.2 to 12, and six strong acid groups that are fully dissociated in solution with pKa ranging from 1.1 to 3.2. This buffering function aids in resisting extreme pH changes during the fermentation process (Veraart et al., 1997). According to the pKa data compiled by Williams (2022), it was found that organic acids such as maleic acid had a pKa ranging from 1.93 to 6.58, tartaric acid from 2.99 to 4.40, malonic acid from 2.83 to 5.69, succinic acid from 4.19 to 5.48, and propionic acid with a pKa at 8.1. Goldberg, Kishore and Lennen (2002) reportedthat the pKa ofacetic acid was 4.76. Hence, this suggests

that phytic acid had a broad range of pKa up to 12, however, the other organic acids, like acetic, maleic, tartaric, malonic, succinic, and propionic, had pKa that fall within a more acidic region. Hence, suggesting that the pH could be shifted to a more acidic region around pH 4.

5.9 Limitation of Study

This study faced several notable limitations that may affect the results and extent of interpretation. Firstly, the specific microbial communities responsible for acetic acid production were not identified. The absence of microbial profiling limits a deeper understanding of which organisms were dominant in the fermentation process and how they influenced acetic acid yield. Secondly, the acceptance of the consumer of the vinegar produced remains uncertain. The marketability and potential acceptance of the product can be well understood through sensory analysis was carried out. Finally, time constraints restricted the duration of the fermentation process and subsequent analysis. As a result, the long-term changes in microbial activity and the evolution of product quality, such as flavor development, stability, and preservation, were not fully explored. These limitations highlighted that microbial characterization, sensory evaluation, and extension of fermentation duration were needed to gain more understanding of the quality attributes of the product and its potential applications.

5.10 Future Study

For future research, microbial community analysis should be conducted at different fermentation stages to identify the populations of microorganisms associated with acetic acid production. The microbial profiling would provide a better understanding of which microorganisms dominate during different fermentation stages and how they contribute to product yield and quality. In addition, sensory evaluation is recommended to evaluate the aroma, taste, and consumer acceptance of the vinegar. This will help to establish the market potential and obtain useful information to improve the product quality. Furthermore, extending the fermentation period beyond 10 days could allow the observation of long-term microbial dynamics and their effects on the stability, flavour, and overall quality of the final product. Besides, alpha amylase can be used to minimize the influence and activity of *Aspergillus oryzae* during saccharification in order to produce a vinegar with better quality.

CHAPTER 6

CONCLUSION

In conclusion, purple sweet potatoes exhibited the highest reducing sugar content after saccharification (28 %wb), followed by the mixed sample (18.28 %wb) and cassava (11.98 %wb). The high reducing sugar of saccharified purple sweet potato is closely linked to the enzymatic activity of koji, whose enzymes were more effective at hydrolyzing α-1,4-glycosidic bonds in sweet potato starch compared to that of cassava. The lower reducing sugar content observed in the mixed sample, compared to pure purple sweet potato indicates that combining the substrates did not increase sugar availability compared to using only purple sweet potato. Hence, purple sweet potato was selected for subsequent fermentation. The free amino acid content of saccharified purple sweet potato, cassava, and mixed samples was 0.07 (%wb), 0.25 (%wb), and 0.14 (%wb), respectively. The free amino acid content of the samples remained unchanged. It was relatively lower than the content of reducing sugar after saccharification because koji is highly specialized in starch hydrolysis rather than protein degradation.

The alcohol content of agarwood and water-treated vinegar was comparable, as agarwood leaves do not contribute additional sugars for yeast activity. This indicates that the incorporation of agarwood leaves does not influence the alcohol content of vinegar. However, the pH value of agarwood-treated vinegar was significantly higher ($p<0.05$) than water-treated vinegar from Day 0 to Day

5 of acetic acid fermentation. The elevation in pH may be attributed to the amino acids present in agarwood leaves, which can take up protons in vinegar. In contrast, the titratable acidity of agarwood-treated vinegar (2.25 g/L) was significantly higher ($p<0.05$) than that of water-treated vinegar (1.69 g/L) at Day 10. This may be due to the bioactive compounds in agarwood leaves with antibacterial and antifungal properties, which inhibit the growth of spoilage microorganisms and thus reduce microbial acid consumption. Moreover, agarwood-treated vinegar showed a significantly higher acetic acid concentration ($p<0.05$) compared to water-treated vinegar between Day 0 and Day 5. Notably, the total phenolic content of agarwood-treated vinegar was significantly higher ($p<0.05$) than water-treated vinegar. This is because a significant amount of phenolic compounds, such as flavonoids, tannins, triterpenoids, and glycosides, present in agarwood leaves, contributed to the phenolic content of the vinegar.

Phytochemical screening revealed that both water and agarwood-treated vinegar contained flavonoids, tannins, and coumarins. This demonstrated that purple sweet potato naturally contains these phytochemical compounds. In addition, quinones were detected in the control water-treated vinegar, possibly due to the oxidation of phenolic compounds in purple sweet potato. However, quinone was not detected in agarwood-treated vinegar because the phenolic compound in agarwood leaves is more stable and resists oxidation to form quinone.

Overall, purple sweet potato is a promising substrate for vinegar production due to its high reducing sugar content. The incorporation ofagarwood leaves did not

affect the alcohol content during alcoholic fermentation, but it can elevate the pH value of vinegar during acetic acid fermentation. However, there was no significant difference ($p>0.05$) between water-treated and agarwood-treated vinegar produced at Day 10 of acetic acid fermentation. Hence, indicates that agarwood leaves do not affect the acetic acid level. The incorporation of agarwood leaves could enhance the biochemical qualities of vinegar in terms of total phenolic content. These suggest that agarwood leaves are a potential ingredient that can enhance the vinegar quality and potentially provide additional health benefits.

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APPENDIX A

Autoclaved Purple Sweet Potato Added with Koji

Saccharified Purple Sweet Potato

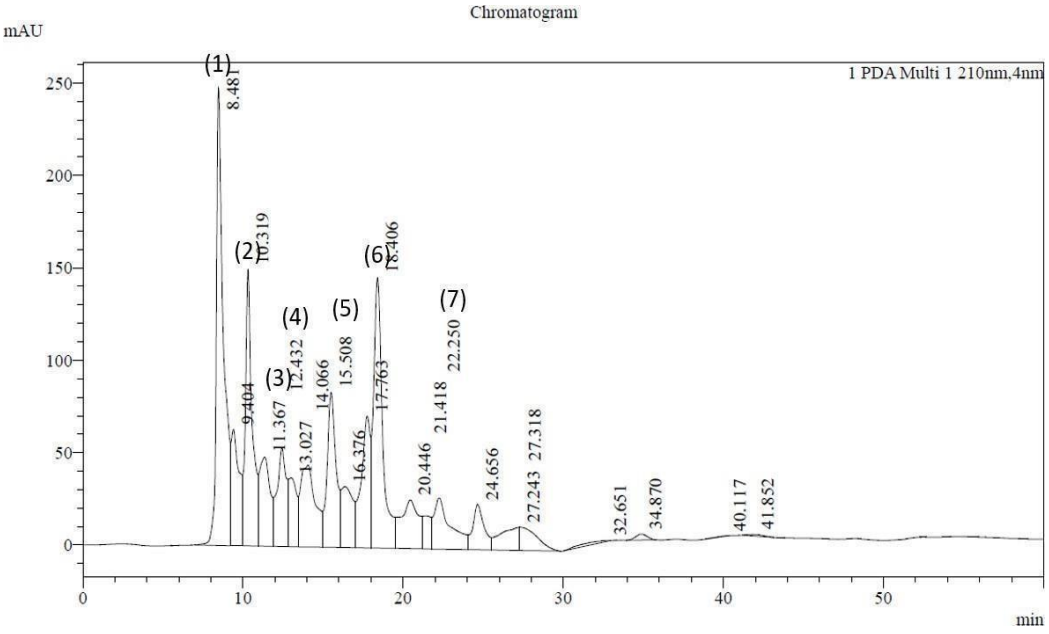
APPENDIX B

Purple Sweet Potato Vinegar Incorporated with Agarwood Leaves

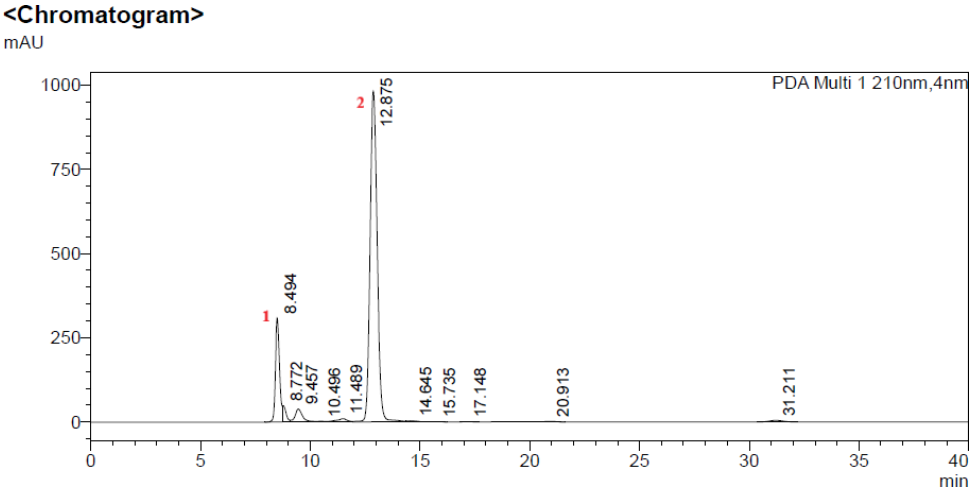
Bragg Apple Cider Vinegar

APPENDIX C

Chromatogram of Organic Acid Content in Agarwood-Treated Purple Sweet Potato Vinegarat Day 10. Peak Identification and Retention Time: (1) Phytic Acid, 8.481 min; (2) Maleic Acid, 10.319 min; (3) Tartaric Acid, 11.367 min; (4) Malonic Acid, 12.432 min; (5) Succinic Acid, 15.508 min; (6) Acetic Acid, 18.406; (7) Propionic Acid, 22.250 min

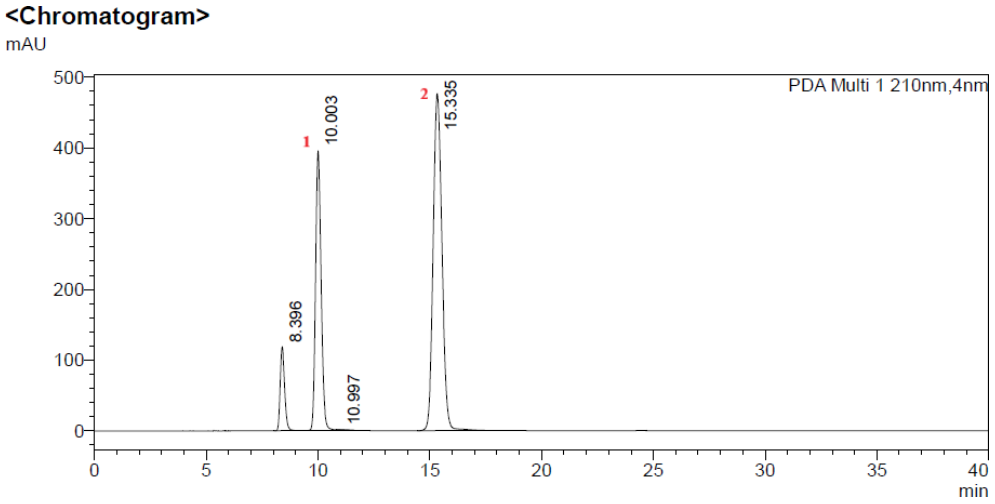


Chromatogram of Phytic Acid Standard with Retention Time of 8.494 min

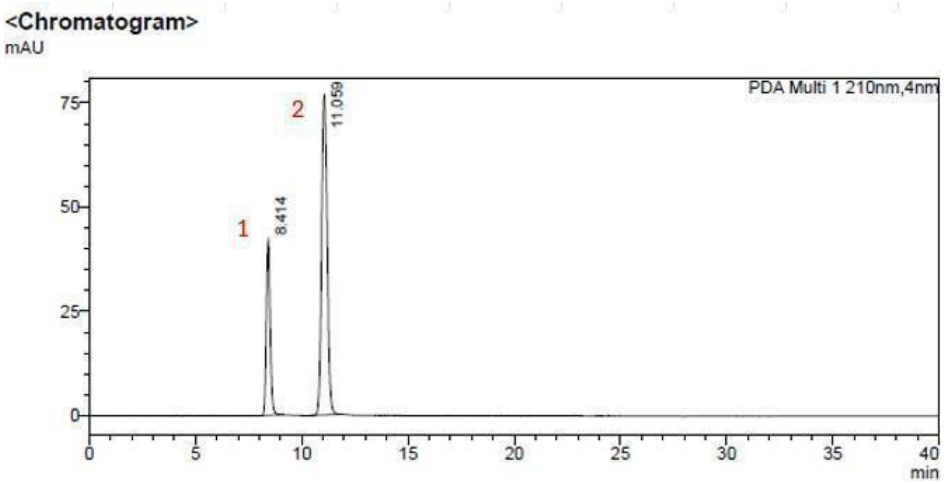


APPENDIX D

Chromatogram of Maleic Acid Standard with Retention Time of 10.003 min

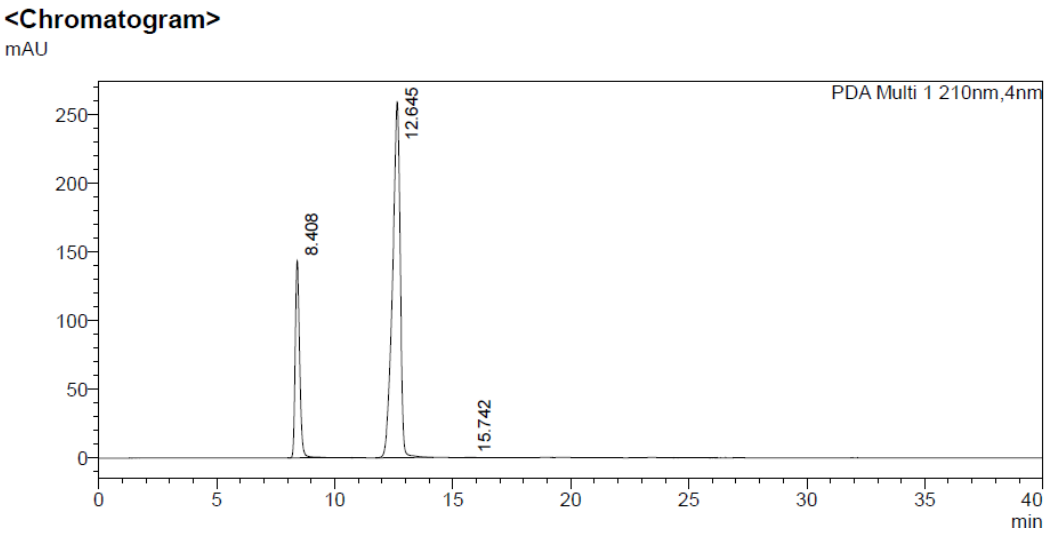


Chromatogram of Tartaric Acid Standard with Retention Time of 11.059 min

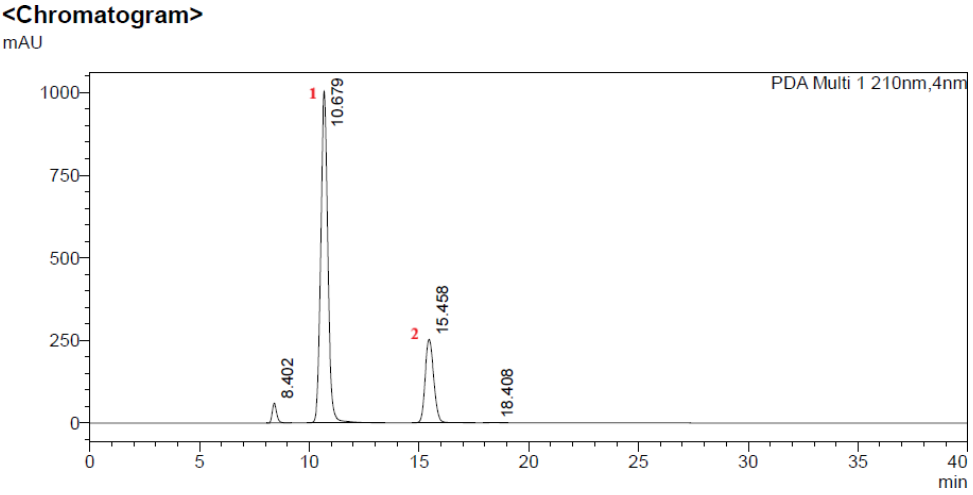


APPENDIX D

Chromatogram of Malonic Acid Standard with Retention Time of 12.645 min

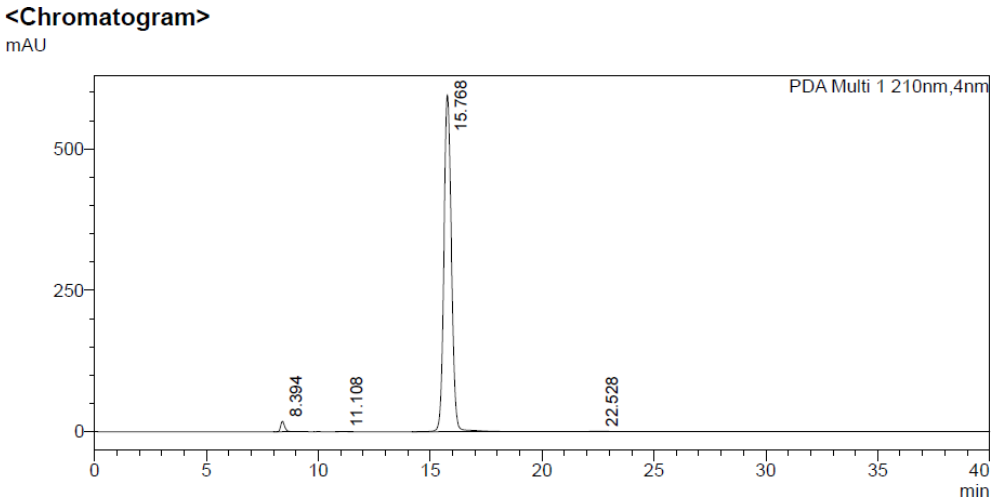


Chromatogram of Succinic Acid Standard with Retention Time of 15.458 min

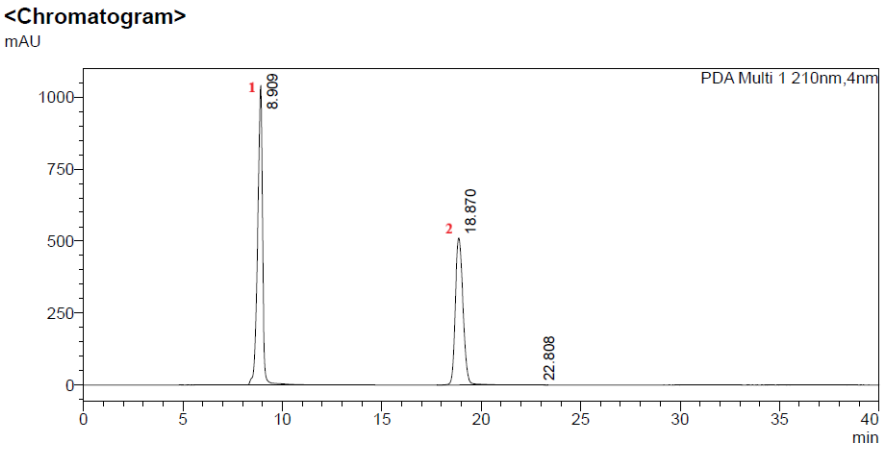


APPENDIX E

Chromatogram of Lactic Acid Standard with Retention Time of 15.768 min

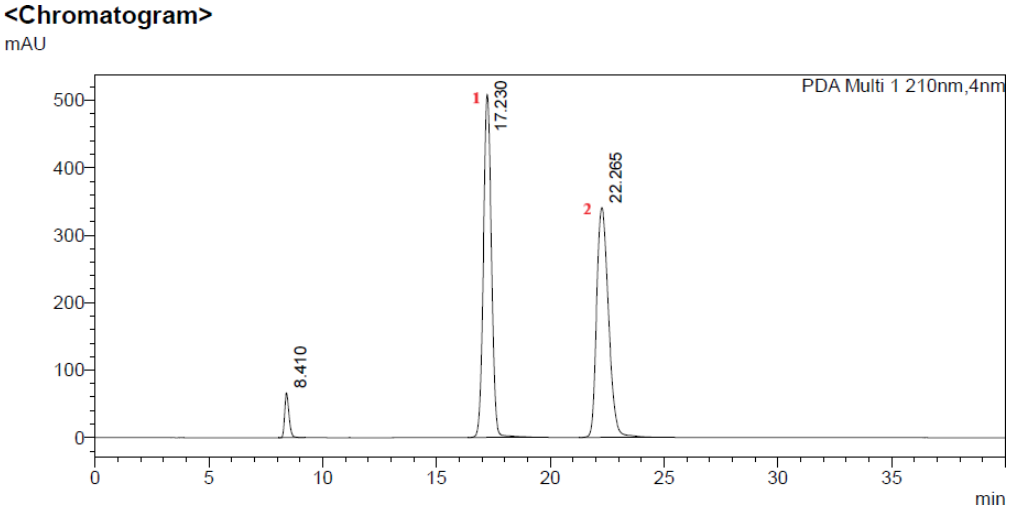


Chromatogram of Acetic Acid Standard with Retention Time of 18.870 min



APPENDIX F

Chromatogram of Propionic Acid Standard with Retention Time of 22.265 min



Normality Test for Reducing Sugar Content

Tests of Normality

Sample Type			Kolmogorov-Smirnov[a]			Shapiro-Wilk		
			Statistic	df	Sig.	Statistic	df	Sig.
Cassava	Reducing Sugar Content (%wb)	Blank	0.348	4		0.832	4	0.172
		Initial	0.200	4		0.978	4	0.889
		Saccharified	0.223	4		0.952	4	0.731
Mixture	Reducing Sugar Content (%wb)	Blank	0.209	4		0.966	4	0.816
		Initial	0.285	4		0.879	4	0.335
		Saccharified	0.288	4		0.794	4	0.092
Sweet Potato	Reducing Sugar Content (%wb)	Blank	0.224	4		0.947	4	0.696
		Initial	0.217	4		0.954	4	0.738
		Saccharified	0.237	4		0.926	4	0.569

a. Lilliefors Significance Correction

APPENDIX G

AVNOVA Test for Reducing Sugar Content

Tests of Between-Subjects Effects

Dependent Variable: Reducing Sugar Content (%wb)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2772.885[a]	8	346.611	534.589	0.000
Intercept	3128.538	1	3128.538	4825.246	0.000
Treatment	1901.265	2	950.633	1466.192	0.000
Sample	770.953	2	385.476	594.533	0.000
Treatment * Sample	100.667	4	25.167	38.816	0.000
Error	17.506	27	0.648		
Total	5918.929	36			
Corrected Total	2790.391	35			

a. R Squared = .994 (Adjusted R Squared = .992)

Post-Hoc Test for Treatment Effects on Reducing Sugar Content

Multiple Comparisons

Dependent Variable: Reducing Sugar Content (%wb)

Sample Type				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Cassava	Tukey HSD	Blank	Initial	-0.4025	0.26169	0.320	-1.1331	0.3281
			Saccharified	-11.2025*	0.26169	0.000	-11.9331	-10.4719
		Initial	Blank	0.4025	0.26169	0.320	-0.3281	1.1331
			Saccharified	-10.8000*	0.26169	0.000	-11.5306	-10.0694
		Saccharified	Blank	11.2025*	0.26169	0.000	10.4719	11.9331
			Initial	10.8000*	0.26169	0.000	10.0694	11.5306
Mixture	Tukey HSD	Blank	Initial	-0.9575	0.45042	0.139	-2.2151	0.3001
			Saccharified	-16.3750*	0.45042	0.000	-17.6326	-15.1174
		Initial	Blank	0.9575	0.45042	0.139	-0.3001	2.2151
			Saccharified	-15.4175*	0.45042	0.000	-16.6751	-14.1599
		Saccharified	Blank	16.3750*	0.45042	0.000	15.1174	17.6326
			Initial	15.4175*	0.45042	0.000	14.1599	16.6751
Sweet Potato	Tukey HSD	Blank	Initial	-3.4725*	0.83737	0.006	-5.8104	-1.1346
			Saccharified	-20.8975*	0.83737	0.000	-23.2354	-18.5596
		Initial	Blank	3.4725*	0.83737	0.006	1.1346	5.8104
			Saccharified	-17.4250*	0.83737	0.000	-19.7629	-15.0871
		Saccharified	Blank	20.8975*	0.83737	0.000	18.5596	23.2354
			Initial	17.4250*	0.83737	0.000	15.0871	19.7629

Based on observed means.
The error term is Mean Square(Error) = 1.402.
*. The mean difference is significant at the .05 level.

APPENDIX H

Post-Hoc Test for Substrate Effects on Reducing Sugar Content

Multiple Comparisons

Dependent Variable: Reducing_Sugar

Treatment		(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Blank	Tukey HSD	Sweet potato	Cassava	5.5630*	1.16581	0.003	2.3080	8.8180
			Mix	4.3780*	1.26918	0.018	0.8345	7.9215
		Cassava	Sweet potato	-5.5630*	1.16581	0.003	-8.8180	-2.3080
			Mix	-1.1850	1.32734	0.658	-4.8909	2.5209
		Mix	Sweet potato	-4.3780*	1.26918	0.018	-7.9215	-0.8345
			Cassava	1.1850	1.32734	0.658	-2.5209	4.8909
Initial	Tukey HSD	Sweet potato	Cassava	9.7900*	0.21316	0.000	9.1949	10.3851
			Mix	8.1125*	0.21316	0.000	7.5174	8.7076
		Cassava	Sweet potato	-9.7900*	0.21316	0.000	-10.3851	-9.1949
			Mix	-1.6775*	0.21316	0.000	-2.2726	-1.0824
		Mix	Sweet potato	-8.1125*	0.21316	0.000	-8.7076	-7.5174
			Cassava	1.6775*	0.21316	0.000	1.0824	2.2726
Saccharified	Tukey HSD	Sweet potato	Cassava	16.4150*	0.94961	0.000	13.7637	19.0663
			Mix	10.1200*	0.94961	0.000	7.4687	12.7713
		Cassava	Sweet potato	-16.4150*	0.94961	0.000	-19.0663	-13.7637
			Mix	-6.2950*	0.94961	0.000	-8.9463	-3.6437
		Mix	Sweet potato	-10.1200*	0.94961	0.000	-12.7713	-7.4687
			Cassava	6.2950*	0.94961	0.000	3.6437	8.9463

Based on observed means.
The error term is Mean Square(Error) = 1.804.

*. The mean difference is significant at the .05 level.

Normality Test of Amino Acid Content

Tests of Normality

Treatment			Kolmogorov-Smirnov[a]		
			Statistic	df	Sig.
Blank	Amino Acid Content (%wb)	Cassava	0.260	2	
		Mixture	0.260	2	
		Sweet Potato	0.260	2	
Initial	Amino Acid Content (%wb)	Cassava	0.260	2	
		Mixture	0.260	2	
		Sweet Potato	0.260	2	
Saccharified	Amino Acid Content (%wb)	Cassava	0.260	2	
		Mixture	0.260	2	
		Sweet Potato	0.260	2	

a. Lilliefors Significance Correction

APPENDIX I

AVNOVA Test of Amino Acid Content

Tests of Between-Subjects Effects

Dependent Variable: Amino Acid Content (%wb)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.047[a]	8	0.006	1079.491	0.000
Intercept	0.263	1	0.263	47779.205	0.000
Treatment	0.012	2	0.006	1098.815	0.000
Sample	0.015	2	0.008	1387.074	0.000
Treatment * Sample	0.020	4	0.005	916.038	0.000
Error	4.946E-05	9	5.495E-06		
Total	0.310	18			
Corrected Total	0.048	17			

a. R Squared = .999 (Adjusted R Squared = .998)

Post-Hoc Test for Treatment Effects on Amino Acid Content

Multiple Comparisons

Dependent Variable: Amino Acid Content (%wb)

Sample Type				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Cassava	Tukey HSD	Blank	Initial	-.0375*	0.00193	0.001	-0.0456	-0.0294
			Saccharified	0.0028	0.00193	0.436	-0.0053	0.0108
		Initial	Blank	.0375*	0.00193	0.001	0.0294	0.0456
			Saccharified	.0403*	0.00193	0.001	0.0322	0.0483
		Saccharified	Blank	-0.0028	0.00193	0.436	-0.0108	0.0053
			Initial	-.0403*	0.00193	0.001	-0.0483	-0.0322
Mixture	Tukey HSD	Blank	Initial	0.0036	0.00166	0.228	-0.0034	0.0105
			Saccharified	-.1427*	0.00166	0.000	-0.1496	-0.1357
		Initial	Blank	-0.0036	0.00166	0.228	-0.0105	0.0034
			Saccharified	-.1462*	0.00166	0.000	-0.1531	-0.1393
		Saccharified	Blank	.1427*	0.00166	0.000	0.1357	0.1496
			Initial	.1462*	0.00166	0.000	0.1393	0.1531
Sweet Potato	Tukey HSD	Blank	Initial	-.0320*	0.00316	0.004	-0.0452	-0.0188
			Saccharified	-.0477*	0.00316	0.001	-0.0609	-0.0345
		Initial	Blank	.0320*	0.00316	0.004	0.0188	0.0452
			Saccharified	-.0157*	0.00316	0.032	-0.0289	-0.0025
		Saccharified	Blank	.0477*	0.00316	0.001	0.0345	0.0609
			Initial	.0157*	0.00316	0.032	0.0025	0.0289

Based on observed means.
The error term is Mean Square(Error) = 1.442E-6.
*. The mean difference is significant at the 0.05 level.

APPENDIX J

Post-Hoc Test for Treatment Effects on Amino Acid Content

Multiple Comparisons

Dependent Variable: Amino_acid

Treatment		(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Blank	Tukey HSD	Sweet Potato	Cassava	-.036500*	0.0015737	0.000	-0.043076	-0.029924
			Mix	-.020650*	0.0015737	0.002	-0.027226	-0.014074
		Cassava	Sweet Potato	.036500*	0.0015737	0.000	0.029924	0.043076
			Mix	.015850*	0.0015737	0.004	0.009274	0.022426
		Mix	Sweet Potato	.020650*	0.0015737	0.002	0.014074	0.027226
			Cassava	-.015850*	0.0015737	0.004	-0.022426	-0.009274
Initial	Tukey HSD	Sweet Potato	Cassava	0.004550	0.0018748	0.179	-0.003284	0.012384
			Mix	-.015150*	0.0018748	0.008	-0.022984	-0.007316
		Cassava	Sweet Potato	-0.004550	0.0018748	0.179	-0.012384	0.003284
			Mix	-.019700*	0.0018748	0.004	-0.027534	-0.011866
		Mix	Sweet Potato	.015150*	0.0018748	0.008	0.007316	0.022984
			Cassava	.019700*	0.0018748	0.004	0.011866	0.027534
Treatment	Tukey HSD	Sweet Potato	Cassava	-.181900*	0.0032393	0.000	-0.195436	-0.168364
			Mix	-.071100*	0.0032393	0.000	-0.084636	-0.057564
		Cassava	Sweet Potato	.181900*	0.0032393	0.000	0.168364	0.195436
			Mix	.110800*	0.0032393	0.000	0.097264	0.124336
		Mix	Sweet Potato	.071100*	0.0032393	0.000	0.057564	0.084636
			Cassava	-.110800*	0.0032393	0.000	-0.124336	-0.097264

Based on observed means.
The error term is Mean Square(Error) = 1.950E-7.

*. The mean difference is significant at the .05 level.

Normality Test of Alcohol Content

Tests of Normality

Duration			Kolmogorov-Smirnov[a]			Shapiro-Wilk		
			Statistic	df	Sig.	Statistic	df	Sig.
Blank	Alcohol Content (%v/v)	Water	0.260	2				
		Agarwood	0.260	2				
Day 2	Alcohol Content (%v/v)	Water	0.287	4		0.871	4	0.302
		Agarwood	0.300	4		0.774	4	0.063
Day 3	Alcohol Content (%v/v)	Water	0.246	4		0.957	4	0.757
		Agarwood	0.201	4		0.960	4	0.781
Day 4	Alcohol Content (%v/v)	Water	0.299	4		0.796	4	0.095
		Agarwood	0.300	4		0.831	4	0.171
Day 5	Alcohol Content (%v/v)	Water	0.224	4		0.929	4	0.589
		Agarwood	0.280	4		0.853	4	0.237
Day 10	Alcohol Content (%v/v)	Water	0.364	4		0.840	4	0.195
		Agarwood	0.288	4		0.935	4	0.624

a. Lilliefors Significance Correction

APPENDIX K

ANOVA Test for Alcohol Content Across Fermentation Durations Under Water and Agarwood Leaves Treatment

Tests of Between-Subjects Effects

Dependent Variable: Alcohol Content (%v/v)

Treatment		Type III Sum of Squares	df	Mean Square	F	Sig.
Water	Corrected Model	17.223[a]	5	3.445	38.129	0.000
	Intercept	361.194	1	361.194	3998.054	0.000
	Duration	17.223	5	3.445	38.129	0.000
	Error	1.445	16	0.090		
	Total	392.608	22			
	Corrected Total	18.669	21			
Agarwood	Corrected Model	19.208[b]	5	3.842	22.275	0.000
	Intercept	331.551	1	331.551	1922.436	0.000
	Duration	19.208	5	3.842	22.275	0.000
	Error	2.759	16	0.172		
	Total	356.827	22			
	Corrected Total	21.968	21			

a. R Squared = .923 (Adjusted R Squared = .898)

b. R Squared = .874 (Adjusted R Squared = .835)

Post-Hoc Test for Alcohol Content Across Different Fermentation Durations (Water Treatment)

Multiple Comparisons

Dependent Variable: Alcohol Content (%v/v)

Treatment				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Water	Tukey HSD	Blank	Day 2	0.4577	0.26030	0.517	-0.3811	1.2964
			Day 3	0.4718	0.26030	0.485	-0.3670	1.3105
			Day 4	0.4323	0.26030	0.574	-0.4065	1.2710
			Day 5	0.4238	0.26030	0.593	-0.4150	1.2625
			Day 10	2.6667*	0.26030	0.000	1.8280	3.5054
		Day 2	Blank	-0.4577	0.26030	0.517	-1.2964	0.3811
			Day 3	0.0141	0.21254	1.000	-0.6707	0.6989
			Day 4	-0.0254	0.21254	1.000	-0.7102	0.6594
			Day 5	-0.0339	0.21254	1.000	-0.7187	0.6509
			Day 10	2.2091*	0.21254	0.000	1.5242	2.8939
		Day 3	Blank	-0.4718	0.26030	0.485	-1.3105	0.3670
			Day 2	-0.0141	0.21254	1.000	-0.6989	0.6707
			Day 4	-0.0395	0.21254	1.000	-0.7243	0.6453
			Day 5	-0.0480	0.21254	1.000	-0.7328	0.6368
			Day 10	2.1949*	0.21254	0.000	1.5101	2.8797
		Day 4	Blank	-0.4323	0.26030	0.574	-1.2710	0.4065
			Day 2	0.0254	0.21254	1.000	-0.6594	0.7102
			Day 3	0.0395	0.21254	1.000	-0.6453	0.7243
			Day 5	-0.0085	0.21254	1.000	-0.6933	0.6763
			Day 10	2.2345*	0.21254	0.000	1.5496	2.9193
		Day 5	Blank	-0.4238	0.26030	0.593	-1.2625	0.4150
			Day 2	0.0339	0.21254	1.000	-0.6509	0.7187
			Day 3	0.0480	0.21254	1.000	-0.6368	0.7328
			Day 4	0.0085	0.21254	1.000	-0.6763	0.6933
			Day 10	2.2430*	0.21254	0.000	1.5581	2.9278
		Day 10	Blank	-2.6667*	0.26030	0.000	-3.5054	-1.8280
			Day 2	-2.2090*	0.21254	0.000	-2.8939	-1.5242
			Day 3	-2.1949*	0.21254	0.000	-2.8797	-1.5101
			Day 4	-2.2345*	0.21254	0.000	-2.9193	-1.5496
			Day 5	-2.2429*	0.21254	0.000	-2.9278	-1.5581

APPENDIX L

Post-Hoc Test for Alcohol Content Across Different Fermentation Durations (Water Treatment)

Agarwood	Tukey HSD	Blank	Day 2	0.9322	0.35965	0.156	-0.2266	2.0910
			Day 3	1.0763	0.35965	0.077	-0.0826	2.2351
			Day 4	1.0763	0.35965	0.077	-0.0826	2.2351
			Day 5	1.1582	0.35965	0.050	-0.0006	2.3170
			Day 10	3.2288*	0.35965	0.000	2.0700	4.3877
		Day 2	Blank	-0.9322	0.35965	0.156	-2.0910	0.2266
			Day 3	0.1441	0.29365	0.996	-0.8021	1.0903
			Day 4	0.1441	0.29365	0.996	-0.8021	1.0903
			Day 5	0.2260	0.29365	0.969	-0.7202	1.1722
			Day 10	2.2966*	0.29365	0.000	1.3504	3.2428
		Day 3	Blank	-1.0763	0.35965	0.077	-2.2351	0.0826
			Day 2	-0.1441	0.29365	0.996	-1.0903	0.8021
			Day 4	0.0000	0.29365	1.000	-0.9462	0.9462
			Day 5	0.0819	0.29365	1.000	-0.8643	1.0281
			Day 10	2.1526*	0.29365	0.000	1.2064	3.0987
		Day 4	Blank	-1.0763	0.35965	0.077	-2.2351	0.0826
			Day 2	-0.1441	0.29365	0.996	-1.0903	0.8021
			Day 3	0.0000	0.29365	1.000	-0.9462	0.9462
			Day 5	0.0819	0.29365	1.000	-0.8643	1.0281
			Day 10	2.1525*	0.29365	0.000	1.2064	3.0987
		Day 5	Blank	-1.1582	0.35965	0.050	-2.3170	0.0006
			Day 2	-0.2260	0.29365	0.969	-1.1722	0.7202
			Day 3	-0.0819	0.29365	1.000	-1.0281	0.8643
			Day 4	-0.0819	0.29365	1.000	-1.0281	0.8643
			Day 10	2.0706*	0.29365	0.000	1.1244	3.0168
		Day 10	Blank	-3.2288*	0.35965	0.000	-4.3877	-2.0700
			Day 2	-2.2966*	0.29365	0.000	-3.2428	-1.3504
			Day 3	-2.1526*	0.29365	0.000	-3.0987	-1.2064
			Day 4	-2.1525*	0.29365	0.000	-3.0987	-1.2064
			Day 5	-2.0706*	0.29365	0.000	-3.0168	-1.1244

AVNOVA Test for Alcohol Content Between Treatments at Different Fermentation Durations

Tests of Between-Subjects Effects

Dependent Variable: Alcohol_content

Duration	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Blank	Corrected Model	.107[a]	1	0.107	1.396	0.359
	Intercept	103.880	1	103.880	1350.747	0.001
	Sample	0.107	1	0.107	1.396	0.359
	Error	0.154	2	0.077		
	Total	104.141	4			
	Corrected Total	0.261	3			
Day 2	Corrected Model	.043[b]	1	0.043	0.302	0.603
	Intercept	154.961	1	154.961	1083.224	0.000
	Sample	0.043	1	0.043	0.302	0.603
	Error	0.858	6	0.143		
	Total	155.862	8			
	Corrected Total	0.901	7			
Day 3	Corrected Model	.153[c]	1	0.153	1.569	0.257
	Intercept	149.441	1	149.441	1529.359	0.000
	Sample	0.153	1	0.153	1.569	0.257
	Error	0.586	6	0.098		
	Total	150.181	8			
	Corrected Total	0.740	7			
Day 4	Corrected Model	.200[d]	1	0.200	0.570	0.479
	Intercept	150.811	1	150.811	429.045	0.000
	Sample	0.200	1	0.200	0.570	0.479
	Error	2.109	6	0.352		
	Total	153.120	8			
	Corrected Total	2.309	7			
Day 5	Corrected Model	.331[e]	1	0.331	5.366	0.060
	Intercept	148.271	1	148.271	2404.100	0.000
	Sample	0.331	1	0.331	5.366	0.060
	Error	0.370	6	0.062		
	Total	148.972	8			
	Corrected Total	0.701	7			
Day 10	Corrected Model	.110[f]	1	0.110	5.178	0.063
	Intercept	36.922	1	36.922	1738.832	0.000
	Sample	0.110	1	0.110	5.178	0.063
	Error	0.127	6	0.021		
	Total	37.159	8			
	Corrected Total	0.237	7			

APPENDIX M

Normality Test of pH Value

Tests of Normality

Duration			Kolmogorov-Smirnov[a]			Shapiro-Wilk		
			Statistic	df	Sig.	Statistic	df	Sig.
Blank	pH value	Water	0.260	2				
		Agarwood	0.260	2				
Control (Day 0)	pH value	Water	0.250	4		0.945	4	0.683
		Agarwood	0.250	4		0.945	4	0.683
Day 2	pH value	Water	0.307	4		0.729	4	0.024
		Agarwood	0.260	4		0.827	4	0.161
Day 3	pH value	Water	0.441	4		0.630	4	0.001
		Agarwood	0.441	4		0.630	4	0.001
Day 4	pH value	Water	0.192	4		0.971	4	0.850
		Agarwood	0.304	4		0.811	4	0.123
Day 5	pH value	Water	0.307	4		0.729	4	0.024
		Agarwood	0.151	4		0.993	4	0.972
Day 10	pH value	Water	0.269	4		0.878	4	0.332
		Agarwood	0.305	4		0.799	4	0.100

a. Lilliefors Significance Correction

ANOVA Test for pH Value Across Fermentation Durations Under Water and Agarwood Leaves Treatment

Tests of Between-Subjects Effects

Dependent Variable: pH value

Treatment		Type III Sum of Squares	df	Mean Square	F	Sig.
Water	Corrected Model	.233[a]	6	0.039	70.536	0.000
	Intercept	363.623	1	363.623	659554.896	0.000
	Duration	0.233	6	0.039	70.536	0.000
	Error	0.010	19	0.001		
	Total	387.633	26			
	Corrected Total	0.244	25			
Agarwood	Corrected Model	.142[b]	6	0.024	62.499	0.000
	Intercept	377.369	1	377.369	999305.008	0.000
	Duration	0.142	6	0.024	62.499	0.000
	Error	0.007	19	0.000		
	Total	401.559	26			
	Corrected Total	0.149	25			

a. R Squared = .957 (Adjusted R Squared = .943)

b. R Squared = .952 (Adjusted R Squared = .937)

APPENDIX N

Post-Hoc Test forpH Value Across Different Fermentation Durations (Water Treatment)

Multiple Comparisons

Dependent Variable: pH value

Treatment				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Water	Tukey HSD	Blank	Control (Day 0)	0.0350	0.02033	0.611	-0.0318	0.1018
			Day 2	-.0900*	0.02033	0.004	-0.1568	-0.0232
			Day 3	-.0925*	0.02033	0.003	-0.1593	-0.0257
			Day 4	-.0975*	0.02033	0.002	-0.1643	-0.0307
			Day 5	-.1600*	0.02033	0.000	-0.2268	-0.0932
			Day 10	-.2775*	0.02033	0.000	-0.3443	-0.2107
		Control (Day 0)	Blank	-0.0350	0.02033	0.611	-0.1018	0.0318
			Day 2	-.1250*	0.01660	0.000	-0.1795	-0.0705
			Day 3	-.1275*	0.01660	0.000	-0.1820	-0.0730
			Day 4	-.1325*	0.01660	0.000	-0.1870	-0.0780
			Day 5	-.1950*	0.01660	0.000	-0.2495	-0.1405
			Day 10	-.3125*	0.01660	0.000	-0.3670	-0.2580
		Day 2	Blank	.0900*	0.02033	0.004	0.0232	0.1568
			Control (Day 0)	.1250*	0.01660	0.000	0.0705	0.1795
			Day 3	-0.0025	0.01660	1.000	-0.0570	0.0520
			Day 4	-0.0075	0.01660	0.999	-0.0620	0.0470
			Day 5	-.0700*	0.01660	0.007	-0.1245	-0.0155
			Day 10	-.1875*	0.01660	0.000	-0.2420	-0.1330
		Day 3	Blank	.0925*	0.02033	0.003	0.0257	0.1593
			Control (Day 0)	.1275*	0.01660	0.000	0.0730	0.1820
			Day 2	0.0025	0.01660	1.000	-0.0520	0.0570
			Day 4	-0.0050	0.01660	1.000	-0.0595	0.0495
			Day 5	-.0675*	0.01660	0.010	-0.1220	-0.0130
			Day 10	-.1850*	0.01660	0.000	-0.2395	-0.1305
		Day 4	Blank	.0975*	0.02033	0.002	0.0307	0.1643
			Control (Day 0)	.1325*	0.01660	0.000	0.0780	0.1870
			Day 2	0.0075	0.01660	0.999	-0.0470	0.0620
			Day 3	0.0050	0.01660	1.000	-0.0495	0.0595
			Day 5	-.0625*	0.01660	0.019	-0.1170	-0.0080
			Day 10	-.1800*	0.01660	0.000	-0.2345	-0.1255
		Day 5	Blank	.1600*	0.02033	0.000	0.0932	0.2268
			Control (Day 0)	.1950*	0.01660	0.000	0.1405	0.2495
			Day 2	.0700*	0.01660	0.007	0.0155	0.1245
			Day 3	.0675*	0.01660	0.010	0.0130	0.1220
			Day 4	.0625*	0.01660	0.019	0.0080	0.1170
			Day 10	-.1175*	0.01660	0.000	-0.1720	-0.0630
		Day 10	Blank	.2775*	0.02033	0.000	0.2107	0.3443
			Control (Day 0)	.3125*	0.01660	0.000	0.2580	0.3670
			Day 2	.1875*	0.01660	0.000	0.1330	0.2420
			Day 3	.1850*	0.01660	0.000	0.1305	0.2395
			Day 4	.1800*	0.01660	0.000	0.1255	0.2345
			Day 5	.1175*	0.01660	0.000	0.0630	0.1720

APPENDIX O

Post-Hoc Test forpH Value Across Different Fermentation Durations (Agarwood Leaves Treatment)

Agarwood	Tukey HSD	Blank	Control (Day 0)	0.0450	0.01683	0.159	-0.0103	0.1003
			Day 2	-0.0350	0.01683	0.402	-0.0903	0.0203
			Day 3	-0.0375	0.01683	0.327	-0.0928	0.0178
			Day 4	-.0925*	0.01683	0.000	-0.1478	-0.0372
			Day 5	-.1000*	0.01683	0.000	-0.1553	-0.0447
			Day 10	-.1975*	0.01683	0.000	-0.2528	-0.1422
		Control (Day 0)	Blank	-0.0450	0.01683	0.159	-0.1003	0.0103
			Day 2	-.0800*	0.01374	0.000	-0.1251	-0.0349
			Day 3	-.0825*	0.01374	0.000	-0.1276	-0.0374
			Day 4	-.1375*	0.01374	0.000	-0.1826	-0.0924
			Day 5	-.1450*	0.01374	0.000	-0.1901	-0.0999
			Day 10	-.2425*	0.01374	0.000	-0.2876	-0.1974
		Day 2	Blank	0.0350	0.01683	0.402	-0.0203	0.0903
			Control (Day 0)	.0800*	0.01374	0.000	0.0349	0.1251
			Day 3	-0.0025	0.01374	1.000	-0.0476	0.0426
			Day 4	-.0575*	0.01374	0.008	-0.1026	-0.0124
			Day 5	-.0650*	0.01374	0.002	-0.1101	-0.0199
			Day 10	-.1625*	0.01374	0.000	-0.2076	-0.1174
		Day 3	Blank	0.0375	0.01683	0.327	-0.0178	0.0928
			Control (Day 0)	.0825*	0.01374	0.000	0.0374	0.1276
			Day 2	0.0025	0.01374	1.000	-0.0426	0.0476
			Day 4	-.0550*	0.01374	0.011	-0.1001	-0.0099
			Day 5	-.0625*	0.01374	0.003	-0.1076	-0.0174
			Day 10	-.1600*	0.01374	0.000	-0.2051	-0.1149
		Day 4	Blank	.0925*	0.01683	0.000	0.0372	0.1478
			Control (Day 0)	.1375*	0.01374	0.000	0.0924	0.1826
			Day 2	.0575*	0.01374	0.008	0.0124	0.1026
			Day 3	.0550*	0.01374	0.011	0.0099	0.1001
			Day 5	-0.0075	0.01374	0.998	-0.0526	0.0376
			Day 10	-.1050*	0.01374	0.000	-0.1501	-0.0599
		Day 5	Blank	.1000*	0.01683	0.000	0.0447	0.1553
			Control (Day 0)	.1450*	0.01374	0.000	0.0999	0.1901
			Day 2	.0650*	0.01374	0.002	0.0199	0.1101
			Day 3	.0625*	0.01374	0.003	0.0174	0.1076
			Day 4	0.0075	0.01374	0.998	-0.0376	0.0526
			Day 10	-.0975*	0.01374	0.000	-0.1426	-0.0524
		Day 10	Blank	.1975*	0.01683	0.000	0.1422	0.2528
			Control (Day 0)	.2425*	0.01374	0.000	0.1974	0.2876
			Day 2	.1625*	0.01374	0.000	0.1174	0.2076
			Day 3	.1600*	0.01374	0.000	0.1149	0.2051
			Day 4	.1050*	0.01374	0.000	0.0599	0.1501
			Day 5	.0975*	0.01374	0.000	0.0524	0.1426

APPENDIX P

AVNOVA Test forpH ValueBetween Treatments at Different Fermentation Durations

Tests of Between-Subjects Effects

Dependent Variable: pH_value

Duration	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Blank	Corrected Model	.012[a]	1	0.012	9.680	0.090
	Intercept	58.064	1	58.064	46451.520	0.000
	Treatment	0.012	1	0.012	9.680	0.090
	Error	0.002	2	0.001		
	Total	58.079	4			
	Corrected Total	0.015	3			
Day 0	Corrected Model	.020[b]	1	0.020	300.000	0.000
	Intercept	113.703	1	113.703	1705548.000	0.000
	Treatment	0.020	1	0.020	300.000	0.000
	Error	0.000	6	6.667E-05		
	Total	113.724	8			
	Corrected Total	0.020	7			
Day 2	Corrected Model	.006[c]	1	0.006	51.857	0.000
	Intercept	119.970	1	119.970	1028314.714	0.000
	Treatment	0.006	1	0.006	51.857	0.000
	Error	0.001	6	0.000		
	Total	119.977	8			
	Corrected Total	0.007	7			
Day 3	Corrected Model	.006[d]	1	0.006	48.400	0.000
	Intercept	120.125	1	120.125	961000.000	0.000
	Treatment	0.006	1	0.006	48.400	0.000
	Error	0.001	6	0.000		
	Total	120.132	8			
	Corrected Total	0.007	7			
Day 4	Corrected Model	.022[e]	1	0.022	44.847	0.001
	Intercept	121.992	1	121.992	248119.729	0.000
	Treatment	0.022	1	0.022	44.847	0.001
	Error	0.003	6	0.000		
	Total	122.017	8			
	Corrected Total	0.025	7			
Day 5	Corrected Model	.005[f]	1	0.005	21.429	0.004
	Intercept	124.189	1	124.189	532237.714	0.000
	Treatment	0.005	1	0.005	21.429	0.004
	Error	0.001	6	0.000		
	Total	124.195	8			
	Corrected Total	0.006	7			
Day 10	Corrected Model	.002[g]	1	0.002	1.207	0.314
	Intercept	131.058	1	131.058	87860.145	0.000
	Treatment	0.002	1	0.002	1.207	0.314
	Error	0.009	6	0.001		
	Total	131.069	8			
	Corrected Total	0.011	7			

APPENDIX Q

Normality Test of Total Titratable Acid

Tests of Normality[a,c]

Duration			Kolmogorov-Smirnov[b] Statistic	df	Sig.
Day 0	Total Titratable Acid	Agarwood	0.260	2	
		Water		2	
Day 2	Total Titratable Acid	Agarwood	0.260	2	
		Water	0.260	2	
Day 3	Total Titratable Acid	Agarwood	0.260	2	
		Water	0.260	2	
Day 4	Total Titratable Acid	Agarwood	0.260	2	
		Water	0.260	2	
Day 5	Total Titratable Acid	Agarwood		2	
		Water	0.260	2	
Day 10	Total Titratable Acid	Agarwood		2	
		Water	0.260	2	

a. Total Titratable Acid is constant when Sample = Agarwood in one or more split files. It has been omitted.

b. Lilliefors Significance Correction

c. Total Titratable Acid is constant when Sample = Water in one or more split files. It has been omitted.

AVNOVATest for Total Titratable Acid Between Treatments at Different Fermentation Durations

Tests of Between-Subjects Effects

Dependent Variable: Total Titratable Acid

Duration		Type III Sum of Squares	df	Mean Square	F	Sig.
Blank	Corrected Model	.031[a]	1	0.031		
	Intercept	11.301	1	11.301		
	Sample	0.031	1	0.031		
	Error	0.000	0			
	Total	11.332	2			
	Corrected Total	0.031	1			
Day 0	Corrected Model	.811[b]	1	0.811	16.000	0.057
	Intercept	45.644	1	45.644	900.000	0.001
	Sample	0.811	1	0.811	16.000	0.057
	Error	0.101	2	0.051		
	Total	46.556	4			
	Corrected Total	0.913	3			
Day 2	Corrected Model	.013[c]	1	0.013	0.200	0.698
	Intercept	38.353	1	38.353	605.000	0.002
	Sample	0.013	1	0.013	0.200	0.698
	Error	0.127	2	0.063		
	Total	38.493	4			
	Corrected Total	0.139	3			
Day 3	Corrected Model	.000[d]	1	0.000	0.000	1.000
	Intercept	36.971	1	36.971	291.600	0.003
	Sample	0.000	1	0.000	0.000	1.000
	Error	0.254	2	0.127		
	Total	37.225	4			
	Corrected Total	0.254	3			
Day 4	Corrected Model	.000[e]	1	0.000	0.000	1.000
	Intercept	36.971	1	36.971	1458.000	0.001
	Sample	0.000	1	0.000	0.000	1.000
	Error	0.051	2	0.025		
	Total	37.022	4			
	Corrected Total	0.051	3			
Day 5	Corrected Model	.013[e]	1	0.013	1.000	0.423
	Intercept	25.674	1	25.674	2025.000	0.000
	Sample	0.013	1	0.013	1.000	0.423
	Error	0.025	2	0.013		
	Total	25.713	4			
	Corrected Total	0.038	3			
Day 10	Corrected Model	.317[f]	1	0.317	25.000	0.038
	Intercept	15.531	1	15.531	1225.000	0.001
	Sample	0.317	1	0.317	25.000	0.038
	Error	0.025	2	0.013		
	Total	15.874	4			
	Corrected Total	0.342	3			

a. R Squared = 1.000 (Adjusted R Squared = .)

b. R Squared = .889 (Adjusted R Squared = .833)

c. R Squared = .091 (Adjusted R Squared = -.364)

APPENDIX R

Normality Test of Total Phenolic Content

Tests of Normality

Duration			Kolmogorov-Smirnov[a]			Shapiro-Wilk		
			Statistic	df	Sig.	Statistic	df	Sig.
Blank	Total Phenolic Content (mg/L)	Water	0.260	2				
		Agarwood	0.260	2				
Control (Day 0)	Total Phenolic Content (mg/L)	Water	0.304	4		0.737	4	0.029
		Agarwood	0.296	4		0.830	4	0.168
Day 2	Total Phenolic Content (mg/L)	Water	0.405	4		0.732	4	0.026
		Agarwood	0.301	4		0.924	4	0.561
Day 3	Total Phenolic Content (mg/L)	Water	0.376	4		0.796	4	0.095
		Agarwood	0.197	4		0.971	4	0.847
Day 4	Total Phenolic Content (mg/L)	Water	0.207	4		0.968	4	0.831
		Agarwood	0.224	4		0.929	4	0.589
Day 5	Total Phenolic Content (mg/L)	Water	0.359	4		0.760	4	0.047
		Agarwood	0.186	4		0.989	4	0.952
Day 10	Total Phenolic Content (mg/L)	Water	0.294	4		0.802	4	0.106
		Agarwood	0.274	4		0.864	4	0.275

a. Lilliefors Significance Correction

ANOVA Test for Total Phenolic Content Across Fermentation Durations Under Water and Agarwood Leaves Treatment

Tests of Between-Subjects Effects

Dependent Variable: Total Phenolic Content (mg/L)

Treatment		Type III Sum of Squares	df	Mean Square	F	Sig.
Water	Corrected Model	454.412[a]	6	75.735	2.507	0.059
	Intercept	25210.341	1	25210.341	834.364	0.000
	Duration	454.412	6	75.735	2.507	0.059
	Error	574.085	19	30.215		
	Total	27632.565	26			
	Corrected Total	1028.497	25			
Agarwood	Corrected Model	152.941[b]	6	25.490	2.372	0.070
	Intercept	51814.811	1	51814.811	4820.655	0.000
	Duration	152.941	6	25.490	2.372	0.070
	Error	204.222	19	10.749		
	Total	55502.268	26			
	Corrected Total	357.163	25			

a. R Squared = .442 (Adjusted R Squared = .266)

b. R Squared = .428 (Adjusted R Squared = .248)

APPENDIX S

Post-Hoc Test for Total Titratable Acid Across Different Fermentation Durations (Water Treatment)

Treatment				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Water	Tukey HSD	Blank	Control (Day 0)	2.5974	4.76039	0.998	-13.0383	18.2331
			Day 2	1.2987	4.76039	1.000	-14.3370	16.9344
			Day 3	4.7727	4.76039	0.947	-10.8629	20.4084
			Day 4	6.7532	4.76039	0.786	-8.8824	22.3889
			Day 5	-0.3896	4.76039	1.000	-16.0253	15.2460
			Day 10	-6.8507	4.76039	0.775	-22.4863	8.7850
		Control (Day 0)	Blank	-2.5974	4.76039	0.998	-18.2331	13.0383
			Day 2	-1.2987	3.88684	1.000	-14.0652	11.4678
			Day 3	2.1753	3.88684	0.997	-10.5911	14.9418
			Day 4	4.1558	3.88684	0.930	-8.6106	16.9223
			Day 5	-2.9870	3.88684	0.985	-15.7535	9.7794
			Day 10	-9.4481	3.88684	0.239	-22.2145	3.3184
		Day 2	Blank	-1.2987	4.76039	1.000	-16.9344	14.3370
			Control (Day 0)	1.2987	3.88684	1.000	-11.4678	14.0652
			Day 3	3.4740	3.88684	0.969	-9.2924	16.2405
			Day 4	5.4545	3.88684	0.794	-7.3119	18.2210
			Day 5	-1.6883	3.88684	0.999	-14.4548	11.0781
			Day 10	-8.1494	3.88684	0.393	-20.9158	4.6171
		Day 3	Blank	-4.7727	4.76039	0.947	-20.4084	10.8629
			Control (Day 0)	-2.1753	3.88684	0.997	-14.9418	10.5911
			Day 2	-3.4740	3.88684	0.969	-16.2405	9.2924
			Day 4	1.9805	3.88684	0.998	-10.7860	14.7470
			Day 5	-5.1624	3.88684	0.831	-17.9288	7.6041
			Day 10	-11.6234	3.88684	0.089	-24.3899	1.1431
		Day 4	Blank	-6.7532	4.76039	0.786	-22.3889	8.8824
			Control (Day 0)	-4.1558	3.88684	0.930	-16.9223	8.6106
			Day 2	-5.4545	3.88684	0.794	-18.2210	7.3119
			Day 3	-1.9805	3.88684	0.998	-14.7470	10.7860
			Day 5	-7.1429	3.88684	0.541	-19.9093	5.6236
			Day 10	-13.6039*	3.88684	0.032	-26.3704	-0.8374
		Day 5	Blank	0.3896	4.76039	1.000	-15.2460	16.0253
			Control (Day 0)	2.9870	3.88684	0.985	-9.7794	15.7535
			Day 2	1.6883	3.88684	0.999	-11.0781	14.4548
			Day 3	5.1624	3.88684	0.831	-7.6041	17.9288
			Day 4	7.1429	3.88684	0.541	-5.6236	19.9093
			Day 10	-6.4611	3.88684	0.647	-19.2275	6.3054
		Day 10	Blank	6.8507	4.76039	0.775	-8.7850	22.4863
			Control (Day 0)	9.4481	3.88684	0.239	-3.3184	22.2145
			Day 2	8.1494	3.88684	0.393	-4.6171	20.9158
			Day 3	11.6234	3.88684	0.089	-1.1431	24.3899
			Day 4	13.6039*	3.88684	0.032	0.8374	26.3704
			Day 5	6.4611	3.88684	0.647	-6.3054	19.2275

APPENDIX T

Post-Hoc Test for Total Titratable Acid Across Different Fermentation Durations (Agarwood Leaves Treatment)

Agarwood	Tukey HSD	Blank	Control (Day 0)	0.3571	2.83926	1.000	-8.9685	9.6828
			Day 2	0.7143	2.83926	1.000	-8.6114	10.0399
			Day 3	-2.2078	2.83926	0.985	-11.5335	7.1178
			Day 4	0.2597	2.83926	1.000	-9.0659	9.5854
			Day 5	0.9740	2.83926	1.000	-8.3516	10.2996
			Day 10	-6.1039	2.83926	0.365	-15.4295	3.2217
		Control (Day 0)	Blank	-0.3571	2.83926	1.000	-9.6828	8.9685
			Day 2	0.3572	2.31824	1.000	-7.2572	7.9715
			Day 3	-2.5650	2.31824	0.918	-10.1793	5.0494
			Day 4	-0.0974	2.31824	1.000	-7.7118	7.5170
			Day 5	0.6169	2.31824	1.000	-6.9975	8.2312
			Day 10	-6.4610	2.31824	0.130	-14.0754	1.1533
		Day 2	Blank	-0.7143	2.83926	1.000	-10.0399	8.6114
			Control (Day 0)	-0.3572	2.31824	1.000	-7.9715	7.2572
			Day 3	-2.9221	2.31824	0.861	-10.5365	4.6923
			Day 4	-0.4546	2.31824	1.000	-8.0689	7.1598
			Day 5	0.2597	2.31824	1.000	-7.3546	7.8741
			Day 10	-6.8182	2.31824	0.098	-14.4325	0.7962
		Day 3	Blank	2.2078	2.83926	0.985	-7.1178	11.5335
			Control (Day 0)	2.5650	2.31824	0.918	-5.0494	10.1793
			Day 2	2.9221	2.31824	0.861	-4.6923	10.5365
			Day 4	2.4676	2.31824	0.931	-5.1468	10.0819
			Day 5	3.1818	2.31824	0.809	-4.4325	10.7962
			Day 10	-3.8961	2.31824	0.636	-11.5104	3.7183
		Day 4	Blank	-0.2597	2.83926	1.000	-9.5854	9.0659
			Control (Day 0)	0.0974	2.31824	1.000	-7.5170	7.7118
			Day 2	0.4546	2.31824	1.000	-7.1598	8.0689
			Day 3	-2.4676	2.31824	0.931	-10.0819	5.1468
			Day 5	0.7143	2.31824	1.000	-6.9001	8.3286
			Day 10	-6.3636	2.31824	0.140	-13.9780	1.2507
		Day 5	Blank	-0.9740	2.83926	1.000	-10.2996	8.3516
			Control (Day 0)	-0.6169	2.31824	1.000	-8.2312	6.9975
			Day 2	-0.2597	2.31824	1.000	-7.8741	7.3546
			Day 3	-3.1818	2.31824	0.809	-10.7962	4.4325
			Day 4	-0.7143	2.31824	1.000	-8.3286	6.9001
			Day 10	-7.0779	2.31824	0.079	-14.6923	0.5365
		Day 10	Blank	6.1039	2.83926	0.365	-3.2217	15.4295
			Control (Day 0)	6.4610	2.31824	0.130	-1.1533	14.0754
			Day 2	6.8182	2.31824	0.098	-0.7962	14.4325
			Day 3	3.8961	2.31824	0.636	-3.7183	11.5104
			Day 4	6.3636	2.31824	0.140	-1.2507	13.9780
			Day 5	7.0779	2.31824	0.079	-0.5365	14.6923

APPENDIX U

AVNOVATest for Total Phenolic Content Between Treatments at Different Fermentation Durations

Tests of Between-Subjects Effects

Dependent Variable: Total_phenolic_content

Duration	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Blank	Corrected Model	141.208[a]	1	141.208	19.875	0.047
	Intercept	6142.891	1	6142.891	864.591	0.001
	Treatment	141.208	1	141.208	19.875	0.047
	Error	14.210	2	7.105		
	Total	6298.309	4			
	Corrected Total	155.418	3			
Day 0	Corrected Model	398.939[b]	1	398.939	9.978	0.020
	Intercept	11376.979	1	11376.979	284.562	0.000
	Treatment	398.939	1	398.939	9.978	0.020
	Error	239.884	6	39.981		
	Total	12015.802	8			
	Corrected Total	638.824	7			
Day 2	Corrected Model	310.878[c]	1	310.878	10.305	0.018
	Intercept	11662.806	1	11662.806	386.593	0.000
	Treatment	310.878	1	310.878	10.305	0.018
	Error	181.009	6	30.168		
	Total	12154.694	8			
	Corrected Total	491.888	7			
Day 3	Corrected Model	711.675[d]	1	711.675	31.878	0.001
	Intercept	11494.828	1	11494.828	514.894	0.000
	Treatment	711.675	1	711.675	31.878	0.001
	Error	133.948	6	22.325		
	Total	12340.450	8			
	Corrected Total	845.623	7			
Day 4	Corrected Model	675.399[e]	1	675.399	58.731	0.000
	Intercept	10185.541	1	10185.541	885.707	0.000
	Treatment	675.399	1	675.399	58.731	0.000
	Error	68.999	6	11.500		
	Total	10929.939	8			
	Corrected Total	744.398	7			
Day 5	Corrected Model	221.319[f]	1	221.319	97.440	0.000
	Intercept	12103.261	1	12103.261	5328.716	0.000
	Treatment	221.319	1	221.319	97.440	0.000
	Error	13.628	6	2.271		
	Total	12338.207	8			
	Corrected Total	234.947	7			
Day 10	Corrected Model	248.035[g]	1	248.035	11.753	0.014
	Intercept	16682.767	1	16682.767	790.476	0.000
	Treatment	248.035	1	248.035	11.753	0.014
	Error	126.628	6	21.105		
	Total	17057.431	8			
	Corrected Total	374.664	7			

APPENDIX V

Normality Test of Acetic Acid Content

Tests of Normality

Duration			Kolmogorov-Smirnov[a]			Shapiro-Wilk		
			Statistic	df	Sig.	Statistic	df	Sig.
Blank	Acetic Acid Content	Water	0.292	3		0.924	3	0.467
		Agarwood	0.356	3		0.818	3	0.158
Control (Day 0)	Acetic Acid Content	Water	0.342	3		0.845	3	0.226
		Agarwood	0.311	3		0.897	3	0.377
Day 2	Acetic Acid Content	Water	0.334	3		0.859	3	0.265
		Agarwood	0.343	3		0.842	3	0.220
Day 3	Acetic Acid Content	Water	0.370	3		0.787	3	0.084
		Agarwood	0.176	3		1.000	3	0.979
Day 4	Acetic Acid Content	Water	0.230	3		0.981	3	0.735
		Agarwood	0.377	3		0.771	3	0.046
Day 5	Acetic Acid Content	Water	0.312	3		0.896	3	0.374
		Agarwood	0.296	3		0.918	3	0.446
Day 10	Acetic Acid Content	Water	0.243	3		0.973	3	0.682
		Agarwood	0.214	3		0.989	3	0.804

a. Lilliefors Significance Correction

ANOVA Test for Acetic Acid Content Across Fermentation Durations Under Water and Agarwood Leaves Treatment

Tests of Between-Subjects Effects

Dependent Variable: Acetic Acid Content

Treatment		Type III Sum of Squares	df	Mean Square	F	Sig.
Water	Corrected Model	17.030[a]	6	2.838	39.655	0.000
	Intercept	148.845	1	148.845	2079.572	0.000
	Duration	17.030	6	2.838	39.655	0.000
	Error	1.002	14	0.072		
	Total	166.876	21			
	Corrected Total	18.032	20			
Agarwood	Corrected Model	23.069[b]	6	3.845	40.024	0.000
	Intercept	132.394	1	132.394	1378.197	0.000
	Duration	23.069	6	3.845	40.024	0.000
	Error	1.345	14	0.096		
	Total	156.808	21			
	Corrected Total	24.414	20			

a. R Squared = .944 (Adjusted R Squared = .921)

b. R Squared = .945 (Adjusted R Squared = .921)

APPENDIX W

Post-Hoc Test forAcetic Acid Content Across Different Fermentation Durations (Water Treatment)

Multiple Comparisons

Dependent Variable: Acetic Acid Content

Treatment				Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Water	Tukey HSD	Blank	Control (Day 0)	-2.0432*	0.21844	0.000	-2.7891	-1.2973
			Day 2	-1.4485*	0.21844	0.000	-2.1944	-0.7026
			Day 3	-1.1614*	0.21844	0.002	-1.9073	-0.4155
			Day 4	-1.6045*	0.21844	0.000	-2.3504	-0.8586
			Day 5	-.7910*	0.21844	0.035	-1.5369	-0.0451
			Day 10	-3.0873*	0.21844	0.000	-3.8332	-2.3414
		Control (Day 0)	Blank	2.0432*	0.21844	0.000	1.2973	2.7891
			Day 2	0.5947	0.21844	0.163	-0.1512	1.3406
			Day 3	.8819*	0.21844	0.016	0.1360	1.6278
			Day 4	0.4387	0.21844	0.451	-0.3072	1.1846
			Day 5	1.2522*	0.21844	0.001	0.5083	1.9961
			Day 10	-1.0441*	0.21844	0.004	-1.7900	-0.2982
		Day 2	Blank	1.4485*	0.21844	0.000	0.7026	2.1944
			Control (Day 0)	-0.5947	0.21844	0.163	-1.3406	0.1512
			Day 3	0.2872	0.21844	0.835	-0.4587	1.0331
			Day 4	-0.1560	0.21844	0.989	-0.9019	0.5899
			Day 5	0.6575	0.21844	0.101	-0.0884	1.4034
			Day 10	-1.6388*	0.21844	0.000	-2.3847	-0.8929
		Day 3	Blank	1.1614*	0.21844	0.002	0.4155	1.9073
			Control (Day 0)	-.8819*	0.21844	0.016	-1.6278	-0.1360
			Day 2	-0.2872	0.21844	0.835	-1.0331	0.4587
			Day 4	-0.4432	0.21844	0.440	-1.1891	0.3027
			Day 5	0.3703	0.21844	0.629	-0.3756	1.1162
			Day 10	-1.9259*	0.21844	0.000	-2.6718	-1.1800
		Day 4	Blank	1.6045*	0.21844	0.000	0.8586	2.3504
			Control (Day 0)	-0.4387	0.21844	0.451	-1.1846	0.3072
			Day 2	0.1560	0.21844	0.989	-0.5899	0.9019
			Day 3	0.4432	0.21844	0.440	-0.3027	1.1891
			Day 5	.8135*	0.21844	0.029	0.0676	1.5594
			Day 10	-1.4828*	0.21844	0.000	-2.2287	-0.7369
		Day 5	Blank	.7910*	0.21844	0.035	0.0451	1.5369
			Control (Day 0)	-1.2522*	0.21844	0.001	-1.9961	-0.5083
			Day 2	-0.6575	0.21844	0.101	-1.4034	0.0884
			Day 3	-0.3703	0.21844	0.629	-1.1162	0.3756
			Day 4	-.8135*	0.21844	0.029	-1.5594	-0.0676
			Day 10	-2.2963*	0.21844	0.000	-3.0422	-1.5504
		Day 10	Blank	3.0873*	0.21844	0.000	2.3414	3.8332
			Control (Day 0)	1.0441*	0.21844	0.004	0.2982	1.7900
			Day 2	1.6388*	0.21844	0.000	0.8929	2.3847
			Day 3	1.9259*	0.21844	0.000	1.1800	2.6718
			Day 4	1.4828*	0.21844	0.000	0.7369	2.2287
			Day 5	2.2963*	0.21844	0.000	1.5504	3.0422

APPENDIX X

Post-Hoc Test forAcetic Acid Content Across Different Fermentation Durations (Agarwood Leaves Treatment)

Agarwood	Tukey HSD	Blank	Control (Day 0)	-1.4449*	0.25307	0.001	-2.3090	-0.5808
			Day 2	-1.5600*	0.25307	0.000	-2.4241	-0.6959
			Day 3	-0.6835	0.25307	0.169	-1.5476	0.1806
			Day 4	-0.5292	0.25307	0.407	-1.3933	0.3349
			Day 5	0.3538	0.25307	0.795	-0.5105	1.2177
			Day 10	-3.0066*	0.25307	0.000	-3.8707	-2.1425
		Control (Day 0)	Blank	1.4449*	0.25307	0.001	0.5808	2.3090
			Day 2	-0.1151	0.25307	0.999	-0.9792	0.7490
			Day 3	0.7613	0.25307	0.102	-0.1028	1.6254
			Day 4	.9157*	0.25307	0.035	0.0516	1.7798
			Day 5	1.7985*	0.25307	0.000	0.9344	2.6626
			Day 10	-1.5617*	0.25307	0.000	-2.4258	-0.6976
		Day 2	Blank	1.5600*	0.25307	0.000	0.6959	2.4241
			Control (Day 0)	0.1151	0.25307	0.999	-0.7490	0.9792
			Day 3	.8765*	0.25307	0.046	0.0124	1.7406
			Day 4	1.0308*	0.25307	0.015	0.1667	1.8949
			Day 5	1.9136*	0.25307	0.000	1.0495	2.7777
			Day 10	-1.4466*	0.25307	0.001	-2.3107	-0.5825
		Day 3	Blank	0.6835	0.25307	0.169	-0.1806	1.5476
			Control (Day 0)	-0.7613	0.25307	0.102	-1.6254	0.1028
			Day 2	-.8765*	0.25307	0.046	-1.7406	-0.0124
			Day 4	0.1543	0.25307	0.995	-0.7098	1.0184
			Day 5	1.0372*	0.25307	0.015	0.1731	1.9013
			Day 10	-2.3231*	0.25307	0.000	-3.1872	-1.4590
		Day 4	Blank	0.5292	0.25307	0.407	-0.3349	1.3933
			Control (Day 0)	-.9157*	0.25307	0.035	-1.7798	-0.0516
			Day 2	-1.0308*	0.25307	0.015	-1.8949	-0.1667
			Day 3	-0.1543	0.25307	0.995	-1.0184	0.7098
			Day 5	.8828*	0.25307	0.044	0.0187	1.7469
			Day 10	-2.4774*	0.25307	0.000	-3.3415	-1.6133
		Day 5	Blank	-0.3538	0.25307	0.795	-1.2177	0.5105
			Control (Day 0)	-1.7985*	0.25307	0.000	-2.6626	-0.9344
			Day 2	-1.9136*	0.25307	0.000	-2.7777	-1.0495
			Day 3	-1.0372*	0.25307	0.015	-1.9013	-0.1731
			Day 4	-.8828*	0.25307	0.044	-1.7469	-0.0187
			Day 10	-3.3602*	0.25307	0.000	-4.2243	-2.4961
		Day 10	Blank	3.0066*	0.25307	0.000	2.1425	3.8707
			Control (Day 0)	1.5617*	0.25307	0.000	0.6976	2.4258
			Day 2	1.4466*	0.25307	0.001	0.5825	2.3107
			Day 3	2.3231*	0.25307	0.000	1.4590	3.1872
			Day 4	2.4774*	0.25307	0.000	1.6133	3.3415
			Day 5	3.3602*	0.25307	0.000	2.4961	4.2243

APPENDIX Y

AVNOVATest forAcetic Acid Content Between Treatments at Different Fermentation Durations

Tests of Between-Subjects Effects

Dependent Variable: Acetic_acid_content

Duration	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Blank	Corrected Model	.149[a]	1	0.149	3.889	0.120
	Intercept	11.292	1	11.292	294.882	0.000
	Treatment	0.149	1	0.149	3.889	0.120
	Error	0.153	4	0.038		
	Total	11.594	6			
	Corrected Total	0.302	5			
Day 0	Corrected Model	.120[b]	1	0.120	0.854	0.408
	Intercept	58.252	1	58.252	413.423	0.000
	Treatment	0.120	1	0.120	0.854	0.408
	Error	0.564	4	0.141		
	Total	58.936	6			
	Corrected Total	0.684	5			
Day 2	Corrected Model	.273[c]	1	0.273	1.774	0.254
	Intercept	49.632	1	49.632	322.682	0.000
	Treatment	0.273	1	0.273	1.774	0.254
	Error	0.615	4	0.154		
	Total	50.520	6			
	Corrected Total	0.888	5			
Day 3	Corrected Model	.040[d]	1	0.040	6.665	0.061
	Intercept	31.582	1	31.582	5296.804	0.000
	Treatment	0.040	1	0.040	6.665	0.061
	Error	0.024	4	0.006		
	Total	31.646	6			
	Corrected Total	0.064	5			
Day 4	Corrected Model	.867[e]	1	0.867	15.911	0.016
	Intercept	35.684	1	35.684	654.857	0.000
	Treatment	0.867	1	0.867	15.911	0.016
	Error	0.218	4	0.054		
	Total	36.769	6			
	Corrected Total	1.085	5			
Day 5	Corrected Model	1.032[f]	1	1.032	46.527	0.002
	Intercept	15.179	1	15.179	684.096	0.000
	Treatment	1.032	1	1.032	46.527	0.002
	Error	0.089	4	0.022		
	Total	16.300	6			
	Corrected Total	1.121	5			
Day 10	Corrected Model	.082[g]	1	0.082	0.482	0.526
	Intercept	117.154	1	117.154	684.759	0.000
	Treatment	0.082	1	0.082	0.482	0.526
	Error	0.684	4	0.171		
	Total	117.921	6			
	Corrected Total	0.767	5			

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FACULTY OF SCIENCE

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ID Number(s)	2207451
Programme / Course	Bachelor of Science (Hons) Food Science
Title of Final Year Project	Evaluation of Dynamic Physicochemical Properties Changes of Purple Sweet Potato (*Ipomoea batatas* (L.) Lam) Vinegar through Stepwise Fermentation

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ChangYP

Signature of Supervisor

Name: Dr. Chang Ying Ping

Date: 8 October 2025